TRACKING AND REPORTING SYNTHETIC PEPTIDE IMPURITIES WITH A COST-EFFECTIVE SINGLE QUADRUPOLE MASS DETECTOR FOR IMPROVED CONFIDENCE IN ANALYSIS

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INTRODUCTION

There has recently been a renewed interest in peptide therapeutics, due in part to overcoming some of the early challenges imparted by their physicochemical properties. New regulatory guidelines effective in 2020 could potentially offer manufacturers using synthetic strategies (versus recombinant) a quicker and more cost-effective entry to market.[1] When following a synthetic approach, impurities that result from the manufacturing process or from degradation during manufacturing or storage are typically assayed by HPLC,[2] which can be susceptible to many user-induced pitfalls. In this study, eledoisin is used as a model analyte to demonstrate automated processing and reporting within a compliant-ready software package, which reduces user error. By incorporating an orthogonal cost-effective mass detector into the analysis, peaks that are out of specification can be readily interrogated, and product purity can be easily assessed for further method optimization. This strategy demonstrates the ability to improve confidence in results by combining optical detection and orthogonal mass detection into a single workflow while maintaining compliance.

Figure 1. System configuration. ACQUITY H-Class Bio System configured with a Tunable UV (TUV) Detector and an ACQUITY QDa Detector.

References

1. HHS, FDA, Biosimilars: Questions and Answers Regarding Implementation of the Biologics Price Competition and Innovation Act of 2009, *Guidance for Industry*, 2015.

2. Control Strategies for Synthetic Therapeutic Peptide APIs, *Pharmaceutical Technology*, 2014.

For more information: Waters Application Notes, 720005967en and 720005968en, 2017.

METHODS

LC conditions: Wavelength: 215 nm; Column: ACQUITY UPLC Peptide CSH C18 130 Å 1.7 µm, 2.1 mm x 100 mm; Column temperature: 60 °C; Sample temperature: 10 °C; Injection volume: 5 uL, 0.4 mg/mL; MPA: H_20 with 0.1% (v/v) FA; MPB: ACN with 0.1% (v/v) FA; Original Gradient: 15—45% MPB, 20 minutes; Optimized Gradient: 16—24% MPB, 30 minutes.

MS conditions: Ionization mode: ES+, centroid; Mass range: 350 – 1250 m/z; Cone voltage: 10 V; Capillary voltage: 1.5 kV; Probe temperature: 500 °C.

Data Management: Empower 3 CDS, SR2.

CONCLUSION

- **Automated data processing and reporting**
- **Readily investigate out of spec results**
- **Decrease method development time**
- **Increased confidence compared to optical-based assays**

RESULTS AND DISCUSSION

1. Screen multiple columns and conditions

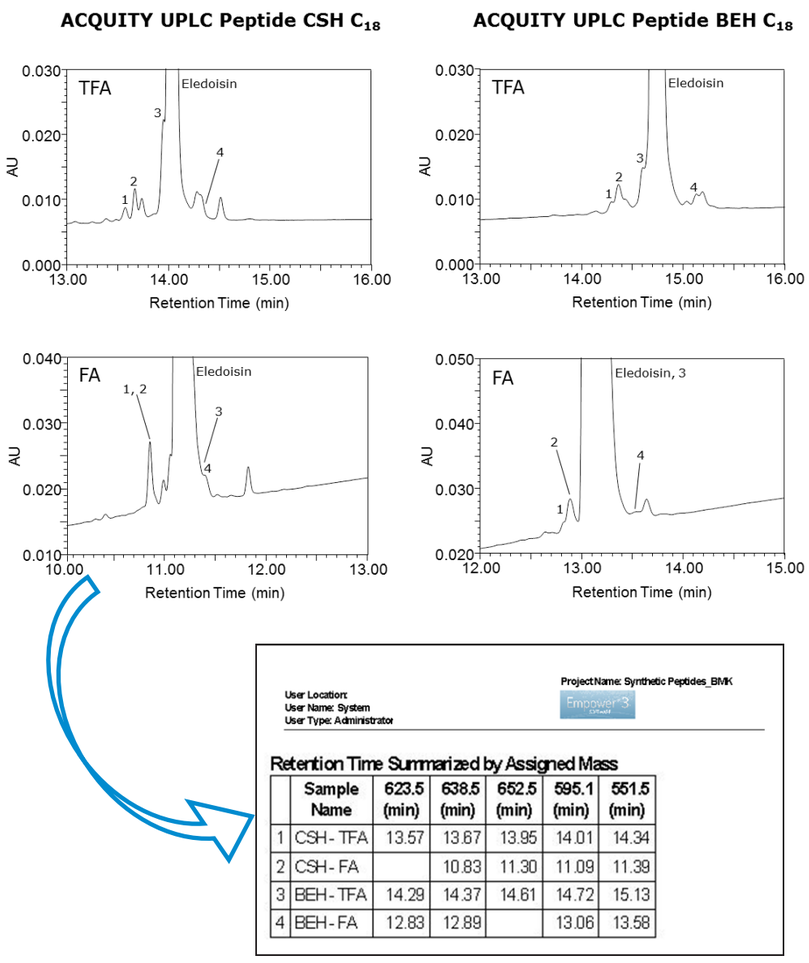
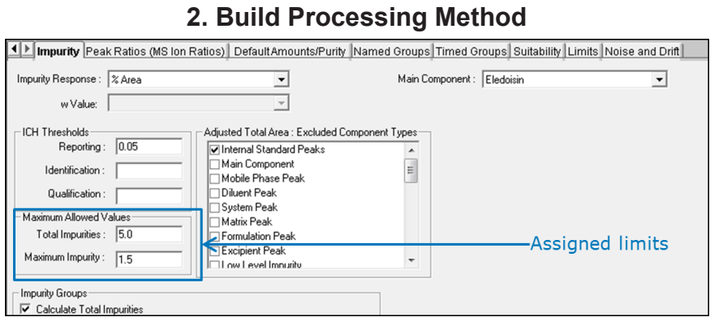


