Scientific Rationale for Determining the Bioequivalence of Inhaled Drugs

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Abstract In recent years, pathways for the development and approval of bioequivalent inhaled products have been established for regulated markets, including the European Union (EU), and a number of orally inhaled products (OIPs) have been approved in the EU solely on the basis of in vitro and pharmacokinetic data. This review describes how these development pathways are structured and their implications for the treatment of airway diseases such as asthma. The EU guidance follows a stepwise approach that includes in vitro criteria as the first step. If all in vitro criteria are not met, the second step is based on pharmacokinetic evaluations, which include assessments of lung and systemic bioavailability. If all pharmacokinetic criteria are not met, the third step is based on clinical endpoint studies. In this review, the scientific rationale of the European Medicines Agency guidance for the development of bioequivalent OIPs is reviewed with the focus on the development of bioequivalent OIPs in the EU. Indeed, we discuss the advantages and disadvantages of the weight-of-evidence and stepwise approaches. The evidence indicates that the EU guidance is robust and, unlike clinical endpoint studies, the pharmacokinetic studies are far more sensitive to measure the minor differences, i.e. deposition and absorption rates, in drug delivery from the test and reference products and, thus, should be best suited for assessing bioequivalence. The acceptance range of the 90% confidence intervals for pharmacokinetic bioequivalence (i.e. 80–125% for both the area under the plasma concentration–time curve and maximum plasma concentration) represent appropriately conservative margins for ensuring equivalent safety and efficacy of the test and reference products.

Key Points

The evidence indicates that the European Union guidance is robust for the approval of orally inhaled products solely based on both in vitro and pharmacokinetic data.

Unlike clinical endpoint studies, pharmacokinetic studies are far more sensitive to minor differences in drug delivery (i.e. deposited dose, absorption rate and the central/peripheral drug deposition ratio) in the test and reference products and, thus, should be best suited for assessing bioequivalence.

1 Introduction

Asthma is a chronic inflammatory disease of the airways leading to airway infiltration by inflammatory cells, subepithelial deposition of collagen, hyperplasia of smooth muscle and goblet cells. Chronic inflammation in the airways may also lead to permanent changes in the airways, a process referred to as airway remodelling. According to the
Asthma is not only associated with patient-specific impairment, but it is also associated with a significant cost to society due to the significant healthcare utilisation associated with this condition. Numerous studies have evaluated the economic burden of asthma on society and individuals [2–7]. For example, Barnett and Nurmagambetov [6] estimated that US$56 billion was spent in direct medical costs and costs due to lost productivity related to asthma in the USA in 2007 [6]. Additionally, in a systematic review in which direct and indirect costs of asthma were evaluated, Bahadori et al. [7] found that the cost of asthma strongly correlated with co-morbidities, age and severity of disease [7]. Studies such as these emphasise the need for asthma treatments that are not only effective but also cost effective.

The GINA guidelines also emphasise the importance of cost as a barrier to adherence and ultimately to achieving asthma control [1]. In this regard, it has only been in recent years that pathways for the development and approval of lower-cost, bioequivalent inhaled products have been established for regulated markets, including the European Union (EU) [8]. Pharmacokinetic studies can directly compare the test product against the innovator product when the drug reaches the target site via systemic circulation. However, evaluation is not straightforward to establish the bioequivalence of products that have a local action such as orally inhaled products (OIPs). It is thus important to understand how these development pathways are structured and their implications for the treatment of airway diseases such as asthma. There are different requirements to establish bioequivalence in different countries; however, they are very different than each other barrering some minor differences. Hence, the purpose of the current article is to review the scientific basis and rationale for the development of bioequivalent OIPs. The focus is on the development of bioequivalent OIPs in the EU; however, we try to compare it in other locations wherever possible.

2 Requirements for Developing Bioequivalent Orally Inhaled Products (OIPs)

The requirements for demonstrating bioequivalence of OIPs have evolved over recent years. In vitro and pharmacokinetic data are currently the key elements in demonstrating bioequivalence of OIPs across European member states. While the need for clinical data in the demonstration of bioequivalence was common prior to the European Medicines Agency (EMA) guidance, this is no longer the case. This is largely due to the recognition that clinical efficacy endpoints are very insensitive for detecting differences between products, which is principally due to the shallow dose–response curves for most inhaled drugs, including inhaled corticosteroids (ICSs) and inhaled long-acting β₂-agonists (LABAs). The high costs involved in the conduct of pharmacodynamic studies also seems to be one of the contributing factors. For these reasons, the EMA’s carefully drafted guidance advocates a stepwise approach for demonstrating therapeutic equivalence of OIPs (Fig. 1) [8, 9].

In this approach, it is possible for formulations that are qualitatively and quantitatively equivalent to be approved on the basis of in vitro data without the need for any comparative in vivo studies. If the test product is not pharmaceutically equivalent to the reference, it cannot be considered similar or equivalent in vitro. However, for drug products that do not meet all the in vitro criteria, there will always be the need for in vivo data (i.e. pharmacokinetic studies). In this regard, the guidance does recognise the validity and sensitivity of pharmacokinetic studies for demonstrating equivalent safety and efficacy of two OIPs, and in the past few years, a number of inhaled products have been approved in the EU solely on the basis of in vitro and pharmacokinetic data (Table 1).

2.1 Step 1: In Vitro Criteria for Determining the Bioequivalence of Two OIPs

As noted earlier, the first step in the EMA stepwise approach requires in vitro characterisation of the test and reference product. Specifically, the EMA guidance outlines nine in vitro characteristics (Table 2) that must be assessed and should be matched (±15%) when evaluating two OIPs.

