

Almac Voice

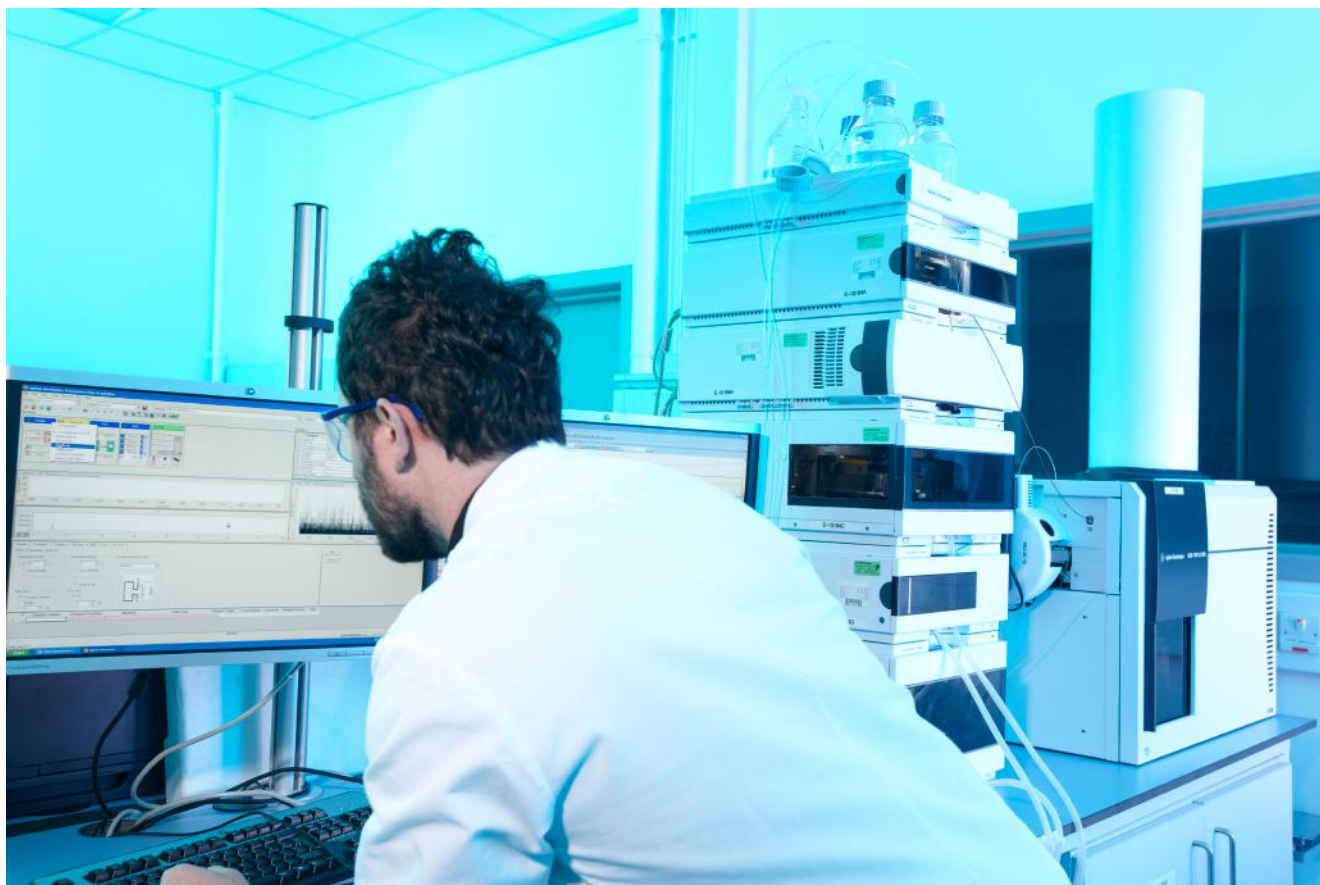
The Power of Spectroscopy in the investigation of unknown impurities



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Spectroscopic tools have long been the go-to analytical technique for impurity identification and with recent advances in instrumentation this toolbox is more powerful than ever. This feature discusses the wide range of GMP spectroscopic tools available at Almac's laboratories and how the power of our team is much greater than the sum of its parts.



Liquid Chromatography Mass Spectrometry (LC-MS) and Gas Chromatography Mass Spectrometry (GC-MS)

The principles of LC-MS and GC-MS are similar but they use different phases to separate mixtures of compounds prior to analysis. They are strong tools with the potential to analyse a wide range of compounds and have a diverse range of uses across the industry.

