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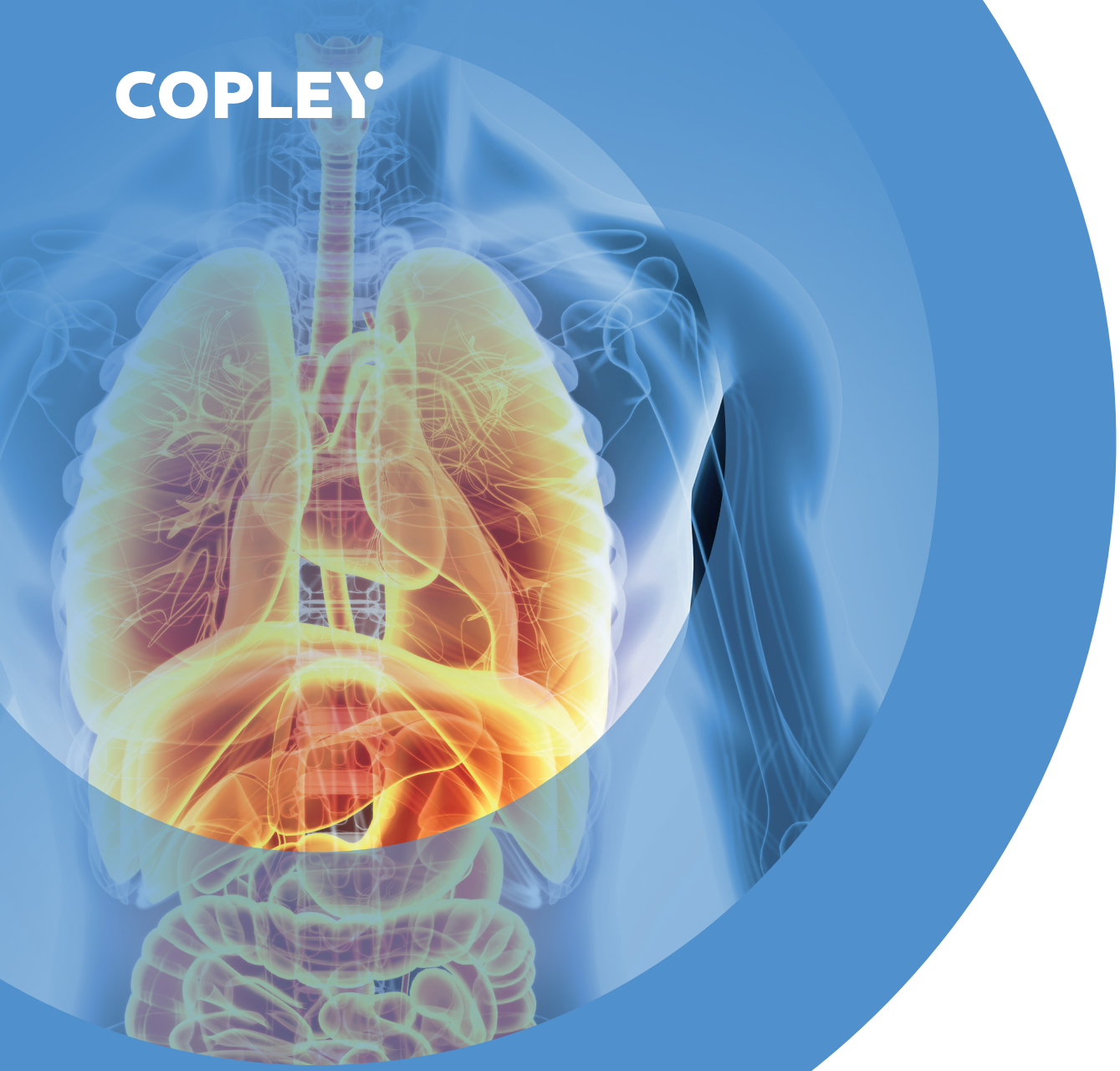


Understanding the links between drug delivery route and *in vitro* test methods

White paper | October 2023

In vitro tests are widely used, from research and development (R&D) through to quality control (QC), to improve the efficacy of drug delivery and confirm product consistency. Simple, quick and inexpensive, highly repeatable and easy to validate – relative to *in vivo* strategies – they are a critical tool for the pharmaceutical industry. Furthermore, as computational capabilities improve, applying *in silico* and *in vitro* methods in tandem is becoming a powerful strategy for faster, more cost-efficient product development. Establishing robust *in vitro in vivo* correlations remains a work in progress but maximising the application and relevance of *in vitro* testing is both ethically and financially sensible.

Ensuring a drug reaches its intended site of action *in vivo*, in an appropriate state, is a crucial first step towards meeting clinical performance goals; *in vitro* testing quantifies the characteristics that influence that performance. Drugs may be delivered through the gastrointestinal tract; via the rectal, vaginal, or oral mucosal membranes; through the surface of the skin; by injection or infusion; or via the surfaces of the nasal cavity or lungs. In each case the requirements for effective delivery are different and the tests applied to assess drug performance differ accordingly.

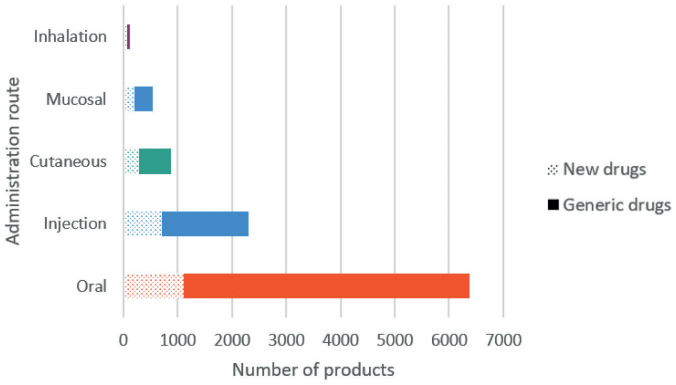
In this white paper, we review the drug product characteristics that define the success of delivery via given routes, and the tests used to evaluate them, using the product types that Copley offers solutions by way of example. A key focus is the link between the mechanisms of drug delivery and the test conditions applied.

Different routes, different challenges

While drug development begins with the identification of a drug candidate with a therapeutically relevant mode of action, delivery route is an early decision in the subsequent product development process.

A clear taxonomic guide for the classification of pharmaceutical dosage forms can be found in **Chapter <1151>** of the US Pharmacopoeia (USP) entitled "Pharmaceutical Dosage Forms". Route of administration is the top tier of this taxonomy and has a defining influence on the formulation of a product and the testing strategies applied in its development and manufacture.