These criteria are important for pharmaceutical development and are more readily matched in the case of solution formulations. For this reason, bio-waivers are often granted for solution formulations [where devices such as dry powder inhalers (DPIs) and pressurised metered-dose inhalers (pMDIs) are not involved], such as nebules without the need for any comparative in vivo studies. However, for complex delivery systems such as solutions and suspensions for pMDIs and dry powders the need for in vivo data (i.e. pharmacokinetic studies) becomes imperative since the
in vitro profiles may be difficult to precisely match in the Anderson cascade impactor, especially for stages (or even groups of stages) where very small amounts of drug are deposited. It is important to highlight that it is essential to be strict because it means to waive the product completely. In contrast, the methodology employed by the US Food and Drug Administration (FDA) to compare the in vitro particle size does not need to be strict because a safety pharmacokinetic study is required. The EU can be strict with the in vitro comparison because, if it fails, Step 2 is still possible. Furthermore, it is essential to be strict and require equivalence in individual stages to assess the particle size distribution (PSD) because similarity in the fine particle dose (FPD) does not ensure equivalence, as shown by Daley-Yates et al. [10]. It is important to mention, however, that demonstration of equivalence (85–118%) of deposition on certain stages or in groups of certain stages may be impossible to demonstrate for certain products due to high variation in the very small amounts of drug deposited on those stages. When the amounts are very small, they may vary from no deposition to small deposition, leading to failure of equivalence between different batches of the same products.

Generally, the in vitro profiles of pharmaceutically equivalent drugs correlate with and predict the in vivo pharmacokinetic profiles of OIPs. For example, in a study by Asmus et al. [11], the 50% difference in respirable dose that resulted from administration of fluticasone propionate (FP) pMDI with two different spacers resulted in approximately 50% differences in the area under the plasma concentration–time curve (AUC) and maximum plasma concentration ($C_{\text{max}}$). Similarly, Woodcock et al. [12] demonstrated that the change in mass median aerodynamic diameter (MMAD) from 2.6 to 1.2 μm, which occurred when beclomethasone dipropionate (BDP) transitioned from a chlorofluorocarbon (CFC)-containing pMDI to an hydrofluoroalkane (HFA) solution pMDI, was associated with systemic exposure changes that could be predicted by the in vitro changes. These types of observations have been made for other OIPs across a range of formulations [13] and indicate that the in vitro profile of an OIP is important in the development of a bioequivalent OIP and that, in general, there is a good correlation between in vitro data [e.g. fine particle mass (FPM)] and in vivo data (e.g. pharmacokinetic data).

In vitro FPM–in vivo (pharmacokinetic) correlations between several products have been occasionally observed. However the reported correlations are generally limited to variations within the same product, rather than across products. Minor differences in formulation and device can offset such correlations. Therefore, an absence of significant quantitative differences in FPM may not ensure in vivo bioequivalence in most complex products. It is perhaps partly for this reason that hitherto the European guidelines have limited the in vitro only approach for approval of qualitatively and quantitatively same for formulations of solutions and suspensions for nebulisation and pMDI where the differences in devices can be minimal and delivery of the drug from the drug product does not depend upon the patient inspiratory effort. As yet, this approach has not been used for approval of any generic DPIs, although the EU guidelines do not exclude approval of generic DPIs based solely on in vitro testing.

There has also been a lively discussion regarding the existence of in vitro–in vivo correlations between standard CI studies and pulmonary deposition studies (e.g. scintigraphy or pharmacokinetic). First, one has to realise that impactors were initially developed as a quality tool for the
production process, before being used to predict the deposition and the outcome of a formulation. Indeed, previous data showed that correlations were not good at all, and that the data obtained with impactors was being overestimated [14, 15]. This was in part due to the use of cut-off points that were too high for small particles. Moreover, some improvements have been shown using casts of anatomic throats instead of the usual ones and by using inhalation profiles that were more realistic (breaths of volunteers) than just a negative pressure applied to the system, and have achieved better prediction by “adapting the system” [15]. However, it remains that this even does not take into account the fact that an aerosol, especially one originating from a pMDI, behaves differently in the airways of a patient than in an impactor, where factors of humidity and hygroscopic growth, interaction between particles (electrostatic forces, cohesion and adhesion) and changes in anatomy due to disease (and its severity) may also interfere. An impactor does not take into account the fact that particles may be exhaled, which may occur, and assumes that once deposited, drug particles dissolve, allowing the drug to interact with receptors and eventually reach the systemic circulation. Indeed, particles that remain longer in the airway mucus can be evacuated by the mucociliary clearance.

Table 1 European Union product approvals based on the stepwise approach of the European Medicines Agency orally inhaled product guidelines

<table>
<thead>
<tr>
<th>Products</th>
<th>Company</th>
<th>Clinical programme</th>
<th>Year of approval</th>
<th>Approving countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmeterol HFA pMDI*</td>
<td>Neolab Ltd</td>
<td>PKs with and without charcoal study and PD safety dose–response study</td>
<td>2011</td>
<td>UK, Poland, Germany and Ireland</td>
</tr>
<tr>
<td>FP/SM DPI (Elpenhaler®)*</td>
<td>Pharos Ltd</td>
<td>PKs with charcoal for both FP and SM</td>
<td>2011</td>
<td>Sweden, Germany, Italy, Portugal, Hungary, Czech Republic and Slovakia</td>
</tr>
<tr>
<td>Fluticasone pMDI</td>
<td>Cipla Ltd.</td>
<td>PKs without charcoal</td>
<td>2013</td>
<td>Sweden, Spain and Germany</td>
</tr>
<tr>
<td>Ipratropium pMDI</td>
<td>Cipla Ltd.</td>
<td>PKs without charcoal</td>
<td>2013</td>
<td>UK and Ireland</td>
</tr>
<tr>
<td>FP/SM pMDI</td>
<td>Cipla Ltd.</td>
<td>PKs with and without charcoal, PD safety dose–response study and PKs with spacer</td>
<td>2014</td>
<td>Sweden, Germany, Czech Republic, Slovakia, Greece, Iceland and Luxembourg</td>
</tr>
<tr>
<td>Budesonide/formoterol DPI (DuoResp Spiromax®), 160/4.5 and 320/9 µg per dose</td>
<td>Teva Pharmaceuticals</td>
<td>PK with and without charcoal studies and PD safety dose response study</td>
<td>2014</td>
<td>EMA approval</td>
</tr>
<tr>
<td>Bufomix (budesonide/formoterol DPI) 160/4.5 µg and 320/9 µg</td>
<td>Orien Corporation</td>
<td>PK study with and without charcoal</td>
<td>2014</td>
<td>Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, Lithuania, Spain, Greece, Hungary, Ireland, Portugal, Poland, Portugal, Romania and Slovakia</td>
</tr>
<tr>
<td>Lifsar (FP/SM DPI) 500/50 µg</td>
<td>Winthrop Pharmaceuticals</td>
<td>PK study with and without charcoal and a PD safety study</td>
<td>2015</td>
<td>UK, Austria, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Iceland, Ireland, Liechtenstein, Lithuania, Latvia, Malta, Norway, Poland, Portugal, Romania, Slovenia and Slovakia</td>
</tr>
<tr>
<td>Sirdupla (FP/SM pMDI) 250/25 and 125/25 µg</td>
<td>Mylan Ltd</td>
<td>PK study with and without charcoal and a PD safety study</td>
<td>2015</td>
<td>UK and Germany</td>
</tr>
<tr>
<td>Tiotropium bromide DPI (Braltus or Gregal) 10 µg per dose</td>
<td>Teva Pharmaceuticals</td>
<td>PK study without charcoal</td>
<td>2016</td>
<td>Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Iceland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden and UK</td>
</tr>
</tbody>
</table>

