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The Advantages of LC/MS in Identifying Residual Impurities in Biopharmaceuticals

The identification of impurities and their subsequent removal constitutes one of the most critical process steps in the development of biopharmaceuticals. The presence of residual impurities has the potential to affect the safety and efficacy of products. As a result, their levels must be effectively controlled and are considered as critical quality attributes.

Fergus Hall, PhD, Section Manager, Pharmaceutical Chemistry at Eurofins BioPharma Product Testing, looks at the complexity of detecting and quantifying residual impurities which are usually present at low concentrations within difficult sample matrices. He will discuss the broad selection of detection methodologies currently available, specifically focusing on the advantages of high performance liquid chromatography mass spectrometry (HPLC/MS) for process validation studies.

Process-related Impurities in Biopharmaceuticals

Biopharmaceuticals make up a class of drugs which are generally produced in living organisms and used for the treatment of a wide variety of diseases, including cancer and diabetes. Examples of biologic products include hormones, enzymes, monoclonal antibodies, vaccines and blood factors, and each present different challenges during drug development and manufacturing. The growth of the biologics market can be attributed to the many advantages they offer over small molecule drugs, such as reduced safety/toxicity issues, the potential to treat complex diseases and high target specificity.

The challenges of developing biopharmaceuticals arise from the structural complexity of these large molecules and their production processes which introduce a range of additives that have the potential to become residual impurities in the process stream. These impurities are potentially toxic and have no benefit to the patient. Consequently, implementation of analytical characterisation processes at each stage of manufacturing is necessary to ensure product efficacy, quality and purity.

Process impurities are related to the manufacturing process and may include cell substrates, such as host cell proteins, host cell deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Other impurities occur as a result of cell culture steps, such as inducers, antibiotics and media components, as well as residual impurities that are introduced downstream from resins, residual solvents and surfactants. There can also be residual impurities that are introduced through chromatographic media used in purification and buffer components, while others are present due to extractable and leachable substances. These include impurities that can migrate from pharmaceutical container closure systems, process equipment and packaging.

In order to protect patients, the level of impurities in biopharmaceuticals must be reduced to acceptable safety limits. However, these process-related impurities are typically present at trace levels in complex sample matrices which makes quantification a challenge.

Regulatory View

The regulatory authorities have introduced extensive guidelines which set out the highly specific and sensitive techniques which offer robust quantification to support process validation and ensure that products meet specification limits for process residuals. The testing of biopharmaceuticals must be conducted in accordance with the International Conference on Harmonization (ICH) Technical Requirements for Registration of Pharmaceuticals for Human Use Q5A to Q5E (Quality of Biotechnological Products) and the Code of Federal Regulations (CFR) Title 21 parts 600, 601 and 610.

Since residuals are typically present at varying levels throughout the process, method development and optimisation can be difficult. For example, extraneous proteins which are known to produce allergenic effects in humans should not be added to the final virus medium of cell culture produced vaccines that are intended for injection. If serum is used at any stage, its calculated concentration in the final medium should not exceed 1:1,000,000 (CFR 21 part 610.15).

Multiple Analytical Techniques

Multiple detection techniques can be used to characterise and classify residual impurities. The most commonly relied-on methodologies utilise liquid chromatography (LC) or gas chromatography (GC) combined with a range of detection methods, such as charged aerosol detector (CAD), evaporative light scattering detector (ELSD), mass spectrometry (LC-MS, GC-MS), flame ionisation detector (GC/FID), enzyme-linked immunosorbent assay; and quantitative polymerase chain reaction (qPCR).

The varying properties of residual protein contaminants in biologic drugs mean that different methods may be used to detect and monitor each. For example, the presence of residual solvents and volatile molecules are frequently determined by GC/FID, while proteins are detected using qPCR. The analysis of compounds where UV detection might be a restriction, e.g. sugars, antivirals, antibiotics, lipids, phospholipids, terpenoids, and alcohols, are typically measured using ELSD.

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Improved Detection Using LC-MS

The industry is continuously exploring more advanced techniques for the detection of residual impurities, particularly at the low concentration levels required for biopharmaceuticals testing. Typically, LC/CAD and LC/MS are used for biologic products. As a cost-effective universal detector, LC/CAD is well suited to limit tests and screening. Highly sensitive and possessing a broad dynamic range, it offers real advantages in the analysis of compounds that lack UV chromophores. It is, however, less robust than other methods and limited by the need for the compound of interest to be non-volatile.

LC/MS, on the other hand, is being widely used to characterise impurities due to exceptional sensitivity down to the picogram (10^{-12}) level. The technique provides high-resolution, accurate mass, selectivity, as well as specificity, positioning itself as a superior detection method that provides more detailed information and a better indication of purity. Additionally, it is able to detect multiple types of impurities. The only stipulation for the use of LC/MS is that the residual impurity being analysed must be ionisable.

When compared to LC/CAD, LC/MS can provide considerably more information on analyte identification. It is one of the most widely-used tools for monitoring and identifying residual impurities and yields both qualitative and quantitative information. It is also the only technique that provides the capability to both identify and quantify residual impurities.

Industry Preference for Outsourcing Models

One area in which LC/MS demonstrates significant drawbacks is the costs associated with implementing the technique. The expense of procuring the instrumentation alone is in the region of $600,000, and this is without even considering the costs associated with the installation, qualification, software validation, and on-boarding of an in-house team to operate the equipment and interpret the resulting data.

Due to the high expense, many manufacturers are turning to third-party vendors that can provide residual impurities analysis using LC/MS as a service. While the choice to outsource can be somewhat of a difficult decision, it is one that can be both analytically and financially rewarding. By carefully considering the services procured and choosing an appropriate outsourcing partner, manufacturers can access expertise and proven experience that offers them the freedom to focus on their core business, all while reducing costs and the need for capital equipment.

Final Thought

As the biologics market continues to strengthen, there is a growing need for manufacturers to take measures to prevent the formation of residual impurities in products, with these being identified and characterised using appropriate detection methods. However, the complexity of new process matrices together with growing demand for increased sensitivity presents a multitude of challenges and calls for advances in detection technologies.

LC/MS represents the most robust analytical technique available and provides the necessary sensitivity and selectivity to detect a broad range of impurities. By identifying and quantifying residuals in biopharmaceuticals with the highest degree of precision, specificity and accuracy, it can help manufacturers streamline their analysis processes and simplify compliance with regulatory guidelines.

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