PEER REVIEWED

Working with Difficult Preparations – Risks, Regulations, and Considerations

Safety testing is a crucial part of drug development and is dictated by pharmacopoeial guidelines which must be followed when performing each step of the process. Ideally, the method development stage is when any potential sample preparation problems are resolved to make the routine analysis easier, however, depending on the product in question, this can become a lengthy process as preparation methods can vary broadly.

The type of test in question may also affect whether a sample is challenging to prepare as any number of roadblocks can occur during the testing process. It can be difficult to determine which method is suitable for each type of product without familiarity with regulatory guidelines and an awareness of how the available methods work with specific product types.

The best place to start is by examining the material safety data sheet (MSDS), which should contain any specific details of the composition of the product and safety concerns.

Drawing on any relevant personal experience, either with a similar sample type or from other types of testing completed with that product already, can also be useful, as well as consulting with other experts such as the manufacturer, or if the sample has already undergone any tests with a different facility, comparing any methods used for that prior testing.

Each product is unique and there are an infinite number of issues related to product type, composition, appearance, sampling size, and hazardous samples that might arise during the testing process.

Inhalation & Aerosol Products

Inhalation products, for example, along with pressurised aerosols can pose an issue with extraction as the extraction method must not affect the natural bioburden or the composition of the product in any way.

Part 2 of the current Ph Eur 2.6.12 Microbiological Examination of Non-Sterile Products: Microbial Enumeration Tests starts with stating that the testing is to be performed under conditions designed to avoid extrinsic microbial contamination of the product to be examined. This includes the area in which the testing is performed but also how to remove the sample from the container and how the subsequent testing is performed.

While extracting the product from its final container, it is important that aseptic technique is applied. One possible extraction method for fluids in aerosol form is to first chill the sample in its container in dry ice for around one hour.

The container can then be aseptically cut open at room temperature, allowing the propellant to escape before collecting the sample.

However, what impact freezing has on the product must be considered, as well as whether the composition may be altered. Whichever approach is chosen must be qualified during method development or if transferring methods, during validation.

To achieve the best results, ideally the manufacturer and technicians conducting the testing should work together to determine a suitable extraction method, ensuring that microorganisms or other contaminants are not introduced in to the test and that the chosen extraction method is safe to perform with that particular product.

It is, therefore, vital to ensure risk assessments for the components

are in place to minimise exposure to propellants and potentially harmful substances during handling, storing and testing of the product.

Insoluble Products

Solubility issues are another factor to consider, as these can affect various tests including microbial limits, sterility and pyrogen testing. The desired outcome of the test itself must be considered, particularly with *in vivo* testing and the route of administration of the sample.

Examples of solubility issues include water soluble products (which may not dissolve completely) and fatty versus non-fatty products which require different approaches to prepare. With a non-fatty product that is insoluble in water, emulsifying agents will need to be added to your diluent to aid in homogenisation of the sample preparation. The pH might also need to be adjusted using sterile acid or alkaline solutions.

With fatty products, the preparation will need to be dissolved in isopropyl myristate or polysorbate 80 or some other emulsifier, and may need to be heated as well but to not more than 40°C or, in exceptional circumstances, to not more than 45°C as detailed in the pharmacopoeias (to ensure that the bioburden is not affected) to aid in preparation of the sample for testing.

Preparation of samples for toxicology testing poses a different set of challenges, given that the dose in the syringe must be a representative dose and therefore issues with solubility cannot be addressed by continued dilutions.

At all stages, particularly in microbiology and toxicology testing, aseptic technique must be observed and any solution used to dissolve the substance should be demonstrated not to alter the composition of the product, provide any potential for growth of microorganisms, nor add to the bioburden of the product.

When acid-based solutions are used in preparation, it's important to be aware of potential issues, as these are used to prepare some of the more insoluble solutions for testing but can inhibit microbial growth, as many organisms prefer to grow in more neutral pH environments. This could impinge on method suitability and cause inaccuracy in reporting of results, so it is vital to ensure you put in place appropriate steps to minimise or negate these effects.