DPI dry powder inhaler, FP/SM fluticasone/salmeterol, HFA hydrofluoroalkane, PD pharmacodynamic, PK pharmacokinetic, pMDI pressurised metered-dose inhaler

* Public assessment reports

$p$ MDI, behaves differently in the airways of a patient than in an impactor, where factors of humidity and hygroscopic growth, interaction between particles (electrostatic forces, cohesion and adhesion) and changes in anatomy due to disease (and its severity) may also interfere. An impactor does not take into account the fact that particles may be exhaled, which may occur, and assumes that once deposited, drug particles dissolve, allowing the drug to interact with receptors and eventually reach the systemic circulation. Indeed, particles that remain longer in the airway mucus can be evacuate by the mucociliary clearance.

However, good in vitro–in vivo correlation may not be observed at all times. A study by Daley-Yates et al. [10] would shed more light on this.
highlighted that two different dry powder formulations of fixed-dose combinations of FP/salmeterol (FP/SM) with seemingly superimposable in vitro profiles were associated with distinctly different pharmacokinetic profiles [10]. It is important to highlight that the study illustrated an example that would not pass comparison of the PSD in individual stages or groups of stages. Despite these differences, however, clinical efficacy measures did not differ in a 12-week clinical study with the two products. In light of such observations, and given the complex site of action relevant to these two anatomic compartments. Specifically, the airways of the lungs, it is often important to directly assess lung and systemic exposure when evaluating bioequivalence of OIPs.

2.2 Step 2: In Vivo (Pharmacokinetic) Criteria for Determining the Bioequivalence of Two OIPs

The OIP guidelines acknowledge that pharmacokinetic studies are valid for demonstrating equivalent safety and efficacy of two OIPs [8]. The objectives of the pharmacokinetic studies in the stepwise approach are thus twofold: (1) demonstration of equivalent total systemic bioavailability of the test and reference products as an indicator of comparative safety; and (2) demonstration of equivalent pulmonary bioavailability as an indicator of comparative efficacy.

Total systemic exposure following administration of an OIP is comprised of both lung and gastrointestinal absorption (Fig. 2) [16]. The in vitro deposition profile of a drug is relevant to these two anatomic compartments. Specifically, the respirable fraction of an inhaled product is the mass fraction of inhaled particles penetrating to the unciliated airways, which generally corresponds to the fraction with particle sizes below approximately 3 μm for adults and 5 μm in children. By contrast, due to high inertia, the fraction with particle sizes more than 5 μm, impacts on the oropharynx and is swallowed after impaction in the oropharynx [17, 18]. Thus, drug concentrations in the blood represent fractions of the aerosolised dose that has reached the blood after absorption from the gastrointestinal tract and the peripheral absorption from the lungs, which determines the safety of the administered drug. The extent to which the swallowed portion is absorbed depends on the oral bioavailability of the drug. For drugs with very low/negligible oral bioavailability (e.g. FP), the amount of drug that reaches the blood via absorption from the lungs is same as the total bioavailability of the drug [19]. In a separate study, it was demonstrated that the bioavailability of monodispersed FP was related to the size of the particles, the total lung dose and the regional deposition of the lung dose [20]. As shown in Table 3, drugs with significant oral bioavailability such as salmeterol and BDP have significant reductions in the AUC from time zero to time t (AUCt) in the presence of oral charcoal blockade, while drugs with essentially no oral bioavailability such as FP do not. An important caveat is that in vivo drug deposition is also dependent on the inhalation flow rate which, in turn, is device specific. For example, an inhalation flow rate of 30–60 L/min is the target for pMDIs. Higher flow rates substantially alter the central/peripheral drug deposition ratio of pMDIs [21].

Within the EMA OIP guidance [8] is the requirement to distinguish pulmonary exposure relevant to efficacy from the total systemic exposure that occurs following administration of an OIP. Methods for differentiating lung exposure from non-lung exposure include scintigraphy and oral charcoal blockade. While scintigraphy (radiolabelling an orally inhaled drug) is appealing, the act of labelling may alter the in vitro profile of inhaled pMDIs and cannot be reliably done for DPIs [22]. As such, scintigraphy is not generally used for the purpose of evaluating lung exposure for two OIPs and provides only supporting information. Furthermore, scintigraphy has not been accepted as a
method of determination of bioequivalence, principally because it requires dismantling and rebuilding of the reference product for radiolabelling.

