Determination of Peptide Peak Purity by Molecular Feature Extraction using Accurate Mass LC-MS

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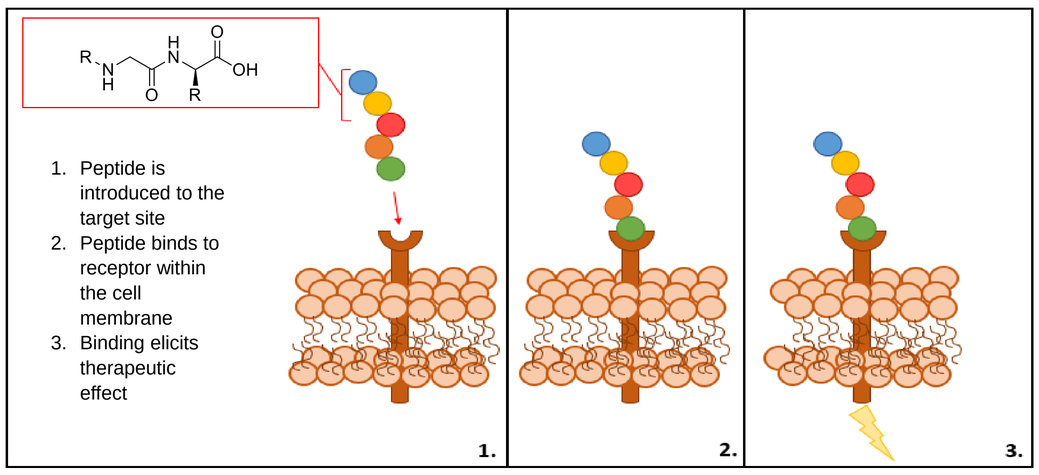
Background

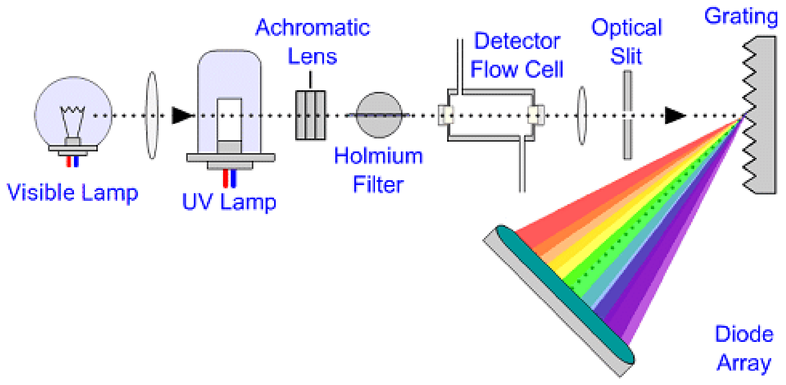
Peak purity is an essential technique to ensure the quality, safety and efficacy of manufactured Active Pharmaceutical Ingredients (APIs) in the pharmaceutical industry. At Almac, the use of Molecular Feature Extraction (MFE) has become an established GMP assured procedure for the identification of co-eluting impurities, particularly in peptide products. Its predecessor, peak purity by diode array detector (DAD), is commonly used for small molecule analyses, but is not capable of sufficiently determining peak purity of biologics products due to similarities between the UV spectra for impurities and API material. Conversely, the MFE algorithm generates a vast quantity of detailed mass information and, alongside expert interpretation, allows analysis of much larger APIs and structurally similar impurities.

Figure 1 - Peptide Structure and Biological Action



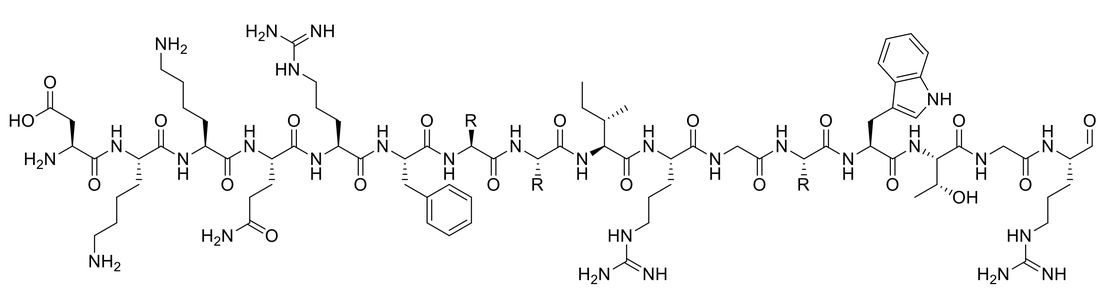
Peptides are a distinctive family of biopharmaceuticals, existing amid traditionally used small molecules and much larger proteins, with characteristic physiochemical properties and therapeutic effects. When compared to small molecule drugs, peptides are more specific, potent, and selective to receptors found on the target cell's surface.
Within the human body peptides hold pivotal roles in acting as signalling or regulatory molecules in processes such as immunogenicity, growth, reproduction and homeostasis. Peptide performance in therapy solutions can be optimised by simultaneously administering a small molecule or antibody to assist targeting the site of action, known as peptide conjugation. This method of delivery is even more specific to the target site of action, therefore less likely to target other in-vivo sites, thus allowing significant control over the efficacy and safety of administration.

