A NEW LC-MS APPROACH FOR SYNTHETIC PEPTIDE CHARACTERIZATION AND IMPURITY PROFILING

INTRODUCTION
- Peptide therapeutics are an emerging group of pharmacologically applicable to a wide range of medical challenges.
- These peptide drugs exhibit relative low toxicity and high biological activity compared to most conventional drugs.
- Chemical synthesis is one of the most common methods of production for synthetic peptides.
- While the low toxicity of these drug products makes them more appealing as therapeutics, the impurities introduced during chemical synthesis of these peptides demand thorough characterization procedures to maintain efficacy and safety of the drug.
- The conventional synthetic peptide impurity profiling methods are mostly LC-based assays relying on the resolution of impurities to baseline for optical detection of low abundance impurities in a challenging. Adding HRMS to the analysis resolves this by improving the detection and identification of low level impurities based on the accurate mass and MS/MS information.

In this work, we have developed a single LC-HRMS based analytical workflow for characterization and impurity profiling monitoring of synthetic peptide drugs.

METHODS

Sample preparation
The synthetic peptide Eledoisin (NH2-CNLSTCVLGKLSQELHKLQTYPRT-CONH2) was purchased from Class Bio System. It was dissolved in water to a final concentration of 0.2 µg/µL for the analysis.

LC condition
- Column: ACQUITY UPLC Peptide CSH C18, 1.7 µm, 2.1 mm x 150 mm ( Waters Corporation, Milford, MA 01757). The column was maintained at 65 °C.
- Mobile phase A: 0.1% formic acid in water, Mobile phase B: 0.1% formic acid in acetonitrile. The gradient was set as follows: 0-1 min, 5% B; 1-34 min, 5-34% B; 34-35 min, 34% B; 35-37 min, 34-5% B; 37-40 min, 5% B.
- Flow rate: 0.4 mL/min.

MS instrumentation:
Vion IMS QTof mass spectrometer - MS condition: Mass range 500-2000 Da, resolution 10,000, Transmission 250 u, Acquisition mode: dynamic range 5, Cones gas: 50 L/hr, Desolvation gas: 1000 L/hr, Desolvation Temp 300 °C.

RESULTS

Validation of API/impurity using MS/MS
The validation of the API/impurity using MS/MS involves the determination of the impurities and their characteristic fragmentation spectra.

Figure 1: The UNIFI review panel displays API and impurities identified. The MS responses used to determine the matched spectra based on the relative% response compared to the MS response of the API.

Peptide Mapping Workflow: Synthetic Peptide/Impurity Characterization
Method editor impurity identification
LC-UN-HRMS for synthetic peptide API/impurity identification

Validation of API and impurity profiling using UNIFI screening workflow
The validation of API and impurity profiling using UNIFI screening workflow involves the characterization of API and impurities.

Figure 2: The workflow for characterizing API and impurities is demonstrated in a practical example. The UNIFI informatics platform can identify, label and verify the API and impurities.

CONCLUSION
- The instrument-informatics platform is a compliant-ready system that can be validated and implemented in regulated laboratory environments.
- The automated peptide mapping workflow identifies impurities based on accurate mass and validates the assignments using MS/MS data.
- The workflow can be used to characterize API and impurity sequences that are linear or cyclic.
- The scientific library can be used to a built custom impurity library for any selected peptide sequence.
- The screening workflow is utilized in robust high throughput profiling therapeutic peptide impurities.

References:
INTRODUCTION

• Peptide therapeutics are an emerging group of pharmaceuticals applicable to a wide range of medical challenges.

• These peptide drugs exhibit relatively low toxicity and high biological activity compared to most conventional drugs.1,2

• Chemical synthesis is one of the most common method of production for synthetic peptides

• While the low toxicity of these drug products makes them more appealing as therapeutics, the impurities introduced during chemical synthesis of these peptides demand thorough characterization procedures to maintain efficacy and safety of the drug.

• The conventional synthetic peptide impurity profiling methods are mostly LC-optical based assays relying on chromatographic separation of impurities.

• Even at optimal chromatographic performance, obtaining baseline resolved peaks for optical detection of low abundance impurities is a challenge. Adding HRMS to the analysis resolves this by improving the detection and identification of low level impurities based on the accurate mass and MS/MS information.

• LC-MS based monitoring of impurity profile provides both accurate mass and relative abundance information that benefits process development and quality control.

• In this study, we have developed LC-HRMS-based analytical workflows for characterization and impurity profile monitoring of synthetic peptide drugs.