Introduction to the OrBiTo decision tree to select the most appropriate *in vitro* methodology for release testing of solid oral dosage forms during development

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Abstract

The EU research initiative OrBiTo (oral biopharmaceutics tools) involving partners from academia, pharmaceutical industry, small medium enterprises and a regulatory agency was launched with the goal of improving tools to predict the absorption of drugs in humans and thereby accelerating the formulation development process. The OrBiTo project was divided into four work packages (WP), with WP2 focusing on characterization of drug formulations. The present work introduces the OrBiTo WP2 Decision Tree, which is designed to assist the investigator in choosing the most appropriate *in vitro* methods for optimizing the oral formulation design and development process. The WP2 Decision Tree consists of four stages to guide the investigator. At the first stage, the investigator is asked to choose the formulation type of interest. At the second stage, the investigator is asked to identify which type of equipment (compendial/modified/noncompendial) is preferred/available. At the third stage, characteristics of the active pharmaceutical ingredient (API) are evaluated and in the fourth stage of the decision tree, suitable experimental protocols are recommended. A link to the living Decision Tree document is provided, and we now invite the pharmaceutical sciences community to apply it to current research and development projects and offer suggestions for improvement and expansion.

Keywords:

Dissolution, decision tree, formulation, oral absorption, PBPK, biopharmaceutics classification system (BCS)

1. Introduction

Over the past two decades, the importance of predictive dissolution testing during formulation development has greatly increased. Since the introduction of biorelevant dissolution media as a milestone in the late 90s, a variety of predictive *in vitro* methods have been developed. Today, numerous publications demonstrating the usefulness of such dissolution methods for predictive investigations can be found in the literature [1-15].

In 2012, the EU research initiative OrBiTo (innovative tools for oral biopharmaceutics, www.orbitoproject.eu/) involving partners from academia, pharmaceutical industry, small medium enterprises and a regulatory agency was launched with the goal of improving tools to predict the absorption of drugs in humans and thereby accelerating the formulation development process. The OrBiTo project was divided into four work packages (WP) focusing on tools regarding the characterization of the API (WP 1) and formulations (WP 2), *in vivo* studies to address gaps in our knowledge of the gastrointestinal tract (WP 3) and the utilization of *in silico* tools (WP 4) (please refer to Figure 1 for an overview). For WP 2, in addition to the eleven tasks which evaluated various dissolution methodologies to address specific drug formulation challenges, a key task was to generate a decision tree to assist investigators in identifying the most appropriate *in vitro* methodology for a given drug/formulation combination.

The aim of this publication is to introduce the web-based decision tree to the scientific community and to invite investigators in the pharmaceutical development to use and participate in revising and extending the decision tree. The current version can be accessed via link here. An overview of the structure of the decision tree is given in the Supplementary Material, along with a Guide for its use. At the moment, the decision tree focuses on the *in vitro* methods which were included in the OrBiTo WP 2 task list as a platform to identify gaps and innovation needs in predictive dissolution testing. However, given the availability of numerous other *in vitro* methods in the literature, the decision tree may serve as a springboard to

extensions in areas not specifically investigated in OrBiTo and thus provide a more comprehensive guide to method selection.

** To open the decision tree document, the free software yEd graph editor is required, which can be downloaded at the following link:* https://www.yworks.com/products/yed.

2. OrBiTo partners and methods

The proposed decision tree represents input from six academic partners, thirteen pharmaceutical companies and one small medium enterprise who all participated in WP 2 of the OrBiTo project (table 1).

The focus of WP 2 was to develop and optimize *in vitro* release methods. In this context, a variety of different dissolution approaches was developed and optimized for immediate (IR), delayed (DR) and extended release (ER) formulations. The investigations included the use of dissolution media with different levels of complexity in terms of their composition, the use of single- vs. multi-compartmental methods such as United State Pharmacopiea (USP) apparatus II-IV or test systems aimed at a more physiological representation of the hydrodynamic forces such as the stress tester or the TIM (Total gastro-Intestinal Model) systems. Where applicable, ring studies were conducted to investigate the reproducibility of the experimental conditions as part of the method validation. An overview of the methods investigated, along with references to associated literature, can be found in table 2.

At this point, the decision tree encompasses the methods investigated in WP 2 of the OrBiTo project. However, the decision tree is intended to be regarded as a living document guiding investigators in the decision process to select the most appropriate *in vitro* test during formulation development and it is anticipated that further methods will be added over time.

3. The general structure of the decision tree

The general structure of the decision tree is depicted in figure 2. In the first stage, the decision tree offers three branches: immediate, extended and delayed release formulations. The investigator chooses one of these branches, depending on which type of oral dosage form is being developed. As a wide variety of different dissolution systems exist, not all of which are readily accessible to all researchers in the industry, at the second stage the investigator is asked to identify which type of dissolution equipment is available/preferred for the release testing. Specifically, the decision tree asks whether compendial-based dissolution systems or methods that are intended to be more physiologically-based, such as the Netherlands Organisation for Applied Scientific Research (TNO) systems, are preferred.

For the decision about which dissolution method is most suitable, not only the formulation type but also the active pharmaceutical ingredient (API) properties highly influence the selection. These aspects are addressed in the third stage of the decision tree. On the one hand, the solubility of the API can have a great impact on the selection of the experimental conditions. It is widely known that the dissolution rate of poorly soluble compounds can be affected by the presence of bile components and lipids, which are present in biorelevant dissolution media, compared to the simple aqueous buffers commonly used as compendial media [15, 36-39]. On the other hand, ionization of the compound may prompt additional investigations. For instance, poorly soluble weak bases have a higher risk for precipitation in the gastrointestinal tract than neutral compounds due to the pH-dependency of their solubility [40]. In such a case, tools for characterization of the API developed in WP 1 e.g. computational models, µDISS measurements, and miniaturized solvent shift techniques can be utilized [41] to make a preliminary assessment about which type of test is needed.

