Listen to a recording of this issue

The Altascientist

SCIENTIFIC JOURNAL

ISSUE NO. 40

ANALYTICAL TESTING–ACCURATE AND COMPLETE CHARACTERIZATION OF YOUR API to maximize bioavailability

The analytical testing of active pharmaceutical ingredients (APIs) is a multi-faceted approach involving various techniques and methodologies to ensure that the API is adequately absorbed in the body to exert its therapeutic effect. By rigorously evaluating and optimizing the chemical, physical, and biological properties of the API, pharmaceutical scientists can enhance bioavailability, leading to more effective and reliable medications.

IN THIS ISSUE

In this issue of *The Altascientist,* we review different analytical testing techniques that support drug product development specific to bioavailability, including a **case study on the manufacturing of a nanosuspension**.

In the preliminary stages of drug development, a full characterization of your API is crucial to optimizing the drug product formulation. Specific to the drug product's bioavailability, understanding your API's particle size and dissolution profile informs downstream formulation decisions, including formulation and dosing for clinical trials. Additionally, different polymorph forms need to be understood, as crystalline structure can impact solubility, which directly impacts bioavailability.

The key components of analytical testing are:

1. Identification and characterization of API
2. Solubility testing
3. Dissolution testing
4. Permeability testing
5. Pharmacokinetic studies
6. Stability testing
7. Formulation testing
8. Advanced analytical techniques

In this article, we focus on points 1, 2, and 3. Maximizing bioavailability is a critical aspect of drug development, ensuring that the maximum therapeutic effect is achieved with the minimum dose. Analytical testing of APIs is essential in this context. Following the full characterization of the API, product development scientists can select appropriate excipients to achieve the desired drug delivery profile and method.

CHARACTERIZATION OF YOUR API

Analytical techniques are used to determine key API properties, including particle size and surface area, dissolution rate, thermal analysis, and crystalline structure.

Dissolution

Oral solid dosage forms are still the most common drug formulation, and the dissolution rate of the drug after it is ingested is a critical design element for product development. The rate of dissolution can significantly affect bioavailability, and dissolution testing is a critical technique used to verify targeted release profiles of different formulations during drug product development. This information can be pivotal for the selection of the dosage form according to desired release profile characteristics.

According to FDA guidance:

> *"The most commonly employed dissolution test methods are:*
>
> 1. *The basket method (Apparatus 1), and*
> 2. *The paddle method (Apparatus 2, Shah 1989).*
>
> *The basket and the paddle methods are simple, robust, well standardized, and used worldwide. These methods are flexible enough to allow dissolution testing for a variety of drug products. For this reason, the official in vitro dissolution methods described in U.S. Pharmacopeia (USP), Apparatus 1 and Apparatus 2 should be used unless shown to be unsatisfactory."*

Particle Size Analysis (PSA)

Particle size is a crucial parameter because it influences surface area, and porosity, and therefore, has an impact on the bioavailability, effectiveness, and shelf life of a drug. Particle size is inversely proportional to surface area; the smaller the particle size, the larger the relative surface area and higher the dissolution rate. The larger the relative surface area of the API particles, the more interaction the API has with its environment, increasing the dissolution rate and solubility, and hence, enhancing bioavailability. Instrumentation is available for all types of particle size analysis, from nanometer to millimeters in particle size.

Common techniques to determine particle size distribution (PSD)

Dynamic Image Analysis (DIA)

The DIA process measures the size and shape of particles ranging in size from 50 µm to 5,500 µm. It is a real-time, non-destructive, non-product contact analytical process that can be used both as a bench-top laboratory instrument and as a process analytical technology in-line. The direct imaging particle size analyzer can measure wet and dry powders as well as bulk solids. As it continuously captures and processes data in real-time, it also allows for the tracking of particle size growth and decrease in the process.

