Optimising the application of *in vitro* test methods for the demonstration of bioequivalence in orally inhaled products

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Orally inhaled products (OIPs) are a commercially compelling target for generic development, with the combined annual revenues of key products such as Seretide®, Spiriva®, and Symbicort® in the region of $10 billion[1]. Diseases of the respiratory system account for 8% of all deaths in the EU, driving demand for treatments that are safe, efficacious and cost-effective. Replicating the performance of an OIP and demonstrating bioequivalence (BE) is complex, largely because OIP behaviour is a function of interactions between the patient, device and formulation. Ensuring the development of an optimal approach to the demonstration of BE is an important step in accelerating safe and effective generic products to market.

Demonstrating BE in any generic product typically relies on a combination of *in vitro*, pharmacokinetic (PK) and pharmacodynamic (PD) studies, though *in vitro* studies alone may be sufficient for certain products and/or in certain circumstances. For OIPs, there is ongoing debate as to the relevance of these different tests and the Food and Drug Administration (FDA) and European Medicines Agency (EMA) guidance differs in this regard. From a commercial perspective, reducing requirements for PK and/or PD studies is an attractive proposition since *in vitro* testing is typically the least expensive option. Maximising the clinical relevance of *in vitro* test methods – an important and ongoing theme in OIP research – supports this goal.

In this article we examine the testing strategies that can be applied to demonstrate the BE of OIPs, their relevance, and the submission approaches outlined by the FDA and EMA, including the information provided by product specific guidance. A key focus is the application of *in vitro* test methods and how these can be modified beyond the standard tests developed primarily for quality control (QC), to give improved *in vitro in vivo* correlations (IVIVCs) that are more useful for BE studies.

Testing for Bioequivalence

A generic product is prescribed interchangeably with the reference product and must therefore deliver closely equivalent clinical efficacy.

In vitro tests, PK and/or PD studies are all routinely used to support claims of BE between a test (T) and reference (R) product, but differ markedly in terms of their:

- Complexity
- Practicality
- Discriminating power
- Clinical relevance
- Cost.

A simple assessment of the way in which inhaled drugs reach the lung and achieve therapeutic action helps to elucidate the relevance, value and limitations of these different testing strategies, and highlights the unique difficulties associated with demonstrating BE between OIPs.

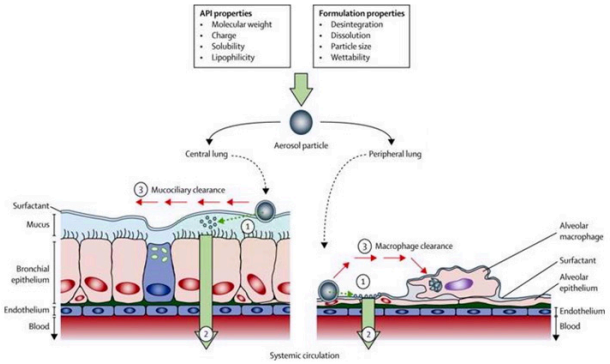

Figure 1: Inhaled drugs typically deliver localised action via a process of (1) deposition and release of API, (2) dissolution and absorption of the API, permeation into the lung tissue and target engagement. (3) Clearance of the undissolved particle. [Reproduced with permission from [2]].

During inhalation, aerosolised particles are drawn from the OIP through the oropharyngeal region, into the main airways of the lung, and potentially into the deep lung. An upper size limit of ten microns is typically assumed for penetration into the upper airways and five microns for deposition in the deep lung, with coarser particles depositing in the mouth/throat and likely entering the bloodstream via the gastrointestinal (GI) tract. Once deposited in the deep lung, inhaled drug particles dissolve in the limited quantities of fluid that line the lungs, although mucociliary clearance (MCC) mechanisms simultaneously act to flush the particles from the body. Permeation into the lung tissue brings the dissolved drug into contact with its intended target, enabling binding and

therapeutic action, which is localised in the case of most OIPs. Any drug absorbed through the lung tissue enters into systemic circulation (see figure 1).

