Effective in vitro bioequivalence studies for dry powder inhalers

A laboratory perspective outlining the complexities involved and presenting solutions

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In the dynamic landscape of pharmaceutical development, in vitro bioequivalence (IVBE) studies serve as a cornerstone in regulatory strategies aimed at establishing bioequivalence (BE) for the abbreviated new drug application (ANDA) of generic orally inhaled products (OIP). Specifically, the US Food and Drug Administration's recommendations for locally acting OIPs are grounded in the comprehensive 'weight-of-evidence' approach, which combines IVBE studies with pharmacokinetic and pharmacodynamic assessments to substantiate equivalence in local delivery.[1]

IVBE studies employ intricate methods, often involving randomisation and blinding, to ensure an objective and unbiased evaluation. This article focuses on specific laboratory challenges encountered in IVBE studies for dry powder inhalers (DPIs), highlighting the resource-intensive nature of aerodynamic particle size distribution (APSD) measurements and the regulatory guidelines. It elaborates on the difficulty of conducting blinded studies and explores solutions to overcome these challenges effectively.

The significant time constraints of IVBE studies

DPI BE studies involve thorough characterisation data on APSD and single actuation content (SAC). These parameters are critical for understanding how drug particles are deposited in the respiratory tract – a key factor in determining the bioequivalence of generic DPIs compared to their reference products.

APSD measurements, which are generally performed with the help of cascade impactors, demand a high level of expertise and consistency from the analyst, as they require meticulous and labour-intensive sample preparation and extraction. Automation possibilities are limited due to the inherent functioning of capsule-based DPIs. Moreover, in vitro techniques are often adapted to add features that account for conditions that mimic 'real-life' use to enhance in vitro-in vivo correlations (IVIVC) and to provide a more realistic data set representing drug delivered to the lung.

To this end, pressure-flow profiles to mimic patient inhalation, the application of humidified air through the impactor, or the utilisation of 'inlets' or throat models designed to closely resemble the anatomical geometry of the patients under examination are often used.[2] While data produced with these refinements may improve IVIVC, its addition often extends the already lengthy turnaround time needed for APSD analysis.

Applicants seeking approval to conduct IVBE studies should select at least three batches each of test and reference products, with a minimum of ten units from each batch tested at three different flow rates. The batches of the test product should come from at least three different batches of drug substances, excipients and device components. The test product should accurately represent the final formulation intended for market release, including both the device and drug constituents. SAC tests should be conducted at the start, middle and end stages of the product's life, while APSD tests should be performed at the beginning and end stages.[3] Even with this limited scope, a total of 320 APSD determinations and 540 SAC determinations need to be conducted.

While the timeframe for measuring SAC and APSD in a capsule-based dry powder inhaler can vary depending on factors such as the chosen method and inhaler type, it is widely acknowledged that a standard APSD test using the next-generation impactor (NGI) for five actuations typically necessitates over a day of dedicated laboratory work. Based on this estimation, more than 64 analyst days are required to perform the minimum number of necessary APSD measurements (**Figure 1**). When factoring in additional essential parameters, such as SAC testing, through-life waste-shot firing, evaluation and statistical analysis, the total duration of a typical IVBE study can extend to six months or more. Although this timeline can vary based

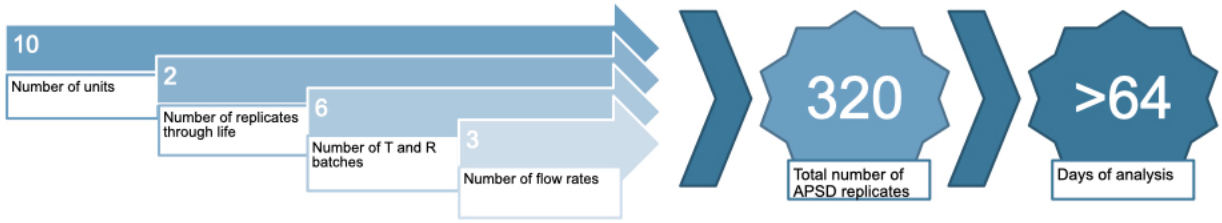

Figure 1. Estimated turnaround time (TAT) for APSD measurement in a standard IVBE study

on various factors, including laboratory expertise, available equipment and analysts, plus study complexity, the overall turnaround time poses a significant challenge for pharma companies and may potentially delay the market entry of their products.

Improving turnaround time

Due to the significant turnaround time (TAT) involved in conducting an IVBE study, the experience of the analytical laboratory conducting the study will be key to improve study delivery timelines and proactively anticipate potential obstacles. As illustrated in **Figure 1**, APSD determinations will likely be a bottleneck due to the intricate preparation, cleaning and maintenance procedures required for the devices.

Therefore it is essential to implement measures aimed at minimising the efforts associated with handling the impactor. In this regard, conducting specialised studies to assess interstage loss (defined as the amount of actuation content lost between the impaction device's sample collection surfaces) and determine the maximum allowable number of actuations in the impactor before oversaturation can substantially reduce the labour-intensive process of cleaning the impactor. Interstage drug loss must adhere to the specifications outlined in USP <601>, which mandates that no more than 5% of the inhaler's total delivered drug mass should be subject to loss.

Ensuring that each impactor can be reused multiple times without excessive loss of products in its wall, and without requiring complex handling and cleaning procedures, can significantly impact the overall TAT of the study. Adopting this method also enables a streamlined, 'assembly line' approach within the APSD analysis process, assigning specific tasks to different analysts across multiple actuations, ranging from pre-separator extraction and stage extractions to chromatographic preparation and data analysis.