In the case of toxicology samples, the acid / alkali balance must be very carefully controlled due to the nature of the testing with both the active dose of ingredients and the dosage rate taken into consideration. It is essential that no more than the maximum human dose is utilised, requiring representative concentration levels prepared specifically for the size / weight of the test subject.

This is an issue that must be considered throughout the toxicology testing process, not just when assessing pH levels, as the dosage size must be calculated correctly to be representative for the size of the laboratory animal versus the human dose, but also must not be so small that there isn't enough drug to challenge the system.

This is relevant with medical device testing as well – the key consideration must be whether the testing is representative of how the drug or device would be used in the end patient, and the test sample should be representative of how the final product will be manufactured and used.

It is a very careful balancing act which requires not only an understanding of the drug product being tested and technical expertise with the test, but also a thorough familiarity with all factors affecting animal welfare.

Products Requiring Filtration

Filtration can also pose problems as a filter or filtration apparatus that is compatible with the product must be chosen. Acidic solutions do not mix well with a dissolvable filter, for instance, as any microorganisms that should be captured on the filter surface could be washed away to waste along with the filter as it disintegrates.

Some samples may also react with the plastic of the filtration apparatus causing it to melt and warp, so in certain instances a glass filtration apparatus might work better. Additionally, samples may need to be pre-filtered prior to testing to remove 'lumps' or, in the case of cell banks, to remove the cell bank cells to prevent blocking of the filter, leaving the smaller microorganisms to pass through and then be captured on the microbial retention filter.

It is also important to consider factors that may be specific to the type of test performed; for example, the microbial limits test. In the microbial limits test, products that are highly antimicrobial or have a high binding ability may result in the product binding itself to the pores of the filter membrane using electrostatic charges.

Such a product may not be able to be washed out of the filter matrix and may then exhibit inhibition to any microorganisms present in the product, giving a falsely low result. This can be overcome by using low binding filter membranes.

The container used for testing must also be considered, such as how a new container might interact with the sample. Whether the material of the container or lid will interfere with the product, including the preservative system, must also be determined in advance of testing. Other concerns to resolve in advance are whether the product is required to be stored in the dark and whether evaporation of the product is likely to occur.

Antibiotic Preparations

Microbiological assays are another challenging series of tests which provide a collective assessment of the potency of the overall biological activity of an antibiotic preparation as compared to a reference microorganism, or standard.

An example of an issue that might need to be considered in this case is whether the sample is hygroscopic, or absorbs water from the environment.

These types of samples must be handled minimally in "sealed" environments where it is possible to minimise the opportunity for water uptake. It may also be prudent to use desiccation sachets. This should be discussed with the manufacturer to ensure the method chosen will not affect the properties of the product.

In addition to environmental controls, you should consider the effect of disinfectants and chemical agents on the sample preparation process. At many other testing facilities, it is usual to decontaminate or sterilise products in their packaging before they are moved into a sterility testing isolator for sterility testing. It is, therefore, important to consider whether a sample container is permeable, for example if using hydrogen peroxide equipment for decontamination purposes, as the hydrogen peroxide in the gassing cycle could potentially permeate into the test sample and adversely affect the sample composition.

In any instance where permeability is a concern, per MHRA and FDA guidance it must be shown through validation that there is no effect on the sample.

In some circumstances, the sterility test itself may be insufficient in proving the impermeability of the sample and a longer-term stability programme may be required.

Coloured Products

Another aspect of the composition of the product which can affect such tests as the bacterial endotoxin or monocyte activation test, is appearance. For example, the kinetic chromogenic endotoxin test is a quantitative kinetic assay based on the development of a yellow colour after cleavage of a synthetic peptide complex. In this test, a coloured sample may provide a false result

and therefore must be diluted and re-assayed to avoid interference with the test, or a different method such as the kinetic turbidimetric endotoxin test chosen instead.