***In vitro* test methods** are used to quantify a number of metrics directly associated with OIP efficacy. Core tests include the amount of drug delivered under standardised/ well-controlled conditions – delivered dose uniformity (DDU) – and the aerodynamic particle size distribution (APSD) of that dose, which is measured using cascade impaction and influences *in vivo* deposition behaviour. Other tests may include spray plume and plume geometry measurements, in the case of metered-dose inhalers (MDIs).

Simple, repeatable, easy-to-use and validate *in vitro* methods are highly differentiating and are heavily relied upon for product QC. However, few good examples of IVIVCs exist for OIPs. This is attributable to factors such as variability in anatomy/ impairment of the lung, and device use and compliance which make it difficult to secure robust relationships between product characteristics and clinical efficacy. As a result, *in vitro* testing is often and necessarily supported by *in vivo* studies (PK/PD) for the demonstration of BE.

The focus of **PK studies** is to determine the fate of the drug substance within the body, primarily by tracking drug concentration in the blood plasma. PK studies are relatively straightforward to implement and can exhibit high discriminating power, particularly if a healthy patient population is used.[3] With regards to demonstrating BE, they are particularly helpful for quantifying total systemic bioavailability to compare drug safety since it is generally accepted that if the systemic concentration measured for T is the same or less than that measured for R then systemic effects will be equivalent or less severe. This conclusion can mitigate the need for PD testing[3].

The use of PK studies as an indicator of pulmonary bioavailability and hence clinical efficacy is more challenging since it can be argued that drug concentration in the blood is the result of pulmonary fate, rather than a reliable indicator of concentration/ effect at the site of action in the lung[3,4,5]. The implications for clinical efficacy of a difference in PK study results may therefore only be robustly resolved via further *in vivo* studies.

PD studies quantify the biological and physiological impact of the drug substance and can be used to investigate both safety and efficacy with a high degree of clinical relevance. However, they can be relatively difficult to implement with a tendency to exhibit high variability, not least because of the need to work with a diseased patient population. Sensitivity can also be an issue. Successful implementation relies on the identification of a measurable, clinically relevant parameter linked with the pharmacological mechanism of the drug – a suitable biomarker. This biomarker must enable the demonstration of a robust dose-response relationship, when tested with representative doses, for a study to be differentiating. If T and R products result in a similar clinical endpoint then this can only be securely taken as being indicative of similar efficacy if a change in dose is rigorously associated with a measurable response[4,5].

The regulatory landscape for generic OIPs

The complexities of PK/PD studies for OIPs, and the difficulty of establishing optimal strategies for their application in the demonstration of BE are arguably reflected in the differences in approach in regulatory guidance in this area from the EMA and the FDA.

The stepwise approach for a generic OIP submission, set out in the latest regulatory guidance released by the EMA[6] indicates that a submission can be accepted on the basis of *in vitro* data alone (see figure 2) with no additional requirement for PK/PD testing.

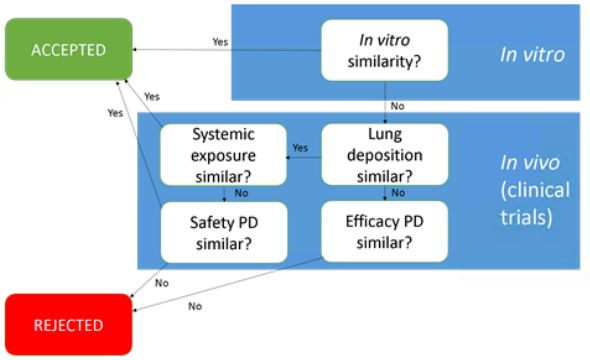

Figure 2: The EMA outlines a stepwise approach to testing in support of generic OIP submission.

The elimination of PK/PD testing substantially streamlines the submission process and is highly appealing from the perspective of time and cost savings, however, few products have yet to be approved on the basis of *in vitro* test data alone. The criteria for demonstrating *in vitro* only BE are demanding and call for the test product to match not only the chemical and formulation characteristics of the reference product, but also for the devices and their behavioural characteristics to be highly similar. This can be severely limiting in practice, especially when commercial and intellectual property considerations are taken into account. Furthermore, the application of a battery of *in vitro* methods[6] with the rigorous comparison of APSD measurements is a core element of such studies[7].

