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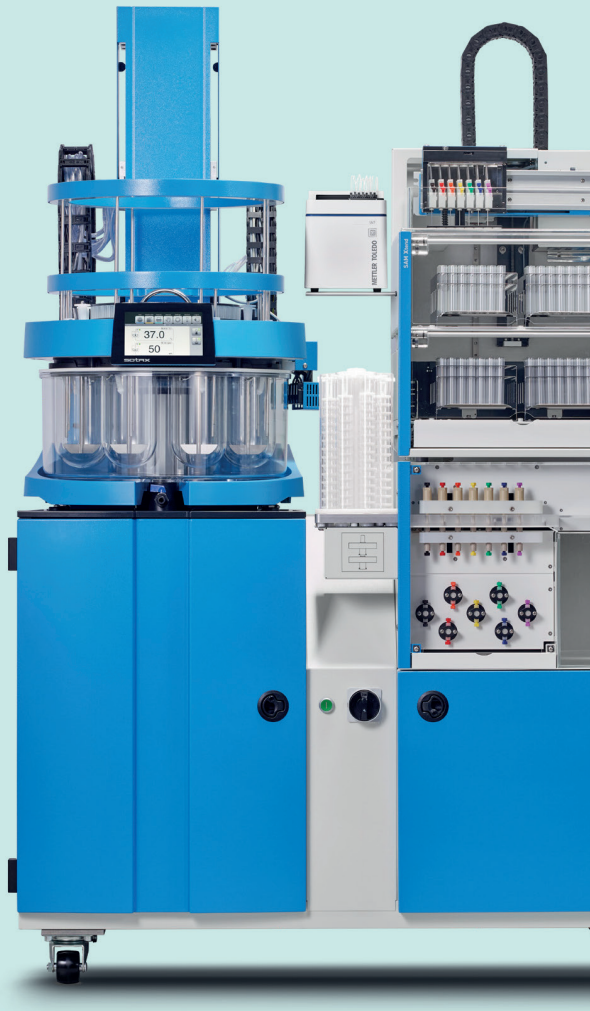

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In Vitro Performance Tests for Continuous Manufacturing: The Impact on the Current Compendial Framework from the Viewpoint of the USP New Advancements in Product Performance Testing Expert Panel

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ABSTRACT

As continuous manufacturing (CM) evolves from an emerging to widely adopted technology by industry in drug product manufacturing, the compendial framework in product performance testing is also being evaluated for its applicability in CM. As such, the CM Working Group of the New Advancements in Product Performance Testing (NAPPT) Expert Panel was convened in 2019 to review the current standard for product performance testing, identify gaps in its applicability to CM, and recommend the development of new standards to support the adoption of advancing technologies industry-wide. This *Stimuli* article discusses the challenges and limitations of the current performance testing by dissolution for CM applications. It also presents recommendations on alternatives or surrogate methods, including in/at-line process analytical technology methods, with a decision tree to support users in identifying an option that is fit for their process. The Expert Panel seeks stakeholder feedback on the recommendations presented in this *Stimuli* article, and requests additional comments on the perceived challenges and limitations of performance testing.

INTRODUCTION

Continuous Manufacturing (CM) is considered one of the most important innovations to modernize the pharmaceutical industry (*1*). Unlike conventional batch processes, which comprise a series of disconnected unit operations, the CM process includes a single integrated process train end-to-end. Starting material is continuously fed into the train, while the finished product gets continuously harvested from the train. Since the first successful application of CM to commercial manufacturing of Orkambi by Vertex in 2015, seven CM products from four different companies have been approved for the market (*2*), with many more in the clinical stage.

Some of the key benefits of CM over batch process include reduced manufacturing footprint, elimination of process scale-up between development and commercial manufacturing, flexible supply with the production duration adjusted according to demand, reduced equipment downtime, and eliminated process intermediate transfer. As a result, up to 50% reduction in manufacturing cost has been demonstrated (*3*). In addition, process control parameters could be varied over pre-defined time intervals during a single development

process to quickly and efficiently execute design of experiment (DOE) to explore the process design space. This agile DOE and real-time process monitoring by process analytical technology (PAT), which is typically an integral part of CM, allows the generation of rich process insights that significantly improves process robustness and final product quality.

However, the continuous nature of CM also poses some unique challenges from both regulatory and technical perspectives, such as batch definition, process validation, and advanced process and product quality control strategies. In this article, the CM Working Group of the New Advancements in Product Performance Testing (NAPPT) Expert Panel discusses some specific challenges around product performance testing and some possible solutions.

CURRENT REGULATORY AND COMPENDIAL FRAMEWORK FOR PERFORMANCE TESTING

Conventional methods for ensuring final drug product quality in standard batch manufacturing such as those in pharmacopeia (United States or any other pharmacopeia) are reliable. Monograph tests, analytical procedures, and acceptance criteria for testing oral drug products are divided into two categories: general product quality attributes and drug product performance tests. Drug product performance tests are designed to assess in vitro drug release from dosage forms (e.g., Dissolution <711> (*4*) and Drug Release <724> (*5*).

The regulatory requirement for the quality of the product in CM remains the same as in conventional batch processing. The drug product performance, which is typically measured with dissolution, is a specific quality attribute that links to bioavailability and bioequivalence studies (*2*). Therefore, the dissolution method should be meaningful, able to characterize the quality of the drug product and capable of distinguishing significant changes in the formulation or manufacturing process that might affect the in vivo performance, and should be sensitive to any changes in product integrity during its shelf life.

Dissolution can also link product quality to in vivo performance through in vivo-in vitro correlations and relationships (IVIVC/IVIVR). This correlation enables the use of dissolution data as a tool for evaluating any post-approval changes to the formulation or manufacturing process, as well as for the development and approval of generic products. It is used as an effective tool to waive in vivo bioequivalence (BE) clinical study requirements, per Scale Up and Post Approval Changes (SUPAC) guidance (see also *In Vitro and In Vivo Evaluation of Oral Dosage Forms* <1088> (*6*) and *Assessment of Solid Oral Drug Product Performance and Interchangeability, Bioavailability, Bioequivalence, and Dissolution* <1090> (*7*)).

The biopharmaceutics classification system (BCS) is commonly applied as a framework for risk assessment when determining the approach for product performance assurance (*7*). For highly soluble drugs, dissolution testing can be replaced by disintegration testing if it is shown that the active pharmaceutical ingredient is highly soluble, the formulation is rapidly releasing (*8*), and a relationship between dissolution and disintegration is established.

Limitations of Current Dissolution Performance Test

While dissolution testing has been widely used in the pharmaceutical industry for formulation development, batch-to-batch quality assurance, product stability and release, and regulatory acceptance of bioequivalence and biowaiver, several limitations associated with the test have also been identified. These limitations can be divided into 3 aspects. The first is related to the test operation. Dissolution testing is time consuming. A normal test run, not including finish detection and data processing, can take up to 1 h for immediate release dosage forms, 3 h for controlled release dosage forms, and much longer for extended-release dosage forms. The test relies on relatively large equipment in a laboratory setting, is not suitable for in-line operation, and can be very challenging for at-line operation. It is a destructive test, and generates a large quantity of aqueous waste, which has an adverse environmental impact.

The second aspect is related to the variability of the test. Dissolution testing can exhibit greater variability than other testing methods for product quality assessment, such as for assay and content uniformity. While some of these potential sources of variance can be reduced or controlled by optimizing the method, they can potentially be reduced even further by substituting PAT data-based dissolution modeling prediction, as will be discussed for use in CM product release.

The third aspect is the biorelevance of the testing. Dissolution testing conditions defined in the pharmacopeia are very different from an in vivo environment, including the volume, media, and mechanisms of agitation. Many dissolution methods developed using compendia! equipment as a quality control tool for manufacturing may not lead to data that can be correlated to in vivo performance. In recent years, significant efforts have been made to develop biorelevant dissolution methods and set clinically relevant specifications (*9*).

Current dissolution testing has other limitations when being considered for use with CM (*10*), which will be discussed in the section below.

ANALYTICAL CHALLENGES OF CONVENTIONAL DISSOLUTION TESTING IN A CONTINUOUS PROCESS

Performance testing of CM batches can be done by off-line traditional dissolution testing per USP <711> or per *Ph. Eur.* 2.9.3 via physical sampling. As CM is still a relatively new technology in drug manufacturing, there hasn't been any well-established documented procedure or guidance defining dissolution testing strategies, specifically in sampling and testing frequency. ICH Q13 (*11*), which is currently in step 3 under public consultation and expected to be officially adopted by the end of 2022, provides guidelines in sampling strategies for process monitoring and control, but it excludes sampling for release testing, especially in the context of physical sampling for offiine testing. This is a unique challenge that CM sponsors face when justifying the testing strategy against expectations from health authorities. For a traditional batch process, dissolution sampling and testing for batch release is typically done post-production, and is achieved by testing a composite sample following well defined and established sampling requirements per USP <711> or *Ph. Eur.* 2.9.3.

However, for a continuous process, the production is defined by time, and the concept of a "composite sample" is quite different from a traditional batch process. There is no well-defined guideline on composite sampling for a CM process, and there may be different expectations from health authorities of different regions. For a sponsor that submits globally, the most complex and conservative sampling procedure usually prevails. The complex sampling procedure may not be an issue through data sampling using in-line measurement if real-time release testing (RTRT) is employed; however, it can be burdensome if sampling is done through physical sampling followed by traditional offline testing. For stratified composite sampling throughout the continuous process, one has to carefully design the sampling probe and sampling point and may need to introduce additional sampling diverter valves in order to not disturb the material flow.

Compared to batch processes, the amount of sampling required by health authorities for a continuous process is generally significantly higher. In one example of a marketing application for a film-coated tablet product, a sampling request of up to 12% of the coating runs was made, which translated to hundreds of tablets for offline dissolution testing. From a practical and economical perspective, the additional and complex sampling throughout the process adds significant resource use and cost to the production. Testing of the composite sample collected through a continuous process for dissolution also faces unique challenges compared to a batch process. Because of the complex sampling design, the number of samples to be tested for dissolution may not be able to follow the staged testing and/or acceptance criteria defined in USP <711>.

The different expectations and requirements from different health authorities create additional challenges for the sponsor to manage a product globally. As there is no harmonized approach across regions, the sponsor would have to manage the dissolution release testing in multiple ways, each specifically tailored to meet different health authorities' requests, as some still follow the pharmacopeia, while others have very specific requirements for sampling and testing.

In this same example above, after rounds of open discussions with the health authority, the final agreed-upon sampling and testing strategy was to sample the process through 12 pre-defined segments, with traceability, and the USP <711> stage 2 criteria were applied for 12 tablets. The agreed-upon sampling and testing plan was based on significant development data and statistical analysis. This sampling and testing strategy, along with the application of USP stage 2 criteria, has subsequently been accepted by multiple major regions.

CM, by design, employs significantly more in-line measurement via PAT, resulting in significantly more process data than a typical batch process. Sampling frequency for a continuous process should take a risk-based approach, and should be determined based on development stage, product and process knowledge (i.e., through quality by design), and fit for the intended use of the data (e.g., making local process-stage vs batch-level quality statements). Once the process is validated for routine commercial production, the role of the physical sample measurements should change from being the primary indicator of quality to solely confirming quality, because quality is ensured by maintaining a state of control with the process parameters within acceptable ranges (*12*). With the amount of in-process monitoring and control implemented in the continuous process, the sampling for release testing should be simplified and harmonized.

The conversion from a batch process to a continuous process could also present challenges in performance testing, especially for a well established product. Can the

same sampling and testing strategy be applied from batch to continuous, or is a completely new set of strategies required, or somewhere in between? This remains a point of uncertainty with regard to requirements from global regulatory bodies.

POSSIBLE ALTERNATIVES OR SURROGATES TO CONVENTIONAL PERFORMANCE TESTING

As mentioned in the previous section, most standard compendia! performance tests are not compatible with the requirements of CM. Suitable performance tests in a CM environment should be real or at least near-real-time, and optimally nondestructive. There are two principal approaches to collecting the required results. The first is on- or at-line tests, such as at-line disintegration test for highly soluble drugs. The second is leveraging data from one or more of the many measurements gathered in the data rich environment associated with CM to create a preferable nondestructive surrogate test. This approach may rely on predictive modeling to convert collected data into the surrogate performance test results. By establishing a relationship/correlation between dissolution and other methods or process parameters, the documented control of this/these parameter(s) during manufacturing will allow for elimination of the requirement for conventional dissolution tests.

As is often the case with analysis of solid oral dosage forms, the nature of the performance tests required to validate product quality correlate to the BCS Classification of the product, and in particular, the solubility. This does not change in a CM environment. Therefore, the recommendations for possible alternative measurement techniques are divided into those for highly soluble and poorly soluble drugs.

Highly Soluble Drugs (BCS Class 1 and 3)

As is also the case in batch manufacturing, one possible approach to testing BCS Class 1 and 3 drugs is to use conventional disintegration testing as a surrogate for the dissolution test (*13*, *14*). To better harmonize the time scale of disintegration testing with CM, one can possibly switch to at-line testing. The advantages of this approach are that it is based on existing, well-defined testing methodologies and that for a product being converted from a batch process to CM, the protocol can be transferred intact. The main disadvantage of this approach is that because disintegration is a destructive test, there is no option for 100% monitoring. Also, while brief, disintegration testing is not real-time, eliminating the possibility of true continuous monitoring.

The alternative method would be a Quality by Design (QbD) approach based on the use of predictive modeling. These models may be based on a single parameter or a combination of measurements. An example of the first approach is to substitute a near-infrared (NIR) spectroscopic measurement for the disintegration test. This virtually eliminates the limits on both the number of units sampled and the time between samples. If required, NIR data can be supplemented or replaced in the model by the addition of particle characterization data on the active pharmaceutical ingredient (API) and excipients, dosage form hardness data, coating thickness data, etc.

BCS Class 2 and 4

For BCS Class 2 and 4 drugs, the available alternative method to conventional dissolution is a QbD approach based on the use of predictive modeling. Again, with sufficient validation, these models may be based on a single parameter or a combination of measurements, with the latter probably being more appropriate for these products. As is common in such modeling (for example, see Reference 10 and all the citations included therein) the required measured inputs for the model will need to account for ongoing variations in characteristics of all the constituent materials, the manufacturing process (wet or dry granulation, hot-melt extrusion, spray-dried dispersion, etc.), and any other process that may affect the final product. These parameters may be part of the existing set of PAT measurements that are already included as part of the rest of the CM control strategy or may require additional readings and sensors to comprise a sufficient set.

Limitations of Alternative or Surrogate Methods

The application of surrogate dissolution testing is a new and rapidly evolving field. Because of this, there are relatively few examples of approved products using surrogate dissolution testing. For predictive modeling, the inputs needed to predict product performance may vary widely depending on the type of process used, and the properties of the drug substance and drug product formulation. This makes it challenging to define standards for surrogate testing. Any standard would need to be flexible enough to encompass both emerging technology and the variety of inputs and models that may be leveraged to predict dissolution performance.

For CM processes, the number of samples needed to demonstrate adequate control and consistent performance is larger than typically required for a batch process. Currently, there is no guidance on approaches to select the appropriate sampling frequency. The larger number of samples can limit the application of on- or

at-line testing. While at-line disintegration testing is commonly applied in a manufacturing setting, the higher frequency of testing needed to assure performance may introduce additional challenges such as the need for additional operator training, waste handling, and long test times relative to the required sampling frequency. These issues are magnified for at-line dissolution testing, which requires larger volumes of medium and spectroscopic or chromatographic analysis endpoints.

The larger number of samples for a typical CM process also leads to uncertainty on how to apply the current USP acceptance criteria for dissolution, which is based on low "n" sampling. A statistical approach may be used to determine the probability of passing each stage based on the larger number of results or predictions from a continuous run. This approach has not yet been standardized.

For predictive dissolution modeling, there is also uncertainty around how much of the profile needs to be predicted to assure adequate control of product performance. While traditional acceptance criteria may only require testing a single time point in the dissolution profile, the ability to predict the entire profile may provide additional assurance of product performance. This, however, has potential to increase the complexity of model development and validation, as well as setting acceptance criteria.

Finally, surrogate performance testing still relies on the existence of a reference dissolution method to act as the surrogate for in vivo product performance. Therefore, the in vivo relevance of the surrogate model can be no better than the in vivo relevance of the reference method. As advances continue in the field of predictive absorption modeling, consideration should be made for the ability to predict absorption directly without the need for the intermediate dissolution prediction.

RECOMMENDATIONS AND CONCLUSIONS

The challenges and limitations of performance testing in a CM process under the current compendial framework are discussed, with alternative surrogates and approaches recommended. To potentially utilize alternative methods to dissolution testing for solid oral dosage forms and enabling RTRT, it is recommended first to clearly examine the dissolution mechanism and understand the risks and factors impacting the dissolution performance of the dosage form. This dissolution mechanism is not only dependent on the solubility and form and particle morphology of the drug but might also depend on the manufacturing process and formulation properties (i.e., excipient selection and properties).

The dissolution mechanism and risk assessment should be used as a guide for selection of the possible surrogate test or dissolution model or if replacing dissolution as a release test is not appropriate. In addition, the overall biopharmaceutic risk for potential dissolution changes on bio performance should be considered when selecting a potential surrogate measure. The decision tree in Figure 1 can be used as starting point for selection of possible alternative methods as RTRT for dissolution.

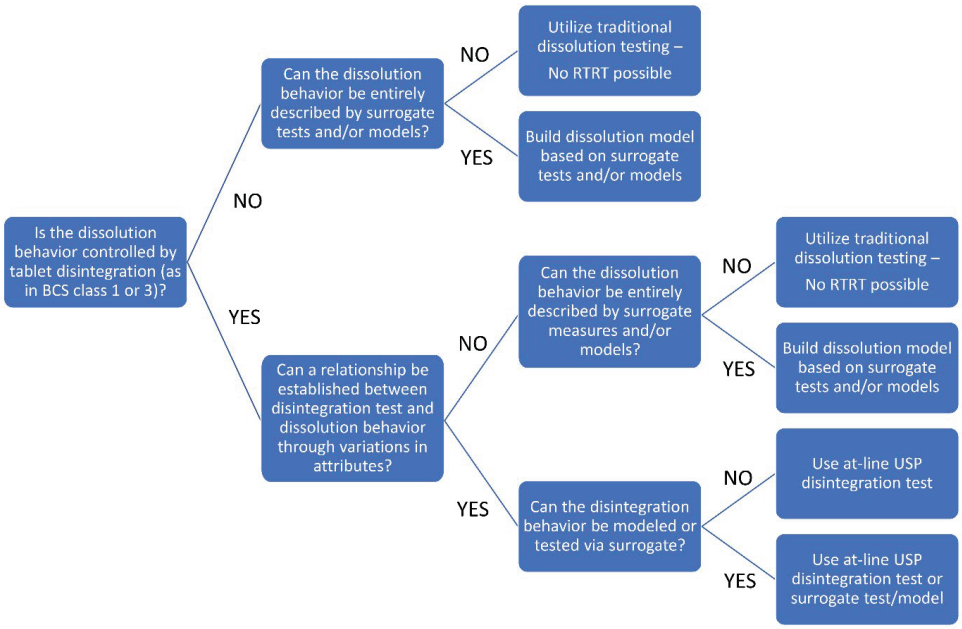

Figure 1. Decision tree for determining if a real-time release alternative or surrogate method to dissolution can be implemented in the continuous manufacturing process for a drug product.

As discussed, sampling for release testing in CM is another challenge that currently doesn't have clear guidance. A risk-based sampling strategy is recommended. Development stage-based sampling may be considered. A harmonized approach on sampling strategy for product performance release testing that is acceptable globally is highly desired. Therefore, this USP Expert Panel recommends that a new standard or addendum to an existing standard be developed that covers the topics of sampling frequency, acceptance criteria application, and bridging the compendia! reference method with the surrogate method.

CONFLICT OF INTEREST

Expert Panel members may have conflicts of interest; the following named author of this article declared a conflict of interest: Carrie A. Coutant is an employee of Eli Lilly and Company.

DISCLAIMER

This article reflects the views of the author and should not be construed to represent the FDA's views or policies.