In contrast, oral charcoal blockade has been widely and successfully used to differentiate lung from non-lung exposure when evaluating OIPs [17, 23], and is the approach recommended in the EMA bioequivalence guidance for evaluating pulmonary deposition of an OIP [8]. In this method, oral charcoal is administered at specified times before and after dosing to block the absorption of an inhaled drug from the oral cavity and the gastrointestinal tract (Fig. 3). Thus, the plasma concentrations of drugs that are measured when an OIP is administered and oral charcoal blockade is used reflect the amount of the drug that reaches the blood circulation via the lungs. This leads to a big challenge while determining the bioequivalence of the fast dissolving and highly permeable drugs and generates the need for a weight of evidence approach. This is defined as the pulmonary available dose (PAD). In summary, oral charcoal blockade is an important and scientifically established technique for differentiating lung exposure from non-lung exposure when evaluating OIPs.

### 3 Relationship Between Lung Deposition and Pharmacokinetics

The lack of confidence in lung deposition data has been due to the fact that many deposition studies have been conducted without determination of clinical effects [24]. Indeed, most clinical studies have been conducted at only one dose level and therefore they are unable to show they were sensitive to detect the existing in vitro difference.

![Fig. 2](image-url) Schematic representation of disposition of a metered inhalation dose of a locally acting respiratory drug product. GI gastrointestinal

![Fig. 3](image-url) Schematic representation of how a charcoal block prevents gastrointestinal absorption of inhaled drugs

Table 3 Systemic exposure following administration of an inhaled product with versus without oral charcoal blockade

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Dose (µg)</th>
<th>With charcoal</th>
<th>Without charcoal</th>
<th>% difference in AUC&lt;sub&gt;t&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmeterol HFA pMDI [67]</td>
<td>200</td>
<td>807</td>
<td>1088</td>
<td>25</td>
</tr>
<tr>
<td>Beclomethasone (B-17-MP) HFA pMDI [68]</td>
<td>1000</td>
<td>1845</td>
<td>6134</td>
<td>31</td>
</tr>
<tr>
<td>Fluticasone HFA pMDI&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1000</td>
<td>210.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1682.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>No difference</td>
</tr>
</tbody>
</table>

**Table 3** Systemic exposure following administration of an inhaled product with versus without oral charcoal blockade

- **AUC<sub>t</sub>**: area under the plasma concentration–time curve from time zero to time t.
- **C<sub>max</sub>**: maximum plasma concentration.
- **HFA**: hydrofluoroalkane.
- **pMDI**: pressurised metered-dose inhaler.
- **<sup>a</sup>**: No difference due to <1% oral absorption from inhaled fluticasone.
- **<sup>b</sup>**: Clearie et al. [63].
- **<sup>c</sup>**: Kunka et al. [39].
addition, the comparison in the response axis of the mean responses is insensitive because the dose response was flat.

Consideration of the ability of the drug to target its specific site of action is also important in determining its clinical effect. The central and intermediate regions encompass the majority of the conducting airways (generations 0–16) [25]. The conducting airway is the region of interest for bronchodilators because the smooth muscles are predominantly located in this region, and it is where a β-agonist should be deposited to achieve effective bronchodilation [26]. Thus, differences in deposition of the bronchodilator in this region (Figs. 4, 5a) would affect its bronchodilator response. Indeed, that is precisely what the results of the study by Usmani et al. [21] demonstrate (Fig. 5b). Various pharmacokinetic and scintigraphy studies that incorporated efficacy variables [27–35] have collectively been inconclusive with regard to the relationship between regional airway drug deposition and therapeutic response. Again, this is due, in part, to the fact that these studies were conducted with doses that are on the flat part of the dose–response curve. Monodispersed scientific pharmacologic aerosols have been successfully used to determine the relationship between in vitro, pharmacokinetic and clinical effects of both a bronchodilator [21] and a corticosteroid [20]. For the bronchodilator, the hypothesis was that the regional airway distribution is important in exerting the bronchodilator response [20].

The results of this study showed a relationship between regional deposition and bronchodilation. Despite its merit, the Usmani et al. [21] study does not address the relationship between particle size, lung deposition, pharmacokinetics and clinical effects, as the plasma concentrations of drug following the various treatments were not measured. However, an extension of the same work [20] in which 50 μg doses of mono-dispersed FP with particle sizes of 1.5, 3 and 6 μm were used did demonstrate a relationship between particle size, lung deposition and the associated pharmacokinetic and clinical effects in subjects with asthma. The FP formulation with the smallest particles (i.e. 1.5 μm) would be expected to be distributed primarily to the peripheral lung and be absorbed faster than the formulation with the largest particles (i.e. 6 μm), which would be expected to be primarily deposited in the central lung and be absorbed less rapidly. This is exactly what was observed (Fig. 6).

The investigators also evaluated the anti-inflammatory effects of FP as assessed by fractional exhaled nitric oxide (FeNO) measurements (Fig. 6) [20]. FENO [total nitric oxide (NO) from the airways] was partitioned into that arising from the conducting large airways (JNO) and that from the small airways [alveolar NO concentration (C_{alv})], and although there was no statistical difference between the particle sizes (as they were secondary outcomes), there were trends seen in the JNO and C_{alv} NO response to large and small FP particle sizes, respectively; i.e. the higher the central deposition, the lower the effect on C_{alv} NO. As expected, there was a predictable relationship between particle size and the C_{max} of FP [i.e. the smaller the particle size (<1.5 and <3 μm) the higher the C_{max} versus the larger particle size (<6 μm)]. The AUC from time zero to 12 h (AUC_{12}) values were higher and relatively similar for smaller 1.5 and 3 μm particles compared with the larger 6 μm particles and this reflects different drug/airway interactions in different lung regions as a function of particle size, in that the 6 μm particles would be more proximally deposited and undergo greater mucociliary clearance into the oropharynx and gastrointestinal delivery with high first-pass metabolism for FP and therefore a lower plasma AUC.