Typically, the preference is to formulate a drug product as a tablet or other oral solid dosage (OSD) form due to relative simplicity and cost effectiveness compared to other dosage forms. However, these are not the only factors to consider. The type of disease being treated, the intended therapeutic effect, the required speed of action, possible side effects, and the nature of the active drug substance must also be taken into account when determining the best delivery route.



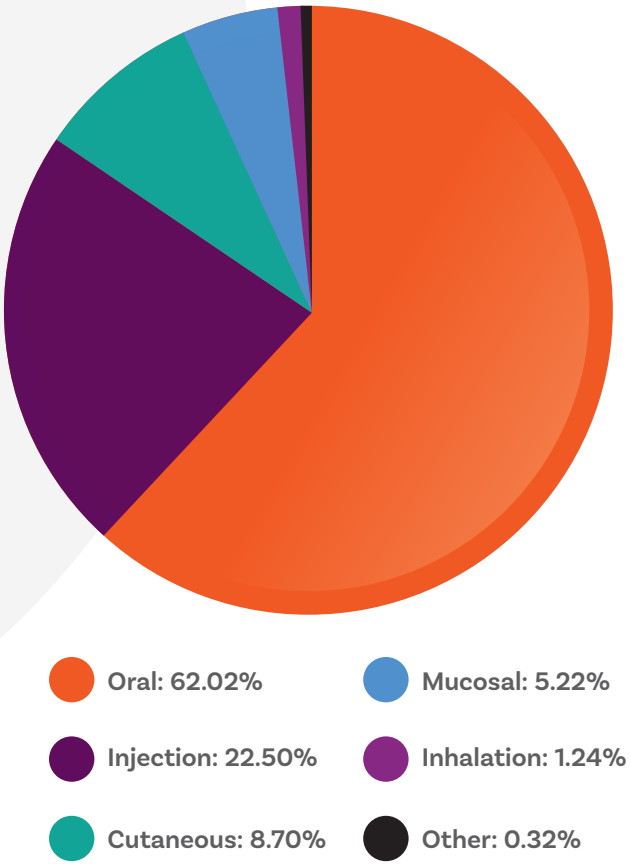


Figure 1: The overall distribution of administration route of all FDA-approval pharmaceutical products (top right) and the ratios in new and generic drugs released in the previous year (above), as of 2018. [1]. Oral and injectable administration accounted for close to 85% of all pharmaceutical products in 2018. These two modes represent the widest range of drugs in the pharmaceuticals market and worldwide, are the most affordable methods; they are especially popular targets for generic development.

Where a drug is specified for local action, for example to rapidly ease breathing during an asthma attack or to alleviate eczema, the preliminary route of delivery for frontline treatments is usually clear, in this case pulmonary delivery and topical skin application, respectively. Associated benefits of local delivery may include rapid symptom relief and the avoidance, or reduction, of some side effects. However, the situation is complex and additional treatment may be necessary, for example, tablets and injectables for asthma can also be prescribed for patients unable to achieve control with inhalers.

Where systemic action is the aim, physiology and the ability to quickly achieve a therapeutic effect may indicate one route above another: growing interest in intranasal drug delivery is a case in point. There is evidence to suggest that this route may by-pass the blood-brain barrier thereby enabling the more effective delivery of drugs to tackle diseases of the brain and central nervous system, from Alzheimer's to depression.

The rich vasculature of the nose has also been associated with extremely rapid drug delivery, as evidenced by nasal drug products for conditions including migraines, seizures and emergency treatments for opioid overdose. The commercial attractions of deriving efficiency gains from reformulation and a switch in delivery route may also be a factor in determining choice and is certainly a driver for the increased use of intranasal drug delivery.

Furthermore, although the gastrointestinal route is a highly effective way of delivering many systemic drugs, as evidenced from the fact that two thirds of all medicines prescribed today are OSD forms, the changing nature of the drug pipeline is increasingly making formulation as a simple tablet unviable. Typically, biologics, from monoclonal antibodies and peptides to gene therapies, are delivered by injection or infusion because of their susceptibility to degradation in the GI tract, though there is intense research underway to enable both oral and inhaled biologics, for greater patient acceptability and compliance. Even for new small molecule drugs poor solubility and/or poor permeability are becoming increasingly common necessitating the use of more sophisticated techniques such as formulation as an amorphous solid dispersion and advanced particle engineering. Efforts to develop more convenient OSD products are also worth mentioning with well-controlled modified release, fixed dose combinations/polypills, and gastroretentive tablets all forming part of an increasingly diverse and complex OSD landscape.

In Quality by Design terms, testing strategies focus on quantifying Critical Quality Attributes (CQA), the variables that determine a product's ability to meet a defined quality target product profile. These are linked with those aspects of the drug delivery process that are critical or rate limiting, for a specific product type. For instance, a drug required to act locally is not necessarily, or even desirably, absorbed into the bloodstream.

As a result, testing focuses on getting the drug substance to the site of action, for example, the delivery of drug particles to the lung in the case of inhalers, or the release of an active from a cream/emulsion matrix in the case of semisolids. Systemic drug administration, in contrast, relies on establishing a therapeutically effective concentration of drug in the blood or lymphatic system. This is potentially a more complex, multi-step process involving consideration of the rate of release from the product matrix and diffusion of the drug through specific biological barriers.

Determining the critical quality attributes for a drug product for any given delivery route therefore calls for an understanding of the factors that will determine its efficacy, and identification of appropriate *in vitro* test methods to generate relevant data. The responsibility for ensuring pharmaceutical products are safe, of adequate quality, and efficacious lies with the various national regulatory bodies, which are supported in their roles by the Pharmacopoeias. The United States Pharmacopoeia (USP), European Pharmacopoeia (Ph. Eur.) and other national pharmacopoeias, define the standards with which the drug formulation shall comply, and the methods by which compliance will be assessed, providing extensive support for test selection and optimisation. These compendial methods are designed for high repeatability and relied upon for product QC. However, in R&D there is growing appetite to refine *in vitro* testing strategies to improve relevance and achieve more precise *in vitro in vivo* correlation. The result may be marginally more complex test methods, but the associated prize is potentially faster, less expensive product development

Gastrointestinal delivery

OSD forms, such as tablets and capsules, are the most popular product type for the delivery of drugs via the gastrointestinal tract, though liquid medicines are a widely used alternative. The prevalence of OSDs is largely attributable to ease of administration and high patient acceptability, but physical and chemical stability is also an important feature.

With these products the amount of drug introduced into the body is relatively easily controlled, by the patient taking a tablet containing a defined dose, but the rate of delivery may be less so, depending on the technology used. Modified or controlled release products are precisely engineered to deliver a drug over an extended time period and maintain a consistent concentration *in vivo* thereby potentially improving therapeutic outcomes while at the same time delivering a treatment regimen that may be easier for patients to comply with.