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Effect of Mannitol Particle Size on Melatonin Dissolution and Tablet Properties using a Quality by Design Framework

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ABSTRACT

The objective of the current study was to develop an optimized formulation for orally disintegrating tablets (ODTs) containing melatonin. Different particle sizes of mannitol (i.e., filler) were used to study the effects on dissolution and tablet properties using a quality by design (QbD) approach. The quality target product profile was identified, then critical quality attributes (CQAs) were determined. Risk assessment was performed using the Failure Mode Effect Analysis (FMEA) method to identify and rank critical material attributes and process parameters. Thirty-four formulations were prepared and tested. Box-Behnken design (BBD) with response surface methodology was applied to assess the effect of independent factors on CQAs. Finally, the most suitable formulation in terms of tablet properties was determined with Minitab. Specification tests were applied to confirm that the optimized formula met USP requirements. Mannitol with a small particle size had the fastest disintegration time and dissolution rate in ODT formulations containing melatonin.

KEYWORDS: Quality by design, orally disintegrating tablet, dissolution, melatonin, optimization, mannitol

INTRODUCTION

Melatonin (MLT) is a natural hormone that can be found in different biological fluids and synthesized in the pineal glands, providing a circadian presence (*1*, *2*). Endogenous pineal MLT demonstrates chronobiotic influence properties by reducing circadian signals of the suprachiasmatic nuclei to induce sleepiness, improve sleep, and induce GABAA-benzodiazepine receptor complex (*3*).

The British Association for Psychopharmacology reported that MLT is the first-choice treatment for insomnia, parasomnia, and circadian rhythm sleep disorders (*4*). Specifically, long-term or time zone travelers can confront circadian rhythm confusions (*5*). MLT reaches a maximum t_{max} value at 30-60 min after oral administration (*6*). Taking MLT just before long flights will help eliminate fatigue and circadian rhythm disorders due to flight (*7*).

The orally disintegrating tablet (ODT) is a solid dosage form that disintegrates rapidly, usually within a matter of seconds, when placed upon the tongue (*8*). It has many advantages such as solid and liquid dosage form along with the particular benefit that dissolving rapidly in saliva causes the drug to be absorbed in the mouth, pharynx, and esophagus, therefore the pregastric absorption of drugs avoid the hepatic metabolism, and thus the bioavailability of the drug can be increased (*9–11*). Accordingly, ODTs are among the most patient-friendly dosage forms (*12*).

Pharmaceutical development is a powerful bridge that links knowledge gained through quality risk management to the improvement of a product and its manufacturing process. Quality by design (QbD) is an effective and systematic approach to pharmaceutical development (*13*), which commences with pre-determined goals, emphasizes product and process understanding, and process control according to sound science and quality risk management (*14*, *15*).

In accordance with ICH guideline Q8(2), quality cannot be examined into products; it should be built-in by design

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(*16*). The process and product design performed via the QbD approach decreases the role of finished product tests and therefore ensures control quality at the design stage (*17*).

QbD consists of elements to provide desired quality, along with a safe and efficient drug product. Identifying the quality target product profile (QTPP) creates the basis of design for the product development and manufacturing process. The next step of the approach identifies critical quality attributes (CQA), which are essential for patients' health as well as the drug's physical, chemical, biological, or microbiological properties within an appropriate limit for desired quality. Understanding the development of the drug product and its manufacturing process depends on establishing functional relationships between CQAs and critical material attributes (CMAs). CQAs are for output materials, including product intermediates and finished drug products, whereas CMAs are for input materials, including drug substances and excipients. Process parameters are referred to as the input operating parameters of process steps, and how their variability impacts the CQAs (*18–20*). A better understanding of the relationship between these variables and product quality aids in risk management, enhances problem detection, raises timely risk control measures (*14*), and maintains a state of control throughout the lifecycle (*21*).

Support can be obtained from various artificial intelligence programs to establish a relationship between all statistical methods, formulation inputs and outputs, and facilitate their evaluation (*22*). Artificial neural networks (ANNs) are applications that learn through experience with appropriate learning examples, not through the program. Moreover, it collects information by identifying patterns and relationships in the data (*23*). Formulation development and optimization studies are carried out using ANNs and have become increasingly more important in drug development studies, especially in the digital era (*24*).

The present investigation was performed to develop and optimize an ODT containing MLT using two different types of mannitol as fillers with varying particle sizes. A QbD framework was used with various statistical tools and multi-objective optimization to understand the dissolution behavior and tableting properties of these excipients.

MATERIALS AND METHODS

Materials

Ready-to-use tablet excipients were received as gifts from suppliers, including Kollidon CL-SF (BASF, Germany), Parteck M100 and M200 (Merck, Germany) with average particle sizes of 70 and 150 µm, respectively, and Parteck LUB (Merck). Melatonin powder was gifted from Swati Spentose PVT. Ltd. (India). All other chemicals and solvents were of analytical grade and high-performance liquid chromatography (HPLC) grade. Water for the study was generated using a Milli Q Water System (EMD Millipore, Germany).

Defining the QTPP and CQAs

The initial step of the QbD framework is defining the QTPP. The desired quality properties of the pharmaceutical product are listed as quantitative attributes (*26*). Therapeutic indication, route of administration, site of activity, dosage form, dose strength, and details of the QTPP elements, and CQAs of MLT ODTs along with justification and reasonable limits to ensure desired product quality are presented in Table 1.

Risk Assessment

Risk assessment methodology was applied to MLT ODTs according to the ICH Q9 guideline (*14*). A risk assessment of the overall process was performed to identify the high-risk procedures that may impact the CQAs of the final drug product. This was achieved using Failure Mode Effect Analysis (FMEA) methodology. Severity, probability, and detectability of possible risks were assessed, and a risk priority number (RPN) was calculated to rank the risks.

Pareto charts were used to identify the critical factors (CMAs and CPPs) that affect quality (CQAs). In addition, a pareto chart helps to identify which factors to focus on (*27*).

Experimental Design

Response surface methodology (RSM) has dependent and independent variables within a particular series of statistical designs that investigates the impact on the response surface of independent process variables (*28*). Variability in the formulations (e.g., lubricant and superdisintegration concentration) and process variables (e.g., tablet compression pressure) may result in product quality failures throughout the shelf life, which may impact patients' health. It is essential to specify CMAs and critical process parameters (CPP) for CQAs in the QbD approach.

For this purpose, ICH Q9 leads risk management, improves problem detection, and promotes timely risk control (*16*). Therefore, a Box-Behnken design (BBD) with RSM was chosen to evaluate the effect of three independent factors, including filler particle size, disintegrant, and tablet compression pressure. Dependent factors were dissolution rate, disintegration time, tablet breaking

Table 1. Quality Target Profile (QTPP) and Critical Quality Attributes (CQAs) for Melatonin (10 mg) ODTs.

QTPP Elements	Target	Justification / Comments	
Therapeutic indication	Sleep disorders, others*	Melatonin is considered the first treatment of insomnia	
Route of administration	Oral	Easy to administer, patient acceptability and compliance	
Site of activity	Systemic	Melatonin is a hormone produced by the pineal gland and has systemic effects	
Dosage form	Orally disintegrated tablet	Fast drug release, fast activity	
Dose strength	10 mg	Commonly accepted strength	
Quality attributes of pharmaceutical product	Product Target	Is it a CQA?	Justification
Apperance	Color and shape acceptable to the patient. No visual tablet defects observed	No	Color, shape, and appearance are not directly related to safety and efficacy. Therefore, they are not critical
Odor	Odorless	No	Odor is not directly related to safety and efficacy, but odor can affect patient compliance
Size	Easily handled by patients	No	Ease of dissolving in the mouth as well as patient compliance
Friability	Below 1.0%	Yes	Drug must have resistance to mechanical activities such as carrying, packaging, etc.
Breaking Force	Appropriate value to be hard enough and not affect the other CQAs (friability, disintegration time, and dissolution)	Yes	Affect friability test, disintegration time, and dissolution test of drug
Disintegration Time	< 30 s (*USP*) < 180 s (*EP*)	Yes	Affect dissolution time
Assay	10 mg ± 5%	Yes	Affect safety and efficacy
Dissolution	According to the USP, no less than 75% dissolution should occur in one hour (*40*, *41*)	Yes	Affect drug bioavailability

** Insomnia, parasomnia.*
ODT: oral disintegrating tablet; USP: United States Pharmacopeia; EP: European Pharmacopoeia.

force, and friability. Knowledge obtained by identifying, perceiving, and controlling inputs (CMAs and CPPs) and outputs (CQAs) and the manufacturing process facilitate establishment of the design space (*16*).

Preparation of Orally Disintegrating Tablets (ODTs)

MLT ODTs were prepared by direct compression technique with sufficient strength and rapid disintegration time under standardized conditions. Two types of mannitol (Parteck M100 and M200, 100–150 mg) were used as fillers due to their compressibility. All formulations contained Kollidon CL-SF (15–30 mg) as a super disintegrant. MLT (10 mg) was used as a model active pharmaceutical ingredient (API). The lubricant agent was Parteck LUB (3.5 mg). The tablet compression pressure was between 3.44 and 10.34 MPa. The quantitative composition and compression pressure of MLT ODT formulations are shown in Table 2. This study was designed as two series using both mannitol concentrations separately, thus comprising 34 formulations.

MLT, mannitol, , and the super disintegrant were weighed on an analytical balance with 0.1 mg accuracy (Sartorius, Germany), then were transferred into the cubic mixer (Aymes, Turkey) and mixed for 10 min at 100 rpm. Lubricant was then added to the mixture and blended for an additional 5 min. At appropriate weight and pressure, the final mixtures were directly compressed in a single punch tablet press (Yeniyurt, Turkey). Pressed tablets were stored in well-closed glass containers.

Characterization of MLT ODTs

Tablet Friability and Breaking Force

Tablet friability was evaluated by a friability test apparatus (Aymes, Turkey). Accurately weighed tablets were placed in the friabilator drum, rotated 100 times at 25 rpm, then reweighed. The difference in weight before and after rotation was calculated. The loss due to abrasion was expressed as a percentage. According to *USP* guidelines, weight loss of less than 1% is generally considered acceptable (n = 10) (*29*).

Table 2. Variables in Box-Behnken Design for Optimization of the Formulation and Composition.

Formulations	Mannitol Filler (Parteck M100 or M200) (mg)	Super Disintegrant (Kollidon CL-SF) (mg)	Tablet Compression Pressure (MPa)	Theoretical Weight of Tablet (mg)
F1*/ F18**	100	22.5	3.44	136.0
F2*/ F19**	125	22.5	6.89	161.0
F3*/ F20**	150	15.0	6.89	178.5
F4*/ F21**	125	15.0	3.44	153.5
F5*/ F22**	100	15.0	6.89	128.5
F6*/ F23**	150	22.5	3.44	186.0
F7*/ F24**	125	22.5	3.44	161.0
F8*/ F25**	125	30.0	6.89	168.5
F9*/ F26**	100	30.0	6.89	143.5
F10*/ F27**	125	30.0	10.34	168.5
F11*/ F28**	150	30.0	6.89	193.5
F12*/ F29**	100	22.5	10.34	136.0
F13*/ F30**	125	30.0	3.44	168.5
F14*/ F31**	125	22.5	10.34	161.0
F15*/ F32**	125	15.0	10.34	153.5
F16*/ F33**	125	15.0	6.89	153.5
F17*/ F34**	150	22.5	10.34	186.0

Note – Melatonin (10 mg) was active pharmaceutical ingredient; lubricant was Parteck LUB (3.5 mg).
**Formulations with Parteck M100 (F1–F17), ** Formulations with Parteck M200 (F18–F34.)*

Tablet breaking force was determined in a diametric compression tester (Sotax HT1, Switzerland) according to *USP* guidelines (n=10) (*30*).

Disintegration Time

A standard USP disintegration test apparatus (Sotax DT2) was used to measure tablet disintegration time. The test was carried out in 1000 mL of distilled water, maintained at 37 ± 0.5 °C. The disintegration time (DT) was determined visually for each formulation when all the tablets disintegrated completely (*31*). The mean value and the standard deviation of these determinations were computed (n = 3).

Dissolution Test Studies

An in vitro dissolution study was performed on a dissolution tester (Sotax AT2) using USP apparatus 2 (900 mL of 0.1 N HCl solution, 50 rpm, 37 ± 0.5 °C). Samples (3 mL) for the dissolution test (n = 3) were collected manually at regular time intervals (1, 3, 5, 10, 15, 20, and 30 min) without replacing the dissolution medium (medium loss was considered during the calculations). Samples were filtered through a 0.45-µm cellulose acetate filter (Alwsci, China) (*32*).

Sample concentration of MLT was determined using a HPLC system (Shimadzu A20, Japan), equipped with a photodiode array (PDA) detector at 220 nm. Chromatographic analyses were carried out at 30 °C on a 5-µm C18 Inertsil ODS-3 column (150 × 4.6 mm, GL Sciences, USA). Separation was achieved by isocratic elution with a flow rate of 1.0 mL/min and injection volume of 100 µl. A mixture of water and acetonitrile (60:40, v/v) was used as the mobile phase. MLT eluted at 3 min with a total run time of 8 min. The method was modified according to the study of Filali et al (*33*).

Statistical Analysis

Data were transferred from Microsoft Excel to Minitab 18 software. Statistical evaluation of the obtained data and effects of the independent variables on CQA parameters were analyzed using Minitab 18 software (Minitab Inc., USA); $p < 0.05$ was considered statistically significant.

Cell Viability Assay

Human colorectal adenocarcinoma Caco-2 cells (HTB-37) were purchased from ATCC (USA). Caco-2 cells were incubated under 5% CO_2 at 37 °C, and cell lines were cultured in Eagle's Minimum Essential Medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin. The cells were passaged with trypsin– ethylenediaminetetraacetic acid (EDTA) solution before confluency. In addition, in vitro cell viability was evaluated by the MTT test. Caco-2 cells were seeded in 96-well plates with a density of 10^4 cells per well with 100 µL Eagle's medium and incubated for 24 h for cells attachment. Subsequently, the culture medium in each well was removed, and MLT

with concentrations of 0.3125, 0.625, 1.25, 2.5, 5, and 10 mg/mL were added to the cells and incubated at 37 °C for 24 h. After this incubation period, 20 µL of MTT solution (5 mg/mL in phosphate buffered saline (PBS) was added to each well, then further incubated at 37 °C for 3 h. The culture medium was discharged, then 100 µL dimethyl sulfoxide (DMSO) was added to visualize MTT formazan purple crystals. The absorbance was measured on a microplate reader spectrophotometer (Biotek, USA) at 570 nm (n = 9).

RESULTS AND DISCUSSION

Risk Assessment of MLT ODTs

In the risk assessment study, a risk score of 200 and above was considered a high risk for failure (*34*). Tablet friability and breaking force, disintegration time, assay, and dissolution were 252, 216, 252, 200, and 250, respectively.

Characterization of MLT ODTs

Tablet Friability and Breaking Force

Friability is impacted by tablet mechanical strength, which defines how easily particles can be displaced from their original locations in the tablet when exposed to an external shear or impact stress (*35*). According to the USP limit, the weight loss for a single evaluation should be less than 1% (*29*).

Figure 1a displays the friability of all formulations. Almost all formulations were within the acceptable limits of weight loss, but a few formulations were more than 1%. Particularly, it was noticed that the friability was higher in formulations containing the smaller particle size of mannitol (M100), which may have poor strength (*36*).

Tablet breaking force results are presented in Figure 1b. The resulting values increased with an increase in tablet compression pressure, which is consistent with published literature (*37*). In addition, tablets compressed at 10.34 Mpa showed the highest breaking force values, as expected.

The particle size of excipients affects interparticulate bonds and the bonding force. For instance, smaller particles lead to an increasing number of bonds per cross-sectional area; hence, the bonding force per particle-particle bridging is larger for coarser particles. In other words, raw material with small particle size does not inevitably lead to higher mechanical tablet strength, for example, owing to changing the porosity or deformation behavior of the particles (*38*).

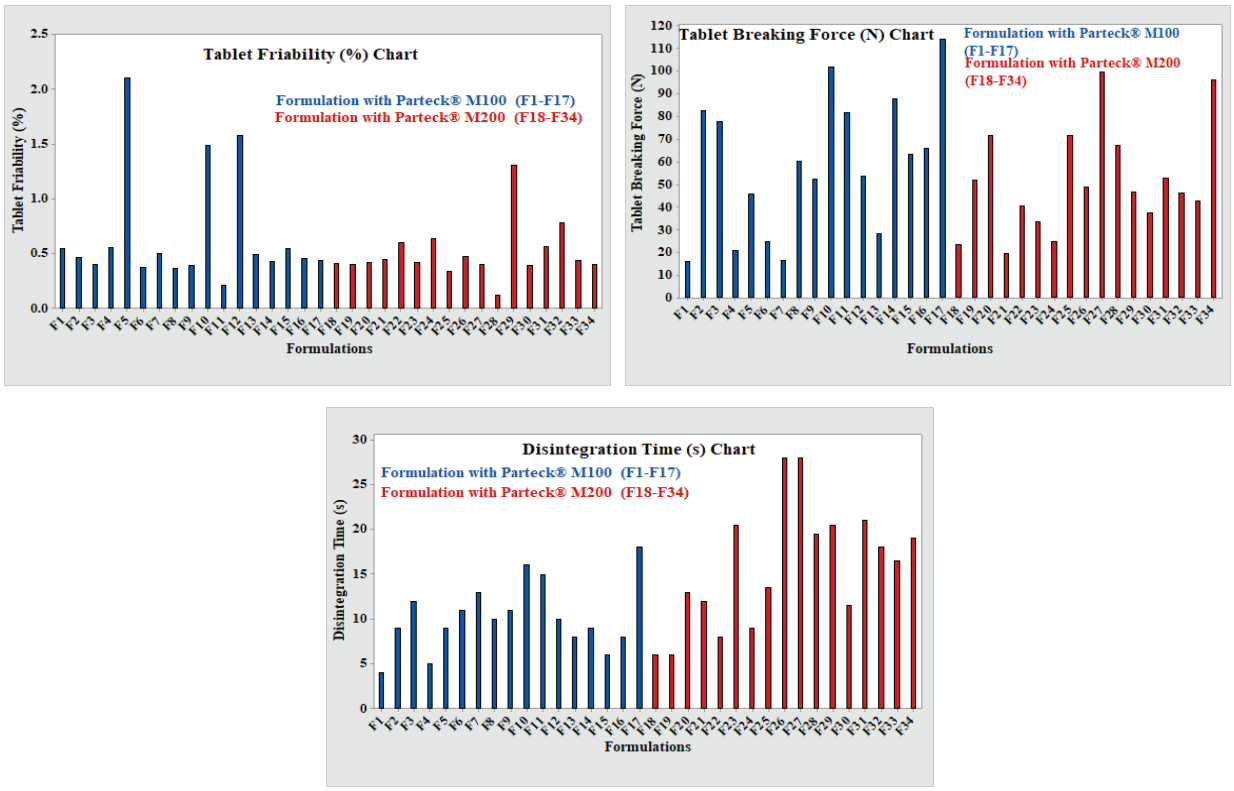

*Figure 1. Results of tablet friability (n = 10) (**a**), breaking force (n = 10) (**b**), and disintegration time (n = 3) (**c**) for all melatonin ODT formulations. Results are mean values. ODT: oral disintegrating tablet.*

Disintegration Time

Disintegration time of the ODT formulations was less than 30 seconds, but formulations containing M100 had a faster disintegration time compared with formulations containing larger mannitol particles (M200), as seen in Figure 1 (*39*). Tablets were prepared using wet granulation followed by tableting, which could produce granules with a smaller size distribution, which can positively impact the mechanical strength of tablets and negatively impact disintegration time and dissolution rate (*40*).

Dissolution Tests

The HPLC method was validated for selectivity, linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ). The validation parameters were found to be linear in a concentration range of 1.0–12 µg/ml (R^2 > 0.9998), accurate (recovery > 98%), precise (intra and inter-day variations < 2%). LOD and LOQ values were 0.07 and 0.21 µg/mL, respectively.

Figure 2 shows the results of dissolution studies. The prepared formulations with M100 showed the fastest dissolution behavior. The formulations comprised of larger mannitol particles (M200) were affected by compression pressure.

Statistical Analysis

All inputs, including filler particle size, different concentrations of super disintegrant, and different compression pressure applications, were found insignificant (p > 0.05) on assay and dissolution behavior at 1, 3, and 5 mins, with low regression values. MLT is BCS class I drug that can rapidly dissolve in the dissolution medium, precisely the parts close to the surface of tablets. Consequently, the inputs had no effect on the dissolution rate within the 5 mins. However, the effects of the inputs on friability, breaking force, disintegration time, and dissolution at 10, 15, 20, and 30 mins were significant (p < 0.05), with different regression values. The findings also suggest that the particle size of mannitol, mainly with tablet compression pressure, makes a significant difference on tablet properties (*27*).