This strategy not only simplifies the workflow for the analysts but also maximises the use of resources and greatly improves throughput. However, coordinating multiple analysts across various devices introduces the risk of traceability issues. To mitigate these concerns, it is essential to implement stringent documentation that is compliant with good manufacturing practice (GMP) standards, guided by the ALCOA principle, which ensures that every step of the process is Attributable, Legible, Contemporaneous, Original and Accurate. By meticulously addressing these factors, analytical laboratories can enhance the efficiency of the IVBE study process, reducing TAT while upholding strict adherence to regulatory standards.

Streamlining investigations

Given the labour-intensive nature of APSD analysis, proactive planning is essential to address unexpected results. This planning is particularly crucial concerning the mass balance criteria outlined in the US Pharmacopeia (USP). Mass balance is defined as the total mass of drug collected in all the components (mouthpiece adapter, the induction port through to the after-filter of the cascade impactor apparatus) against the target delivered dose (TDD) of the product. Discrepancies between a product's TDD and its actual performance are often the cause of mass balance failures.

While GMP laboratories typically have established procedures for out-of-specification results, mass balance is not a direct test of the product itself, but serves to ensure the validity of the results. Incorporating investigation steps for mass balance failures directly into the study protocol through a predefined process flow is a proactive approach (**Figure 2**). This involves outlining the investigation and reporting rules clearly, which can significantly streamline the documentation and investigation process, while also improving transparency and consistency of documentation and investigation into such events.

Ensuring study randomisation

Another indispensable aspect of a successful IVBE study lies in the implementation of study randomisation and ensuring that all involved analysts are blinded to the identity of the samples. Analyst blinding serves as a crucial measure to mitigate conscious or unconscious biases that may arise from prior knowledge of the treatment assignment, thus upholding the integrity and reliability of the study outcomes. However, maintaining blinding can be particularly challenging in IVBE studies for DPIs, where subtle visual cues or other characteristics of the formulations may inadvertently reveal their identities to the analyst. In order to effectively address this

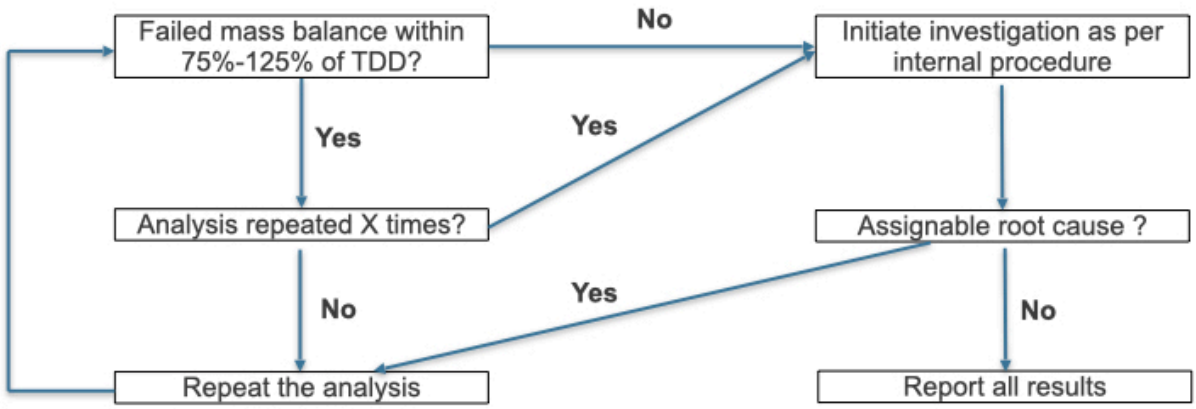

Figure 2. Example of investigational flow chart for mass balance failures outside USP specifications (85%-115% of TDD)

challenge, stringent protocols must be established to standardise the study setting, sample labelling and handling procedures, ensuring consistency and maintaining blinding throughout the study duration.

One approach to bolstering blinding efforts is through the utilisation of anonymised labelling codes, facilitated by laboratory information management systems (LIMS), which play a pivotal role in managing and tracking sample information while preserving confidentiality. Additionally, incorporating independent oversight by specialised study managers can provide an extra layer of verification to ensure the integrity of the blinding process. By adhering to these rigorous protocols, any observed differences between test and reference products can be confidently attributed solely to product performance, thus enhancing the validity and credibility of the IVBE study findings.

Robust chromatographic methods for automatic integration

Developing robust chromatographic methods and evaluation system is essential for ensuring the integrity and reliability of data obtained through IVBE studies. Manual integration, while sometimes unavoidable, should be justified strictly based on scientific necessity and conducted with caution to minimise potential errors. The use of chromatography data systems (CDS) has been a focal point of regulatory attention since the Able Laboratories fraud case in 2005, and excessive reliance on manual integration methods will be challenged by regulatory authorities during the audits following up the submission of an ANDA.[4]

Therefore, laboratories conducting IVBE studies must prioritise the development and implementation of robust chromatographic methods with automated peak picking and integration, which ensures data integrity and reliability, improves efficiency and mitigates the risk of adverse compliance findings.

Conclusion

In conclusion, the labour-intensive and complex nature of IVBE studies for DPIs imposes significant time constraints and can challenge even the most well-equipped and staffed laboratories. Experienced analytical laboratories can address these challenges effectively by adherence to best practices, from proactive planning, optimised and streamlined execution and strict adherence to best practices with regard to documentation and compliance. This will benefit sponsors, regulators and patients by speeding up study timelines, delivering reliable, robust and accurate data and ensuring the highest levels of quality and compliance.

References

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