**Fig. 4** a Regions of interest, where a 5 × 8 matrix was closely fitted to the krypton ventilation-scan lung boundary and superimposed on the posterior thorax aerosol deposition images, which was partitioned into a central zone, a 2 × 3 matrix zone centred on the lung hilum; an intermediate zone, a 3 × 5 matrix excluding the central zone; and a peripheral zone defined as the remaining lung field within the lung outline. Posterior thorax images of technetium-99m-labelled albuterol aerosol deposition in the lungs for the 1.5 μm particles (b), the 3 μm particles (c) and the 6 μm particles (d). Red areas indicate regions of highest radioactivity and black areas indicate the regions of least radioactivity. Reprinted with permission of the American Thoracic Society. Copyright © 2016 American Thoracic Society; Usmani et al. 2005 [21]. The American Journal of Respiratory and Critical Care Medicine is an official journal of the American Thoracic Society.
These data indicate that pharmacokinetic studies are sensitive to differences in regional lung deposition among different formulations of the same inhaled product. Importantly, these data also indicate that when appreciable particle size differences between or among inhaled products are present, they will be reflected in pharmacokinetic measures and potentially in clinical measures as well. However, the particle size of the active pharmaceutical ingredients (APIs) is not the only criterion that will reflect in the clinical measures—the droplet size of the pMDI formulation and differing lactose fines in the DPIs might also show differences in aerodynamic PSD (APSD) and in the regional deposition.
3.1 Step 3: Pharmacodynamic/Clinical Endpoint Studies Versus Pharmacokinetic Studies for Assessing Safety and Efficacy of Bioequivalent Inhaled Products

Bioequivalence in a pharmacokinetic study is established when the 90% confidence interval (CI) for the ratio of the test and reference product for AUC and \( C_{\text{max}} \) values falls within the range of 80–125%. This specified range for determining bioequivalence is a globally applicable regulatory concession to the known variability (inter- and intra-subject) inherent to assessing drug products in vivo. Bioequivalence based on these criteria for AUC and \( C_{\text{max}} \) values ensure equivalence of both safety and efficacy [36].

In evaluating these criteria, it is important to examine how useful they are for determining the equivalence of the test and reference product as they relate to safety and efficacy. With respect to efficacy, the comparability of two products is assured by the lower bound of the 90% CI. Similarly, the safety comparability of two products is assured by the upper bound of the 90% CI [8]. As is discussed in Sect. 5, the 90% CI of 80–125% can be considered a conservative margin for ensuring that the efficacy and safety of the test and reference product are not different.

The basis for this conclusion is derived from two lines of evidence:

1. Relationship between dose, exposure and efficacy/safety pharmacodynamic effects.
2. Relationship between pharmacokinetic and efficacy/safety pharmacodynamic effects for two products at the same dose.

These lines of evidence are considered separately here regarding the safety and efficacy of two major classes of inhaled drugs—namely, LABAs and ICSs.

4 Assessment of Long-Acting \( \beta_2 \)-Agonist (LABA) Safety

There are relatively few published studies that have simultaneously evaluated the pharmacokinetics and pharmacodynamics of inhaled drugs. As shown in Table 4, there is an expected increase in systemic exposure as the dose increases for LABAs such as salmeterol and formoterol. However, the relationship between systemic exposure and pharmacologically predictable safety effects of LABAs (e.g. increases in heart rate and decreases in serum potassium) is not linear, and supratherapeutic doses of LABAs need to be administered before these pharmacologically predictable effects are manifested (Table 4).

The implication of these data is that differences in exposure within the therapeutic range are very unlikely to manifest as differences in pharmacologically predictable safety adverse effects. This is, in fact, what the upper bound of the acceptance range of the 90% CI assesses. It is also important to recognise that systemic effects for a test and reference product are generally observed only after administration of multiple inhalations (i.e. at doses that far exceed the established therapeutic dose range). However, this is the model that is widely used to assess whether differences in systemic exposure are associated with pharmacodynamic safety differences. In this regard, despite nearly 50% higher values for \( C_{\text{max}} \) and AUC for salmeterol CFC relative to salmeterol HFA, the differences in heart rate (approximately 1–7 bpm) and serum potassium (approximately 0.1–0.3 nmol) were small and not clinically relevant [37] (Table 4).

Similarly, while the \( C_{\text{max}} \), AUC from time zero to 90 min (AUC\(_{90\text{ min}}\)) and AUC from time zero to 8 h (AUC\(_{8\text{ h}}\)) were approximately 15–25% higher for formoterol HFA pMDI versus formoterol DPI, there were no appreciable differences in pharmacologically predictable pharmacodynamic measures [38] such as glucose (difference of approximately 4%) and potassium (difference of approximately 3%) [38].

### Table 4: Dose versus exposure versus pharmacodynamic effects: long-acting \( \beta_2 \)-agonists

<table>
<thead>
<tr>
<th>Test Product</th>
<th>Dose 1</th>
<th>Dose 2</th>
<th>Dose 3</th>
<th>Dose 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmeterol HFA pMDI&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50 µg</td>
<td>150 µg</td>
<td>300 µg</td>
<td></td>
</tr>
<tr>
<td>PD effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>70</td>
<td>75</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>Serum potassium (mmol/L)</td>
<td>3.9</td>
<td>3.8</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>PK effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( C_{\text{max}} ) (pg/mL)</td>
<td>224</td>
<td>700.8</td>
<td>1482</td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;c&lt;/sub&gt; (pg·h/mL)</td>
<td>80.6</td>
<td>558.9</td>
<td>1612.7</td>
<td></td>
</tr>
<tr>
<td>Salmeterol CFC pMDI&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50 µg</td>
<td>150 µg</td>
<td>300 µg</td>
<td></td>
</tr>
<tr>
<td>PD effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>74</td>
<td>82</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>Serum potassium (mmol/L)</td>
<td>3.8</td>
<td>3.5</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>PK effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( C_{\text{max}} ) (pg/mL)</td>
<td>541.1</td>
<td>1286.6</td>
<td>2005.5</td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;c&lt;/sub&gt; (pg·h/mL)</td>
<td>278.6</td>
<td>1233.3</td>
<td>2254.5</td>
<td></td>
</tr>
<tr>
<td>Formoterol DPI&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12 µg</td>
<td>24 µg</td>
<td>48 µg</td>
<td>96 µg</td>
</tr>
<tr>
<td>PD effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>69</td>
<td>71</td>
<td>72</td>
<td>77</td>
</tr>
<tr>
<td>Serum potassium (mmol/L)</td>
<td>3.6</td>
<td>3.5</td>
<td>3.5</td>
<td>3.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Kempsford et al. [37]  
<sup>b</sup> Burgess et al. [38]