Figure 2 - Peak Purity in the Pharmaceuticals



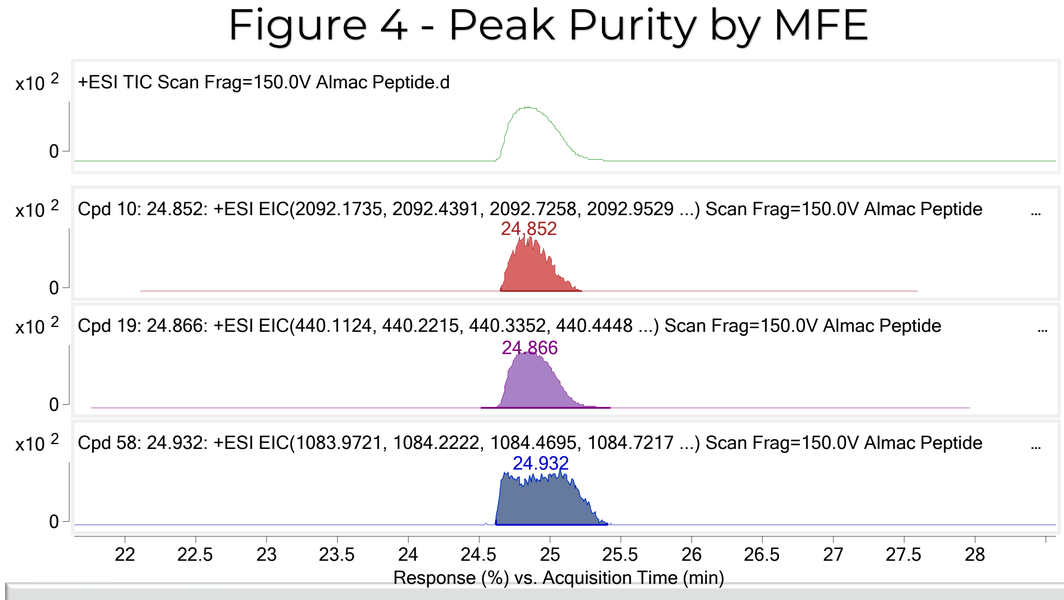
Regulatory authorities advise the use of peak purity to determine if the main analyte chromatographic peak is purely attributable to one component or if there are co-eluting impurities present. For small molecules this has typically been performed using LC-UV, specifically a diode array detector (DAD) capable of detecting multiple wavelengths at the same time, thereby generating orthogonal data. Chromatographic software is employed with algorithms capable of extracting all absorbance spectra within the retention window, or peak, of interest and assessing their similarity. If spectra are identified to be different, this will suggest there are co-eluting impurities within the peak. This is a powerful analytical tool, but only if the impurities are spectrally different to the main analyte peak.

Figure 3 - Almac Peptide Partial Structure



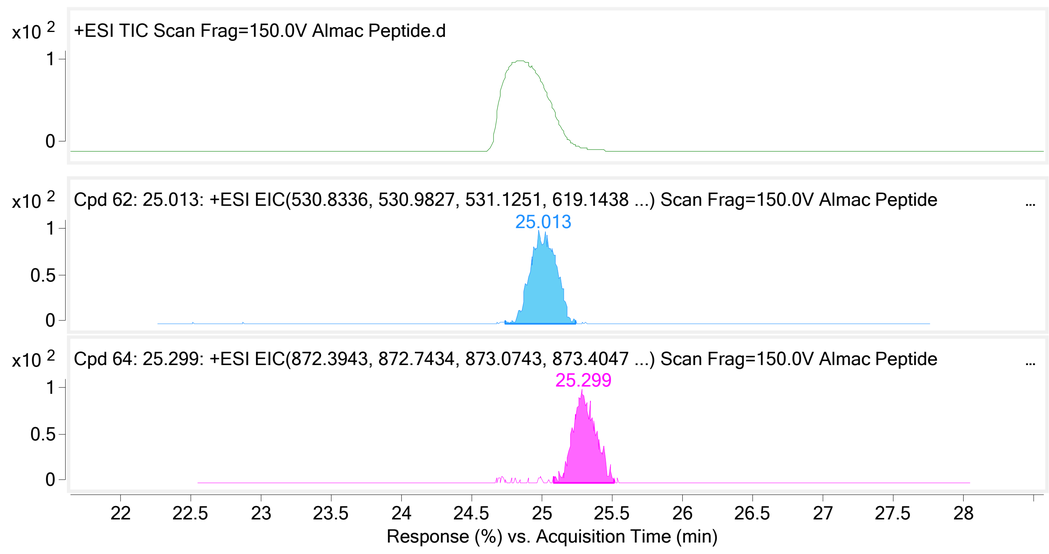
Due to the structural complexity of peptides, such as Almac Peptide (approximately 50% of its sequence shown above), there are many forms of impurities that may arise during or following manufacture. Amongst these impurities include truncations or side reactions formed during the formulation process or degradants formed from storage of the drug (such as oxidation or deamidation). Unlike with traditional small molecule drugs, impurities such as deamidation or isomerism result in compounds that are effectively undiscernible by traditional detection methods such as LC-UV as they are spectrally similar and unlikely to be resolved. Therefore, new technologies must be assessed and equipped to detect and quantify these impurities. At Almac, the use of Molecular Feature Extraction (MFE) has been extensively used to interrogate peptide peak purity.