AccuSizer A7000 SIS system

Limitations of the DIA process:

- Limits of detection of particles.
- Requires algorithm optimization by user to obtain accurate measurements for dark particles. Optimization is a trial-and-error process and can be time-consuming.
- Relies on direct illumination of sample for particle identification within the algorithm, and therefore, transparent materials such as glass and some polymers cannot be accurately measured.
- Highly reflective particles are difficult to measure.
- The focal length of the system is limited, and therefore, samples must be sufficiently close to appear in focus and measurable by the system.
- The depth of field is small; thus, material with a wide sample range distribution will be difficult to measure at the extremes of the distribution curve.

Laser Diffraction (LD)

Laser diffraction is a technique for determining the PSD of a sample material by analyzing the scatter pattern of light from the sample. When light interacts with particles, it creates a scatter pattern, which an array of sensors detects and measures. Because the angle and intensity of the scatter pattern are proportional to particle size, it is possible to mathematically derive particle size information from the scattered light of an observed sample.

Particle size information for a given sample is presented as a volume fraction distribution based on the diameter of the observed particle's sphere of equivalent volume.

Limitations of LD:

- Measurement accuracy depends on user inputs; inaccuracies can occur if the refractive indices used to build the optical model are not correct.
- Assuming that all particles are perfect spheres can reduce measurement accuracy; samples with high eccentricity reduce measurement accuracy.
- Non-homogeneous sample analysis can result in incorrect measurements due to changing refractive index values.
- The measurement time can range from 2 to 10 minutes.
- Testing must be done offline, limiting the use of laser diffraction as an in-process control method.

Dynamic Light Scattering (DLS)

Dynamic light scattering, also referred to as quasi-elastic light scattering (QELS) and photon correlation spectroscopy (PCS), is an optical method for determining particle size properties in a dispersed sample by observing the change in scattered light intensity over time.

The random motion of colloidal particles due to physical interactions/collisions with molecules in a solution creates a constantly changing particle position and a changing scattered light intensity for a stationary solution. The rate of change of light intensity is related to particle size distribution in the sense that small particles fluctuate more frequently, resulting in a faster change in scattered intensity.

Unfortunately, there is no direct method of determining the mass distribution as the density of particles needs to be known. Additional methods must be utilized to determine the mass distribution.

Limitations of DLS analysis:

- The sample must be in solution.
- It is an offline method.
- Low resolution with closely spaced size populations with a difference of size of less than a factor of three; DLS will not precisely characterize a polydisperse sample.
- It is typically employed for particle sizes in the range 0.002 to 2 µm.
- Multiple light scattering when one particle is scattered by another before reaching the detector can hinder accurate calculation of particle size.
- Very sensitive to temperature, solvent viscosity, and refractive index.
- Sensitive to contamination (e.g., from dust).

Sieve Particle Analysis

This process separates fine particles from more coarse particles by passing the material through several sieves of different mesh sizes, sorting particles into specific sieve bin sizes. Wet sieving is suitable for particle sizes from 20 µm up to 3 mm while dry sieving can be used from 30 µm up to 125 mm.

Limitations of sieve particle analysis:

- For elongated and flat particles, a sieve analysis will not yield reliable mass-based results.
- It does not account for particle shape effects of different particles.
- Sieve analysis may not provide high resolution for a sample with a narrow distribution because of the limited range of mesh sizes available.
- No further particle shape information is available from sieve analysis without additional measurement methods.
- This method does not have the ability to analyze single particles.
- Sieve analysis has longer measurement times and a lower measurement speed than LD or DIA.
- Sieves are prone to blinding when the sieve openings are blocked by the material that is being analyzed. This can result in particles that are smaller than the sieve apertures being trapped on that sieve, which greatly affects the mass distribution and accuracy of the PSD.
- It is an offline method of particle size distribution and is unable to monitor processes in real-time.
- No particle images can be obtained using sieve analysis.