The p-value was below 0.05 for all variables whose modeling capability (R^2) was also above 0.50. This finding provides evidence that inputs used in this study were critical parameters, having a significant effect on outputs. The greatest effects of inputs were on tablet friability, breaking force, and dissolution at 3 min (Fig. 3).

The parameters that had the highest effect on the breaking force were the tablet compression pressure, (M100 and tablet compression pressure), (M200 and tablet compression pressure), (tablet compression pressure and tablet compression pressure), and (super disintegrant and tablet compression pressure), respectively. M200 and tablet compression pressure were the main parameters affecting the friability. The most significant input parameters for dissolution at 3 min were tablet compression pressure and tablet compression pressure.

M100 and M200 (CMAs) and tablet compression pressure (CPP) affected disintegration time and breaking force (CQAs), as shown in contour plots (Fig. 4).

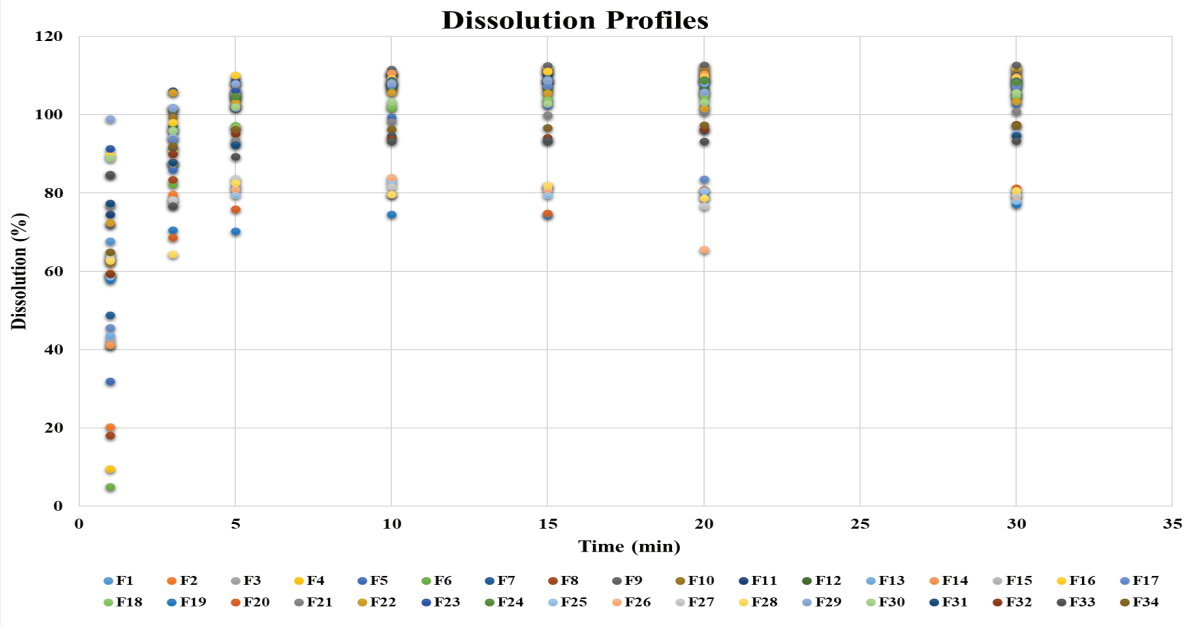

Figure 2. Dissolution results of melatonin ODT formulations (F1–F34). ODT: oral disintegrating tablet.

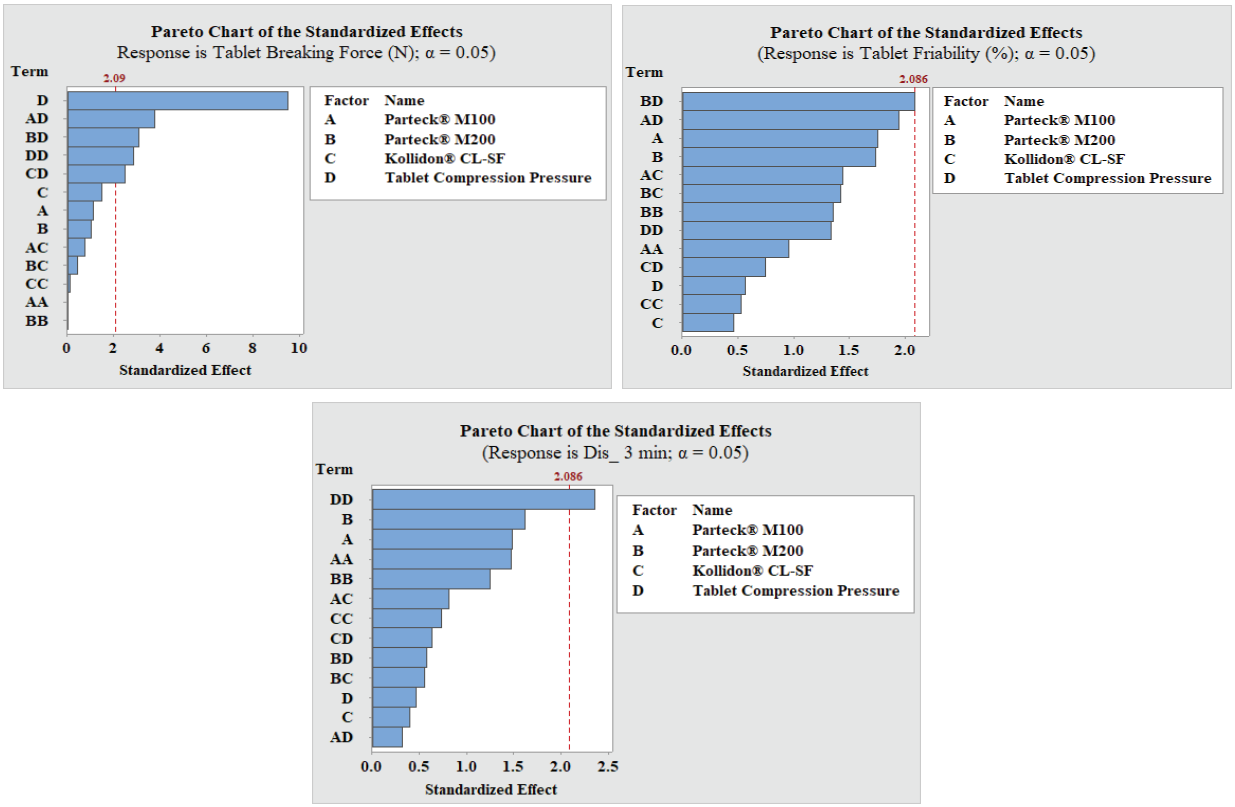

Figure 3. Pareto chart analysis of tablet breaking force, friability, and dissolution (Dis) 3 mins for melatonin ODT formulations. ODT: oral disintegrating tablet.

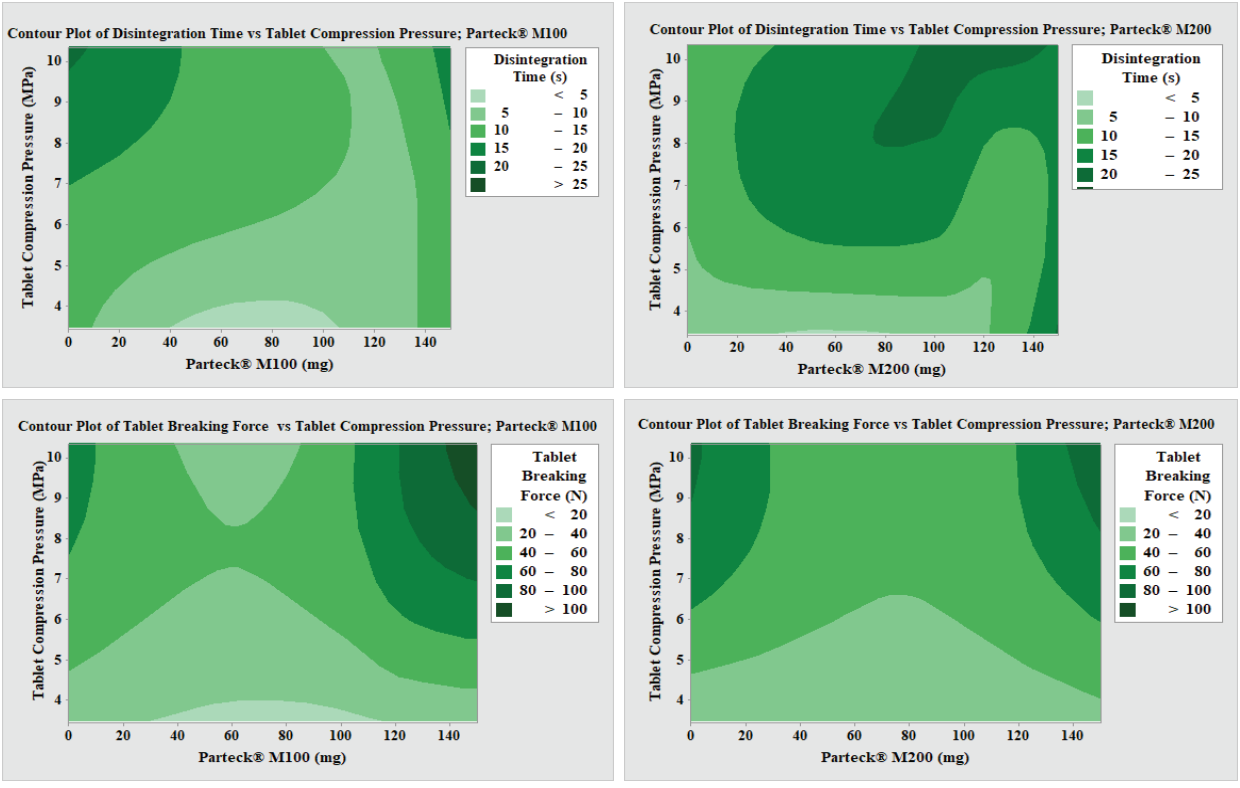

Figure 4. Contour plots of disintegration time and tablet breaking force for melatonin ODT formulations. ODT: oral disintegrating tablet.

Particle size and tablet compression pressure also affected disintegration time (Fig. 4).

As shown in Figure 4, M100 and M200 exhibited various behaviors, specifically in the disintegration time. For instance, M200 with a large particle size resulted in a longer disintegration time as tablet compression pressure increased.

CMAs and CPPs of the Optimized MLT ODT Formulation

As a result of the optimization analysis for MLT ODTs, the values that should be applied to formulation content and process parameters are: M100: 91.795 mg, M200: 0.054 mg, super disintegrant: 20.472 mg, and tablet compression pressure: 3.44 and 3.64 Mpa.

The disintegration time, friability, and breaking force tests were within the pharmacopeial limits (15 s, 0.48%, and 30 *N*, respectively). The dissolution profile of MLT ODTs showed rapid release around 1 min, a plateau around 5 mins, and more than 80% of drug was released at 30 min.

Cell Viability Assay

The cell viability of MLT was evaluated using the MTT test in Caco2 cells. The IC_{50} value was calculated as 11.6925 mg/mL. Cell viability decreased in a concentration-dependent manner. It was observed that 2.5, 5, and 10 mg/mL of MLT significantly reduced cell viability compared to the control group.

CONCLUSION

MLT ODTs were prepared using two different types of mannitol (Parteck M100 and M200), Kollidon CL-SF, and various tablet compression pressures. ODTs were successfully prepared, characterized, and optimized using the QbD approach. Mannitol showed different tablet characteristics according to particle size. Tablets with smaller particle size mannitol had a fast disintegration time and high friability and breaking force compared with tablets having larger mannitol particles. Tablet compression pressure had the greatest effect on the tablet characteristics. The study reveals how different CMA and CPP parameters affect dissolution studies (e.g., different types of the same excipient can affect different effects on dissolution), which is a primary decision criterion in determining bioavailability of drugs. Evaluation of the effects of both material and process parameters for product specifications can be accomplished using the QbD approach.

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CONFLICT OF INTEREST

The authors disclosed no conflicts of interest related to this article.

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Investigating the Influence of HPMC K4M and Eudragit L 100-55 on Guanfacine-Loaded Extended-Release Tablets

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ABSTRACT

This study aims to optimize the concentrations of hydroxypropyl methyl cellulose (HPMC) K4M and Eudragit L100-55 (methacrylic acid) in formulation of extended-release tablets containing guanfacine hydrochloride by employing a 3^2-factorial design approach. Extended-release tablets of guanfacine hydrochloride reduce the need for frequent dosing to achieve the desired therapeutic outcomes. Cumulative drug release after 1, 8, and 20 hours of dissolution were taken as target responses and concentrations for both polymers as the variables. 3D response surface and polynomial equations were generated for choosing the optimized formulation with the most favorable response. Suitability of the drug and excipients was assessed during preliminary evaluation and compatibility studies using an accelerated thermal stress study. The release kinetics of the formulations followed Hixson Crowell and Higuchi models, indicating slow erosion of polymer to release the drug over an extended time period. Validated optimization techniques confirmed predictability of the model. The stability study verified superiority of the optimized formulation after 3 months of storage.

KEYWORDS: Guanfacine hydrochloride, extended-release tablets, hydroxypropyl methyl cellulose (HPMC), Eudragit, response surface methodology, kinetic modeling

INTRODUCTION

Guanfacine hydrochloride is an effective α-adrenergic blocker that is useful in individuals with attention deficit hyperactivity disorder (ADHD) and hypertension (*1*, *2*). It is a BCS class I drug that gets absorbed rapidly following oral administration. Immediate-release tablets of guanfacine hydrochloride require frequent dosing (up to 4 times a day), which might reduce patient compliance and increase risks of undesirable responses. These issues can be resolved by preparing an extended-release product to be administered in a single daily dose (*3*). The innovator product (INTUNIV, Shire US Inc) is a once daily prolonged-release tablet of guanfacine hydrochloride. This product is FDA approved and currently indicated for the treatment of ADHD in children and adolescents (*4*, *5*). Some studies have shown that once daily extended-release formulation of guanfacine hydrochloride is more effective than the immediate-release dosage form (*6*–*8*).

Extended-release dosage forms can reduce frequent dosing, optimize release rates, improve patient compliance, and minimize adverse effects (*9*, *10*). Polymer matrices comprising of hydrophilic polymers are widely used in extended-release formulations. In matrix systems, the active pharmaceutical ingredient (API) is homogeneously dispersed using one or more polymers, such as microcrystalline cellulose, sodium alginate, carbopol, etc. (*11*, *12*). Hydroxypropyl methyl cellulose (HPMC) is a type of hydrophilic polymer that is widely used in the preparation of polymer matrices to extend drug release. It remains stable at pH 3–11 and withstands enzymatic degradation (*13*). Eudragit L 100-55 is a versatile methacrylic acid-based synthetic polymer that is available as a solid powder with faint odor. It is used for efficient coating of tablets and other solid dosage forms to develop extended-release or controlled-release pharmaceutical products (*14*).

In this study, HPMC K4M and methacrylic acid (Eudragit L100-55) were used as a hydrophilic matrix to prepare extended-release tablet formulations to release drug

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for 24 hours. An appropriate combination of these polymers is expected to extend the release of guanfacine hydrochloride. Eudragit L100-55 controls pH-dependent release of the drug as the polymer does not dissolve in acidic medium, while HPMC K4M retards the release rate throughout the gastrointestinal region (*13*, *14*). The optimized combinations may also be useful in formulation of extended-release tablets containing other APIs with short half-life and low bioavailability. A 3^2-factorial design was applied in the study to investigate the effect of two independent factors, such as concentration of HPMC K4M and amount of Eudragit L100-55, on the dependent variables, i.e., drug release at 1, 8, and 20 hours. Design expert software (version 13) was employed to provide information on the values essential for generating preferred responses and probable interactions between the independent and dependent variables.

METHODS

Materials

Guanfacine hydrochloride was obtained from Intas Pharmaceuticals Ltd.(Ahmedabad, Gujarat). HPMC K4M (molecular weight: 1261.4 g/mol, degree of substitution: 20–24% of methoxyl and 7–12% of hydroxypropyl substitutions) was procured from Samsung Fine Chemicals Co., Ltd (Korea). Eudragit L100-55 (methacrylic acid) was purchased from Evonik Industries Signet Chemical Corporation Pvt. Ltd (Maharashtra, India). Microcrystalline cellulose PH-102 was from FMC Asia – Pacific, Inc. (Maharashtra, India). Isopropyl alcohol was obtained from Rankem (Gujarat, India). Lactose monohydrate was obtained from Tiwari Chemicals and Tiwari Pharma (Himachal Pradesh, India). Citric acid and fumaric acid were obtained from Thirumalai Chemicals (Maharashtra, India). Glyceryl behenate was obtained from Gattefosse, Ltd. (India). Lake of Indigo carmine and ferric oxide yellow were from Colorcon (West Point, PA, USA). All chemicals and reagents used were of analytical grade.

Precompression Evaluation

The ratio of the weight of powder to the bulk volume is known as bulk density. It consists of the solid portion of the particles and the space between them. Bulk density is important in determining the size of equipment needed for handling and processing. Tapped and untapped bulk density measurements can estimate the compressibility of a material. Flow rate, particle size distribution, and cohesiveness of the powder are the factors on which the compressibility of the powder is dependent. Powders that possess more than 20% of Car's index (compressibility index) value exhibit poor flow properties. From the values of bulk density and tapped density, Car's index and Hausner's ratio were calculated.

Particle size distribution and shape affects the chemical and physical properties of the drug substance. It also effects biopharmaceutical behavior, content uniformity, solubility, and stability. A Malvern analyzer (Mastersizer 3000) was used to measure the particle size distribution of guanfacine hydrochloride (*15–17*).

Compatibility Study

Drug-excipient compatibility studies of guanfacine hydrochloride with different commonly used excipients were carried out with an accelerated thermal stress study. The blends of the drug substance with different excipients in a 1:1 (w/w) ratio were used for the compatibility study. Samples were stored at accelerated conditions of 40 °C and 75% relative humidity (RH) in open and closed vials (Sigma Aldrich, 20 mL vial with size of 21 × 61 mm) and checked for any physical changes after 2 weeks and for chemical changes after 4 weeks (*18*, *19*).

Preparation of Extended-Release Tablets

Formulations were prepared using a wet granulation method. All ingredients were sifted through 40 mesh. Drug and excipients were mixed uniformly and granulated using purified water. The cohesive mass was dried, and granules were sized by passing through 20 mesh. Granules were lubricated using either glyceryl palmitostearate (formulation F1 and F2) or glyceryl behenate (formulation F3–F8). Finally, the blend was compressed using an 11 × 6 mm-oval BL/BL punch and tablet compression machine (Rimek, Mini Press I) (*20, 21*). Each compressed tablet contained 4 mg of guanfacine hydrochloride (all formulations).

Physical Characterization of Tablets

The prepared guanfacine hydrochloride tablets were evaluated for physical parameters such as weight variation (Metter Toledo), hardness, thickness, friability (Labtronics), and content (% assay) according to *United States Pharmacopoeia* (USP) (*22*). Weight, friability, and drug content results were reported as mean and standard deviation.

Experimental Design

Based on the results obtained with preliminary formulations, a randomized 3^2-factorial design approach was used to identify the optimized formulation. In this design, two factors were evaluated, each at three levels, and experimental trials were performed for all nine possible combinations. The composition of all formulations is shown in the Table 1. The concentration of HPMC K4M and the amount of methacrylic acid were selected as

independent variables. As dependent variables, drug release (%) was measured after 1, 8, and 20 hours of dissolution. The release profiles of the formulations were estimated utilizing the Electrolab (Edt 08lx) dissolution tester. The outcomes of the experiment were evaluated statistically for the response variables using Design Expert (version 13, Stat-Ease Inc., Minneapolis, MN, USA).

Kinetic Modeling and Similarity Factor Analysis

Dissolution profiles for each formulation were fitted to various kinetics models including zero order, first order, Higuchi, Hixson Crowell, and Korsmeyer-Peppas to ascertain the kinetics of drug release (*23–25*). The best fitting kinetic model of drug release was determined based on the regression coefficient. Kinetic modeling is a model-dependent approach. In controlled drug delivery formulations, swelling, diffusion, erosion, and dissolution-controlled drug release are the most important rate-limiting mechanisms. The diffusion system, dissolution system, and osmotic system are mechanisms for delivering the drug in a controlled manner. Formulations containing swelling polymers show swelling as well as diffusion mechanism because the kinetics of swelling includes relaxation of polymer chains and imbibition of water, causing the polymer to swell and changing it from a glassy to rubbery state.