Tablets are comprised of a mixture of active drug substances and excipients, usually in powder form, which are blended and then compressed to form the finished tablet. The quantity of active drug tends to be very low with the bulk consisting of: diluents, binders or granulating agents; glidants and lubricants to ensure efficient tableting; disintegrants to promote tablet break-up in the digestive tract; sweeteners or flavours to enhance taste; and pigments to make the tablets visually attractive. Increasingly sophisticated OSD products may also include excipients that form polymer matrices, that impart enzyme activation or buoyancy, or that confer mucoadhesion to control or target drug release; nanotechnology is an increasingly important part of the formulation armoury.

A polymer coating is often applied to make the tablet easier to swallow and more resistant to environmental degradation, and for primary control over release rate. Capsules differ from tablets in that they contain the drug formulation in a polymeric shell, typically hard gelatin. The associated manufacturing process therefore involves capsule filling rather than compression, a factor that directly influences the excipients used in the formulation. However, testing strategies for the two product types are closely similar.

Characteristics impacting product performance and the speed of drug delivery

For tablets and capsules, product stability, from manufacturing through packaging and transportation is a primary goal, to ensure intact delivery to the patient - for consistent dosing - and a practical shelf life.

Mechanical integrity therefore requires precise control. Once the tablet has entered the body then its break down profile influences the rate of release, bioavailability, and absorption of the drug. This process involves disintegration of the original OSD form, followed by dissolution of the resulting, smaller particles to produce a solution that can diffuse into the bloodstream as required with respect to speed and location.

Core testing requirements

Friability testing

Friability is the tendency for a tablet to chip, crumble or break and is one of the parameters used to quantify the physical integrity and stability of an OSD.

Optimal friability gives the tablet sufficient physical stability for transport and storage while at the same time allowing it to break down readily in the gastrointestinal tract.

Methods and equipment for the friability testing of uncoated tablets are detailed in **Ph. Eur. Chapter 2.9.7** and **USP Chapter <1216>**. Testing involves weighing a sample of tablets (10 in total), rotating them 100 times in a drum of closely defined specification (the Roche friability drum) at a set speed, removing any loose dust/chips that have broken off from the tablets, and then reweighing the sample. The percentage weight loss quantifies friability, with a figure of <1% usually taken as the limit of acceptability.

For harder tablets and capsules, and for granules and spheroids, the level of attrition that occurs in standard test equipment is insufficient to give a meaningful result. Here alternative testing strategies are required, as described in **Ph. Eur. Chapter 2.9.41**, to apply a higher degree of abrasive action and create a measurable change in surface area, and by extension weight. With the equipment described in Method B: Oscillating Apparatus, for example, the horizontal shaking movement of an oscillating arm causes samples to rub against and collide with one another, and with the internal surfaces of the sample container, to promote breakage.

Hardness testing

Hardness, more correctly defined as breaking force (USP) or crushing strength (Ph. Eur.), is used alongside friability to quantify the physical stability of an OSD. Excessive hardness may result in long disintegration times and poor dissolution performance, while low hardness may be associated with a higher number of defective products and unacceptable weight variation.

Hardness testing is described in **Ph. Eur. Chapter 2.9.8** and **USP Chapter <1217>** and involves placing the tablet between two platens or jaws, one attached to a load cell, the other to a motor which provides the mechanical drive. During testing the motorised jaw presses the tablet against the fixed jaw which measures the force at which the tablet breaks.

Disintegration testing

Disintegration is the first step in the breakdown of the tablet/capsule *in vivo*. Conditions within the gastrointestinal tract vary from patient to patient and assessment of the extent of disintegration can be subjective but the apparatus and methods described in **Ph. Eur. Chapter 2.9.1** and **USP Chapter <701>** provide a reproducible and standardised method for assessment for all OSD forms.

Figure 2: *A simple apparatus for standardised, reproducible disintegration testing*

During disintegration testing the tablet or capsule is held in a tube within a basket assembly which moves up and down in a vessel containing a defined volume of simulated gastric fluid, held at 37°C (see **figure 2**). A plastic disc inserted in the tube, along with the sample, assists disintegration. The lower end of the tube is covered by a sieve mesh and the tablet is deemed to have passed the test if no residue remains on this mesh after a certain time period; 30 minutes is typical for ordinary tablet; 60 minutes for enteric coated tablets.

Dissolution testing

Dissolution testing is the primary *in vitro* method for investigating and comparing the bioavailability associated with different OSDs, i.e. the amount of drug that the product makes available to the body. Measurements of dissolution rate support the optimisation of bioavailability and consequently therapeutic efficacy, and are also used to assess bioequivalence, for generic products, and for the confirmation of batch-to-batch equivalence, in QC.

The progressive optimisation of dissolution testing for different OSD forms has led to the introduction of a range of different apparatuses and techniques as detailed in **Ph. Eur. Chapter 2.9.3** and **USP Chapter <711>**. Factors that influence the results obtained include: the composition and de-aeration state of the dissolution media; the precise physical dimensions of the test apparatus; and the test conditions applied, most especially whether these ensure that the tablet is dissolving into sink conditions, i.e. that dissolution is not inhibited by a high localised concentration of drug substance. Furthermore, dissolution rate can change as the tablet dissolves since this process naturally changes the exposed surface area. Intrinsic dissolution testing is a distinct method which directly addresses this issue via constant surface area testing.

The most common apparatuses used for dissolution testing are **Basket (Apparatus 1)** and **Paddle (Apparatus 2)**. A dissolution tester consists of a cylindrical vessel that holds the simulated gastric juice dissolution media, and is partially immersed in a water bath to maintain the dissolution apparatus at 37°C. In the Basket method, the tablet or capsule is contained in a cylindrical mesh basket, whereas in the Paddle method, it simply sinks to the bottom of the vessel below a paddle (see **figure 3**). During testing, the basket or paddle is rotated at a specified speed, and samples of the dissolution media are extracted at predefined time intervals to determine the percentage of dissolved drug present, typically via HPLC. These results enable the generation of a dissolution profile, a plot of drug release as a function of time. Other techniques specified in the USP for dissolution testing include: **Reciprocating Cylinder (Apparatus 3)**, **Flow-Through Cell (Apparatus 4)** and **Reciprocating Holder (Apparatus 7)**. These are typically only required for highly specialised dosage forms.