Figure 4 - Peak Purity by MFE



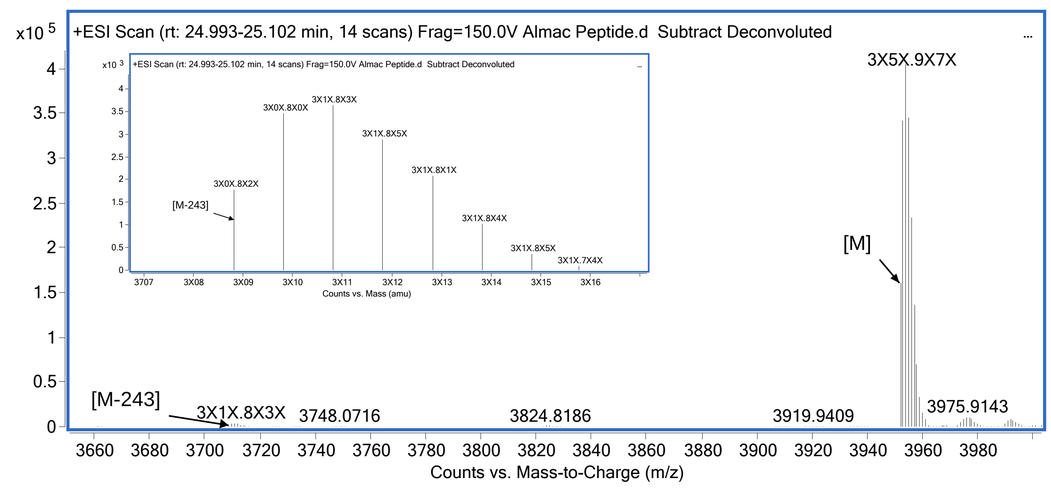
MFE is an algorithm, developed by Agilent Technologies, which relies on 'compound' finding within a peak of interest. In the region of interest, the algorithm will extract individual molecular features or "compounds", regardless of chromatographic complexity or compound resolution. MFE assesses all acquired spectra and identifies compounds that are related to the peak in question, based on charge state envelope, isotopic distribution, adducts and clustering. An operator can then study the output to determine if these signals are related to the peak of interest, by assessing the peak profile and, in particular, the apex compared to the main peak. Compounds can exhibit "saddling", as seen with Cpd 58 above – where competitive ionisation in the MS source results in suppression of signal. This phenomenon is discernible from truly co-eluting impurities by projecting the peak profile with saddling inverted and comparing to the main peak.

Figure 5 – Co-Eluting Impurity Profiles



In contrast to adducts, clusters or in-source fragmentation products like Cpds 10, 19 and 58, co-eluting impurities do not share peak profile and apex with the main peak. Cpds 62 and 64 are examples of genuine co-eluting impurities found in Almac Peptide, found at respective levels of 0.18% and 0.04%. Without the aid of MFE, an analyst would not be capable of making raw spectra distinctions due to the vast quantities of mass data they would need to interrogate. Furthermore, the computational algorithms can detect low level impurities that would effectively be undetectable to an analyst.

Figure 6 – Co-Eluting Impurity Spectrum



Deconvoluted spectra for co-eluting impurities above established both impurities as resulting from peptide truncation. At Almac, peak purity is used in subsequent forced degradation studies as an impurity monitoring technique, allowing route cause determination of co-eluting impurities as arising from either synthetic processes or peptide degradation.

Conclusion

Peak purity by MFE using accurate mass LC-MS offers superior impurity profiling when compared to historic LC-UV techniques. Considering the advancing complexity of pharmaceutical products and the transition away from small molecule development, the reliance on more multifaceted data-mining software is only expected to increase accordingly. Without the use of MFE for this Almac Peptide, it would not have been possible to identify the extremely low level co-eluting impurities observed. With full GMP compliance, Almac has integrated a more scrutinous and robust method of ensuring optimal purity of both internal and external client pharmaceutical products.