For modified-release dosage forms, SUPAC guidelines use the similarity factor (f_2), which is used to compare dissolution profiles. The dissolution profiles of all formulations were compared to the innovator using a f_2. An f_2 value between 50 and 100 indicates similarity among the dissolution profiles (*26*).

In Vitro Drug Release of the Optimized Formulation

The drug release profile of the optimized formulation was measured in dissolution media representing three distinct pH conditions, i.e., HCl buffer pH 1.2, acetate buffer pH 4.5, and phosphate buffer pH 6.8. A sample (10 mL) of each solution was withdrawn at 1-hour intervals for 24 hours, with the replacement of fresh dissolution medium at each timepoint.

The samples were passed through a 0.45-µm membrane filter and diluted to a suitable concentration with the specific medium. The absorbance of these solutions was measured at 220 nm using a UV-Vis scanning spectrophotometer (Shimadzu UV-1800, Japan). The dissolution test was performed using USP apparatus 2 (paddle method) (Model: TDT-08L1202085, Electrolab, India).

Stability Study

The optimized formulation was subjected to stability study according to ICH guidelines (*27*). The stability study was conducted using the Thermo Fischer Scientific stability chamber (model no. 3940). All tablets were packed in aluminum foil at the end of every week. The tablets were visually examined for any physical changes and for chemical changes in drug content for 3 months. During this period, aluminum foils were subjected to different storage conditions including 40 °C and 75% RH, 30 °C and 65% RH, and 25 °C and 60% RH (*27*). The tablets were evaluated for drug content, loss on drying (LOD), hardness, weight, and impurities (single and total) at the end of each month.

Table 1. Composition of Formulations as per Factorial Designs

Ingredients	Amount (mg)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Guanfacine hydrochloride	4	4	4	4	4	4	4	4	4
Avicel PH 102	60.21	80.21	90.21	80.21	70.21	75.21	65.21	85.21	70.21
HPMC K4 M	35	25	25	35	35	30	30	30	25
Eudragit L 100-55	80	70	60	60	70	70	80	60	80
Ludipress	40	40	40	40	40	40	40	40	40
Fumaric acid	18	18	18	18	18	18	18	18	18
Glyceryl behenate	26	26	26	26	26	26	26	26	26
Lake of Indigo carmine	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Ferric oxide yellow	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
$\%DR_1$	16	22	23	15	18	20	26	24	26
$\%DR_8$	66	75	82	86	87	71	75	85	69
$\%DR_{20}$	85	101	102	92	95	98	91	100	85

$\%DR_1$, $\%DR_8$, and $\%DR_{20}$ are percent drug release in 0.1 N HCl at 1 h, 8 h, and 20 h, respectively. HCL: hydrochloric acid.

RESULTS AND DISCUSSION

Precompression Evaluation

Results from the precompression evaluation showed that guanfacine hydrochloride exhibits poor flow properties because Carr's index and Hausner's' ratio were 37.44% and 1.5, respectively. The particle size distribution of guanfacine hydrochloride indicated that around 90% of powder exhibited particle size higher than 355 µm, i.e., within coarse size range.

Compatibility Study

The accelerated thermal stress study indicated no significant physical changes in the excipients compared to guanfacine hydrochloride alone. The level of impurities found in the blend after completion of 4 weeks was also not significantly different from the initial levels.

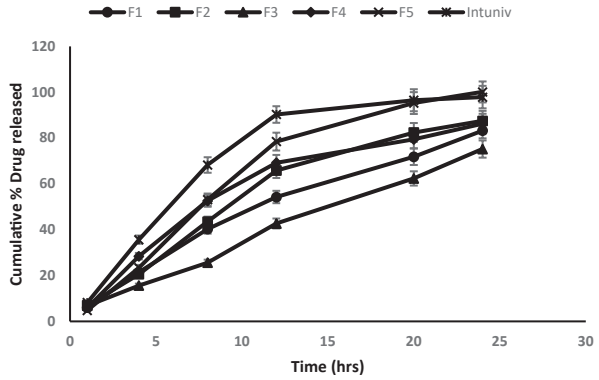
Physical Characterization of Tablets

All formulations conformed to pharmacopeial specifications. The average weight and hardness of all formulations were 265 mg and 110 N, respectively. Tablet thickness was 4.2–4.7 mm. The assay results varied among batches, i.e., 100.2 ± 5.01% for F6 to 79.82 ± 3.99 for F3. Friability results were less than 1% for all formulations, indicative of optimum physical strength.

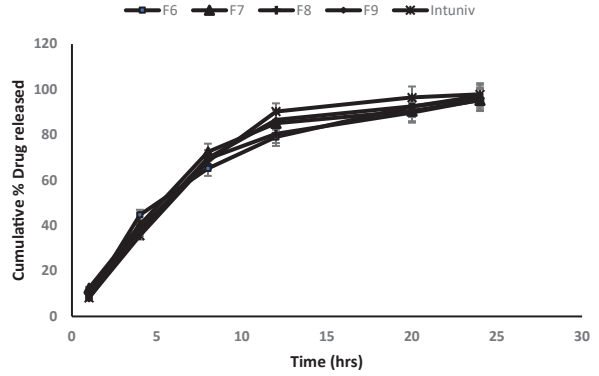
Kinetic Modelling and Similarity Factor Analysis

Release rate kinetics and outcomes of the f_2 analysis are displayed in the Table 2. The dissolution profile for F6–F9 were best fitted to that the Innovator, with f_2 values of 68–85. The F9 formulation showed superior fit (f_2 = 85) in comparison with the release profiles of the other tablets.

On the basis of linearity, the in vitro release of guanfacine hydrochloride from the innovator tablet and all test formulations was best delineated by the Hixson Crowell equation, followed by Higuchi and Korsmeyer–Peppas. This means that dissolution predominantly takes place through gradual decrease in surface area of the tablets as per Hixson Crowell equation, and subsequently slow diffusion of drug from the formulation is explained by Higuchi's equation. The diffusion exponent (np) of all the formulations and the innovator are within the range of 0.7454 to 0.966, which depicts that the release of drug follows anomalous diffusion, i.e., the drug release occurs by both erosion and diffusion mechanisms.



(a)



(b)

Figure 1. Dissolution profiles for formulations F1–F5 (a), F6–F9 (b), and INTUNIV.

Optimization of Experimental Design

The 3^2 factorial designs employed two independent factors: quantity of HPMC K4M (X_1) and quantity of methacrylic acid (X_2) varied at three levels (high [+1],

Table 2. Kinetic Modeling and Similarity Factor Analysis of Dissolution Data

Model	F1	F2	F3	F4	F5	F6	F7	F8	F9	INTUNIV
R_0	0.7979	0.9322	0.9922	0.8858	0.9095	0.8403	0.7897	0.8235	0.9702	0.9682
R_1	0.6114	0.7743	0.8939	0.6633	0.7029	0.6000	0.6322	0.6374	0.7718	0.691
R_H	0.9235	0.9755	0.9601	0.9738	0.9718	0.9542	0.9184	0.941	0.996	0.9822
R_{HC}	0.9357	0.9787	0.9901	0.9652	0.9673	0.9837	0.9288	0.961	0.9995	0.9938
R_{KP}	0.9398	0.9877	0.9827	0.963	0.9762	0.9286	0.9486	0.9513	0.9946	0.9881
n_p	0.966	0.8509	0.7856	0.864	0.7454	0.935	0.9629	0.891	0.8147	0.914
f_2	40.5	46.75	33.1	51.02	59.37	67.97	74.24	71.87	85.42	Ref

R_0, R_1, R_H, R_{HC} and R_{KP} are the correlation coefficients of the zero order, first order, Higuchi, Hixson Crowell, and Korsmeyer-Peppas equations; n_p is diffusion exponent; and f_2 is similarity factor.

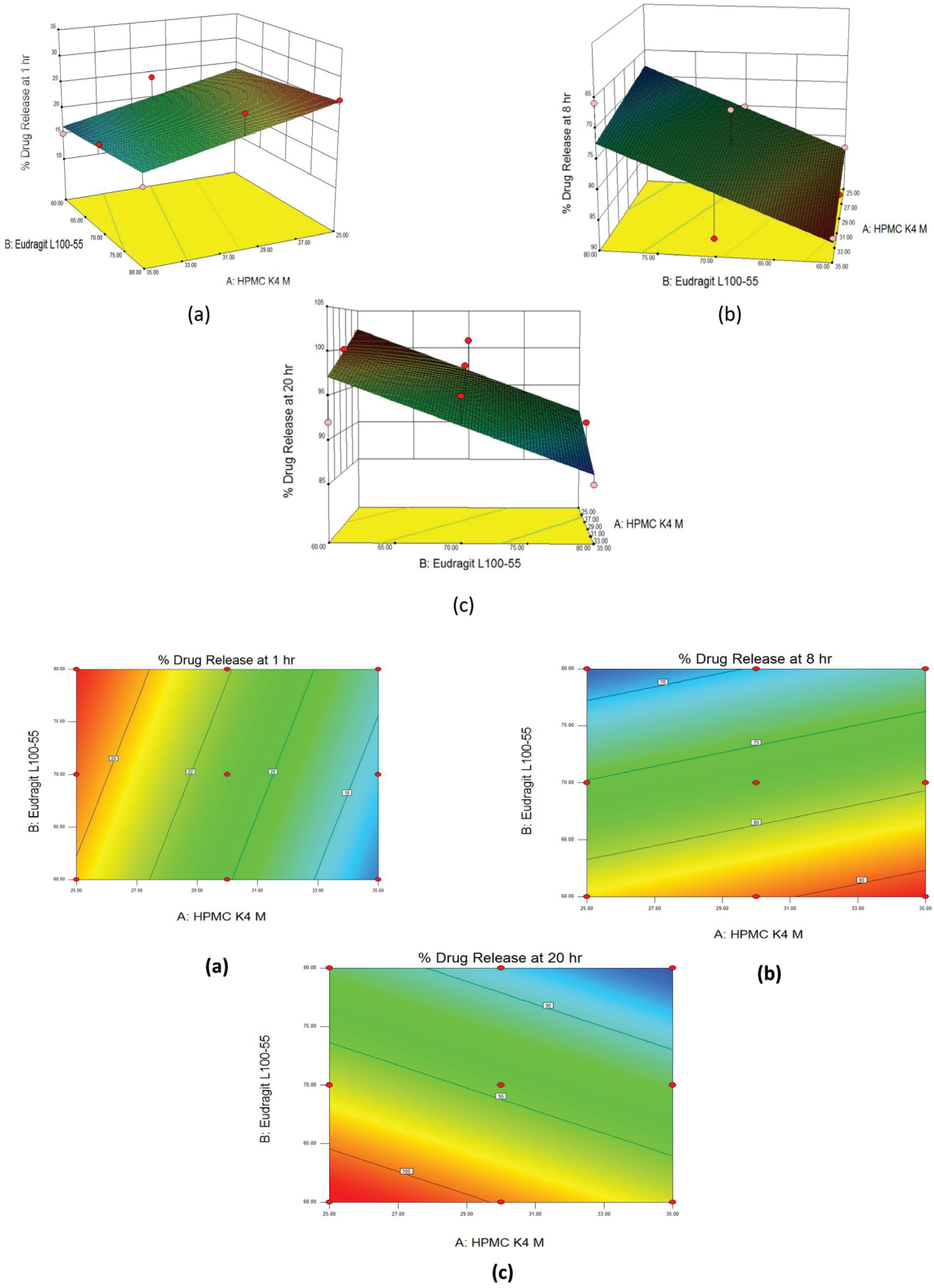


Figure 2. 3D response plots (top) and contour plots (bottom) of cumulative drug release (%) in 0.1 N HCl at 1 h (a), 8 h (b), and 20 h (c).

medium [0], and low [-1]). The impact of these factors was studied on response parameters (dissolution at 1, 8, and 20 h; Y_1 [%DR_1], Y_2 [%DR_8], and Y_3 [%DR_{20}], respectively) in the present investigation. The outline of trials and response outcomes are presented in Table 2. The polynomial model equations were generated from the software, including the main factors and interaction factors after putting the data. The optimized equations are given below in Equations 1–3, respectively.

$$Y_1 = 4.94 - 4.02\,X_1 - 1.05\,X_2 + 0.42\,X_1X_2 + 1.30\,X_1^2 + 0.025\,X_2^2 \quad \text{Eq. (1)}$$

$$Y_2 = 96.02 + 4.08\,X_1 + 0.82\,X_2 - 0.45\,X_1X_2 - 1.63\,X_1^2 - 0.75\,X_2^2 \quad \text{Eq. (2)}$$

$$Y_3 = 45.18 + 3.83\,X_1 + 0.97\,X_2 - 0.43\,X_1X_2 - 1.39\,X_1^2 - 0.14\,X_2^2 \quad \text{Eq. (3)}$$

Coefficients β_1 and β_2 were significant for Y_1, Y_2, and Y3; β_1 and β_2 were negative for Y_1, but positive for Y_2 and Y_3. Drug release in HCl Buffer pH 2.2 decreased with increasing concentration of X_1 and X_2. ANOVA results are depicted in the Table 3, showing that all models were significant for all the studied responses. Design Expert software was employed to produce 3D response surface plots (Figure 2), which show a downward inclination of the wire mesh at higher level (+1) and upward inclination at the lower level (-1) for the concentration of both X_1 and X_2. The plot trend showed the combined effect of X_1 and X_2 in retardation of drug release in the acidic medium. However, higher concentrations of X_1 and X_2 increased drug release owing to increased elasticity of the film and pore formation.

Table 3. Analysis of Variance for Dependent Variables from Full Factorial Design

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-ratio	*P*-value
Cumulative drug release(%) in 0.1 N HCl at 1 h					
Linear Model	2	86.67	43.33	5.391	0.0457
X_1	1	80.66	80.66	10.036	0.0194
X_2	1	6.00	6.00	0.746	0.0408
Cumulative drug release (%) in 0.1 N HCl at 8 h					
Linear Model	2	336.33	168.16	6.241	0.0342
X_1	1	28.16	28.16	1.045	0.0360
X_2	1	308.16	308.16	11.437	0.0148
Cumulative drug release (%) in 0.1 N HCl at 20 h					
Linear Model	2	224.16	112.08	5.805	0.0395
X_1	1	42.66	42.66	2.210	0.0177
X_2	1	181.50	181.50	9.401	0.0220

To optimize the responses, contour plots were generated (Fig. 2). Interestingly, the associated degrees from zero outside of the bounds is within the range of at least one goal. The concentrations of independent variables that depicted maximum desirability are close to 1.

Therefore, the statistically optimized formulation was F9 with 25 mg HPMC K4M and 80 mg methacrylic acid.

Release Profile of the Optimized Formulation

The dissolution profile of the optimized formulation (F9) is presented in Figure 3. The cumulative mean ± SD amount of drug released in HCl buffer pH 1.2, acetate buffer pH 4.5, and phosphate buffer pH 6.8 was 92.16% ± 3.59%, 92.44% ±3.12%, and 99.42% ± 3.72%, respectively, after completion of 20 hours (Fig. 3). Evidently, the percentage of drug released from the optimized tablet formulation was affected by changes in pH, primarily due to the presence of methacrylic acid as the delayed-release polymer. Methacrylic acid has low solubility at acidic pH conditions, therefore the amount of drug released was significantly lesser in acidic (pH 1.2 and 4.5) media. Its high solubility in phosphate buffer pH 6.8 resulted in almost complete release of guanfacine hydrochloride in 20 hours (Fig. 3). Thus, optimized combination of HPMC K4M and methacrylic acid as the matrix attained extended release of drug throughout the day.

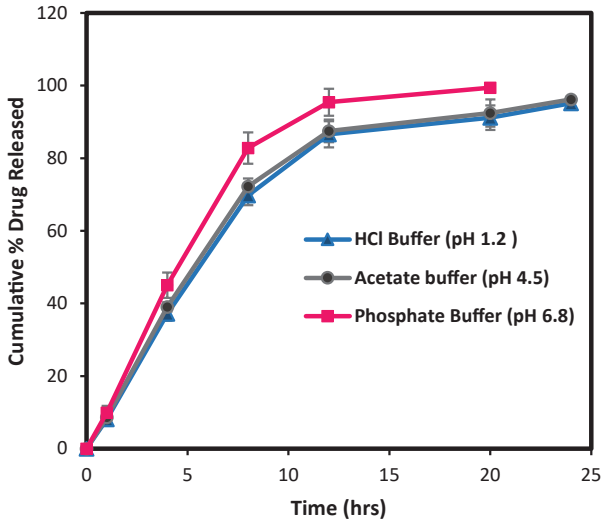


Figure 3. Comparative plot of cumulative drug release of the optimized formulation (F9).

Stability Study

The stability study of the optimized formulation (F9) showed no indications of change in the appearance of tablets, assay, % drug release in acidic medium, etc. The results of stability study in various conditions and the % drug release after 3 months.

CONCLUSION

This investigation focused on the effect of varying concentrations of HPMC K4M and methacrylic acid (Eudragit L 100-55) in designing the extended-release tablets of guanfacine hydrochloride. The precompression evaluation and compatibility studies indicated suitability of the chosen excipients. Physical characterization parameters of the compressed tablets were within the acceptable range. The release kinetics of the formulations best fit the Hixson Crowell and Higuchi's equation, owing to slow erosion of tablet surface. The optimized formulation was found by employing 3^2 factorial designs to identify the most suitable concentration of HPMC K4M and methacrylic acid in formulation F9, which met all requirements with regards to desired rate of release and high f_2 value. Dissolution of the optimized formulation was considerably higher in phosphate buffer pH 6.8 compared with HCl buffer pH 1.2 and acetate buffer pH 4.5. There were no signs of instability after 3 months of storage. The formulation was successful in delaying the release of drug, which may be useful in protecting drugs from destabilizing in the acidic environment of the stomach. The formulation is expected to show prolonged duration of action in future in vivo studies.

CONFLICT OF INTEREST

The authors disclosed no conflicts of interest related to this article.

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Evaluation of Critical Quality Attributes of Dapsone Gel

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ABSTRACT

The purpose of this study was to evaluate critical quality attributes (CQAs) of dapsone gel compared to its marketed reference gel, in accordance with *United States Pharmacopeia* and draft guidance established by United States Food and Drug Administration, which are based on suggestions about quality and performance of the semi-solid dosage forms in recent years. In this context, pH analysis, microscopic analysis, x-ray diffraction analysis, and rheological analysis were used to evaluate quality attributes of the test products towards the reference product. In vitro release tests, in vitro permeation tests, and stratum corneum tape-stripping studies were performed to examine pharmaceutical quality and performance of the gels. In addition, stability of the test product was investigated in terms of visual appearance, pH, viscosity, quantification assay, and drug release following storage at 25 ± 2 °C and 60% ± 5% relative humidity for 6 months. The quality tests indicated that the test product was similar to the reference product. The gels exhibited significantly similar diffusion coefficients and equivalent amounts in the skin layers for all products. The test product was stable for 6 months, physically and chemically. Overall, it is possible to conclude that dapsone gel is of comparable quality and performance to the marketed reference gel.

KEYWORDS: Topical product, semi-solid dosage form, critical quality attributes (CQAs), quality tests, performance tests

INTRODUCTION

Critical quality attributes (CQAs) are physical, chemical, biological, or microbiological properties or characteristics that should be within an appropriate limit, range, or distribution to ensure the desired product quality, as described in the International Council for Harmonization (ICH) Q8 Guideline (*1*). CQAs of the finished product have critical importance because they influence the product performance in terms of quality, efficacy, and safety. These attributes may affect specifications such as impurity, potency, stability, drug release and microbiological properties (*2*). In recent years, the United States Food and Drug Administration (FDA) has been interested in identifying CQAs of topical semi-solid dosage forms that require continuous monitoring to ensure of microstructural similarity. At this point, the quality attributes of topical semi-solid dosage forms, which may be essential for therapeutic performance, include pH, globule size, drug particle size, rheological behavior, drug polymorphic form, dissolved/undissolved drug ratio, and others (*3*, *4*). In the draft guidance on dapsone, the US FDA recommends the same components, same amounts of same components, evaluation of CQAs that define the microstructure of semi-solid products as well as the in vitro equivalent rate of drug release and permeation to enable an efficient comparison of the proposed test product with reference product (*5*).