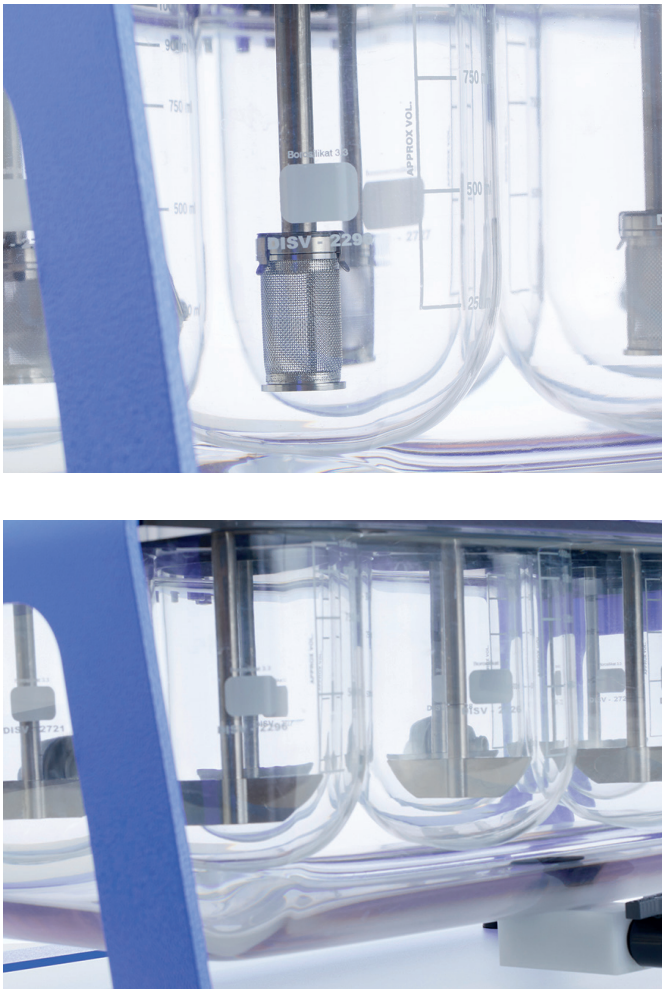


Figure 3: The most common types of dissolution testing apparatus are Apparatus 1 (Basket) [Top] and Apparatus 2 (Paddle) [Bottom]

Dermal delivery – topical and transdermal

Pharmaceutical products applied directly to the skin may be designed for topical action or for systemic delivery, and the test methods applied are differentiated accordingly.

Most topical drugs are classified as semisolids, a group of products which includes creams, ointments, lotions and gels. These are typically hydrocarbon-based systems or oil in water emulsions incorporating additional ingredients such as emulsifiers, stabilisers, pH buffers, preservatives, absorption promoters and perfumes, and are applied to the skin for immediate relief.

In contrast, transdermal drug products (TDPs), most often patches, are designed to release an active ingredient through the skin into the bloodstream, over a prolonged period; primary examples are products for hormone delivery and smoking cessation. Transdermal patches contain a reservoir of drug held within a physical device incorporating multiple polymeric membranes, or as a solid matrix; incorporating the drug within the adhesive used to apply the patch is also an option. TDPs with active delivery systems utilise, for example, microneedles or alternative mechanisms to promote transfer across the skin. A successful product must control the rate of release of the drug while simultaneously maintaining close contact with the skin.

Transdermal products enjoy a high degree of patient acceptance/compliance and are easy to use. In addition, semisolids are often formulated to deliver a moisturising effect, which can enhance topical relief and efficacy, while TDPs offer the important advantages of avoiding first pass metabolism in the gastrointestinal tract and enabling controlled release over a prolonged period. However, the skin is a highly efficient barrier against the outside environment so ensuring that a drug substance reaches the intended site of action can be a defining challenge for systemic delivery.

Characteristics impacting product performance and the speed of drug delivery

Transdermal products are subject to both product quality and performance testing. Product quality tests assess general physical attributes while performance tests focus on the release of the drug substance from the formulation matrix.

For semisolids, product quality tests are detailed in **USP Chapter <3>** and address issues such as apparent viscosity, which impacts ease of use, and product uniformity over the defined shelf life. Performance testing involves measurement of the amount of drug released and the rate of release from the emulsion.

Product quality tests for TDPs include the measurement of tack and adhesion, which is crucial for keeping the product in place and, as for semisolids, are detailed in **USP Chapter <3>**. TDP performance is more complex to assess. For absorption into the bloodstream the drug must diffuse out of the layered matrix of the product then through the layers of the skin to reach the capillaries that provide access to the bloodstream. Diffusion from the product is controlled by the design of the patch while the rate of diffusion through the skin is influenced by physical and chemical properties of the drug such as: liposolubility; molecular weight; and electronic structure. Methods for testing TDPs have consequently been expanded beyond the simple measurement of dissolution rate across a solid-liquid interface to include the kinetics of membrane transfer.

Core testing requirements

Semisolids

Performance tests for semisolids are detailed in **USP Chapter <1724>** which describes three different apparatuses for the determination of drug release: Vertical Diffusion Cell (VDC); Immersion Cell; and Flow Through Cell (Apparatus 4). Of these the VDC is emerging as the preferred option, due to its simplicity and reproducibility.

Figure 4: *Designs are available for testing different volumes of semisolids.*

A VDC comprises a sample holder and a reservoir containing the receptor medium, which is typically maintained at 32°C to approximate normal skin conditions (37°C for vaginal preparations). These two parts are separated by a membrane which contains the test sample while at the same time keeping it in contact with the receptor medium. Over time – a typical test period is 6 hours, with no fewer than 6 samples extracted - the drug substance diffuses from the sample, through the membrane into the receptor medium.

As with dissolution testing, the extraction and analysis of samples of receptor medium therefore enables the generation of a drug release profile. Topping up the receptor reservoir as sampling proceeds keeps the sample in contact with receptor medium at all times, maintaining the diffusion process.

TDPs

Compendial methods for measuring the drug release performance of TDPs are closely analogous to the techniques used for OSD dissolution testing with three alternative apparatuses to choose from: Paddle Over Disk; Rotating Cylinder; and Reciprocating Holder (Ph. Eur. only). The Paddle Over Disk method is a modified version of dissolution test Method 2 (Paddle Method) and preferred on account of its simplicity. It is described in **USP Chapter <724> Method 5** and **Ph.Eur. Chapter 2.9.4. Method 1**.

The Paddle Over Disk method makes use of standard dissolution testing apparatus, together with a disk assembly comprising a stainless steel screen and holder. Different disks are available for testing differently sized patches. The TDP is mounted onto the disk, release side up, using a suitable adhesive, and the disk assembly is then placed at the bottom of the dissolution vessel, which is filled with preheated, degassed media held at 32°C to simulate skin conditions. During testing the paddle is rotated at a defined speed and samples are extracted from the dissolution vessel to determine a release profile for the drug substance.