Figure 2: Screening columns and mobile phase using a 20 minute gradient from 15% - 45% acetonitrile containing 0.1% (v/v) TFA or 0.1% (v/v) FA as indicated. Because of differences in column selectivity, the resolution and elution order varies between each set of conditions. The Empower 3 MS Peak Tracking feature organizes data based on the retention time for a specific peak across injections, which avoids the additional step of having to run independent standards to determine elution order, resulting in more efficient method development.

2. Build Processing Method



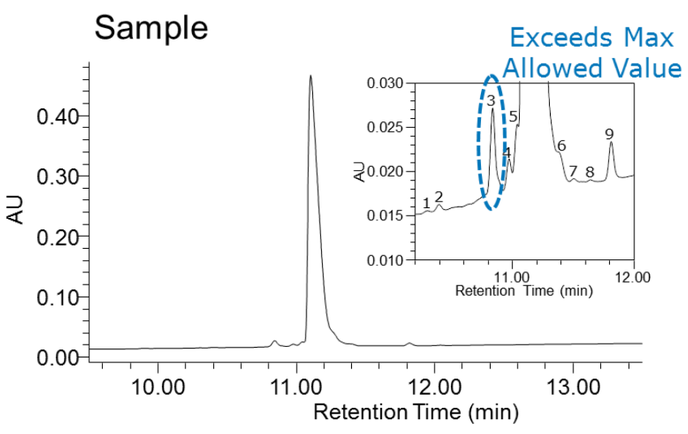
Acceptance Criteria: Assigned Limits

Any individual impurity: NMT 1.5%

Total Impurities: NMT 5.0%

Figure 3. Processing method parameters under Impurity tab. Impurity response is determined as peak area percent. ICH Thresholds may be entered, in this case, a reporting limit of 0.05 is used. From the acceptance criteria, any individual impurity is to be NMT 1.5%, and the total impurities must be NMT 5.0% These values are entered into the Maximum Allowed Values fields. The user also has the option of excluding component types from the total area if needed.

3. Acquire Data



4. Automate Reporting

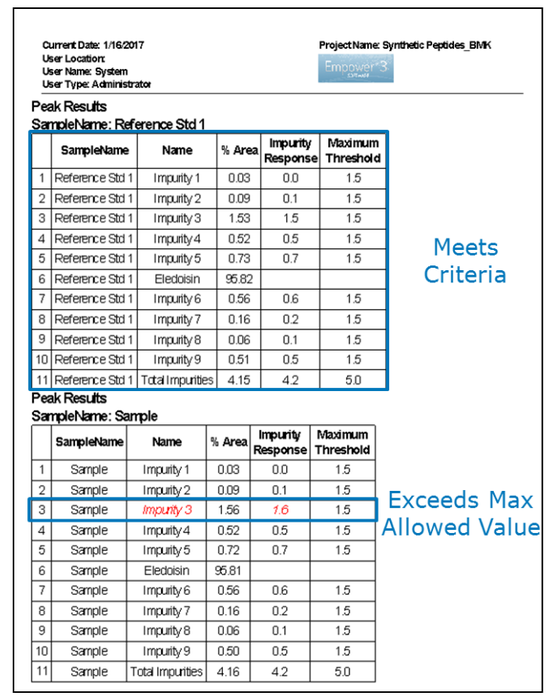
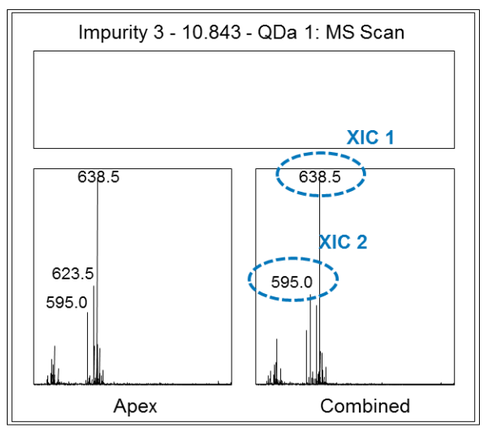
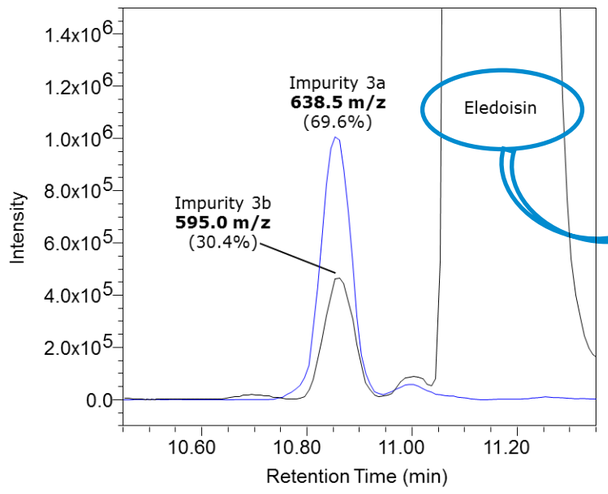


