USING COMPLIANT-READY SOFTWARE FOR SYNTHETIC PEPTIDE IMPURITY TRACKING AND REPORTING WITH ADDED MASS DETECTION FOR IMPROVED CONFIDENCE IN RESULTS

Brooke M. Koshel, Ximo Zhang, Robert Birdsall, Ying Qing Yu, Joe Fredette, Scott Berger, and Asish Chakraborty
Waters Corporation. Milford MA

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

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. Because peptides are not readily classified as small molecules or biologics, the regulations surrounding their development is not straightforward. 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]

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.

Although impurity reporting traditionally relies on optical detection, the FDA and ICH encourage new technologies to be considered when these technologies offer greater understanding and confidence of product quality.[3,4] By incorporating an orthogonal cost-effective mass detection strategy, peaks that are out of specification can be readily interrogated, and product purity can be readily 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.

3. FDA, Analytical Procedures and Methods Validation for Drugs and Biologics, 2015.

4. ICH, ICH Q6B Specifications, 1999.

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

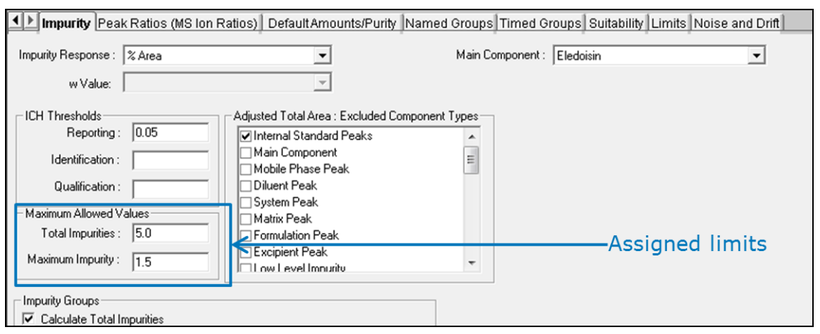
Data Management
Empower 3 CDS, SR2

Analyte (eledoisin) sequence
pGlu-Pro-Ser-Lys-Asp-Ala-Phe-Ile-Gly-Leu-Met-NH_2

RESULTS

Impurity tracking and reporting can be automated in LC-UV workflow using customizable acceptance criteria

1. Build Processing Method



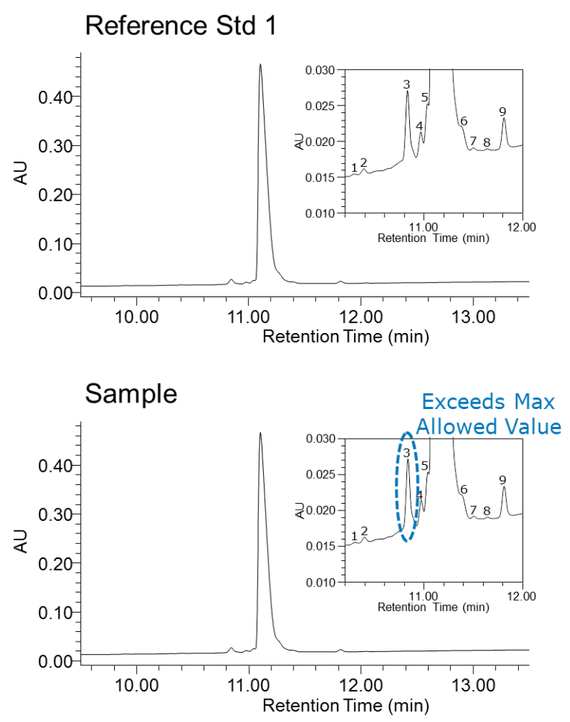
Acceptance Criteria: Assigned Limits

Any individual impurity: NMT 1.5%

Total Impurities: NMT 5.0%

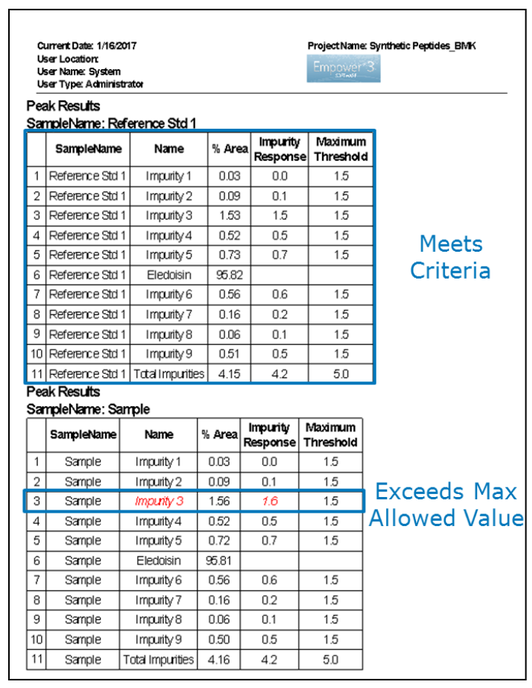
Figure 2. Processing method parameters under Impurity tab. Impurity response is determined as peak area percent. ICH Thresholds may be entered, in this case, a reporting limit of 0.05 is used. From the acceptance criteria, any individual impurity is to be NMT 1.5%, and the total impurities must be NMT 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.