X-Ray Diffraction (XRD)

XRD analysis is a non-destructive technique used to determine crystalline structure; it measures the arrangement of atoms within a crystal, based on the angle of diffraction of the x-ray beams when they interact with the electrons around the atoms. This allows for identification and quantification (typically amount of amorphous material) of the crystalline structure of the API. These aspects influence the solubility of the API, and therefore, the dissolution rate.

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry analysis measures the heat-flow density into or out of a molecule as a function of temperature. DSC helps identify the melting point, crystallization temperature, glass transition temperature, and other thermal properties of a sample through controlled heating. This data can provide information regarding the stability of the crystalline structure of the API and inform drug product formulation decisions.

High and Ultra Performance Liquid Chromatography (HPLC and UPLC) for Analysis

HPLC separates the components in a sample for qualitative and quantitative analysis. HPLC is used to separate different constituents of an API using high pressure to push solvents through a column and is the most widely used technique to identify, quantify, and separate components of a mixture or compound. UPLC uses the same HPLC process but operates at higher pressure to deliver increased sample throughput, chromatographic efficiency, sensitivity, and decreased run time. UPLC systems operate at pressures up to 15,000 pounds per square inch (psi), while HPLC operates at pressures of 6,000 psi. Both technologies are widely used for the analysis of constituents of pharmaceutical actives, drug products, and many other substances. For bioavailability, HPLC equipment is used for the backend analysis portion of dissolution, to determine the extent of dissolution at varying timepoints.

FORMULATION APPROACHES TO MAXIMIZE BIOAVAILABILITY

Once an API has been fully characterized and determined to be a good candidate for development, formulation decisions are made. Often, the formulation approach serves to maximize the bioavailability of the API, and below we discuss some of the most common.

1. Nanomilling

Nanomilling uses a high-energy wet mill to physically break down coarse particles, reducing their size to less than 1,000 nanometers (nm)—usually in the 100 to 200 nm range, sometimes even smaller when a smaller milling media is used. High energy and shear forces are generated through the impact of the milling medium with the drug. This further disintegrates microparticulate drug into nanosized particles. With modern mills, the milling times may be only a few minutes, making it a very efficient technique.

To manufacture a drug nanosuspension with the desired particle size and appropriate storage stability, it is essential to select the optimal program parameters (stabilizer formulation and process/equipment framework) for the wet media milling process.

Advantages of Nanomilling

Nanomilling's universal applicability is a major advantage over other formulation approaches for poorly water-soluble APIs. Nanomilling can be applied to just about any insoluble API fairly easily, making it an appealing first-line approach to solubilization. Other advantages include:

- No harsh organic solvents or pH extremes: most nanomilled suspensions are aqueous-based
- High API concentrations: 5 to over 40 percent API (w/w)
- Easy scale-up: commercial nanomilling equipment allows batch sizes to increase without changing process variables
- Reproducibility: once a nanomilling process is optimized, there is minimal variation in particle size between batches

2. Liquid-Filled Hard Capsules (LFHCs)

Many APIs have high melting points and poor aqueous solubility, which directly impact their dissolution and bioavailability. In an LFHC, readily metabolized lipid-based solutions are used as liquid carriers, providing optimized API absorption.

The capsule's drug formulation and polymer composition can be customized to accommodate APIs with many different properties and desired dissolution profiles. For example, drugs with high levels of hygroscopicity can be formulated in capsules with a low water content to avoid clumping. Opaque capsule shells protect the API and filling from light intrusion. The final formulation, including the drug substance, must be screened for compatibility before the formulation is finalized.

Advantages of LFHCs

- LFHCs are pre-made dosage forms, in that the shell itself does not require formulation. Liquid filling can be done quickly (same day) and relatively easily.
- LFHCs are ideal for low doses. Small quantities can be filled in-house with lab-scale equipment and just a few grams of formulation for preclinical or early-phase clinical trials.