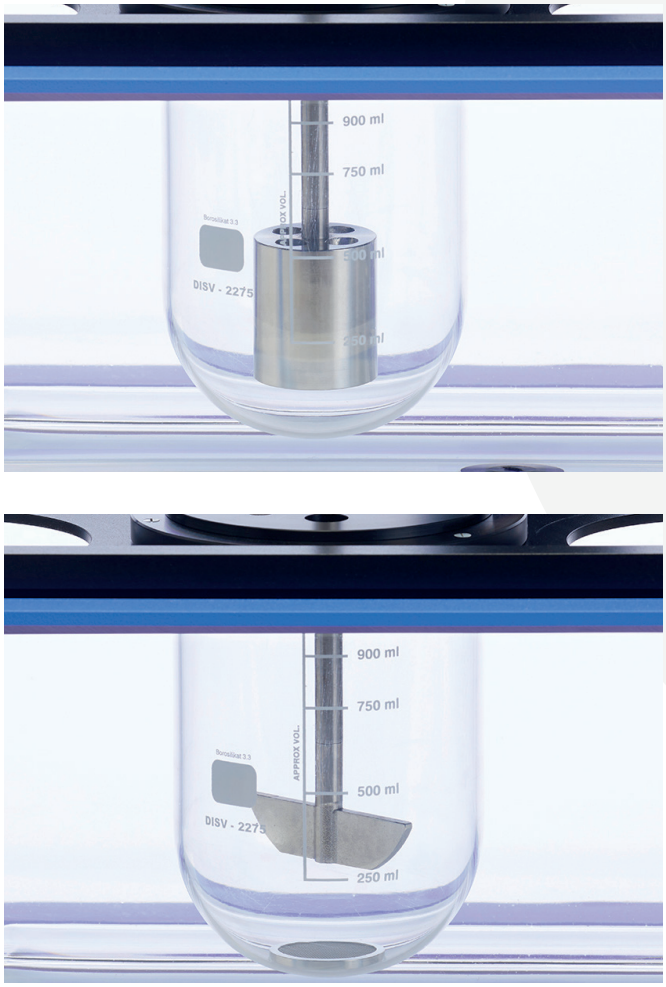


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Figure 5: *Methods for performance testing for TDPs use modified OSD dissolution testing apparatus and include: Rotating Cylinder (top) and Paddle over Disk (bottom).*

Delivery via the rectal and vaginal mucosal membranes

Delivering drugs via the rectal or vaginal mucosal membranes advantageously avoids digestion in the gastrointestinal tract, in the same way as transdermal or inhaled drug delivery.

Suppositories, solid formulations that are inserted into the body cavity, are the most common form of product for delivery via this route and share many of the same attributes as tablets.

They may be hydrophilic or lipophilic in nature, depending on the intended application, and can be used to achieve topical action or for systemic drug delivery, the delivery of contraceptives being a primary application. However, suppositories have relative low patient acceptability and convenience, and drug absorption can be relatively unpredictable.

Suppositories contain an active drug substance formulated in a solid matrix. Hydrophilic products are formulated with a water-soluble base such as polyethylene glycol and, once inserted into the body, disintegrate, and then dissolve into the rectal or vaginal fluids. Lipophilic suppositories, on the other hand, have a greasy base such as cocoa butter, which melts at body temperature to release the drug.

Characteristics impacting the success and speed of delivery

As with OSD forms, suppositories reliably introduce a defined dose of drug into the body, so it is the rate of release that is less easily controlled and the focus of testing.

For hydrophilic products, disintegration is an important part of the drug release process, while for lipophilic formulations softening and melting times are key; no single method of drug release testing is suitable for all types of suppositories.

Core testing requirements

The suppository is a more common and accepted dosage form in Europe than in the USA, which may explain why Pharmacopoeial references to specific test methods for suppositories, are mainly confined to the Ph. Eur.

The rate of dissolution of hydrophilic suppositories can be measured using the standard **Paddle**, **Basket** or **Flow Through** methods described in **USP Chapter <711>** and **Ph.Eur. Chapter 2.9.3**. The European Pharmacopoeia 8th Edition also includes a disintegration method for these products in **Chapter 2.9.2**. To quantify disintegration a sample is inserted into a cylindrical sample holder that is immersed in a glass vessel contained within a water bath controlled at 37°C. Every 10 minutes, during testing, the sample is inverted through 180 degrees to promote disintegration which should occur within a predetermined time

Figure 6: *Disintegration testing apparatus for hydrophilic suppositories can also be used to measure the softening times of lipophilic products.*

Methods described for measurement of the dissolution rate of lipophilic suppositories include a modified **Basket** method, a **Paddle** method using a sinker and a modified **Flow Through Cell** with dual chamber which is described in **Ph. Eur. Chapter 2.9.42**. The softening time of lipophilic suppositories can be measured using the same apparatus as for hydrophilic disintegration testing but with alternative attachments, as described in **Ph. Eur. Chapter 2.9.22**.

Inhaled delivery

Pulmonary drug delivery is the most popular choice for the topical treatment of respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD). However, as the inhaled route avoids digestion of the drug substance it may also be used for systemic therapies, antibiotics being a prime example.

A primary point to recognise about inhaled drug delivery is that the dose delivered to the patient is not precisely controlled. Rather it is a function of features of the inhalation device, of the formulation, and of the physiology and inhalation technique of the patient. This differentiates inhalation from any other delivery route, excepting intranasal drug delivery (see below), and defines the testing landscape for orally inhaled products (OIPs).

The successful delivery of drugs to the lungs requires the generation of particles of a respirable size and numerous device types are employed to achieve this, for different types of formulation. Dry powder inhalers (DPIs) contain the drug substance (or a combination of drug substances) in powder form either in isolation or mixed with larger excipient particles, typically lactose. As the patient inhales, air is drawn through the dose, aerosolising it and dispersing the particles, which are then pulled into the lung. Ensuring adequate dispersion using only the energy provided by the inhalation manoeuvre of the patient is the defining challenge in developing DPIs with high drug delivery efficiency.

Metered dose inhalers (MDIs), in contrast, deploy an active drug delivery method, using a propellant to atomise a fixed volume of liquid solution or suspension. This means that inhalation and dose release are not naturally coordinated, so with these products the efficiency of drug delivery may be compromised by the patient failing to inhale at an optimal point. This issue of technique is routinely addressed through the use of spacers, valved holding chambers (VHCs) or novel breath-actuated devices.