For many years, dapsone has been used orally to treat leprosy and dermatitis herpetiformis. Furthermore, the potential of oral dapsone administration to treat acne vulgaris has well established, but the possibility of significant hematological side effects, even at low doses, has restricted its use in the relatively healthy population with acne. In 2005, the US FDA initially approved a topical formulation of dapsone in 5% strength (Aczone, Allergan, Inc., Irvine, CA, USA) for the treatment of acne vulgaris (*6*). Then, it approved a stronger topical formulation of dapsone at 7.5% (Aczone, Amirall, Inc., Exton, PA, USA) as a new drug application (NDA) in 2016. Generic versions of Aczone (Taro and Taro Pharma Pharmaceuticals Industries

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Ltd., Brampton, Canada) have not yet been commercially available because of drug patents and/or drug exclusivity were approved by the FDA in 2017 and 2019, respectively (*7*).

We aimed to evaluate CQAs by utilizing the microstructure similarity of dapsone test gel and its reference product (Aczone gel) as a proof-of-concept in vitro. The relationship between CQAs and microstructure of the reference and test gels was assessed using physicochemical characterization analysis including determination of pH, microscopic images, polymorphic form, rheological behavior, in vitro release test (IVRT), in vitro permeation test (IVPT), and stratum corneum (SC) tape-stripping studies according to the current draft guidance on dapsone (*5*). Furthermore, stability of the test gel in aluminum tubes was monitored for 6 months under room temperature.

MATERIALS AND METHODS

Materials

Dapsone was provided by Assos Pharmaceuticals (Istanbul, Turkey). Two lots of dapsone test gel were formulated in our laboratory. The composition of "test gel" is confidential because of a commercial agreement with Istanbul University and Assos Pharmaceuticals. The reference product (Aczone gel) was supplied from a pharmacy in the United States. Methanol (HPLC grade) was purchased from Sigma-Aldrich (Missouri, USA). All other chemicals were of analytical grade and used as received.

Gel Appearance

The appearance (i.e., consistency, color, odor) of reference and test gels was assessed qualitatively as suggested in the draft guidance on dapsone (*5*).

pH Analysis

The pH of reference and test gels was measured using a calibrated pH meter (Eutech Instruments, Landsmeer, Netherlands) at 25 ± 2 °C.

Microscopic Analysis

The particle size distribution and crystal habit of dapsone in the reference and test gels were observed using a polarized light microscope (Nikon Instruments, New York, USA). A small quantity of the gel was placed between a cover slip and glass slide, and then the images were viewed at a magnification of x10.

X-Ray Diffraction (XRD) Analysis

X-ray diffraction (XRD) data were acquired using an x-ray diffractometer (LabX, XRD 6000, Shimadzu, Japan) equipped with a Cu-K radiation source (λ = 1.54060 A°). The scanning angle ranged from 2° to 40° in 2θ steps of 0.02° and a counting time of 0.6 s/step. A generator tension of 40 kV and current of 30 mA were used for XRD analysis of the gels.

Rheological Behavior Analysis

The rheological behavior of reference and test gels was demonstrated using a rheometer (RheoStress 1, Haake, Germany) equipped with a temperature controller and a cone/plate geometry (35-mm diameter, 1° cone angle, gap width of 0.053 mm). For each test, approximately 1.0 g of gel was put on the lower plate, and then the cone was slowly lifted down. After 5-min relaxation time, the measurements were performed at 25.0 ± 0.2 °C. Then, the following procedures were sequentially conducted to characterize rheological characteristics on each sample.

Shear Flow Test

To determine flow properties and viscosity (η) values of the gels, the flow curves were carried out with shear rates (ɣ) in the range of 0–100 s^{-1} for 100 s, by fitting to the Ostwald de Waele model, indicating the highest determination coefficient ($r^2 > 0.99$).

Thixotropy

The thixotropy of the samples was demonstrated with a shear rate from 0–200 s^{-1} and again down to 0 s^{-1} during 200 s^{-1}, while the mean momentary dynamic viscosity was measured at a constant shear rate of 200 s^{-1} for 30 s.

Oscillatory Analysis

First, the linear viscoelastic region was calculated for each sample following a stress sweep of 0.01–100 Pa at a constant frequency of 1 Hz, as the region where stress was directly proportional to strain and the storage modulus (G') remained constant. All frequency sweep measurements were conducted over the frequency range of 0.1–100 Hz following application of a constant shear stress (0.1 Pa). Then, the storage modulus (G'), loss modulus (G") and loss tangent (tan δ) were determined.

All the rheological parameters were calculated using Rheology Solutions (Haake RheoWin Software, Germany).

High-Performance Liquid Chromatography Analysis

The samples obtained from chemical stability, in vitro release and permeation studies were quantified by a high-performance liquid chromatography (HPLC) system equipped with UV detector (Shimadzu, Japan). A C_{18} column (5 µm, 3.9 × 150 mm, Thermo Scientific, USA) was utilized to quantify the drug. The mobile phase was a mixture of 0.03 M potassium dihydrogen phosphate solution and methanol (70:30, v/v). The detection was performed with an injection volume of 20 µL at

295 nm with a run time of 10 min. The flow rate and column temperature were set at 1.0 mL min^{-1} and 25 °C, respectively (*8*).

The method was validated for selectivity, linearity, accuracy, and precision. The regression value (r^2) of calibration curve was more than 0.999. Accuracy, expressed as a percentage of mean recovery, was 95–105%; precision was less than 2% relative standard deviation (RSD). Comparison of chromatograms of samples from release medium and extracted skin exhibited no interfering peaks with dapsone, confirming selectivity of the analytical method.

In Vitro Release Tests (IVRTs) and Kinetics

In vitro release tests (IVRTs) were conducted using dialysis membrane and Franz diffusion cells with a diffusion area of 1.77 cm^2 and a receptor volume of 12 mL (Permegear, USA) (*9*). The phosphate buffer (pH 7.4) containing 2% of Tween 80 (w/v) was used as the receptor medium and was maintained at 32.0 ± 0.5 °C for 6 hours.

The receptor medium was filled into the diffusion cells after degassed in an ultrasonic bath. The dialysis membrane was mounted between donor and receptor compartments of diffusion cells. Approximately 1 g each of the reference and test gels was placed onto the donor compartments. At specific intervals from 30 minutes up to 6 hours, 1-mL samples were removed from the receptor compartments and replaced with fresh receptor medium at same temperature and volume. The samples were filtrated via a 0.45-µm PTFE membrane filter (Millex-LCR, Merck Millipore, Massachusetts, USA). The released amount of dapsone from the products was determined by a validated HPLC method.

Drug release kinetics were fitted to Higuchi matrix model using the following equation (*10*): $C = kt^{1/2}$, where C is drug concentration released at time t, and k is the Higuchi release rate constant.

In Vitro Permeation Tests (IVPTs) and Stratum Corneum Tape-Stripping Studies

In vitro permeation tests (IVPTs) were carried out using dorsal porcine skin and Franz diffusion cells for 24 hours (*11*). The phosphate buffer (pH 7.4) containing 1% of bovine serum albumin (w/v) was used as receptor medium to maintain sink conditions. Following IVPTs, the skin samples were cleaned carefully, and adhesive tapes (Scotch 3M, 19 × 40 mm) were applied onto the treated skin 20 times, pressing with a roller to avoid from effects of furrows and wrinkles. Each tape strip was removed with a quick movement. The tape strips, including stratum corneum and residual skin, were extracted in acetonitrile for 24 hours. Afterwards, the samples were vortexed for 5 minutes and filtered via a 0.45-µm PTFE membrane filter. Dapsone amounts in stratum corneum, residual skin, and the receptor medium were quantified by a validated HPLC method.

Stability of Dapsone Gel

Stability was assessed for test gels stored in aluminum tubes at room temperature (25 ± 2 °C and 60% ± 5% relative humidity [RH]) for 6 months (*12*). Stability assessment of test gels included examination of visual appearance, pH, dynamic viscosity, quantification assay, and IVRT (i.e., diffusion coefficients) after 0-, 3- and 6-months of storage.

For quantification assays, 0.125 g of the gels was dissolved in 25 mL of the mobile phase and diluted with 50 mL of the mobile phase. Then, the samples were filtered via a 0.45-µm PTFE membrane filter, and drug concentration was quantified by a validated HPLC method.

The dynamic viscosity of reference and test gels was measured using a rheometer as described previously. Also, pH and IVRT studies were performed as described previously.

Statistical Analysis

Results are presented as mean values of at least three experiments ± SD. Statistical analysis was performed using a one-way analysis of variance (ANOVA) with GraphPad Prism Software (version 6.05, La Jolla, California, USA). A multiple comparison test was used to compare the formulations, and $p \leq 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Assessment of Gel Appearances

An organoleptic test (i.e., appearance, color, odor, etc.) is useful to rapidly compare the gels and ensure no separation of phases, no extrusion of water from the gels, and color/odor changes during storage. Both two lots of the test and reference products were whitish, homogenous, and odorless gels at the time of formulation and after 6 months of storage.

pH of Gels

The pH can affect stability of drug molecules, rheological behavior of semi-solid products, and effectiveness of preservatives in the products (*13*). The pH values of the reference and test gels were between 6.0 and 6.6 at the time of formulation and after 6 months of storage.

Microscopic Observation

In all products, dapsone particles had similar size and uniform distribution. However, dapsone in the test gels

exhibited rhombus-shaped crystals (Fig. 1c and 1d), whereas the reference gel contained needle-shaped crystals (Fig. 1a). Both rhombus- and needle-shaped crystals were observed in the test gel-Lab scale (Fig. 1b). This difference could be based on batch size or manufacturing process of the gels.

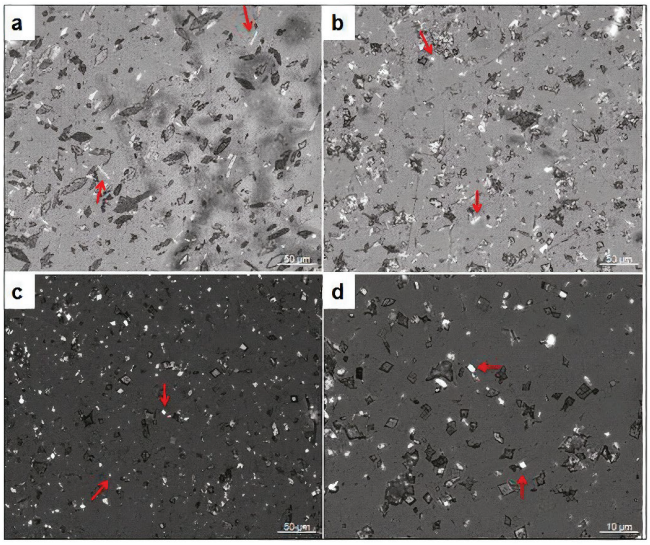

Figure 1. Microphotographs of dapsone matter (arrows) in Reference gel (a), Test gel - Lab scale (b), Test gel - Lot no. 1 (c), and Test gel - Lot no. 2 (d).

XRD Analysis

It is well known that numerous drugs could exist in more than one crystalline form with different stability, solubility, and bioavailability characteristics (*14*, *15*). Analysis of dapsone's polymorphic forms in the drug product is a crucial parameter to assess because dapsone could transform into any one of five crystalline forms during the pharmaceutical manufacturing process (*16*). As shown in Figure 2, XRD patterns displayed numerous sharp peaks on the same positions, indicating the same crystalline polymorphic form in the products. Additionally, the magnitude of the prominent peak at approximately 21° in the test gels was similar to the peak in the reference gel.

Rheological Behavior

The rheological behavior is an essential feature of semi-solid dosage forms that exhibit non-Newtonian flow. However, viscosity of that product is poorly determined by a single shear rate-based method. Therefore, viscosity of the products was demonstrated as a flow curve reflecting shear stress as a function of shear rate (*17*). Further, dapsone-specific guidance recommends evaluation of quality and performance across the range of attainable shear rates until low or high shear plateaus are identified (*5*). In addition, viscoelasticity of the products is a crucial factor, which is presented as a frequency sweep, reflecting the storage (G') and loss (G") moduli at increasing frequencies (*17*).

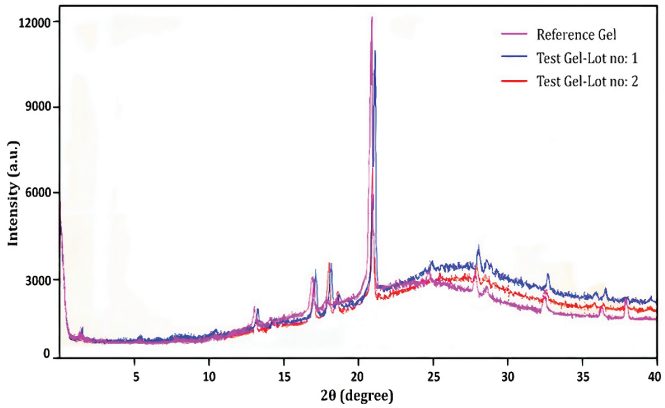

Figure 2. X-ray diffractograms of the reference and test products (dapsone gel).

Figure 3A shows that the reference and test gels exhibited relatively similar flow curves of shear stress (or viscosity) vs. shear rate of flow. Figure 3B shows that the reference and test products displayed pseudo-plastic flow known as shear thinning, and thixotropic behavior that is characteristic of plastic and pseudo-plastic systems. Oscillation data revealed G' values (> 450.4 Pa) greater than G" values (< 214.9 Pa) over all frequency ranges for each product, indicating viscoelastic behavior with strong gel structure. The value of loss tangent (tan δ = G"/G') was less than 1 (range: 0.277–0.362); as tan δ became smaller, elasticity of the gel increased and viscosity decreased. Overall, rheological behavior of the test gels was similar to the reference gel.

IVRTs and Kinetics

Calculation of diffusion coefficients (drug release rate) in is a requirement to predict quality and performance of drug products (*18*). In Figure 4, in vitro drug release profiles of the reference and test gels show linear characteristics according to the Higuchi kinetics model (r^2 > 0.98). Moreover, there is no difference between diffusion coefficients for the reference (145 ± 6.57 µg/cm^2/h$^{-1/2}$) and test gels (141 ± 5.98 and 141 ± 6.67 µg/cm^2/h$^{-1/2}$ for Lot no. 1 and 2, respectively), indicating equivalent drug release rates and kinetics (p > 0.05).

IVPTs and Stratum Corneum Tape-Stripping

As IVPTs predicts drug permeation through the skin, tape-stripping studies show drug accumulation in the outermost skin layer (stratum corneum) (*19*). Results are shown in Figure 5. IVPTs revealed that no dapsone was detected in the receptor medium for the reference and test gels, indicating no permeation of drug to the blood circulation through the skin. The tape-stripping studies

also indicated that dapsone was localized in equivalent amounts in the stratum corneum and residual skin for all tested products ($p > 0.05$).

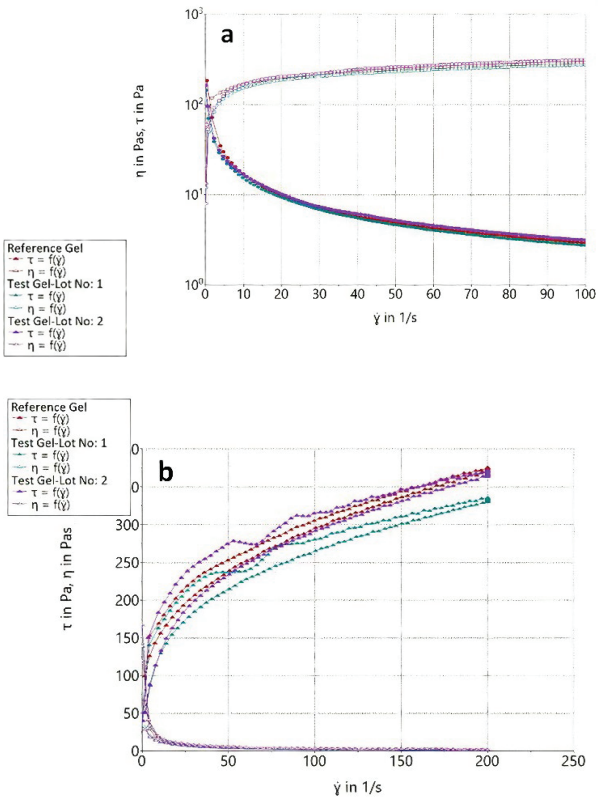

Figure 3. Flow curves (a) and thixotropic behavior (b) of the reference and test products (dapsone gel).

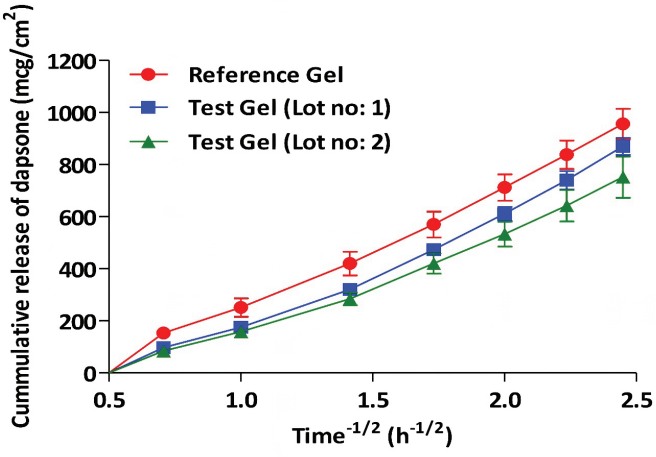

Figure 4. In vitro drug release profiles of the reference and test products (dapsone gel) (n = 3).

Stability

Stability studies are an essential part of the pharmaceutical development process, and regulatory agencies require examination of stability for establishing and sustaining high-quality products (*20*). In the present study, the stability data showed no noteworthy change in visual appearance, pH, and viscosity values of test gels after 6 months of storage at 25 ± 2 °C and 60% ± 5% RH (Table 1). Similarly, the assay and diffusion coefficient results exhibited no significant differences after 6 months of storage, indicating physical and chemical stability of the gels.

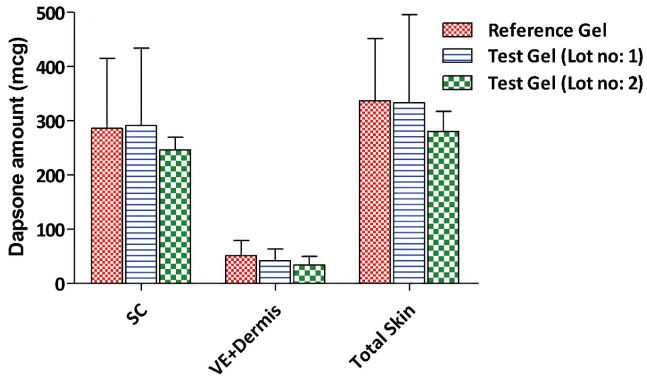

Figure 5. Amount of dapsone localized in stratum corneum (SC), residual skin (viable epidermis [VE] plus dermis), and total skin (n = 3).

Table 1. Stability Data of Test Gels at 25 ± 2 oC and 60% ± 5% Relative Humidity

Critical Quality or Performance Attribute	0 months	3 months	6 months
	Test Gel - Lot no. 1		
Appearance	Homogenous, whitish, odorless	Homogenous, whitish, odorless	Homogenous, whitish, odorless
pH	6.50	6.60	6.55
Viscosity (Pa · s)	11.50	11.60	11.70
Assay (%)	101.4	100.3	99.8
Diffusion coefficient* (μg/cm²/h^-1/2)	141 ± 5.98	136 ± 12.81	142 ± 10.62
	Test Gel - Lot no. 2		
Appearance	Homogenous, whitish, odorless	Homogenous, whitish, odorless	Homogenous, whitish, odorless
pH	6.40	6.50	6.55
Viscosity (Pa · s)	11.70	11.80	11.90
Assay (%)	102.5	101.7	100.6
Diffusion coefficient* (μg/cm²/h^-1/2)	121 ± 13.12	129 ± 5.05	141 ± 6.67

**Experiment was performed using at least three samples. Specifications for pH: 6.0–6.6; viscosity: 7.00–15.00 Pa · s; assay: 90–110% label claim.*

CONCLUSION

CQAs include physical, chemical, biological, and microbiological properties or characteristics that should

be within an appropriate limit, range, or distribution to ensure acceptable quality of a drug product. In the recent years, regulatory agencies have focused on identifying CQAs of topical semi-solid dosage forms on the basis of microstructure similarity. In this study, critical quality and performance attributes of dapsone test gels were compared to that of the reference product (Aczone gel) in accordance with USP and FDA guidelines. The results demonstrated that dapsone gel has comparable quality and performance with the reference gel. The test product was physically and chemically stable when stored at room temperature for 6 months; future studies will assess stability for up to 24 months.