The investigator can choose from recommended *in vitro* protocols for the respective drug/formulation combination. In the fourth and most detailed stage of the decision tree, experimental protocols are made available. The general structure of these documents is shown in figure 3.

For each of the *in vitro* methods presented, a short introduction to the experimental setup, exemplary experimental conditions as well as references for further information are given. Where possible, limitations of the utilization of the respective method are indicated. The decision tree thus provides an overview of dissolution methods appropriate for a given drug/formulation combination as well as the practical information required to perform the dissolution experiments.

It is further recommended, based on OrBiTo experience, that information gained from the release tests can be incorporated into *in silico* tools from WP 4, such as PBPK modeling, in order to get a deeper understanding of the relative importance of the various factors affecting drug absorption *in vivo* [42].

4. Application of the OrBiTo WP 2 Decision Tree

In this section we provide three examples of how the decision tree would roll out in different development scenarios.

4.1. Scenario 1: Immediate release formulation of a poorly soluble weak base

In the first case example, let us assume that the investigator is asked to conduct dissolution studies on a poorly soluble weak base (BCS II) formulated (or to be formulated) as a standard immediate release formulation.

As a first step, the investigator would enter the IR branch of the decision tree. Due to limited access to more physiologically-based dissolution apparatus, in the second step it is decided to use a compendial-based equipment. Preformulation studies have indicated that the compound is a weak base and demonstrates a poor dose-solubility ratio in FaSSIF (media simulating the fasted state conditions in the small intestine). For that reason, the weak base decision tree is chosen.

Further preliminary investigations on the drug substance have revealed that the solubility in the intestinal dissolution medium FaSSIF is lower than in the corresponding gastric medium FaSSGF, indicating a risk of precipitation (depending on the dose) when the drug enters the small intestine. The decision tree therefore recommends investigation of supersaturation/precipitation by using e.g. two-stage tests or transfer experiments. Reaching to the deepest level of the decision tree,

the investigator is provided with specific information on the appropriate setup as well as literature references for further information and application examples.

4.2. Scenario 2: Extended release formulation of a poorly soluble drug

In the second application example (see Figure 4), let us assume that the investigator is asked to examine the risk of food effects on a poorly soluble compound formulated as a capsule containing extended release microparticles (particle diameter 250 µm).

This time, the investigator chooses the extended release branch of the decision tree and has access to methods utilizing the compendial dissolution systems USP II-IV (paddle, BioDis, flow through cell). Due to the poor solubility of the compound and the changing dissolution environment along the gastrointestinal tract in both the fasted and fed state, the investigator chooses to use a multi-compartmental dissolution approach, e.g. USP apparatus III or IV, both of which can be used to simulate the passage of the dosage form through the gut.

The investigator enters the decision tree for extended release formulations using USP apparatus III and IV and follows the decision tree for poorly soluble compounds. As a next step, he is asked to provide the size of the dissolution rate controlling units of the formulation. In the USP apparatus III, the formulation is contained in a glass cylinder which has both a top and bottom mesh, preferably with a mesh opening of 420 µm. As the multi-particulate formulation under consideration has a particle size smaller than the mesh openings, the dissolution test will likely result in under-recovery of the drug due to undissolved particles passing through the mesh. These particles would then be lost when the reciprocating cylinder moves to the next row of glass vessels [12].

Since the particle size of the microgranules is smaller than 420 µm, it is recommended to perform the dissolution studies in the flow-through cell, which mitigates the risk of losing drug particles. In the deepest level of the decision tree, the investigator is provided with detailed information as to the selection of the appropriate dissolution media and experimental conditions representative of the fasted and fed states.

4.3. Scenario 3: Immediate release, lipid-based enabling formulation of a poorly soluble drug

In the third case example, the formulation team decides to pursue a lipid-based formulation approach to overcome the solubility-limited absorption of a poorly soluble drug. The investigator is asked to rank order different lipid-based formulations using *in vitro* release studies.

For this purpose, the investigator enters the IR branch of the decision tree. Due to the higher complexity of the formulation compared to conventional IR tablets, the investigator is recommended to consider extra experiments for the investigation of enabling formulations (see Figure 5).

In this context, the investigator receives an overview of potential tools for the *in vitro* characterization including two-step digestion models, which allow the estimation of the effect of lipid digestion on the release profile of lipid-based formulations.

In the experimental protocol provided, specific information about how to set up the experiments, as well as pertinent literature references, are given.

5. Summary and Outlook

With an increasing variety of *in vitro* methods available in the literature, it is becoming more and more important to understand the range of application of each method. The present work introduces the OrBiTo decision tree, which is designed to assist the investigator in choosing the appropriate experimental conditions and thus use the most appropriate methodology to optimize the formulation design and development. Currently, the decision tree focuses on the *in vitro* methods developed as part of the OrBiTo project. However, it is anticipated that, with feedback from the pharmaceutical scientific community, the decision tree will be revised and extended in the future to cover a broader range of experimental methods, and that, going forward, an independent consortium committee consisting of members experienced in formulation development in the pharmaceutical industry will supervise the continuation and optimization of the decision tree. Based on OrBiTo experience, it is further recommended that

information gained from the release tests be incorporated into *in silico* tools, such as PBPK modeling, in order to get a deeper understanding of the relative importance of the various factors affecting drug absorption *in vivo.*

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