Nebulisers, the third general classification of OIPs, continuously atomise a drug formulation, once loaded, and the patient inhales the formulation by breathing normally through a mouthpiece or mask. This arguably makes them the easiest inhaled product to use, however, nebulisers are far from being the most convenient as traditionally they are relatively large and deliver a dose over a relatively long timescale. Innovation remains strong in the field of nebulisers and the development of handheld and smart nebulisers are addressing some of these factors regarding ease of use. With both MDIs and nebulisers there also remains the challenge of designing device and formulation to ensure consistent, well-controlled dispersion.

Aqueous droplet inhalers (ADIs, otherwise referred to as soft mist inhalers, SMIs) are a relatively new class of OIPs that seek to combine the advantages of MDIs and nebulisers. ADIs actively aerosolise the drug formulation using comparable technology to a nebuliser to release a respirable mist. In this way they eliminate the environmental impact of propellant use associated with MDIs. In other respects, they are similar to MDIs though with a reduced requirement for coordination and corresponding success in delivering a higher fine particle fraction than either MDIs or DPIs. As with all multi-dose systems microbial contamination can be an issue and novelty also equates to high cost, relative to MDIs.

Characteristics impacting the success and speed of delivery

As delivered dose is not directly controlled in inhaled drug delivery, it is one of the primary metrics measured to assess efficiency and clinical efficacy.

The other critical characteristic for OIPs is particle size since this influences deposition behaviour in the lungs. Generally, particles greater than 10 µm will fail to deposit in the lung, but will instead remain in the mouth and throat, while particles less than 5 µm will reach the deep lung and be therapeutically available due to the presence of receptors. This requirement for very fine particles to penetrate the defences that keep harmful material out of the lung explains why drug release *in vivo* has historically been considered a secondary issue, with particles in this size range typically assumed to dissolve relatively rapidly, even in the suboptimal dissolution conditions of the lung. This situation is now changing with the *in vivo* fate of inhaled particles now subject to far greater scrutiny, notably for the delivery of drugs for systemic action; dissolution testing for inhaled drugs has become an important area of focus for drug developers and regulators alike.

Beyond these broad requirements specific tests vary from product to product, reflecting differences in the way each delivers a drug. With nebulisers, for example, there is an additional requirement to measure the amount of drug substance delivered as a function of time. The impact of patient physiology is also reflected in some tests with nebulisers characterised under conditions that reflect the inhalation profiles of the intended patient group – neonate, infant, child or adult – and DPIs tested at flow rates that correspond with the resistance to inhalation that they present.

Core testing requirements

Delivered dose uniformity

The delivered dose is the total amount of drug emitted from the inhaled product and, in the case of MDIs and DPIs, is measured using a Dosage Unit Sampling Apparatus (DUSA) in accordance with the methods described in **USP Chapter <601>** and **Ph. Eur. Dosage Forms 0671**.

Separate chapters describe specific test methods for nebulisation - **USP Chapter <1601>/ Ph. Eur. Chapter 2.9.44** - while guidance for the testing of MDIs with spacers and VHCs is presented in draft **USP Chapter <1602>**.

To measure delivered dose, the inhaled product is actuated into the DUSA through which air is drawn at a defined flow rate using a vacuum pump. The DUSA consists of a sample collection tube with a filter at one end. The delivered dose is collected in the tube and on the filter and the amount of drug substance in both is then determined from a chemical assay, typically by HPLC.

The principal way in which the various methods for the measurement of delivered dose differ is the test conditions applied during dose capture, most especially test flow rate, which reflects the delivery mechanism of the device. For an MDI, testing is carried at a constant flow rate of 28.3 L/min (or 30 L/min depending on impactor of choice for aerodynamic particle size measurement – see below) while for DPIs the flow rate applied is that which results in a 4 kPa pressure drop across the device.

DPIs with a higher resistance to air flow are therefore tested at lower flow rates than those that are easier to inhale through. This is to ensure that the data gathered is more representative of the performance that will be observed in the clinic. It is also important to note that for DPIs testing is carried out under critical flow conditions, to ensure flow rate stability.

With nebulisers a sinusoidal breathing pattern is used to simulate use, with the dose being collected similarly on a filter. The dimensions of this pattern – total volume, frequency and inhalation to exhalation ratio – depend on the patient group (i.e. neonate, infant, child or adult) for which the product is intended. Furthermore, the measurements made include the active substance delivery rate and the total active substance delivered by emptying the reservoir of the nebuliser. Delivered dose testing for MDIs with VHCs and spacers is also carried out under tidal breathing conditions.