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CONFLICT OF INTEREST

The authors disclosed no conflicts of interest related to this article.

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Predicting Dissolution of Entecavir Using the Noyes Whitney Equation

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ABSTRACT

The dissolution rate of a drug directly affects its absorption and utilization in vivo. The dissolution test is used to evaluate the quality of formulation and production process. Entecavir is approved by the United States FDA for the treatment of chronic hepatitis B. Entecavir monohydrate (ETV-H) is used in commercial ETV tablets. The anhydrous form of entecavir (ETV-A) often appears as an impurity polymorph during the preparation process. This study aims to investigate the dissolution behavior of ETV-H in four dissolution media (water, pH 1.2, pH 4.0, and pH 6.8) and compare with those of ETV-A. The dissolution rates of ETV-H at pH 6.8, pH 4.0, and ultrapure water were faster than those of ETV-A, resulting in faster complete dissolution of ETV-H. To save time in the dissolution testing, an analytical method based on the Noyes Whitney equation is proposed to obtain the fitted (predicted) dissolution curve. Differences (loss values) between the predicted and experimental dissolution curves for ETV-H at pH 6.8 and pH 1.2 were 0.0013 and 0.016, respectively. The proposed analytical method can save up to 75% of experimental time and can be used for dissolution testing of active pharmaceutical ingredients in the production of pharmaceutical crystals.

KEYWORDS: dissolution, analytical method, entecavir, Noyes-Whitney equation

INTRODUCTION

Drug dissolution refers to the rate and extent of drug release from solid preparations (such as tablets, capsules, or granules) in a specific medium (*1*). Because the dissolution rate directly affects the absorption and utilization of drugs in vivo, the dissolution test has become one of the indicators to evaluate the quality of drug production (*2–4*). Dissolution is widely used in the development of solid dosage forms, raw materials, and new drugs (*5*, *6*). The main factors affecting dissolution are variations in the crystalline form, preparation form, formulation and excipients, manufacturing process, and drug interactions (*7–9*). The surface free energy of different crystal forms affects the dissolution rate and bioavailability of drugs. Studies have shown that different crystalline forms of the same drug have different absorption in vivo (*10*, *11*). Dissolution is an important quality control indicator used to evaluate the consistency of different production batches. The dissolution test is applicable not only to solid dosage forms but also to active pharmaceutical ingredients (APIs) (*12*).

The United States FDA approved Entecavir (ETV) (Baraclude, Bristol-Myers Squibb Co. Ltd) for the treatment of chronic hepatitis B in 2005 (*12*). ETV is a carbocyclic 2'-deoxyguanosine analogue, which can phosphorylate into a triphosphate form that can inhibit hepatitis B virus (HBV) in active cells (*13*). ETV is chemically defined as 2-amino-1, 9-dihydro-9- ((1S,3R,4S) -4-hydroxy-3-(hydroxymethyl)-2-methylenecyclopentyl)-6H-purin-6-one. Entecavir monohydrate (ETV-H) is the API used in commercial ETV tablets (*14*). As a polymorphic impurity of ETV-H, entecavir anhydrate (ETV-A) may occur in the ETV-H production process (*15*).

Most patients who take ETV are elderly or infirm, and the pH value and motility of the gastrointestinal environment are quite different from those of young people. To investigate the dissolution of ETV-H and ETV-A, four dissolution media with pH values of 1.2, 4.0, 6.8, and 7.0 (ultrapure water) were selected for this study. The dissolution medium at pH 1.2 was usually used to simulate the gastric acid environment, pH 4.0 was used to simulate the gastric environment of elderly or weak patients, and

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pH 6.8 was used to simulate intestinal environment.

In previous literature, the dissolution of ETV-H was investigated by HPLC (*16*, *17*) and Raman spectroscopy (*18*). In this paper, the dissolution of ETV-H was investigated with UV-Visible spectrophotometry. To save time with dissolution testing of APIs with polymorphic impurities such as ETV, an analytical method based on the Noyes Whitney equation was developed. The method was validated with dissolution of ETV-H in different media.

MATERIALS AND METHODS

Materials

ETV-H was obtained from Zhejiang Ausun Pharmaceutical Co., LTD (purity > 99.9%). ETV-A was recrystallized by dissolving ETV-H solid powder in a methanol solvent using a cooling crystallization method. Standard Entecavir (Batch No. 101248-201503) was purchased from China Institute for Food and Drug Control, and the calculated C12H15N5O3 content was 93.8%.

Hydrochloric acid (HCl), potassium hydroxide (KOH), sodium dihydrogen phosphate (NaH_2PO_4), sodium hydroxide (NaOH), acetic acid (CH_3COOH), sodium acetate (CH_3COONa), potassium chloride (KCl), and boric acid (H_3BO_3) were used for solubility and dissolution determination. All reagents were purchased from Aladdin Ltd. (Shanghai, China), were of analytical grade, and were used without further purification. Ultrapure water was prepared by Arium Mini ultrapure water system (Sartorius, Goettingen, Germany).

Preparation of Tablets for Dissolution Determination

ETV-H powder and ETV-A powder (150 mg of each crystal form) were pressed respectively for 1 minute with YP-15 manual powder tablet press (Tianjin Zhongshi JOSVOK Technology Development Co., Ltd., China). The tablet diameter was 10 mm, and the pressure was 25 MPa. The measured thickness of the compressed tablet was about 1 mm. A total of 12 tablets were pressed for each crystal form.

All weighing operations were performed in XS205DU electronic balance (METTLER TOLEDO Instrument Co., Ltd, Switzerland) with an accuracy of 0.01 mg.

Preparation of Dissolution Media

The HCl solution at pH 1.2 was prepared according to the *Chinese Pharmacopoeia* (ChP). HCl with a mass concentration of 37% (7.65 mL) was transferred to a 1000-mL volumetric flask, then ultrapure water was added to the tick mark.

The acetate buffer solution at pH 4.0 was prepared according to ChP. Acetic acid (114 mL) was added to a 1000-mL volumetric flask, then ultrapure water was added to tick mark to obtain a 2 mol/L solution. Sodium acetate (1.22 g) and the acetic acid solution (20.5 mL) were added to a 1000-mL volumetric flask, and then ultrapure water was added to the tick mark.

To prepare the phosphate buffer solution at pH 6.8, NaOH (8.0 g) was transferred to a 1000-mL volumetric flask and ultrapure water was added to the constant volume to obtain the NaOH solution with a concentration of 0.2 mol/L. Potassium dihydrogen phosphate (6.8 g) and sodium hydroxide solution (0.2 mol/L, 112 mL) were transferred to a 1000-mL volumetric flask, and then ultrapure water was added to dilute to the constant volume.

All dissolution media were degassed under vacuum before use with a ZKT-18F vacuum degasser (Tianjin Tianda Tianfa Technology Co., Ltd.) at 0.05 MPa vacuum degree for 30 minutes.

Dissolution Experiments

To obtain the dissolution curves for comparison of the two substances, we used the same weight of ETV-A and ETV-H. The moles of ETV in ETV-A and ETV-H were not the same, so, the UV spectrum of ETV-H and ETV-A was slightly different.

Standard Entecavir (7.5mg) was added to a 250-mL volumetric flask, diluted with ultrapure water to volume. A solution with a concentration of 30 µg/mL was obtained. Then the solution was diluted to a series of ETV-H solutions with concentration of 25, 20, 15, 10, and 5 µg/mL. According to the content of C12H15N5O3 in standard entecavir (93.8%), a series of ETV-A solutions were prepared with concentrations of 30, 25, 20, 15, 10, and 5 µg/mL. All standard solutions of ETV-H and ETV-A were measured at 253 nm against blank solution to obtain absorbance, and each concentration was measured three times. Finally, the standard curves of ETV-H and ETV-A were plotted according to the UV absorption intensity at 253 nm of different concentrations (Figs. 1c and 1d). The UV spectrum of ETV-H and ETV-A in HCl solution is shown in Figure 1e, and the absorption peaks of ETV-H and ETV-A appeared at 255 nm (the standard curves are shown in Figs. 1f and 1g). The absorption peaks of ETV-H and ETV-A at pH 4.0 and 6.8 were consistent with that in water. The standard curves of ETV-H and ETV-A in pH 4.0 and pH 6.8 were also obtained referred to the above method (not shown).

All dissolution experiments were performed using the

paddle dissolution apparatus and RCZ-8M drug dissolution meter (Tianjin Tianda Tianfa Technology Co., Ltd., China), which had eight dissolution vessels (1000-mL capacity) and eight paddle shafts. Concentration of the solution was analyzed with a Cary 60 UV-Vis spectrophotometer (Agilent, USA).

The dissolution tests were conducted at 37 ± 0.5°C with a speed of 250 rpm in 900 mL of dissolution media. Samples (5 mL) were withdrawn at different time points and filtered through 0.45-µm nylon filters (Sarrtorius, Germany). The dissolution medium was replaced with the same temperature and volume (5 mL). Then samples were analyzed at the wavelength of 253 nm by the UV-Vis spectrometer. Experiments were conducted in triplicate, and the relative standard deviation (RSD%) value was calculated at each time point. The concentration at each time point was obtained according to the standard curve. The experiment was stopped when ETV-H or ETV-A tablets were completely dissolved.

The analytical method was performed using Matlab R2017b (Mathworks Inc., MA, USA).

Characterization of Entecavir Polymorphs

ETV-H and ETV-A were characterized by powder x-ray diffraction (PXRD) to verify the purity of both crystal forms. A Rigaku D/Max-2550 powder diffractometer (Rigaku Co., Japan) was used, with a CuKα radiation source, λ = 1.54059Å, at 40 kV and 250 mA. The scans ran from 3.0° to 50.0° (2θ), with an increasing step size of 0.02° (2θ) and count time of 2 s per step. Data were processed using MDI Jade software (version 9.0).

RESULTS AND DISCUSSION

Calibration Curve

The PXRD results of ETV-H and ETV-A showed that these two crystal forms had distinct characteristic peaks (Fig. 1a). ETV-H and ETV-A had no change in crystal form after tablet pressing. Both ETV-H and ETV-A had UV absorption peaks at 253 nm (Fig. 1b).

Dissolution Curves

The dissolution test results are shown in Figure 2.

At pH 1.2, the sampling time points were 3, 6, 9, 12, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, and 90 minutes (Fig. 2a). The dissolved amount of ETV-H and ETV-A both reached 90% at 15 minutes, indicating rapid dissolution at pH 1.2. There was no significant difference in the dissolution rate between ETV-H and ETV-A.

At pH 4.0, the sampling time points were 3, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 100, 120, 140, 160, 180, 200, 220, 240, 280, 300, and 320 minutes (Fig. 2b). The cumulative release of ETV-H exceeded 90% at 120 minutes and 99% at 180 minutes. The cumulative dissolution of ETV-A exceeded 90% at 240 minutes and 99% at 300 minutes. The dissolution rate of ETV-H at pH 4.0 was faster than that of ETV-A.

At pH 6.8, the sampling time points were 3, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320, 340, and 360 minutes (Fig. 2c). The cumulative release of ETV-H was over 90% at 160 minutes and 99% at 260 minutes. The dissolution of ETV-A exceeded 90% at 220 minutes and 99% at 260 minutes. At pH 6.8, ETV-H dissolved faster than ETV-A before cumulative drug release reached 90%.

In ultrapure water, the sampling time points were 3, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 110, 120, 160, 180, 200, 220, 240, 260, 280, 300, and 320 minutes (Fig. 2d). The cumulative release of ETV-H and ETV-A exceeded 90% at 140 and 220 minutes, respectively, and complete dissolution was reached at 160 and 280 minutes, respectively. The dissolution rate of ETV-H was faster than that of ETV-A in pure water.

To sum up, under the same experimental conditions, the dissolution rate of ETV-H was higher than that of ETV-A in dissolution medium of pH 4.0, pH 6.8, and ultrapure water. The dissolution profiles at pH 1.2 differed from those at pH 4.0, pH 6.8, and ultrapure water, which may be due to the protonation of ETV in the dissolution medium at pH 1.2 (*19*). Therefore, after the API dissolved at pH 4.0, pH 6.8, and in ultrapure water, the pH value of the dissolution solution remained constant, and the pH of dissolution medium may change at pH 1.2.

Analytical Method for Predicting the Dissolution Curve

The earliest report of the dissolution rate equation was jointly proposed by Noyes and Whitney in 1897 (*20*).

During the dissolution process of the drug, the surface area of the drug is constantly changing. Assuming that the initial volume of the tablet is V_t, and the volume of drug dissolved in the dissolution medium at time t is V_d, then, during the drug dissolution process, the undissolved solid volume of the drug at time t is $V_u = V_t - V_d$. Dissolved solid volume correlates with the drug surface area.

When the drug is a cylindrical solid tablet, the tablet's surface area is $S = \pi r^2 + 2\pi rh$, and the tablet's volume is $\pi r^2 h$, where r represents the radius of the bottom surface of the tablet, and h is the height of the tablet. Assuming that the height of the tablet is βr, then $S = \pi r^2 (1 + 2\beta)$ and

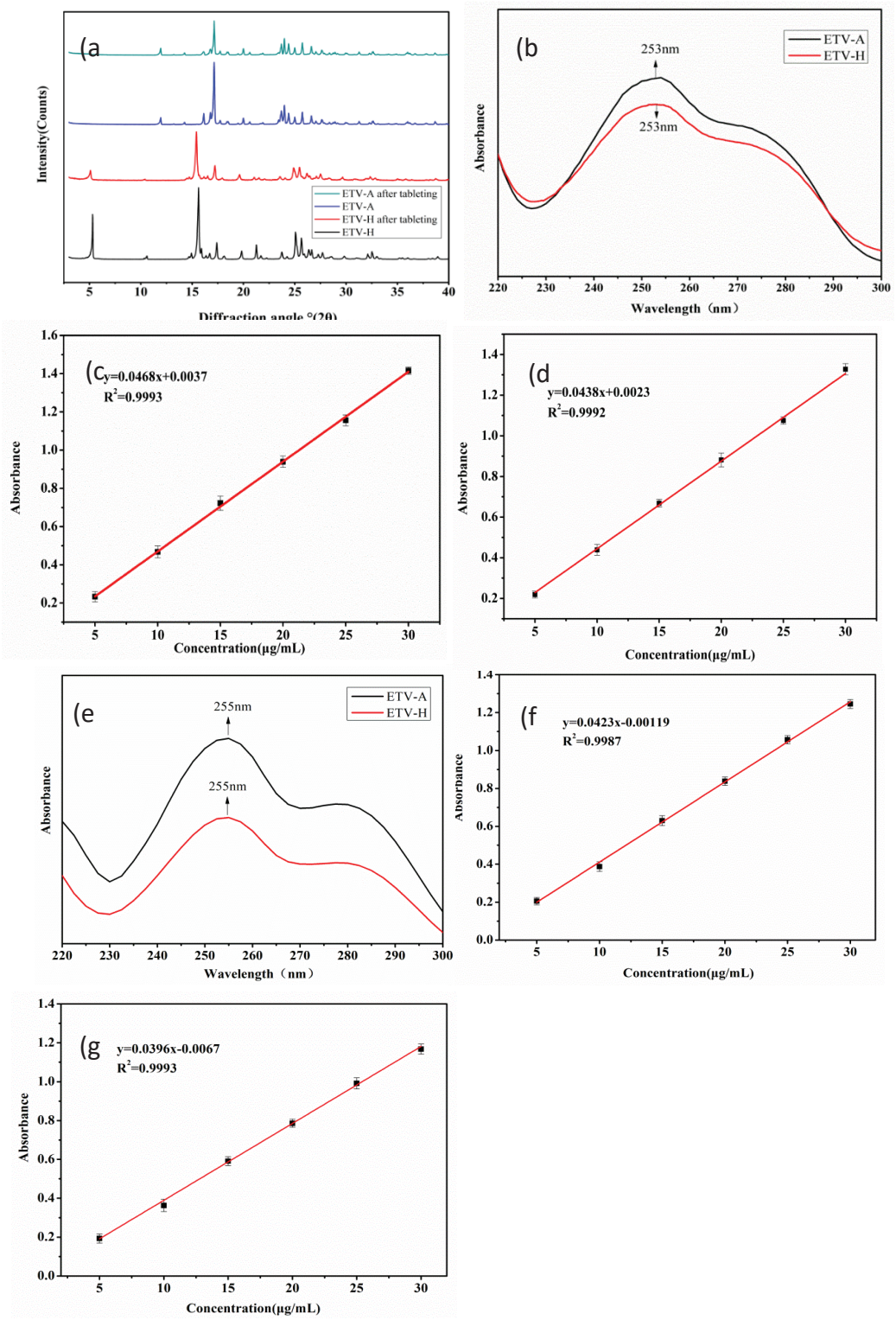

Figure 1. Powder x-ray diffraction of ETV-A and ETV-H (a); UV spectra of ETV-A and ETV-H in water (b); UV absorption standard curve of ETV-A in water (c); UV absorption standard curve of ETV-H in water (d); UV spectra of ETV-A and ETV-H at pH 1.2 (e); UV absorption standard curve of ETV-A at pH 1.2 (f); and UV absorption standard curve of ETV-H at pH 1.2 (g). ETV-A: entecavir anhydrate; ETV-H: entecavir monohydrate; UV: ultraviolet.

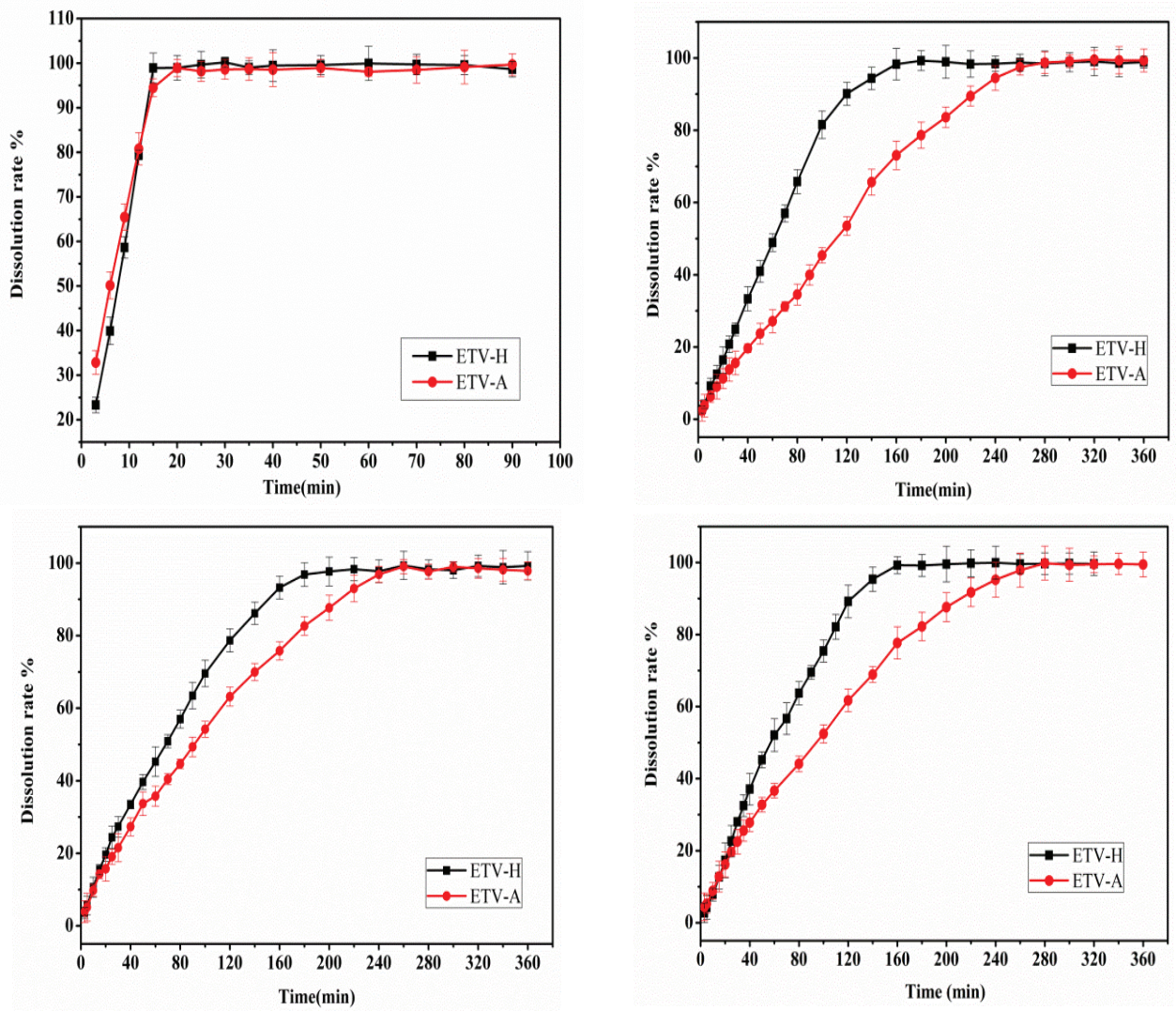

Figure 2. Dissolution curves of ETV-H and ETV-A at pH 1.2 (a), pH 4.0 (b), pH 6.8 (c), and in pure water (d). ETV-A: entecavir anhydrate; ETV-H: entecavir monohydrate.