At the time of writing the FDA has no comparable formalised guidance, however the FDA 505(j) and 505(b)(2) pathways for generic and supergeneric OIP submissions respectively, call for a quite different "weight of evidence" approach (see figure 3). This involves qualitative and quantitative formulation sameness, device similarity, PK (comparative systemic exposure studies) and PD studies, in addition to *in vitro* tests. Beyond this general guidance, the FDA also offers a steadily increasing number of product specific guidances for popular generic targets[8]. These too typically indicate a requirement for both *in vitro* and *in vivo* (PK/PD) testing.

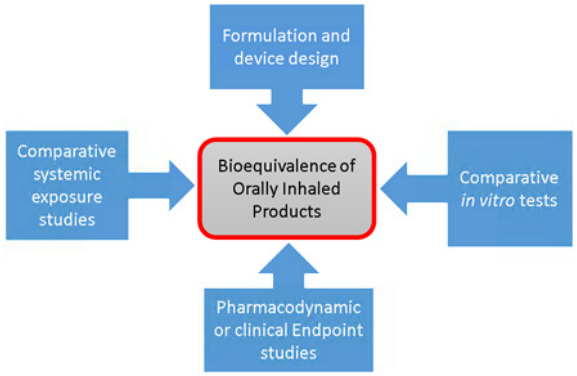

Figure 3: FDA guidance outlines a 'weight of evidence' approach.

The current regulatory position is that most approved generic OIPs have been subjected to some form of *in vivo* testing. Such PK/PD studies not only add in time and cost but may also introduce additional risk, with poor correlations between *in vitro* and *in vivo* data complicating the robust demonstration of BE. Improving the clinical relevance of *in vitro* methods to access better IVIVCs and enable the greater reliance on *in vitro* methods for BE testing is an important goal for the industry.

Moving towards better IVIVCs

When it comes to refining the existing test methods towards greater clinical relevance, it has been concluded that new models should be selected on the basis of their ability to reflect *in vivo* predictability – the primary aim - rather than anatomical correctness, where there is a choice to be made between the two[9]. Practicality is also an important consideration, both from the perspective of ease of use and of production. Simplifying models as far as is possible within the constraint of not compromising predictability helps to realise this goal. Adopting these strategies has already resulted in the development of a number of products that can be used to improve IVIVCs. These include:

- A more realistic throat model
- More representative breathing profiles
- The use of face models when testing add-on devices with face masks
- Dissolution testing.

A more realistic throat model

Figure 4: Use of an Alberta Idealised Throat (AIT) (bottom) more accurately reflects the amount of drug captured in the throat than a standard USP/Ph.Eur. induction port (top).

In a standard set-up for measuring the APSD of an OIP by cascade impaction (see figure 4) the product is interfaced to the impactor via the standard USP/Ph.Eur. induction port. This accessory has a simple right-angled geometry that is easy to use and allows reproducible drug recovery. However, it has been shown to capture less of the dose delivered by an OIP than would be deposited in the mouth-throat region during routine clinical use[10,11].

From the perspective of anatomical representativeness the use of a throat cast is a solution to this issue. However, throat casts are patient specific, difficult to manufacture reproducibly, problematic to interface with the device/impactor and tend to suffer from other practical limitations such as poor durability. The Alberta Idealised Throat (AIT) on the other hand is an alternative induction port (see figure 4) with a standard, idealised geometry developed from CT patient scans.

The AIT is reproducibly manufactured from aluminium, for compatibility with a wide range of solvents. It can be fully opened for drug recovery and to coat the internal surfaces to more closely simulate *in vivo* deposition. Adult and child versions are available for representative testing for specific patient groups. The AIT has been validated against clinical data over a period of around ten years and experimental data shows that it more accurately quantifies deposition than the standard USP/Ph.Eur. induction port[12]. This accessory is therefore a good example of a practical design that delivers enhanced predictability.