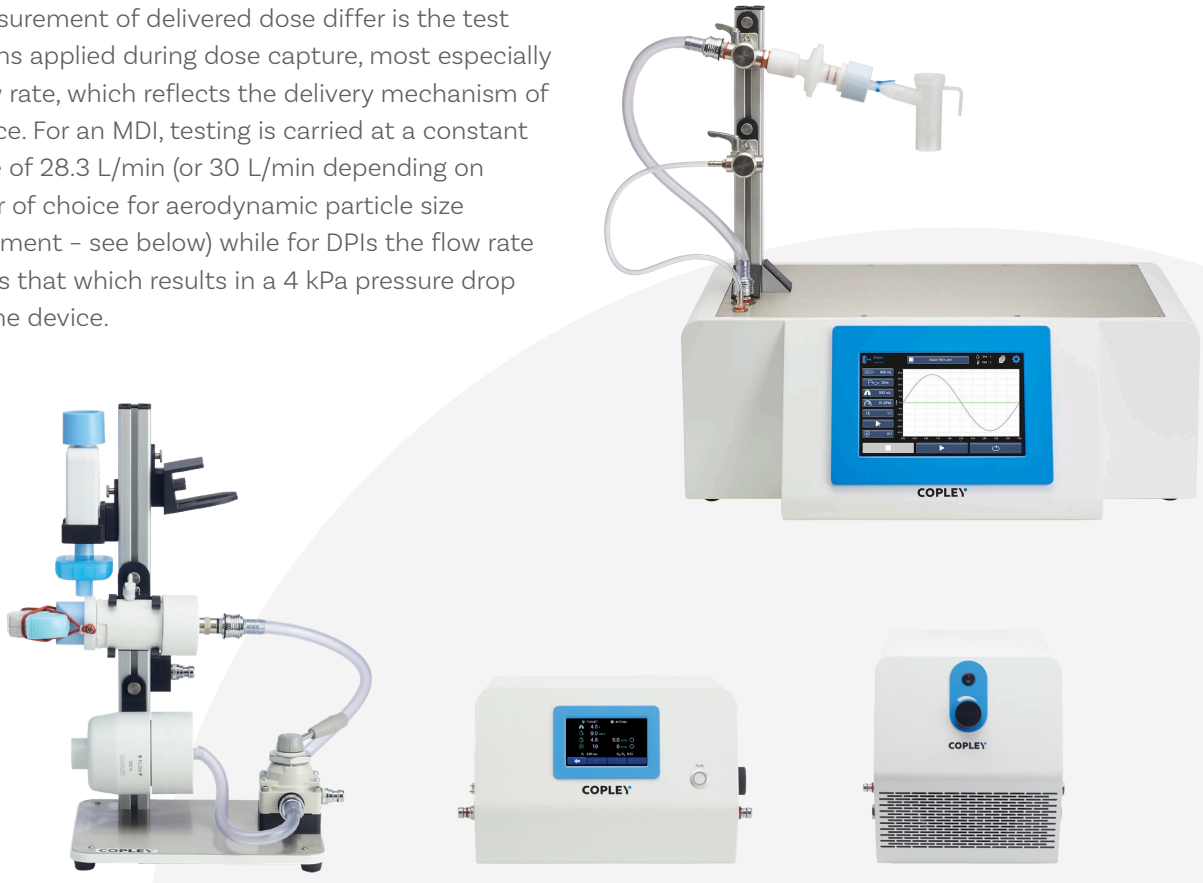

Figure 7: *DUSA set-ups vary for different OIPs. A standard set-up for DPIs (above) and nebulisers (top right) is shown here.*

Aerodynamic particle size distribution (APSD) measurement

Compendial methods for the measurement of particle size specify the technique of cascade impaction.

Unlike other particle sizing techniques, cascade impaction generates a particle size distribution for the drug substance, rather than the formulation as a whole, and, has the added advantage of measuring aerodynamic particle size, a parameter of intuitive relevance in the specification of OIPs. The pharmacopoeias recommend several commercially available impactors for testing MDIs and DPIs, but the three most widely used impactors - common to both **Ph.Eur. Chapter 2.9.18** and **USP Chapter <601>** - are the Next Generation Impactor (NGI); Andersen Cascade Impactor (ACI); and Multi-Stage Liquid Impinger (MSLI). In the case of **Ph.Eur. Chapter 2.9.44** and **USP Chapter <1601>** for nebulisers, only the NGI features due to its lower range of calibration flow rates.

A full description of the technique of cascade impaction lies beyond the scope of this paper – see reference [2] - but in summary APSD measurement involves actuating the OIP into the cascade impactor which then separates the dose on the basis of particle inertia, a function of particle size, shape, density and velocity. Once the separation is complete the particle mass on each stage is recovered using a suitable solvent and then analysed, usually by HPLC to determine the amount of drug present and generate an APSD for the drug substance. There is considerable guidance and indeed equipment available to optimise cascade impactor test set-ups for relevance and to maximise data integrity.

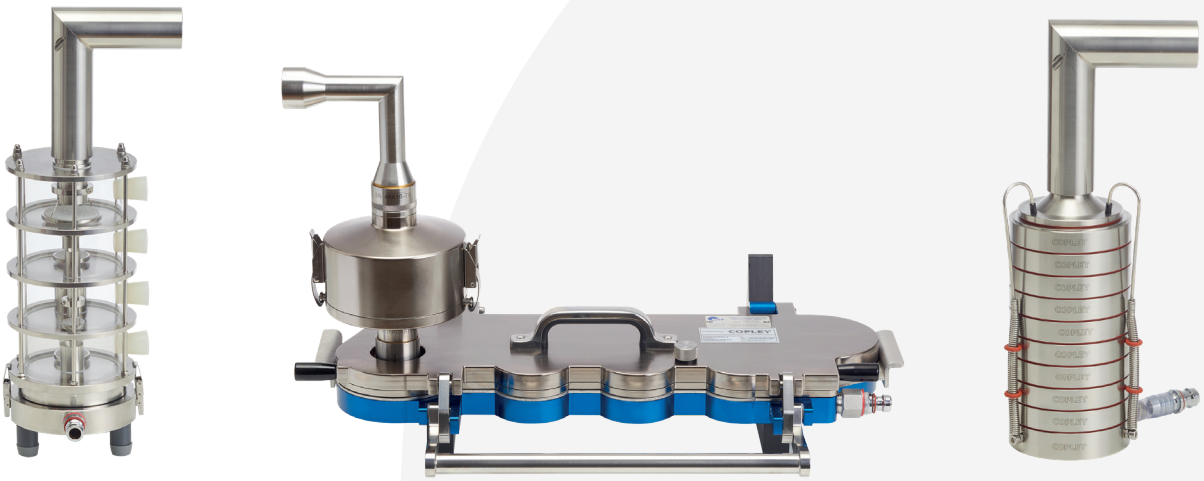

Figure 8: *The MSLI (left), NGI (centre) and ACI (right) are all referenced in compendial methods for determining the APSD of OIPs but the NGI and ACI are the instruments of choice for the majority of testing.*

A key feature of cascade impactors is that the separation performance that they deliver is flow rate dependent, because of the correlation between particle inertia and velocity. This means a constant, well-defined, and known air flow rate must be applied during testing. The flow rates specified are usually identical to those used for delivered dose testing for each device, for obvious reasons, except for when a sinusoidal pattern is specified. For nebulisers APSD measurement is carried out at 15 L/min, a figure deemed representative of the flow during normal tidal breathing. MDIs with spacers and VHCs are tested at the standard flow rate for MDIs – 28.3 L/ min (30 L/min when using the NGI which has calibrated performance at this flow rate) for adult use, but a lower flow rate may be more suitable for other patient categories such as paediatrics. A further compendial requirement for nebulisers, which is also recommended for ADIs, is to cool the cascade impactor to 5°C during APSD measurement to prevent droplet evaporation and a consequently erroneous result.

Intranasal drug delivery

Intranasal drug delivery is used routinely for the delivery of topical therapeutics for the treatment of allergies, rhinitis, colds and flu.

These products account for the majority of the intranasal drug delivery market and are a primary target for generic developers. However, intranasal drug delivery for systemic action via the dense blood vessel network at the back of the nasal cavities is currently an area of intense research activity. Vaccines, prophylactics for respiratory illness, new therapeutics for areas of unmet clinical need associated with the central nervous systems, and emergency medications are all important features of the modern nasal drug product landscape.

As with pulmonary drug delivery, the dose delivered via intranasal drug delivery is determined by the device used, the formulation and the physiology and technique of the patient, in combination. The goal is retention within the nasal cavity rather than dripping from the nostrils or passing through the nasopharanyx to the back of the throat – issues associated with coarser particles/droplets - or conversely, pulmonary deposition, an issue associated with fine particles.