Product Availability

While not an issue with the sample itself, another aspect of testing that might need to be considered would be if there is only a small sample size available for testing due to manufacturing issues, or limited availability of materials such as a small batch or small amount of product per test item.

In some circumstances, a reduced number of samples can be used for validation or method suitability but it is essential to put in place a robust justification for 'scaling down' any testing that doesn't meet pharmacopoeial regulations.

Although price is always an issue from a commercial standpoint and it might therefore be tempting to consider this in assessing sample sizes for testing, it is not a reasonable excuse to scale down samples in the eyes of regulators for most pharmacopoeial testing.

Hazardous Products

Cytotoxic, carcinogenic and other teratogenic substances can be difficult in a different way, as the preparation of the sample itself may not be inherently difficult, but there are specific safety precautions which must be followed given these drugs affect normal cells alongside the tumour cells they are targeted toward.

These substances can produce significant side-effects in anyone exposed to them and their preparation and administration must be carefully considered. When working with laboratory animals, the risk to staff is heightened as working with hazardous substances in a live system has additional risk and complications versus a cleanroom environment.

One such issue is dosing, as this can be a difficult process regardless and requires good control at all stages to avoid additional risk to technicians.

These types of products must be tested in a safety cabinet and may be restricted to testing by only technicians of a certain age or gender. A code of practice should be put in place that covers the safe handling of these drugs, including sample receipt, testing and disposal, which are all considerations which should be evident from a thorough review of the MSDS.

Any such code of practice for cytotoxic substances should require all samples to be marked clearly as such and that appropriate personal protective equipment be in place at all times during handling. All workspaces and materials should be thoroughly cleaned to ensure neutralisation of any cytotoxic material once the sample is dealt with (booked in or tested) and such samples stored securely and separately from other samples to avoid cross-contamination.

Additionally, with these types of teratogenic drugs, any laboratory animals used for their safety evaluation or the waste produced from such tests must not be handled by any staff who are pregnant, planning a pregnancy or breastfeeding due to the increased risk. These steps must be taken to ensure that the testing can be carried out effectively with no harm to any personnel involved.

Product Method Transfer

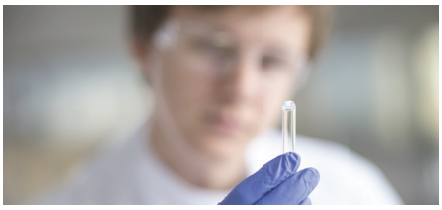
It's also important to mention the potential testing and sampling issues that can arise when transferring methods, particularly from in vivo to in vitro tests such as the rabbit pyrogen versus the monocyte activation test, where there may be a different metabolic effect. An example of this would be a sample contained within a liposomal sheath requiring toxicology testing. The difficulty lies in how to determine when the drug breaks down as if it hasn't broken down completely, the full potency of the drug may not have been tested.

While this may seem like a testing issue rather than sampling, when switching from using a live system to an in vitro method, it must be considered what needs to be done to the drug in the preparatory stages to make it break down fully with the exact same mechanism as would happen in the body. There can be no changes to the chemical makeup and no reduction of the reaction that would occur in the original test.

These examples illustrate that a vital part of any drug development lifecycle is conducting a risk assessment of the product and any potential sampling issues in advance of initiating actual testing or method development.

The process of preparing a risk assessment can be difficult and time-consuming, which is why many manufacturers and drug developers choose to outsource this to an experienced laboratory facility. The human element of this is particularly important and the risk to the workforce must be considered in advance when dealing with high-risk samples such as toxic or cytotoxic substances, given that accidents do happen, no matter how much care is taken.

A thorough examination of any relevant safety information and product details in combination with reliance on the technical expertise gained through familiarity with common sampling issues can help to ensure that you avoid costly delays due to testing failures.



John McKenzie

Chief Executive Officer, Wickham Laboratories Ltd. He has an extensive background in the life sciences industry with over 20 years of management experience across the European and Asian continents.

Email: mail@wickhamlabs.co.uk