The majority of Active Pharmaceutical Ingredients (API) and drug products have an HPLC or GC method detailed on the release specification to demonstrate the purity. An HPLC or GC purity method could, however, highlight impurities in a batch. LC-MS or GC-MS can be used to identify any unknown peaks which may appear in the chromatograms. For HPLC methods, some up-front work may be required to bring the method in line with LC-MS requirements (e.g. removal of phosphate buffers in the mobile phase).

2 Dimensional-LC-MS (2D-LC-MS), which is available at Almac, negates the requirement to carry out this extra step. This means that client chromatography that is not compatible with MS can be reproduced on the first dimension and the unknown peak of interest can be diverted to the second dimension, utilising MS compatible mobile phase for MS (or MS/MS see below) analysis. These techniques can also remove the need for expensive isolation of impurities and can be used on small quantities of material.

An added advantage of LC-MS and GC-MS is the availability of a NIST library, which can generate a number of potential structures from the MS and MS/MS data.

Tandem Mass Spectroscopy (MS/MS)

For small molecules MS can be sufficient in providing a structure, but for more complex molecules further interrogation may be required.

MS/MS analysis provides a further dimension of scrutiny of the ions generated during MS analysis. MS/MS further breaks down the ions of interest produced in MS analysis, allowing a more detailed interpretation and potentially an identification of a peak.

High Resolution Mass Spectrometry (HRMS)

Advances in technology have enabled MS instruments to become more sensitive and provide higher resolution and accuracy, allowing a more detailed scrutiny of ions. LC-HRMS features both high resolution and high mass accuracy and is growing in popularity, based on its ability to efficiently offer a wealth of multi-attribute information that can be used to characterise compounds more efficiently. The increased resolution of HRMS instrumentation makes it possible to distinguish isotopic distributions and improve the accuracy of chemical formula prediction. The instrumentation (including both TOF and Orbitrap technologies at Almac Sciences) can generate mass data accurate to at least 4 decimal places. These accurate masses can be used to facilitate the ID of compounds with similar chemical compositions.

Analysis of biopharmaceuticals can benefit from this improved data quality, which can lead to improved drug characterisation with higher confidence and less ambiguity. LC-HRMS has significant advantages over more simple chromatographic techniques, particularly during the development of biotherapeutics such as large proteins and Monoclonal Antibodies (MAb's). It can be used to analyse many product quality attributes, including molecular weight, amino acid sequence, post-translational and chemical modifications, as well as both product-related and process-related impurities. The Thermo Scientific Q Exactive BioPharma MS/MS Orbitrap mass

spectrometer in Almac takes full advantage of the high-resolution, accurate-mass capabilities of the Orbitrap mass analyser to enable key protein characterisation workflows such as denatured and native MS intact analysis, subunit and top/middle-down analysis, and peptide mapping. Combined with experience in sample preparation, the Q Exactive BioPharma provides a complete solution for the rigorous structural characterisation of biotherapeutic compounds.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

ICP-MS is a relatively new technology to pharmaceutical analysis which is used to detect elemental impurities in samples. It came into mainstream use with the introduction of USP chapters on elemental impurities. This technique is the gold standard test for identification of trace elemental impurities in pharmaceutical products. It is a powerful technique that can scan for a range of elemental impurities and at the same time further scrutinise the sample to provide quantitative analysis of impurities down to ppb levels. ICP-MS is routinely used during pharmaceutical development and is an important tool in the identification of any unknown contaminants. Contamination of a batch could be down to the presence of residual metals, either from a catalyst that has not been sufficiently purged, or through contact with metal manufacturing parts.

Nuclear Magnetic Resonance (NMR)

NMR has long been the workhorse identification test in the pharmaceutical industry, providing reliable structural data of raw materials, active ingredients and impurities. Whilst NMR is traditionally applied to ^1H nuclei, Almac have cryoprobe technology which allows the detection of a range of different nuclei in the range ^{31}P to ^{15}N , including ^{13}C and ^{19}F .

Detection and identification of impurities are possible at low levels with the enhanced sensitivity achieved from the cryoprobe technology allowing vastly reduced time and resource for impurity identification.

Quantitative NMR is an absolute analytical method, where an unrelated commercially available reference standard is used to quantify the compound of interest using quantitative ^1H NMR experiments. Cryoprobe technology allows quicker NMR run times and increased sensitivity, which is often a critical parameter in assay and impurity quantification. A single QNMR analysis can provide relative or absolute quantitative analysis of a target compound in a sample. This is particularly useful for crude mixtures from manufacturing streams, polymers and natural products.



Conclusion

Whilst availability of all the spectroscopic instrumentation is useful when an unknown impurity arises it can be difficult to predict which tool will be most suitable for analysis and identification. By having dedicated spectroscopy laboratories and a very experienced spectroscopy team with easy access to the instrumentation, the most appropriate technique can easily be identified, or multiple techniques can be accessed in a short space of time.

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