More representative breathing profiles

With many OIPs the breathing manoeuvre of the patient directly influences drug delivery. These include dry powder inhalers (DPIs), where aerosol generation is typically driven solely by the inhalation manoeuvre of the patient, and nebulisers and MDIs with spacers/valved holding chambers (VHCs), which are operated with a tidal breathing pattern. Changes to the pharmacopoeial test methods for nebulisers, and more recently for MDIs with spacers/VHCs, reflect this with defined breathing profiles now specified to simulate product use by certain patient groups[13,14].

Beyond the pharmacopoeial methods there remains potential to improve IVIVCs by further extending the use of more representative breathing profiles, particularly for passive DPIs, which can be sensitive to inhalation flow profiles. Cascade impactors are constant flow rate devices and the breathing profiles specified for APSD measurement have a rectangular (flow versus time) wave form. DPIs are tested at the flow rate that generates a 4 kPa pressure drop across the device, but the instantaneous application of this flow is poorly reflective of the ramp up to the peak inspiratory flow rate (PIFR) that occurs when a patient breathes 'strongly and deeply'. Crucially, this unrealistically rapid ramp rate is recognised as enhancing aerosolisation and dispersion in the first few fractions of a second of drug delivery – the dose emission phase – relative to what will likely occur in clinic. Pharmacopoeia methods for DPIs further call for testing at a 4 litre (or 2 litre) inhalation volume, which are not necessarily reflective of true patient values, especially for chronic disease states. This may also have consequences for device emptying.

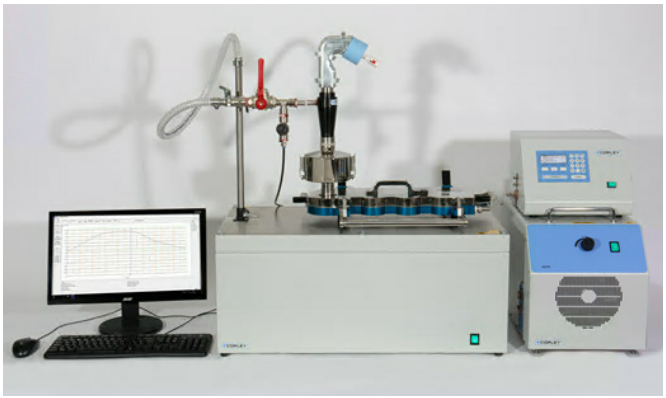

Figure 5: Breath simulators can be used as part of advanced testing methods to improve IVIVCs and allow better understanding of product performance, beyond the standard pharmacopoeial tests.

Breath simulators (see figure 5) are a cost-efficient solution for investigating how breath profiles impact drug delivery performance with commercially available systems offering the flexibility to vary defining characteristics such as inhalation or tidal volume, inhalation/exhalation ratio, frequency and waveforms. Such studies help to elucidate the clinical efficacy that may be observed in different patient groups and are very much aligned with a Quality by Design (QbD) approach to product development and a robust demonstration of BE. Indeed, confirming similarity between the performance of a T and R DPI across a range of flow rates supports claims that the products can be used interchangeably by all patients.

The use of face models when testing add-on devices with face masks

The correct use of MDIs, the most widely prescribed OIP for common respiratory illnesses, requires the patient to begin to inhale immediately prior to actuation thereby drawing the aerosolised dose directly into the lungs on the incoming breath. Certain patient groups, such as paediatrics, can find this level of coordination difficult to achieve and as a result tend to use MDIs with spacers and valved holding chambers (VHCs). These inexpensive and easily retrofitted devices eliminate the need for coordination by providing a dead volume into which the dose is aerosolised, and from which the patient inhales the drug, by breathing tidally. However, the introduction of dead volume can impact both the amount and APSD of the delivered dose.