Figure 4: Empower reporting. Peak results can be summarized to contain data of the user's choice. Here, % Area, Impurity Response, and Maximum Threshold are shown. Results are compared for a reference standard solution of eledoisin (not shown) and a sample solution of eledoisin. The reference standard solution meets both the individual impurity requirement (NMT 1.5%) and the total impurities requirement (NMT 5.0%). The sample solution, however, contains a peak that is outside of the maximum allowed value, which appears in red text.

Added mass detection provides complementary data for improved confidence in results

Investigate OOS Results





Assess Product Purity

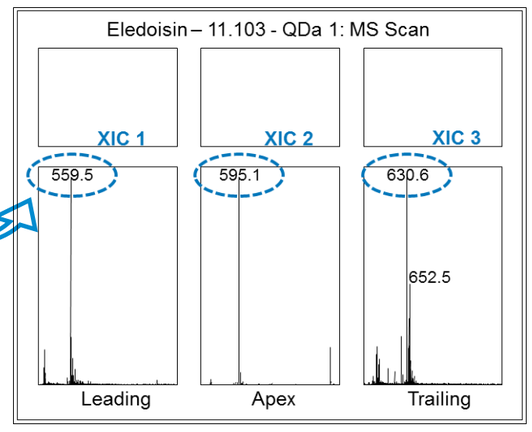


Figure 5. Determining the composition of the out of spec Peak 3 impurity. 5A) Screen capture from Empower Software of the Mass Analysis Window. Mass data is displayed for both the highest point (Apex) and the average (Combined) spectrum of Impurity 3. XICs of the two most dominant m/z values from the combined scan were used to determine the composition of Impurity 3, as a combined scan is more representative of the overall peak composition. 5B) XICs of 638.5 m/z (Impurity 3a) and 595.0 m/z (Impurity 3b). From integration of the XICs, Impurity 3 is composed of 69.6% 3a and 30.4% 3b.

Figure 6. Product purity assessment. Mass data is displayed for the leading edge, apex, and trailing edge of eledoisin. The m/z values from the leading edge and trailing edge spectra are not charge states of eledoisin, but instead are impurities that are not resolved from the main peak.

Decrease Method Development Time

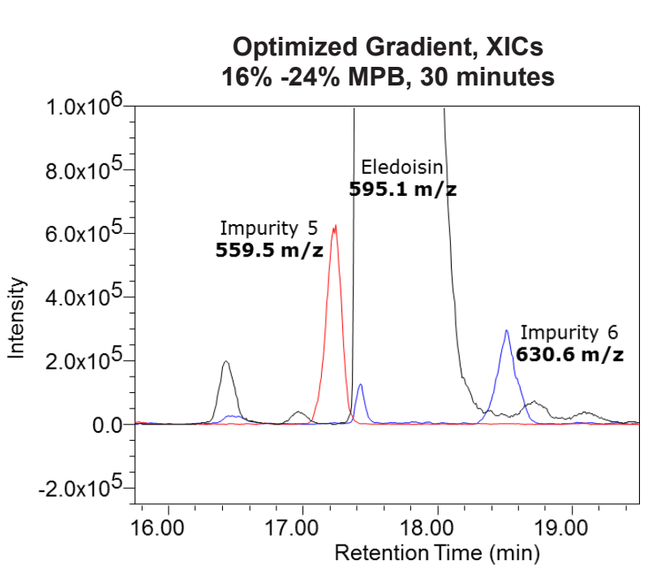


Figure 7. Optimized gradient. A focused gradient was used to improve the separation of the main peak from impurities (based on mass data from Figure 6) for determining product purity more reliably.

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