$V_t = \pi r^2 \times \beta r$. Then $r = (V_t / \pi\beta)^{2/3}$. The tablet surface area can be expressed as $S = 2\pi (1 + 2\beta) (V_t / \pi\beta)^{2/3}$. If $\alpha = 2\pi (1 + 2\beta) (1 / \pi\beta)^{2/3}$, then S is transformed to $S = \alpha (V_t)^{2/3}$.

If the volume of the tablet remains a cylinder during the dissolution process, then the Noyes-Whitney equation is converted into:

$$\frac{dC}{dt} = k\alpha(V_t - V_d)^{\frac{2}{3}}(C_s - C_t)\,, \qquad \text{Eq. (1)}$$

where C_s refers to the saturated solubility of the drug, k is the dissolution rate constant, S means the surface area of the drug, and C_t denotes the solubility of the drug in the solvent at time t, which is the instantaneous concentration. At time t, V_d is determined by C_t, the tablet density ρ, and solvent volume V_s. Then Eq. (1) is converted as follows:

$$\frac{dC}{dt} = k\alpha\left(\frac{V_t \times \rho}{V_s} - C\right)^{\frac{2}{3}}\left(\frac{V_s}{\rho}\right)^{\frac{2}{3}}(C_s - C_t)\,, \qquad \text{Eq. (2)}$$

Assuming that the concentration of the drug after complete dissolution in the dissolution medium is C_d, it can be calculated by the following equation: $C_d = (V_t \times \rho) / V_s$. Then Eq. (2) can be transformed into:

$$\frac{dC}{dt} = k\alpha(C_d - C)^{\frac{2}{3}}\left(\frac{V_s}{\rho}\right)^{\frac{2}{3}}(C_s - C_t)\,, \qquad \text{Eq. (3)}$$

During the dissolution process, V_s is always 900 mL by replenishing the solution. So, in Eq. (3), k, α, V_s, ρ are constants. If $K = k\alpha (V_s / \rho)^{2/3}$, then Eq. (3) can be converted to:

$$\frac{dC}{dt} = K(C_d - C)^{\frac{2}{3}}(C_s - C_t)\,, \qquad \text{Eq. (4)}$$

where K is a constant. The key to solving Eq. (4) is to find the value of K. The analytical method refers to predicting the whole dissolution curve through a small amount of dissolution test data using Eq. (4), which is derived from the Noyes Whitney equation.

As an example, the dissolution data of ETV-H in the pH 6.8 medium at different time points (including the time point and drug concentration in the dissolution medium at this time point) were imported into Matlab. The K value in the dissolution curve was obtained by selecting the data for a certain number of time points, then K was substituted into Eq. (4) as follows.

According to the existing literature, the saturated solubility of ETV-H in the dissolution medium of pH 6.8 is 2.5 mg/mL at 37 °C (*19*). Saturated solubility can be calculated as follows. Assume that the drug concentration in the dissolution medium is C_{t1} at time $t1$ and C_{t2} at time $t2$. Then the concentration difference is divided by the time difference to get the dissolution rate $k1$ between two adjacent points, which is equal to the dC / dt value: $k1 = dC / dt = (C_{t2} - C_{t1}) / (t2 - t1)$. The constant K in Eq. (4) is calculated as $K = k1 / (C_d - C_{t1})^{2/3}(C_s - C_{t1})$. The constant K between time $t1$ and $t2$ is $k1$. It can be inferred that between time $t2$ and time $t3$, the constant K is $k2$, and the constant K between time t–1 and time t is kt–1. Substituting $k1$, $k2$, …, kt–1, the obtained average value is equal to K in Eq. (4), and the fitted dissolution curve can be obtained. During the calculation, the dissolution curve obtained from K fluctuates in the instantaneous concentration values near the time point of complete dissolution. Thus, Eq. (4) can be converted to:

$$\frac{dC}{dt} = K(|C_d - C|)^{\frac{2}{3}}(C_s - C_t) \qquad \text{Eq. (5)}$$

By comparing the fitted (predicted) curve with the actual (experimental) dissolution curve, the sum of the squares of the concentration difference between the two curves can be obtained. The number of data points corresponding to the smallest loss value can be chosen as the optimal number of samples to predict the dissolution curve.

Figure 3 shows the comparison between the predicted and experimental curves for ETV-H at pH 6.8. When the number of data points was eight (i.e., 3, 5, 10, 15, 20, 25, 30, and 40 min), the loss value was the smallest at 0.0013. At this point, the predicted curve was closest to the experimental curve. For these eight data points, the calculated average K value was 0.00195, and the standard deviation was 2.66×10^{-5}.

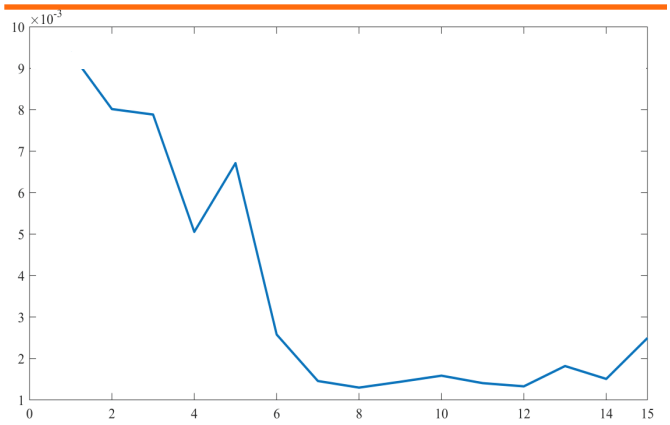

Figure 3. Determination of required number of experimental data points needed to predict the dissolution curve for ETV-H at 6.8. Loss values reflect differences between fitted (predicted) and actual (experimental) results. ETV-H: entecavir monohydrate.

As shown in Figure 4, the predicted dissolution curve tended to agree with the actual dissolution curve at pH 6.8. Therefore, only the dissolution sampling results within 40 minutes were needed to obtain the predicted dissolution profile according to Eq. (5). Thus, this analytical method can greatly reduce the experimental time.

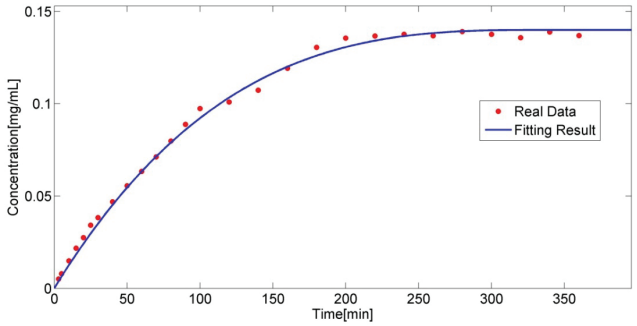

Figure 4. Comparison of predicted dissolution curve (blue line) and experimental results (red dots) of ETV-H in pH 6.8. ETV-H: entecavir monohydrate.

For the dissolution of ETV-H at pH 1.2, the loss value between the predicted with actual dissolution curve was obtained by selecting different data points. As shown in Figure 5, the loss value was the smallest (0.016) when taking the first four experimental data (i.e., 3, 6, 9, 12 min). These four data points were used to predict and fit the dissolution curve, as shown in Figure 6. The average K value was 0.002, and the standard deviation was 6.67×10^{-5}. The predicted dissolution curve was in good agreement with the experimentally obtained dissolution curve. The saturated solubility of ETV-H was approximately 25 mg/mL at 1.2 at 37 °C (*19*).

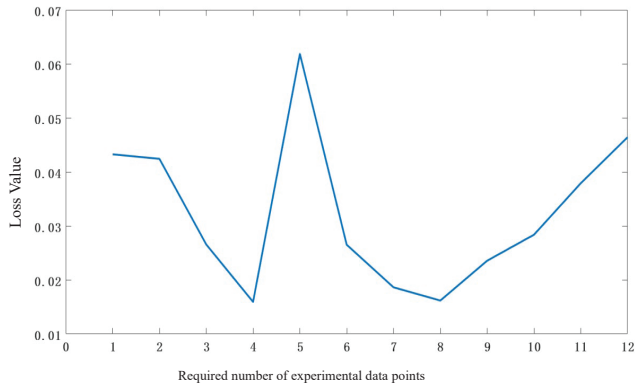

Figure 5. Determination of required number of experimental data points needed to predict the dissolution curve for ETV-H at 1.2. Loss values reflect differences between fitted (predicted) and actual (experimental) results. ETV-H: entecavir monohydrate.

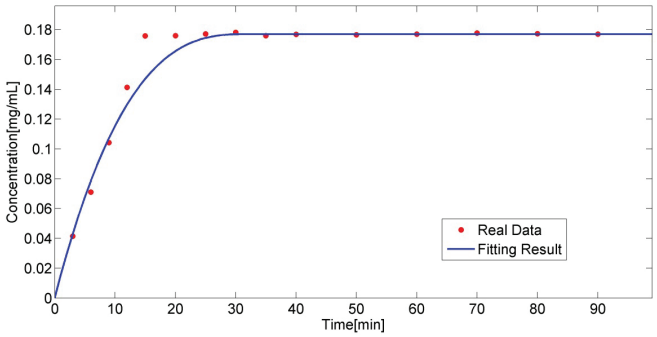

Figure 6. Comparison of predicted dissolution curve (blue line) and experimental results (red dots) of ETV-H at pH 1.2. ETV-H: entecavir monohydrate.

CONCLUSIONS

The dissolution behavior of ETV-H in four dissolution media was comprehensively investigated and compared with that of ETV-A. The dissolution rate of ETV-H in pH 6.8, pH 4.0, and ultrapure water was faster and the complete dissolution time was shorter than that of ETV-A. A soft-sensor analytic method for predicting the ETV-H dissolution curve using the Noyes Whitney equation was proposed. The dissolution curves of ETV-H in acidic and weak alkaline media (pH 6.8 and 1.2) were predicted. The predicted curves were consistent with the experimental results, differences (loss values) of 0.0013 and 0.016 at pH 6.8 and 1.2, respectively. This dissolution test method can save up to 75% of the experimental time for dissolution testing of bulk APIs.

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CONFLICT OF INTERESTS

The authors disclosed no conflicts of interest related to this article.

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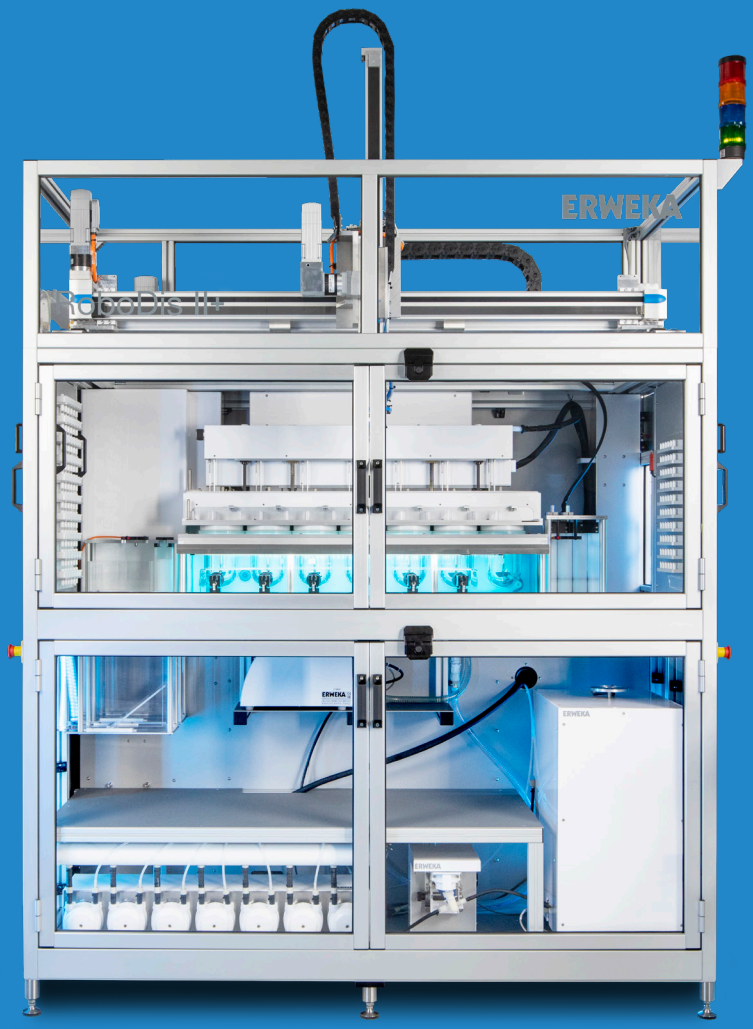

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Question & Answer Section

*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 What is the recommended approach for the performance verification test (PVT) for the qualification of USP Apparatus 3. The USP general chapter <711> Dissolution currently does not state any guidance. The manufacturer of my equipment notes the use of USP Chlorpheniramine Maleate extended-release calibrator tablets; however, I am unable to source these tablets.

A The USP Chlorpheniramine Maleate Extended-Release Tablets Reference Standard catalog #1123102 was discontinued in 2013. The last lot sold, G1J218, expired on February 28, 2014. It was discontinued due to a revision to <711> that became official in USP 36-NF 31. Please refer to the currently official chapter in the USP-NF Online for test details. Currently, the principle of the approach used to qualify the instrument is through mechanical calibration and verification of the individual components of the system.

Q I am trying to trace some history on USP Apparatus 4 (Flow through Cell). In the past, probably between 2003 and 2006, USP Apparatus 4 was included in USP<724> Drug Release, whereas currently it is in USP<711> Dissolution. Could you please provide additional information about this change.

A USP Apparatus 4 was transferred from <724> Drug Release to <711> Dissolution in the Second Supplement of USP 29 with an official date of Aug 1, 2006 to align with other pharmacopeias.

Q We are developing a product as an immediate-release soft gelatin capsule. We would like to know when Tier 2 dissolution conditions are needed.

A Tier 2 dissolution testing is done only when there is evidence of cross-linking in the gelatin capsules. Evidence of cross-linking can include the formation of a pellicle or thin membrane, which prevents the contents from releasing or prevents the capsule from rupturing. Refer to USP general chapter <711> Dissolution and <1094> Capsules – Dissolution Testing and Related Quality Attributes for further guidance.

Q In the USP monograph for Alfuzosin Hydrochloride Extended-Release Tablets, Dissolution Test 2, at the 1-hour time point, the acceptance criteria is not in the range form, it is NMT 20%. How can the Acceptance Table 2 from the USP general chapter <711> Dissolution be applied in this case?

A In such a situation, NMT 20% can be expressed as 0-20%.

Q Why there is no dissolution test in the USP monograph for Sitagliptin Tablets?

A Dissolution test is not mandatory. In certain cases, with appropriate justification, the dissolution test can be replaced with a disintegration test. See USP general chapter <1711> Oral dosage Forms – Performance Tests for more information.

Q Is it necessary to correct the dissolution results for water content?

A Typically, the dissolution results are not corrected for the water content in the samples. When using USP reference standards to prepare standard solutions, some standards require correcting for water content to accurately determine the final solution concentration; however, the possible implications of the water content in the dissolution results

should be evaluated during the product development and dissolution method validation on a case-by-case basis.

Q What is the tolerance in sampling time for USP apparatus 1 and 2?

A The tolerance for the sampling time is ± 2%. See USP general chapter <711> Dissolution, Procedure, Apparatus 1 and Apparatus 2, Immediate-Release Dosage Forms, Time.

Q What are the reasons for the the inclusion of a Blank solution to be made with one capsule dissolved in medium in the USP monograph for Cefdinir Capsules?

A The color of the capsule for the product marketed in the USA interferes with the quantitative procedure. As a result, the sample solutions are read using the capsule solution as blank to correct for the interference from the capsule components. This procedure should be evaluated using a case-by-case approach during the dissolution method validation and is required if the color or other capsule components interfere with the analytical procedure.

Q There is a USP monograph that states to do a background correction in the UV determination of the amount of drug substance released. The text in the monographs is "Analytical wavelength: 230 or 231 nm; use a suitable wavelength for background correction" What is the purpose of this correction and how is it done?

A The formulation that generated the dissolution test in the USP monograph has an interference from the placebo that can only be accounted for by performing a background correction at the specified wavelength. The absorbance measurement of the sample solution at 450 nm is subtracted from the absorbance obtained at 231 nm. As part of the dissolution method validation, the interference of placebo contents at the analytical wavelength of the active ingredient must be determined as well as the appropriate background correction wavelength. Determining the "suitable" wavelength for background correction will depend on the makeup of the placebo.

Q How should Simulated Intestinal Fluid (without enzyme) and Simulated Gastric Fluid (without the enzyme) be prepared?

A The instructions on how to prepare Simulated Intestinal Fluid and Simulated Gastric Fluid are in the Test Solutions section of USP-NF. Simply omit the addition of pancreatin to the intestinal fluid or pepsin in the case of gastric fluid.

Q When running a disintegration test, should we ensure that the immersion fluid start temperature is 37 ± 2° or is the intent to have a start and end temperature to ensure that this temperature is maintained throughout the testing?

A The temperature of the disintegration medium must be kept at 37 ± 2° during the entire test. The ability of the instrument to maintain temperature throughout the entire test should be part of the instrument qualification. Refer to the instructions recommended by the manufacturer of the disintegration instrument.

Q Why, in some USP monographs, is the quantitative procedure in the dissolution test different from the method used in the assay test?

A The quantitative procedure used in dissolution testing must be linear, precise, and accurate for entire dissolution profile. In most cases, the linearity range in dissolution is from around 5–15% up to 120–150% of the product label claim (considering the upper limit of the uniformity of dosage units test), this range is broader than that used for the assay test. It may be the case where the quantitative method used in the assay test does not provide the appropriate linearity, precision, and accuracy for this range, so another quantitative procedure must be used. In addition, because the assay sample and dissolution samples are prepared in different ways, issues such as the placebo interference, the effect of dissolution or extraction media, and other factors may be different in each case. Interference from various sources must be considered when selecting the appropriate quantitative procedure for each test.

Q **In the USP monographs there is no mention of the type of sampling, automated or manual, used in the dissolution test. Which sampling procedure is the most appropriate?**

A The sampling procedure is decided by the laboratory during the dissolution method validation process. There are no general rules other than the sampling method should meet the tolerance for the sampling time (± 2%) and sample volume accuracy (± 1%) as described in the general chapter <711> Dissolution. If automated sampling is going to be used, it must be validated for each product separately.

Q **USP general chapter <701> Disintegration states that disks can only be used if specified or allowed in the monograph. We purchased new disintegration equipment that has disks with sensors that automatically detect the endpoint of the disintegration test. These disks must remain in the tubes for all tests during the entire test. How can we justify the use of the disks in all methods?**

A To use this type of equipment, a validation of the new disintegration method using the disks should be performed. The goal of the method validation would be to demonstrate that the presence of the disks does not effect the disintegration evaluation for each of the products previously evaluated without disks.