3. Solutions and Suspensions

Liquid formulations, such as solutions, suspensions, and emulsions, assist with bioavailability because the drug is already in solution or suspended in the liquid. Despite the advantages, there are challenges associated with liquid dosage, such as stability and palatability, which need to be considered in the design. Formulating the right oral liquid dosage form in early-stage development depends on the art of pharmacy.

4. Spray-Dried Dispersion (SDD)

An SDD is an amorphous molecular dispersal of a drug in a polymer matrix, created by dissolving the drug and polymer in a solvent and then spray drying the solution. The process can reduce particle size compared to the original particle size of the starting product. Spray drying increases the surface area of the particles and improves dissolution rates. SDD is not widely available and only works for feeds that can be atomized. Often, dilutions and solvents can overcome atomization problems, but not always. Despite the brief exposure to high heat, there are also some substances that will melt once they come into contact with hot gas in the dryer.

Advantages of SDD

- Enhanced bioavailability of poorly soluble compounds
- Consistent particle size distribution
- Long-term stability
- Robust, scalable process
- Higher drug loading
- Enables taste masking or controlled release dosage forms

Disadvantages of SDD

- Environmental concerns: spray drying requires organic solvents that can be difficult to recycle. Some solvents are also toxic and flammable, so they may need to be stored in separate facilities.
- Challenges with small scale: spray dryers may not perform well in small laboratories, and extra care may be needed to avoid false negatives.
- Powder properties: spray-dried powders can have low density, compressibility, and poor flow. This can make it difficult to fill capsules, so additional excipients may be needed to improve processability. The compatibility of these excipients with the spray-dried powder should be tested.
- Encapsulated SDDs: encapsulated SSDs can have limited drug payload and may gel within the capsule, which can make it hard for the drug to dissolve.

SUMMARY

There are many important considerations to make during drug product formulation development. A well-characterized API with an appropriate testing regiment will identify critical characteristics to support a successful program. Mastering manufacturing techniques used to achieve appropriate particle size can be a critical aspect of a drug product development program to ensure targeted bioavailability.

Robust analytical testing is a crucial part of an IND drug submission, and careful attention should be given to the methods chosen and the documentation of results.

ALTASCIENCES' CASE STUDY

Altasciences was contracted by a client to develop a nanosuspension for their clinical trials. A nanosuspension was selected because particle size reduction significantly increases the surface area of the API and leads to enhanced bioavailability. The project required milling the material over multiple batches with differing particle sizes and identifying the appropriate excipients for the formulation. An in-process particle size test was conducted on each sample pulled to assess the reduction in size over time using HORIBA's LA-960 particle size analyzer.

- **Drug Development Phase:** R&D preclinical
- **Class of Drug:** Small molecule API, PB-ECL Category 2
- **Indication:** HIV treatment
- **Delivery Method:** Subcutaneous/intramuscular
- **Services Provided:** Formulation of a nanosuspension and analytical services

The study's purpose was to manufacture a nanoparticulate suspension formulation using the client's API. Altasciences selected additional components to optimize the formulation by reducing particle size to improve bioavailability and increase stability.

Methods

- Standard nanomilling conditions employing a jar mill
- Sampling timepoints: 0, 1, 2, 4, 8, 16, 24, and 48 hours
- API concentration 25%
- Milling media, YTZ® beads
- Aqueous phase: 6 aqueous phases, each implementing a distinct surfactant
- Analytical: laser diffraction particle size analysis using HORIBA's LA-960 particle size analyzer

Results

A total of six distinct formulations were manufactured using a target yield of 30 grams. Each formulation consisted of 25% (w/w) API and common excipients used in the manufacture of oral dispersions. Carboxymethylcellulose was used as a thickening agent to obtain an appropriate viscosity, ascorbic acid was included as an antioxidant, and an appropriate surfactant was used in each formulation to observe the effect on particle size.