Metered-dose, multi-dose nasal sprays are used widely, to deliver both solutions and suspension formulations. Unit-dose devices – single or double – are also commonplace, notably for the delivery of vaccines and emergency medications. Nasal aerosols are the other alternative for liquid formulations and, like metered-dose inhalers, use a propellant for dose aerosolization and delivery. Commercial nasal powders are an alternative option but currently relatively few in number. They offer the opportunity for preservative-free delivery and long retention times and are therefore particularly suitable for the delivery of hormones, antigens, and peptides.

Characteristics impacting the success and speed of delivery

Just as with inhaled drug delivery, delivered dose (or single actuation content) is one of the primary metrics measured to assess the efficiency and clinical efficacy of nasal drug products. Similarly, particle size is also an important metric.

A target median particle size is typically within the 30 – 120 µm range to ensure retention in the nose, with sub-10 µm fine particles the primary concern with respect to pulmonary delivery.

In addition to these requirements spray pattern and plume geometry provide further insight into deposition behaviour while priming and repriming behaviour is investigated to assess the reliability of the product in use. The issue of desposition behaviour is highly pertinent for nasal drug products and an important area of focus for research activities. For topical products the goal is simply retention in the nose but for systemic delivery there is a growing need to target different areas of the nasal cavity such as the olfactory region for drug delivery to the central nervous system. Understanding how currently measured characteristics relate to drug deposition behaviour in the nasal cavity is important for exploitation of the full potential of intranasal drug delivery.

Core testing requirements

Delivered dose uniformity

For nasal drug products, delivered dose is sampled across the lifetime of the product (as defined by the label claim) in accordance with methods described in **USP Chapter <601>** and **Ph. Eur. 10.5 (Nasal Preparations)**.

To measure delivered dose, the nasal drug product is actuated into a suitable receiver to capture a representative dose which is subsequently subject to chemical assay to determine drug content.

While test methods are analogous to those used for MDIs, several issues specific to nasal drug products are worth highlighting. Firstly, there is a requirement to ensure complete retention of the dose in the sampling apparatus while at the same time firing in the 'vertical/near vertical, valve-up position' that patients are required to use. Use of the standard MDI DUSA is stated in the pharmacopoeias but there are specific accessories now available that make it easier to meet this dose retention requirement when the DUSA is not used, such as when conducting bioequivalence testing for a generic where the reference product was not originally registered with DUSA data. Secondly, there is an indication to use 'a mechanical means of actuating the pump assembly', a requirement that mitigates towards automated product actuation. The need to prime the product in accordance with the patient information leaflet is also highlighted.

For nasal sprays the requirement is only to determine the amount of drug in the entire impactor-sized mass while for nasal aerosols, which typically produce higher levels of fines, a full APSD is required.

Aerodynamic particle size distribution (APSD) measurement

Cascade impaction plays a complementary role to laser diffraction in the characterisation of nasal drug products, relevantly quantifying drug in the sub-10 µm fraction that could potentially penetrate through the lung.

Interfacing the impactor and nasal drug product via a glass expansion chamber (see **figure 9**) ensures that the dose is fully dispersed prior to sampling in the impactor, with chambers of different size used to maximise the impactors sized mass for assessment of the worst-case scenario. Testing is carried out at the same flow rate as used for MDIs (28.3 L/min or 30 L/min when testing with an NGI as previously mentioned).

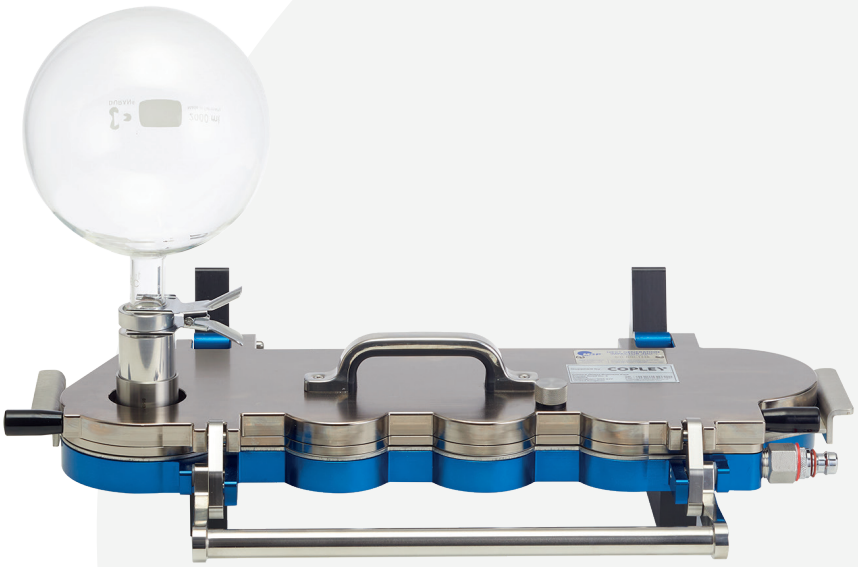

Figure 9: *The NGI shown here with a 2 litre glass expansion chamber is used to assess the amount of drug in small particles or droplets in respect of nasal sprays and aerosols.*

Final thoughts

Understanding the factors that influence the clinical efficacy of different pharmaceutical products provides insight into why different *in vitro* methods are applied to test them, and the criticality of specific test conditions.

In vitro methods are crucial, from R&D through to QC, because of their ability to cost-efficiently provide information for the development of new drugs and for the confirmation of product quality. Optimisation remains an ongoing challenge and tests are refined on an ongoing basis with new introductions helping to enhance *in vitro in vivo* relationships and thereby improve relevance.

Such developments are a particular focus for 'newer', more complex drug delivery methods such as inhalation where, for example, the use of anatomically realistic interfaces and breathing simulators is paying dividends in delivering more representative data than can be achieved using standard compendial test methods. Here, the issue of *in vitro in vivo* relationships continues to draw the attention of the regulatory bodies, as evidence by the current USP stimuli paper: "Testing the *In Vitro* Product performance of Inhalation and Nasal Drug Products: Views of the USP Expert Panel" exploring how to incorporate more advanced testing into the submission requirements. Ultimately the more reliably an *in vitro* method can quantify the critical aspects of drug delivery, for any product, the greater its value in supporting *in silico* studies, accelerating products to market and ensuring ongoing manufacture to the very highest standards.

References

[1] Hao Zhong, Ging Chan, Yuanjia Hu, Hao Hu and Defang Ouyang 'A comprehensive map of FDA-approved products' Pharmaceutics 2018, 10(4), 263.

[2] Copley, M. (2007) Understanding cascade impaction and its importance for inhaler testing. Copley Scientific.

For more information on any aspect of inhaled product testing please refer to: Driving Results in Inhaler Testing 2021 Edition, Copley Scientific.

For more information on testing for all other pharmaceutical products discussed in this paper please refer to: Driving Results in Pharmaceutical Testing 2020 Edition, Copley Scientific.

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