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 15–16, 2023

MIDD+ 2023 Conference - Improving Health Through Innovative Solutions in Model-Based Drug Discovery
Location: Online
Registration: https://www.simulations-plus.com/events/midd-2023/

February 22–24, 2023

Biowaiver, Bioequivalence, and Dissolution Testing
Sponsored by AAPS IVRDT Community and College of Pharmacy University of the Philippines Manila
Location: Online from 8 am -12 pm each day, Philippine time (PHT)
For information, email: vagray@rcn.com

February 23, 2023

Dissolution Discussion Group Quarterly Online Meeting—Revisions to USP chapter <1724> Dissolution testing of semisolids
Location: DDG Online Meeting at 10:30 am ET
Registration: https://www.agilent.com/chem/dissolution-webinars

March 3, 2023

European Complimentary Introduction to GastroPlus® Workshop
Location: Online
Registration: https://www.simulations-plus.com/events/european-gastroplus-introductory-workshop/

March 6–8, 2023

Introduction to GastroPlus Workshop
Location: San Diego, CA, USA
Registration: https://www.simulations-plus.com/events/introduction-to-gastroplus-workshop/

March 9–10, 2023

Advanced GastroPlus DMPK and Clinical Pharmacology Workshop
Location: San Diego, CA, USA
Registration: https://www.simulations-plus.com/events/advanced-gastroplus-dmpk-and-clinical-pharmacology-workshop/

March 9–10, 2023

Advanced GastroPlus Pharmaceutical Development Workshop
Location: San Diego, CA, USA
Registration: https://www.simulations-plus.com/events/advanced-gastroplus-pharmaceutical-development-workshop/

March 31, 2023

25th Annual Conference, AAPS-Northeastern regional discussion Group
Location: St. John's University, Queens Campus, 8000 Utopia Pkwy, Queens, NY, USA
For registration & more info: https://aaps-nerdg.org/

April 3, 2023

Complimentary Introduction to GastroPlus® Workshop
Location: Online
Registration: https://www.simulations-plus.com/events/complimentary-introduction-to-gastroplus-workshop-7/

April 20–21, 2023

Comparative Clinical Endpoint and Pharmacodynamic Bioequivalence Studies for Generic Orally Inhaled Drug Products – Considerations and Alternatives
Location: Online and in person, Rockville, MD, USA
For information, visit info@complexgenerics.org

May 25, 2023

Dissolution Discussion Group Quarterly Online Meeting—Looking Ahead: The dissolution lab of the future

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

July 24–28, 2023

Controlled Release Society 2023 Annual Meeting

Location: Las Vegas, NV, USA
For information, visit http://www.controlledreleasesociety.org/meetings/annual

July 27, 2023

Dissolution Discussion Group Quarterly Online Meeting—Go with your gut: A biorelevant dissolution media discussion

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

October 12, 2023

Advances in PBPK Modeling and its Regulatory Utility for Oral Drugs Product Development

Location: Online and in person, College Park, MD, USA
For information, visit info@complexgenerics.org

October 22–25, 2023

PharmSci 360 AAPS Meeting

Location: Orlando County Convention Center, Orlando, FL, USA
For information, visit https://www.aaps.org/pharmsci/annual-meeting

November 13–15, 2023

Eastern Analytical Symposium and Exhibition

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

November 23, 2023

Dissolution Discussion Group Quarterly Online Meeting—Dissolution Qualification: The PQ vs MQ debate. What's right for your lab?

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

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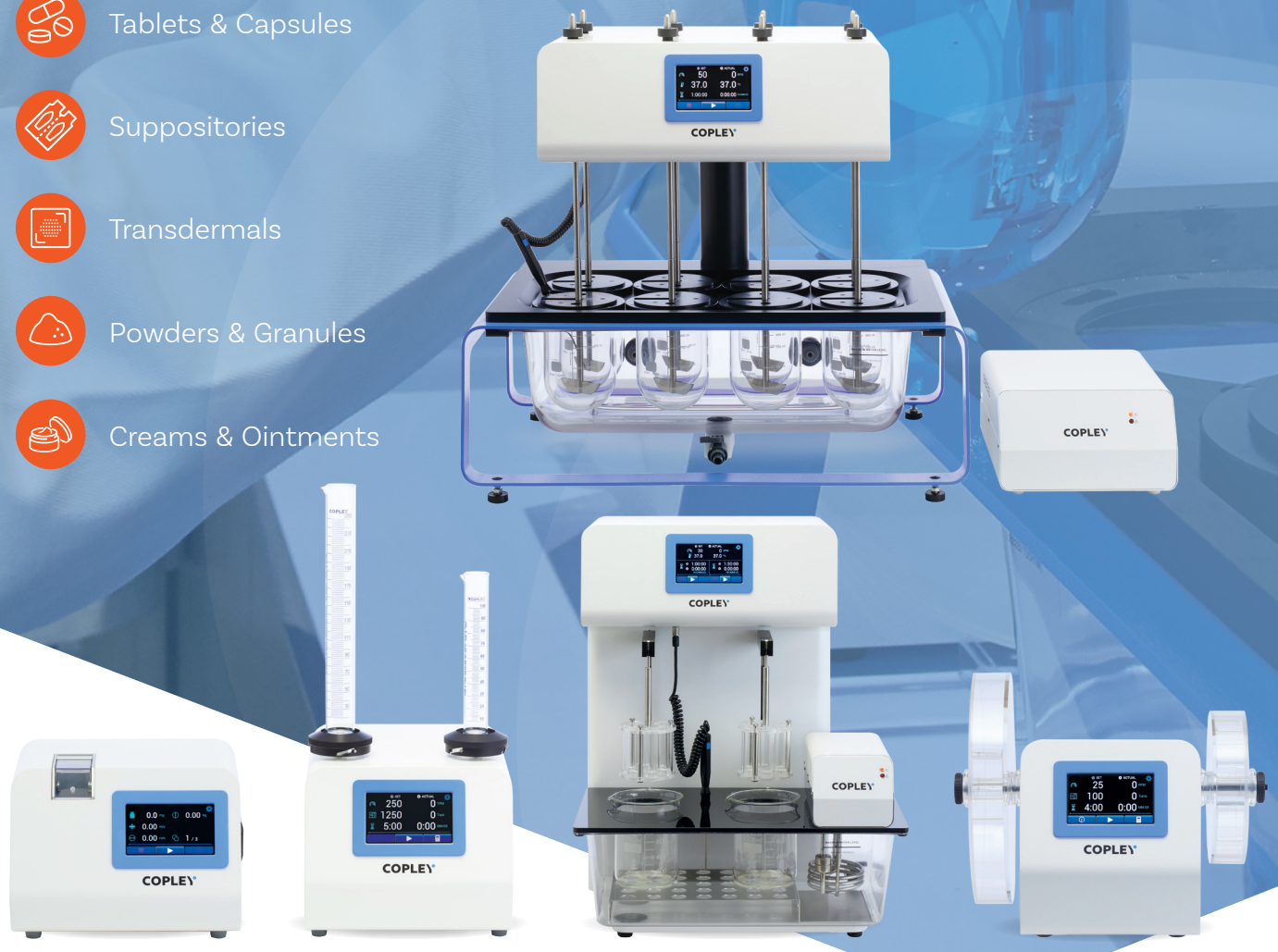

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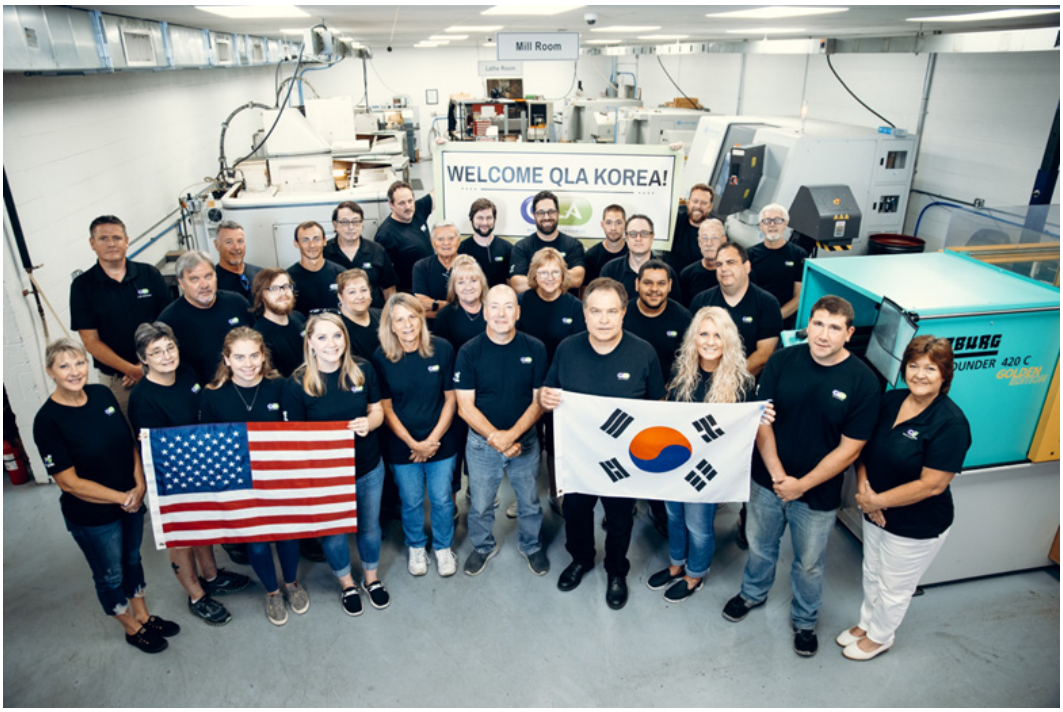
Industry News

Quality Lab Accessories Launches QLA Korea

Quality Lab Accessories (QLA) is proud to announce our QLA brand expansion into the Asian market with the introduction of QLA Korea. QLA Korea is headquartered in South Korea and operated by Gyujong Lee.



About QLA

For over 25 years, QLA has been a leading manufacturer and supplier of Dissolution Accessories and Consumables, Calibration Tools and Services to Pharmaceutical Laboratories, worldwide. We pride ourselves on being the only company in our industry that not only has a dedicated sales force and Lab Services division but also a fully staffed machine shop and modern manufacturing facility located in Telford, PA. Our products are engineered and manufactured according to the precise specifications of the USP and Original Equipment Manufacturer (OEM). We are the "go-to" company for custom fabrication and we solve unique lab problems with innovative designs and common-sense solutions. QLA is ISO 9001 certified (Cert# FM595556). QLA's main goal is to give our customers the ultimate customer experience in our industry.

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Senior Appointments at Copley Signal Start of New Phase of Growth

03 January 2023; Nottingham, UK: Copley begins 2023 with news of two key appointments to the senior team: Jamie Clayton as Managing Director and Matthew Fenn as Head of Business Development. The appointments follow a period of sustained growth for Copley, which ended 2022 as Nottinghamshire Business of the Year winners, and reflect ambitious plans for the future.

Known across the pharmaceutical industry for his role in propelling the FT4 Powder Rheometer to global prominence, Clayton brings complementary knowledge and widely respected expertise to the top table. With close to 20 years of experience in the healthcare sector, Fenn will focus on customer needs, advancing education and training opportunities and delivering ever more innovative product and service solutions.

Mark Copley (left) welcomes to Copley Jamie Clayton (middle) as Managing Director and Matthew Fenn (right) as Head of Business Development.

"Copley has grown substantially over the last five years," said Mark Copley, CEO, "and we continue to welcome new recruits at all levels to augment our well-established team. Securing senior talent is an essential element of our long-term strategy but relies on identifying experienced individuals that combine a 'Copley' mindset with complementary and relevant experience. Both Jamie and Matthew are an ideal fit and I'm excited to begin working with them."

As Managing Director, Clayton will manage all day-to-day operations at Copley from finance through to product development. A primary focus will be ongoing growth of the team and embedding the company culture, to ensure optimal practice and an exceptional customer experience as expansion continues.

"Jamie has a proven track record of company management, a customer-centric approach and in-depth technical expertise in all aspects of powder technology, from dry powder inhaler formulation to tablet manufacture," said Mr Copley. "His appointment is a major boost for Copley that will release me to explore and progress new investment opportunities and strategic partnerships, a vital element of our ongoing plans."

Fenn joins Copley from Jabil, the world's largest manufacturing solutions provider for the healthcare industry with experience that includes a long spell at 3M Health Care. With a background in medical devices, pharmaceutical delivery systems and consumer health products, Matthew is well-equipped to help Copley to define needs, refine services and set new goals for innovation.

"Matthew's key objective is to build the business development team and customer relationships that we need to drive growth," said Mr Copley. "The aim is to get closer to our customers to understand exactly what they need to succeed and how best to deliver it. Copley is growing by remaining true to our core enduring values and at the same time open to new thinking. Both Jamie and Matthew can be sure of the warmest of welcomes and an exciting time ahead."

About Copley Scientific

Copley Scientific is widely recognised as the world's leading manufacturer and supplier of inhaler test equipment and is a major provider of testing systems for other pharmaceutical dosage forms. The company also supplies equipment for detergent testing.

Copley's pharmaceutical product range includes test equipment for all types of orally inhaled and nasal drug products - metered-dose inhalers, dry powder inhalers, nebulisers and nasal sprays - with a particular focus on solutions for delivered dose uniformity and aerodynamic particle size distribution measurement. It also includes testers for tablets (dissolution, disintegration, friability and hardness) capsules, powders, suppositories, semisolids and transdermals.

Used from R&D through to QC, this extensive range of equipment is supported by a full validation and aftersales service. Copley works in partnership with specialist distributors to extend localised support across the world. This network provides expert help and training to every customer, directly enhancing the application of all Copley products. www.copleyscientific.com

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Logan Instruments launches the Inhaler Testing System

Logan Instruments Corp. is pleased to introduce the newest automated AIR-1300 Delivery Dosage Uniformity (DDU) Inhaler testing system and semi-automated next generation impactor (NGI) and Andersen cascade impactor (ACI) systems.

AIR-1300 is a fully automated benchtop system that can test metered dose inhaler (MDI), nasal spray, and nasal aerosol products with extreme precision and repeatability. While allowing for the option to use any standard industry test procedures, it provides total control over testing techniques.

- Support for all types of apparatus
- Temperature and relative humidity measurement functions are available
- Built-in analytical balance enables automatic equipment weighing
- Continuous testing of up to 13 samples with high efficiency

VAC-2000 is the most advanced vacuum pump technology available, offering great performance and minimum maintenance. It is designed for the best performance at the flow rates required for MDI and dry powder inhaler (DPI) testing. The range of the vacuum pump is in accordance with pharmaceutical regulations for inhaler testing.

ACI-800 is an 8-stage cascade impactor that is specifically made for measuring the aerodynamic particle size distribution (APSD) produced by MDIs and DPIs. An aerosol stream passes through each stage, larger particles stay at that stage's collection plate, while smaller particles will move on to the next impaction stage. The flexibility of 4 to 12 stages is offered to help with the testing of various medications.

NGI-800 is a high-performance, precision impactor designed specifically for pharmaceutical inhaler testing. To evaluate dry-powder and metered-dose inhalers as well as other inhaled medication delivery systems including nebulizers and nasal sprays, Logan NGI-800 classifies aerosol particle sizes into size fractions. Logan provides a specially designed refrigerator to ensure temperature control.

CAF-1200 enables automated flow settings on the intuitive touchscreen control. Temperature and relative humidity sensors are available to offer precise information about environmental conditions.

Pictured: AIR-1300 (left), NGI system (middle), and ACI system(right).

For more information, contact info@loganinstruments.com or visit www.loganinstruments.com.

LOGAN INSTRUMENTS CORP.

Simulations Plus and University of Florida Awarded New FDA Contract

New Contract to Support Development and Regulatory Assessment of Inhaled Products

LANCASTER, CA, November 2, 2022 - Simulations Plus, Inc. (Nasdaq: SLP), a leading provider of modeling and simulation software and services for pharmaceutical safety and efficacy, today announced that, through a joint proposal with the University of Florida's College of Pharmacy, it has been awarded a new funded contract from the U.S. Food and Drug Administration (FDA) to advance in vitro and (patho)physiology-based pharmacokinetics (PBPK) models to understand and predict pulmonary absorption and tissue retention of inhaled drugs.

For this award, Dr. Rodrigo Cristofoletti, Assistant Professor in the Department of Pharmaceutics, and his lab at the University of Florida will generate in vitro data from different systems, including cells from healthy subjects as well as patients with asthma and COPD, to assess the mechanistic components of pulmonary absorption for different drugs. The scientific team at Simulations Plus will apply these datasets, along with additional pathophysiology information for asthma and COPD populations, to validate the enhanced Pulmonary Compartmental Absorption and Transit (PCAT™) model within the GastroPlus® platform for different orally inhaled drug products (OIDPs). The resulting outcome will provide the foundation of a viable alternative to in vivo studies for the establishment of bioequivalence for OIDPs.

Mr. James Mullin, Associate Research Fellow at Simulations Plus and co-Principal Investigator for this project, said: "Our novel physiologically based biopharmaceutics (PBBM)/PBPK modeling approach within GastroPlus for pulmonary delivery was initiated in 2009 through an industry-funded collaboration, and to date there have been over 25 peer-reviewed journal publications validating the platform across a wide range of drugs and chemicals. This partnership with Dr. Cristofoletti, his team, and the FDA will expand and improve upon our current cutting-edge technology. Ultimately, the strategies that we are outlining and implementing for applying in vitro systems and in silico models hold potential to lower regulatory burden and minimize the need for animal and human studies."

FDA scientific and program staff will actively collaborate with the University of Florida and Simulations Plus. Mr. Mullin, with assistance from Dr. Maxime Le Merdy, Ms. Farah AlQaraghuli, and Dr. Viera Lukacova, will coordinate modeling and simulation activities of the contract.

"We are very excited about the funding of this FDA contract that allows us to collaborate with Dr. Guenther Hochhaus, Dr. Jürgen Bulitta, and Simulations Plus," said Dr. Cristofoletti. "Our goal is to allow realization of new models that will bridge the gap between in vitro and human data and guide the development of biopredictive methods to improve the mechanistic understanding of drug performance locally within the lung."

Funding for this collaboration is made possible by the Food and Drug Administration through contract 75F40122C00182. 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.

SimulationsPlus

Simulations Plus and University of Bath Awarded New FDA Grant

LANCASTER, CA, November 15, 2022 - Simulations Plus, Inc. (Nasdaq: SLP), a leading provider of modeling and simulation software and services for pharmaceutical safety and efficacy, today announced that, through a joint proposal with the University of Bath's Department of Life Sciences, it has been awarded a new funded grant from the U.S. Food and Drug Administration (FDA) to advance state-of-the-art, physiologically-based biopharmaceutics (PBBM)/pharmacokinetics (PBPK) modeling approaches that can inform regulatory decisions on innovator and generic products for locally acting drugs in the gastrointestinal (GI) tract.

For this award, Dr. Nikoletta Fotaki, Professor in Pharmaceutics, her collaborators (Dr. Bernardo Castro Dominguez, Assistant Professor, Department of Chemical Engineering) and her lab at the University of Bath will generate in vitro data from different systems for selected commercial formulations, including conditions simulating healthy, ulcerative colitis (UC), and Crohn's disease (CD) GI environments, to assess drug release and characterize the critical quality attributes (CQAs) of the marketed products to generate and evaluate formulation variants. The scientific team at Simulations Plus will apply these data sets, along with additional pathophysiology information for UC and CD populations, to enhance and validate the Advanced Compartmental Absorption and Transit (ACAT™) model within the GastroPlus® platform and determine the impact of changes to CQAs and physiological variables on local and systemic exposure in patient groups. The resulting outcome will provide the foundation of a viable alternative to in vivo studies for the establishment of bioequivalence for locally acting GI products.

Dr. Haiying Zhou, Sr. Director, Simulation Technologies at Simulations Plus and co-Principal Investigator for this project, said: "The story of GastroPlus started in 1998, and for nearly 25 years it has been, time and time again, independently verified as the preeminent software for predicting oral drug absorption for pharmaceuticals and chemicals alike. This new collaboration with Dr. Fotaki, her team, and the FDA will look to build upon our foundation to improve the accuracy of drug concentrations predicted locally within gut tissue and outline novel strategies for applying in vitro systems and in silico models to lower regulatory burden and minimize the need for animal and human studies as new formulation approaches are evaluated for addressing GI diseases."

"Thanks to this FDA-funded partnership with Simulations Plus, the future is coming much quicker than we predicted, and this project will be a great way for both industry and academia to make that leap from research into real life applications, with potential benefits of tangible patient outcomes," said Dr. Fotaki. "Our goal is to allow realization of new models that will bridge the gap between in vitro and in vivo data, and the final framework and best practices developed under this contract will be meaningful for both the FDA as well as the end users in the companies that develop therapies for this disease space."

Funding for this collaboration is made possible by the Food and Drug Administration through grant award 1U01FD007660-01. 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.

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