Each formulation was milled using a jar mill containing the API, excipients, and YTZ milling media. The formulations were milled over 48 hours, and samples were removed at 0, 1, 2, 4, 8, 16, 24, and 48 hours. An in-process particle size test was conducted on each sample pulled to assess the reduction in size over time. Each formulation was created using a unique surfactant to determine if it impacted the reduction in particle size. We evaluated the following:

1. Kolliphor P338
2. Kolliphor EL
3. Kolliphor HS 15
4. Kollisolv PEG 300 G
5. Dioctyl Sulfosuccinate Sodium, 96%
6. Vitamin E TPGS 1000 NF

The initial particle size of the T0 samples exhibited D90 values in the range of 9,000 nm to 15,000 nm. In two of the six formulations, particle size was effectively reduced during the 48-hour milling time to a D90 less than 400 nm. The formulations using Kolliphor® EL and vitamin E surfactants exhibited the most significant decrease in particle size.

Conclusion

Altasciences demonstrated that a nanosuspension could be successfully formulated for the client using their API. Expert analytical testing confirmed that two formulations matched the target particle size range, and viable options were provided to the client to support their program before clinical trials.

HOW ALTASCIENCES CAN HELP

Altasciences provides contract HPLC/UPLC method development and validation, raw material, in-process, finished product, and stability testing for your drug development programs. We take pride in the development of robust and rugged analytical procedures to ensure the quality of your products, and qualification and validation of analytical procedures to meet both your requirements and those of regulatory agencies worldwide.

Our analytical lab and testing service offering includes:

Development and Validation of Critical Methodologies

- Cleaning methods for the detection of API on manufacturing equipment
- API methods for assay/related substance
- Finished dosage products (assay/degradation, dissolution)

Stability Testing

- ICH environment stability chambers

Controlled Substance Testing

- DEA Analytical License (Schedules I-V)

Excipient and Active Pharmaceutical Ingredients (APIs)

- Compendial method verification and validation studies
- Monograph release testing per the USP, EP, BP, and JP

Drug Product Release Testing

- HPLC and UPLC
- Gas Chromatography (GC)
- Dissolution and disintegration
- Moisture analysis (gravimetric and Karl Fischer [KF] titration)
- Melting point determination
- Residue on ignition
- Spectroscopy (ultraviolet/visible [UV/VIS] and infrared [FTIR])
- Total organic carbon (TOC)
- Viscosity
- Particle size analysis
  - HORIBA analyzer
  - AccuSizer® (USP <788>)

ALTASCIENCES' RESOURCES

Webinars/Podcasts

The Development of Nanosuspensions for Poorly Soluble Drugs

Altasciences' Manufacturing Solutions

From Concept to Market: Overcoming the Challenges of Manufacturing and Clinical Trials

Tips to Ensure Successful Formulation for Nonclinical Testing

Scientific Journals

Nanomilling for Better Solubility and Improved Bioavailability

The Applications of Liquid-Filled Hard-Shell Capsules in Drug Development

Maximizing Drug Formulation for First-in-Human Trials

Achieving Optimal Preclinical Formulation and Drug Product Manufacture

Videos

Step Inside Altasciences' Manufacturing Facility

The Art of Pharmaceutical Formulation

Webpage

Altasciences' Comprehensive Suite of Manufacturing Services

Fact Sheet

Pharmaceutical Contract Manufacturing

ABOUT ALTASCIENCES

Altasciences is an integrated drug development solution company offering pharmaceutical and biotechnology companies a proven, flexible approach to preclinical and clinical pharmacology studies, including formulation, manufacturing, and analytical services. For over 25 years, Altasciences has been partnering with sponsors to help support educated, faster, and more complete early drug development decisions. Altasciences' integrated, full-service solutions include preclinical safety testing, clinical pharmacology and proof of concept, bioanalysis, program management, medical writing, biostatistics, clinical monitoring, and data management, all customizable to specific sponsor requirements. Altasciences helps sponsors get better drugs to the people who need them, faster.

ALTASCIENCES

CONTACT US

altasciences.com | contact@altasciences.com | Tel: 514 246-4191

Follow us on LinkedIn