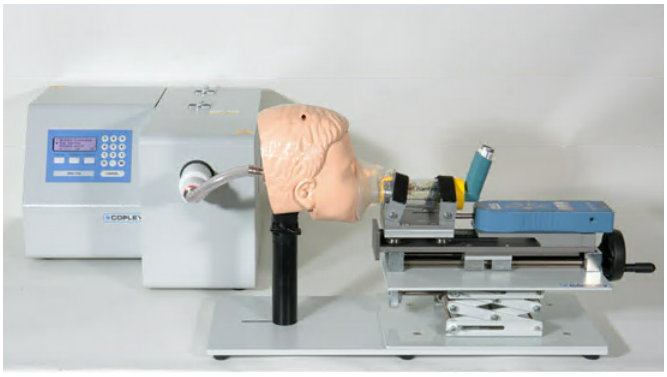

Figure 6: Newly introduced test methods for MDIs with spacers and VHCs specify the use of realistic face models when testing the face masks used with these add-on devices.

Newly released USP Chapter <1602>[13] represents the latest thinking regarding the testing of MDIs with add-on devices and details test methods that are highly relevant for the robust demonstration of BE for MDIs. In cases where the spacer or VHC features a facemask, interfacing it with the test apparatus presents a significant challenge.

The chapter allows for the use of face models that have the following clinical relevant characteristics:

- Appropriate facial dimensions for the intended user age range
- Ability to apply the facemask with the predicted amount of dead space when it is applied with a clinically relevant force to the model
- Physiological accurate soft facial tissue modelling around the chin, cheeks, and nose where the facemask makes contact
- Means of correctly mounting the spacer/VHC so that the facemask is oriented with the correct alignment to the face, as would occur when in use by the patient.

Commercially available systems exist (see Figure 6) to allow interfacing of infant, child and adult face models with apparatus for DDU and APSD testing of MDIs with spacers and VHCs, with some organisations choosing to develop their own face models to more closely match the facial structure of their intended patient population.

Dissolution testing

There are as yet no pharmacopoeial requirements for dissolution testing for OIPs though this is clearly the subject of FDA interest[15]. Where inhaled particles are very small then dissolution may be extremely rapid, however, for poorly soluble drugs, dissolution testing potentially has value for achieving a better understanding of *in vivo* behaviour. That said, the dissolution testing of inhaled drugs is challenging. For example, the amount of fluid in the lung is very limited and its composition varies depending on disease state and region of the lung.

A number of methods have been proposed for dissolution testing including the McConville/Copley methodology[16] which uses existing USP/Ph. Eur. tablet dissolution testing apparatus with sample captured using an NGI with modified cup and membrane holder (an alternative insert for the Andersen Cascade Impactor is also available). This set-up allows particles to be collected at defined impaction stages such that specific fractions of the APSD can be used for dissolution testing. Simulated lung fluid can be used as the dissolution medium.

There is evidence that dissolution testing can distinguish between formulations of the same drug and it is a particularly promising tool for investigating the performance of modified release formulations, or poorly soluble drugs. However, there remain practical challenges to overcome in the development and application of suitable, robust methodologies, not least in order to gather data that can be clearly correlated with *in vivo* behaviour and clinical efficacy and that can consequently play a role in the demonstration of BE.

In conclusion

At the heart of many current debates around the analytical strategies used to demonstrate BE in OIPs is the need to balance simplicity, practicality and reproducibility with clinical relevance. This is evident in issues associated with PK and PD studies and in moves to develop *in vitro* methods so as to improve their correlation with *in vivo* behaviour. Improving clinical relevance often involves the introduction of complexity and increased variability. Greater variability translates into lower differentiating power so a test that may be more clinically relevant for the demonstration of BE may be less able to detect a difference between a T and R product.

In vitro tests are the simplest of those that can be applied to demonstrate BE and their rigorous and robust development towards better IVIVCs has much to offer in terms of helping to streamline generic OIP submissions. Much progress has been made in this area over the last decade and current activities will undoubtedly deliver further advances. Optimising the application of *in vitro* methods will help to cut the time and cost of generic development while at the same time ensuring the safety and efficacy of new products.

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