\( C_{\text{max}} \): area under the plasma concentration–time curve from time zero to time \( t \).  
\( CFC \): chlorofluorocarbon.  
\( C_{\text{max}} \): maximum plasma concentration.  
\( DPI \): dry powder inhaler.  
\( HFA \): hydrofluoroalkane.  
\( PD \): pharmacodynamic.  
\( PK \): pharmacokinetic.  
\( pMDI \): pressurised metered-dose inhaler.
Together, these data indicate that the safety dose–response for LABAs is flat and that even at doses (and systemic exposures) that exceed the established therapeutic dose range, appreciable effects on pharmacologically predictable adverse effects are minimal or cannot be demonstrated [37, 38]. Thus, it can be concluded that the upper bound of the acceptance range of the 90% CI for pharmacokinetic bioequivalence is a conservative margin for assuring that the safety of a test and reference LABA are equivalent.

5 Assessment of Inhaled Corticosteroid (ICS) Safety

As for LABAs, there are studies that have simultaneously evaluated the pharmacokinetics and pharmacodynamics of ICSs. However, there an expected increase in systemic exposure pharmacokinetic as dose increases for ICSs such as FP (Table 6; Fig. 7) [39–44]. Of note, the relationship between systemic exposure and pharmacologically predictable effects of ICSs (e.g. cortisol suppression) has been shown to be log-linear (Fig. 8; Table 6) [45–52].

![Dose related AUC response](image_url)

**Fig. 7** Relationship between inhaled fluticasone propionate hydrofluoroalkane pressurised metered-dose inhaler dose and systemic exposure as assessed by AUC (single dose); data from Kunka et al. [39] and Mollmann et al. [40]. AUC area under the plasma concentration–time curve.
drugs such as steroids which work via a receptor that can eventually be saturated. Once saturated, the effect cannot normally be increased any further, which is the $E_{\text{max}}$. Of course, when expressed logarithmically, we get a line, but in fact this relationship just expresses that once a maximum is almost reached, a further increase can only be obtained by increasing the dose manyfold. Thus, once 100% suppression is achieved with corticosteroids, further suppression cannot be attained. Certainly, 80–90% suppression can be achieved with inhaled fluticasone 2000 μg in healthy subjects [55, 56]. Indeed, the best way to assess systemic effects is 24 h collection of urinary cortisol and serum cortisol profiles.

The implication of these data is that since systemic exposure and cortisol suppression are both dose related and approximately proportional once a threshold dose has been reached, potential differences in safety between two products can readily be predicted from exposure differences between products. In this regard, a pharmacodynamic study to assess cortisol suppression is required to determine the differences in safety profiles, if any, between products when the upper bound of the 90% CI exceeds the acceptance range for bioequivalence (i.e. 125%). These data also provide assurance that the very small variations in systemic exposure that can occur when two products lie within the 80–125% bounds of the acceptance range of the 90% CI for equivalence will not result in appreciable differences in pharmacologically predictable effects such as cortisol suppression. Hence, it can be concluded that the upper bound of the acceptance range of the pharmacokinetic 90% CI for bioequivalence is also a conservative margin for assuring that the safety of test and reference ICSs are equivalent.

6 Assessment of LABA Efficacy

Previous studies have shown an expected increase/decrease in the systemic exposure/pharmacokinetics of LABAs as the LABA dose increases/decreases [37, 39]. The fact that the LABA dose-response for efficacy is flat, as evident from LABA dose-ranging studies (Table 7), indicates that even the marked changes in systemic exposure that occur at successively higher doses are generally not accompanied by appreciable changes in efficacy, whether at low, middle or high doses. Pharmacodynamic assessments of β-agonists, such as the methacholine provocation test, can be relatively sensitive when studies are designed correctly. However, pharmacokinetics can differentiate between formulations that differ in their regional deposition, whereas pharmacodynamic studies are insensitive to these differences. This can be substantiated further by the outcome of the previous studies [57, 58] in which three different formulations of salbutamol were made with 15, 27 and 67% FPMs of the labelled dose. There was no difference in the effects observed, which could be because the selected dose had already reached the plateau of the dose–response curve. However, adverse drug reactions were seen more with the higher FPM, especially lower serum potassium levels and an increased heart rate. These levels are indeed the amount of drug that reaches the systemic circulation through lung absorption. In a different study, Usmani et al. [59] also demonstrated that a large FPM caused extensive and faster absorption that could be confirmed by pharmacokinetic measurements; however, efficacy and even ADRs

Fig. 8  Relationship between inhaled fluticasone propionate dose and percentage suppression of cortisol production (dashed blue line indicates the threshold for clinically significant cortisol suppression [Szefler et al. [61]); Grahnen et al. [55]—fluticasone propionate dry powder inhaler (7 days’ dosing); Donnelly et al. [60]—fluticasone propionate pMDI (5 days’ dosing); Boorsma et al. [66]—fluticasone propionate pMDI (4 days’ dosing); Szefler et al. [61]—fluticasone propionate pMDI (6 weeks). pMDI pressurised metered-dose inhaler
were similar among all three FPM groups of 26, 29 and 40% of the labelled dose [59].

