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Almac Voice

Peptide UHPLC development

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Ultra high-performance liquid chromatography (UHPLC) is a commonly used analytical technique to separate, identify, and quantify each component in a mixture. Standard UHPLC testing uses pumps to transfer a pressurised liquid, which contains the sample mixture, through a column filled with a solid absorbent material.

UHPLC remains the workhorse analytical method of choice for assay, purity and related substances for release of API (Active Pharmaceutical Ingredients) and drug product. This is a technique that works well across a range of various products, and is equally applicable for small molecules, peptides and biologics. However, UHPLC peptide method development is a vastly different prospect than small molecule.

Coming from a small molecule background, the first step into peptide UHPLC method development is to unlearn everything you think you know! Small molecule method development typically covers a range of known impurities, and predictable degradation products. With peptides the added complexity of secondary and tertiary structures results in a significant impact on the analytics, which means that in terms of method development all bets are off!

There are so many unknowns in the world of peptide analytics that it can be difficult to know where to start. Although it is difficult to predict the type of method that will work, it does not mean that there is not a logical path forward.

Almac's solution:
Almac take a systematic approach using method screening to extract as much data as possible with a small set of experiments. Previously, method development required significant effort to test a range of mobile phases and columns to try and obtain a good chromatograph. Whilst no two peptide methods are alike, they typically encounter the same challenges:

- Resolution
- Purity of the main peak caused by co-eluting impurities
- Methods with long run times, and limited solution stability

We have undertaken extensive work with column managers to design a screening approach; 4 columns have been selected which provide reliable results with peptide test articles. This, coupled with a range of pH, solvents and concentrations of TFA provide a good overview of the state of the analytics of a specific peptide, and generate around 40 chromatographs.

Prior to this approach it may have taken up to 3 months to generate this data, but through close collaboration with equipment manufacturers, Almac has created a dedicated work package to provide a 3-day peptide pre-screen. The aim of this screen is to provide a quick, high-level indication of the best parameters to select for a full method development program. Whilst this fast approach will not provide a final method suitable for validation, it will inform Almac and the client of the complexity in developing a robust analytical method.

A further advantage of this system is that it interrogates the peptide from several different angles to provide good coverage on the number of impurities. Given the range of conditions screened it can easily identify which impurities are missing from which system. The newest iteration of this system incorporates a QDA detector (a single-quadrupole mass detector with an electrospray ionization source), which gives the mass of the impurities and allows tracking across multiple chromatographs.

This system generates up to 44 chromatographs which are then reviewed by the peptide analytical SMEs at Almac. Post-review, a development plan is created and shared with the client detailing a comprehensive list of recommendations to improve the method.

Almac are currently installing a software add-on to this system which will allow an automated review of these chromatographs saving further

time. This Fusion software utilises QbD to model chromatography to enhance method development. The predictions made by this software will be reviewed by our SMEs and used to inform the extend of further development required.

Key considerations:

- Peak purity is a key issue when it comes to peptide UHPLC methods, but fortunately at Almac we host a mass spectroscopy suite which encompasses a range of different instruments including a high-resolution LC-MS. The interrogation of peak purity for peptide methods is a key step in development, and Almac's state-of-the-art facilities and technical expertise means we are well-positioned to handle this.
- Material availability is often an issue with peptides, and this system typically uses around 10mg of peptide to demonstrate how problematic the development may be. Prior to analysis, stress testing of the sample is performed (using conditions advised by our manufacturing colleagues) to give an initial indication if the method is likely to be stability indicating.
  This technique is incredibly powerful and provides a huge volume of data using minimal time and effort. Whilst this does not replace the need for a full program of method development, it can give an early indication of potential issues with methods, and how problematic they may be to overcome. It can also provide a window of opportunity for the client to manufacture additional material if required.
- Budgets for analytical method development can be very open-ended. The longer spent in development, the more improvements can be made to a method. However, the Almac QbD systematic approach provides our clients with a significant advantage on method development and is the ideal starting point for a multitude of complex products.

Almac has a group of ~20 specialist peptide analytical chemists who work on a multitude of different projects. This group has ~10 years' experience and has worked on >50 different peptides in this time. The deep understanding of this group has led to the development and validation of particularly complex analytical methods for a range of peptides.

The familiarity built up over the years has been instrumental into the design and implementation of our analytical screening system, making use of their acquired knowledge to provide a robust system to accelerate method development.

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