The implications of these data from the LABA dose-ranging studies in asthma is that it would be very difficult, if not impossible, to detect any appreciable efficacy differences between products at recommended doses when a test and reference product are within, or even considerably outside, the acceptance range of the 90% CI for pharmacokinetic bioequivalence. Thus, it can be concluded that the lower bound of the acceptance range of the 90% CI for bioequivalence is a conservative margin for assuring that the efficacy of a test and reference LABA are equivalent.

7 Assessment of ICS Efficacy

Previous studies have shown an expected increase/decrease in systemic exposure of ICSs as the ICS dose increases/decreases (Fig. 8; Table 6) [39, 40, 55, 60, 61]. The fact that the ICS dose response is very flat, as evident from ICS pharmacokinetics. These differences were not translated into clinical efficacy data such as peak expiratory flow (PEF), forced expiratory volume in 1 s (FEV1) and use of rescue medication, highlighting the insensitivity of the pharmacodynamic approach. Nevertheless, more pronounced suppression of urinary cortisol and a higher heart rate were noted in the group with higher pharmacokinetic data.

In contrast, Clearie et al. [63] used the relative potency approach, which ensures sensitivity to detect differences. However, the slope was so flat that the width of the 90% CI of the relative potency was extremely wide, making the study inconclusive and it was not able to show equivalence within normal limits (80–125% or 66–150%). Clearie et al. [63] compared the effects of two similar nominal doses of an ICS of two formulations; however, even the lower dose in this study was too high to detect differences between doses and hence between formulations. Moreover, the

Table 7 Long-acting β2-agonist dose response in phase IIb asthma registration studies

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Study (year)</th>
<th>Endpoint</th>
<th>Placebo/ baseline</th>
<th>Dose 1</th>
<th>Dose 2</th>
<th>Dose 3</th>
<th>Dose 4</th>
<th>Dose 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmeterol pMDI</td>
<td>GSK Clinical Study Register [study ID SLGH04] (2005) [41]</td>
<td>FEV₁ AUC (L/h)</td>
<td>Baseline 1.61</td>
<td>12.5 µg 1.99</td>
<td>25 µg 2.11</td>
<td>50 µg 2.13</td>
<td>100 µg 2.16</td>
<td></td>
</tr>
<tr>
<td>Formoterol DPI</td>
<td>Dahl et al. (2004) [42]</td>
<td>FEV₁ AUC (L/h)</td>
<td>Placebo 5 µg 2.38</td>
<td>10 µg 2.54</td>
<td>15 µg 2.60</td>
<td>30 µg 2.61</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indacaterol DPI</td>
<td>FDA medical review (application no. 022383) [43]</td>
<td>FEV₁ AUC (L/h) (change from placebo)</td>
<td>0.06</td>
<td>18.75 µg 0.06</td>
<td>37.5 µg 0.14</td>
<td>75 µg 0.16</td>
<td>150 µg</td>
<td></td>
</tr>
<tr>
<td>Vilanterol DPI</td>
<td>FDA medical review (application no. 204275) [44]</td>
<td>Trough FEV₁ (L)</td>
<td>Placebo 3 µg 2.38</td>
<td>6.25 µg 2.45</td>
<td>12.5 µg 2.15</td>
<td>25 µg 2.51</td>
<td>50 µg 2.50</td>
<td></td>
</tr>
</tbody>
</table>

Italicised dose is the approved dose for asthma/chronic obstructive pulmonary disease; the lowest approved dose of formoterol is 12 µg in the USA and 6–12 µg in the European Union

AUC area under the plasma concentration–time curve, DPI dry powder inhaler, FEV₁ forced expiratory volume in 1 s, GSK GlaxoSmithKline, pMDI pressurised metered-dose inhaler

Adis
PD20 (provocation dose causing a 20% decline in FEV\textsubscript{1}) of methacholine itself is a relatively insensitive endpoint regarding sensitivity to ICS, and it is possible that the PD20 of adenosine would have been a far better choice, illustrating that with an inappropriate design it is difficult to make conclusions in terms of dose equivalence. Thus, if one decides to go to step 3, it is essential to demonstrate that the study would detect the differences with a relative potency calculation. In this regard, it is also important to highlight that Busse et al. \[65\] also used the approach of relative potency, and with a better study design including three doses; nevertheless, it is also important to note that an adequate sample size should be used to show equivalence within 80–125% or 66–150%.

Similarly, in a study by Needham et al. \[64\] comparing two FP/SM DPI combination products, the day 1 FEV\textsubscript{1} AUC\textsubscript{12} was similar for the two products despite an approximately 40% difference in AUC\textsubscript{t} for salmeterol between products. In a study by Daley-Yates et al. \[10\] comparing two FP/SM DPI products [FP/SM administered by the Diskus device or the reservoir powder inhalation device (RPID)], there was no statistical or clinically meaningful difference in PEF between the two DPI products in a 12-week study involving 270 asthma patients, despite the fact that systemic exposure pharmacokinetics with FP/SM administered via the Diskus device were nearly 50% lower than with FP/SM administered via the RPID. A study by Busse et al. \[65\] compared a CFC and an HFA formulation of beclomethasone and suggested that an approximate 2.5-fold dose reduction in the HFA was required to demonstrate equivalent efficacy of both formulations. It is important to note that although pharmacokinetics were not measured in this study, pharmacokinetic measures would have differed by

### Table 8  Inhaled corticosteroid dose response in phase IIb registration studies

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Study (year)</th>
<th>Endpoint</th>
<th>Placebo</th>
<th>Dose 1</th>
<th>Dose 2</th>
<th>Dose 3</th>
<th>Dose 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluticasone propionate DPI</td>
<td></td>
<td>FEV\textsubscript{1} (L)</td>
<td>–0.22</td>
<td>0.43</td>
<td>0.47</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>Fluticasone propionate pMDI</td>
<td>Pearman et al. (1997) [45]</td>
<td>FEV\textsubscript{1} (L)</td>
<td>0.14</td>
<td>0.40</td>
<td>0.51</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>Budesonide DPI</td>
<td></td>
<td>FEV\textsubscript{1} (L)</td>
<td>–0.10</td>
<td>0.19</td>
<td>0.26</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Beclomethasone pMDI</td>
<td></td>
<td>FEV\textsubscript{1} (L)</td>
<td>–0.03</td>
<td>0.13</td>
<td>0.28</td>
<td>0.29</td>
<td>0.31</td>
</tr>
<tr>
<td>Ciclesonide pMDI</td>
<td></td>
<td>FEV\textsubscript{1} (L)</td>
<td>0.17</td>
<td>0.28</td>
<td>0.29</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Mometasone DPI</td>
<td></td>
<td>FEV\textsubscript{1} (L)</td>
<td>0.16</td>
<td>0.41</td>
<td>0.41</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Fluticasone furoate DPI</td>
<td></td>
<td>FEV\textsubscript{1} (L)</td>
<td>0.13</td>
<td>0.23</td>
<td>0.26</td>
<td>0.34</td>
<td>0.36</td>
</tr>
</tbody>
</table>

The doses given are the total daily dose; the italicised dose is the approved dose in adults for asthma/chronic obstructive pulmonary disorder DPI dry powder inhaler, FDA US Food and Drug Administration, FEV\textsubscript{1} forced expiratory volume in 1 s, pMDI pressurised metered-dose inhaler

### Table 9  Changes in the methacholine doubling dose with budesonide hydrofluoroalkane pressurised metered-dose inhaler versus chlorofluorocarbon pressurised metered-dose inhaler

<table>
<thead>
<tr>
<th>PK/PD effects [63]</th>
<th>HFA/CFC—200 μg [T/R (95% CI limits)]</th>
<th>HFA/CFC—800 μg [T/R (95% CI limits)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD effects</td>
<td>Methacholine (PC\textsubscript{20})</td>
<td>0.92 (0.76–1.13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HFA/CFC—1600 μg [T/R (90% CI limits)]</td>
</tr>
<tr>
<td>PK effects</td>
<td>(C_{\text{max}}) (pg/mL)</td>
<td>0.89 (0.77–1.04)</td>
</tr>
<tr>
<td></td>
<td>AUC\textsubscript{t} (pg h/mL)</td>
<td>1.03 (0.9–1.18)</td>
</tr>
</tbody>
</table>

\(AUC\textsubscript{t}\) area under the plasma concentration–time curve from time zero to time \(t\). CFC chlorofluorocarbon, CI confidence interval, \(C_{\text{max}}\) maximum plasma concentration, HFA hydrofluoroalkane, \(PC_{20}\) provocative concentration of methacholine needed to produce a 20% fall in forced expiratory volume in 1 s, PD pharmacodynamic, PK pharmacokinetic, T/R test/reference

\(\Delta\) Adis
approximately 2.5-fold despite the fact that these same doses resulted in equivalence for the efficacy endpoints [10]. Together, these data indicate that the efficacious dose response for ICSs is flat at recommended doses and, despite marked differences in dose and/or systemic exposure, it is difficult to show efficacy differences between doses for a given product or between different products at the same nominal doses. These data also indicate that clinical endpoint studies are very insensitive for detecting differences between products while pharmacokinetic data are highly sensitive for discriminating between products. Thus, it can be concluded that the lower bound of the acceptance range of the 90% CI for bioequivalence is a conservative margin for assuring that the efficacy of a test and reference ICS are equivalent.

In summary, these data indicate that, unlike clinical measures, pharmacokinetic measures are very sensitive for detecting differences between doses of a given inhaled product and between different products at equivalent doses. Hence, when two OIPs have been shown to be bioequivalent based on adequately conducted pharmacokinetic studies, equivalence in the safety and efficacy profile of the test product relative to the innovator product can also be assured.

8 Conclusions

There is a strong need for bioequivalent OIPs for the treatment of asthma and other airway diseases such as chronic obstructive pulmonary disease. Bioequivalent products help overcome an important barrier to achieving cost-effective asthma control. However, for such barriers to be effectively removed, it is critical that the bioequivalent products that are developed are cost effective and have efficacy and safety profiles that are comparable with those of the innovator products. In this regard, the EMA guidelines, which no longer rely on expensive clinical endpoints since they are essentially insensitive for detecting differences between OIPs, do just that. Namely, the EMA bioequivalence guidance for OIPs utilises a stepwise approach for determining bioequivalence of OIPs. The process is robust and, in most instances, requires sensitive pharmacokinetic studies to be undertaken. The acceptance range of the 90% CIs required for the demonstration of bioequivalence (i.e. 80–125% for both the AUC and $C_{\text{max}}$) can be considered appropriately conservative margins for ensuring that the safety and efficacy profiles of the test and reference product will also be same.

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Compliance with ethical standards

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Conflict of interest Dr. Usmani reports having received consulting fees, honorarium, institutional grants and payments for lectures from Boehringer Ingelheim, AstraZeneca, Chiesi, Napp, Mundipharma, Aerocrine, GlaxoSmithKline (GSK), Sandoz, Takeda, Edmond Pharma, Zentiva and Cipla outside of the submitted work. Dr Moli-mard reports having received consulting fees from Novartis Pharma, GSK and Mundipharma outside of the submitted work. Dr Derom reports that he has received travel grants from Boehringer Ingelheim, GSK and AstraZeneca to attend international congresses, participated in advisory boards by Boehringer Ingelheim, Chiesi, Cipla and Astra Zeneca, for which a fee was given, and received speaker’s fees from Boehringer Ingelheim, GSK, Astra Zeneca and Menarini to give scientific presentations to Belgian general practitioner groupings (all of which are not related to this work). His clinical department received financial support from Boehringer Ingelheim and Novartis to perform clinical studies. Drs Gaur, Gogtay, Singh and Malhotra are full-time employees of Cipla Ltd, Mumbai, India.

References


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