2. Acquire Data



3. Automate Reporting

Figure 3: 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 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.



Current Date: 1/16/2017
User Location:
User Name: System
User Type: Administrator
Project Name: Synthetic Peptides_BMK

Peak Results
SampleName: Reference Std 1

	SampleName	Name	% Area	Impurity Response	Maximum Threshold	
1	Reference Std 1	Impurity 1	0.03	0.0	1.5	
2	Reference Std 1	Impurity 2	0.09	0.1	1.5	
3	Reference Std 1	Impurity 3	1.53	1.5	1.5	
4	Reference Std 1	Impurity 4	0.52	0.5	1.5	
5	Reference Std 1	Impurity 5	0.73	0.7	1.5	
6	Reference Std 1	Eledoisin	96.82			
7	Reference Std 1	Impurity 6	0.56	0.6	1.5	
8	Reference Std 1	Impurity 7	0.16	0.2	1.5	
9	Reference Std 1	Impurity 8	0.06	0.1	1.5	
10	Reference Std 1	Impurity 9	0.51	0.5	1.5	
11	Reference Std 1	Total Impurities	4.15	4.2	5.0	Meets Criteria

Peak Results
SampleName: Sample

	SampleName	Name	% Area	Impurity Response	Maximum Threshold	
1	Sample	Impurity 1	0.03	0.0	1.5	
2	Sample	Impurity 2	0.09	0.1	1.5	
3	Sample	Impurity 3	1.56	1.6	1.5	Exceeds Max Allowed Value
4	Sample	Impurity 4	0.52	0.5	1.5	
5	Sample	Impurity 5	0.72	0.7	1.5	
6	Sample	Eledoisin	95.81			
7	Sample	Impurity 6	0.56	0.6	1.5	
8	Sample	Impurity 7	0.16	0.2	1.5	
9	Sample	Impurity 8	0.06	0.1	1.5	
10	Sample	Impurity 9	0.50	0.5	1.5	
11	Sample	Total Impurities	4.16	4.2	5.0	

Added mass detection provides complementary data for improved confidence in results

Investigate OOS Results

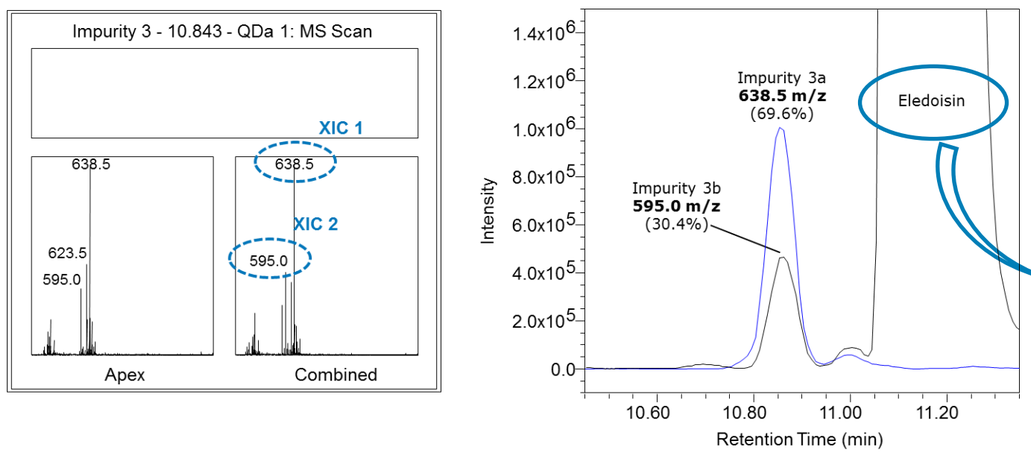


Figure 4. Determining the composition of the out of spec Peak 3 impurity. 4A) Screen capture from Empower Software of the Mass Analysis Window. Mass data is displayed for both the highest point (Apex) and the average (Combined) spectrum of Impurity 3. XICs of the two most dominant m/z values from the combined scan were used to determine the composition of Impurity 3, as a combined scan is more representative of the overall peak composition. 4B) 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.

Assess Product Purity

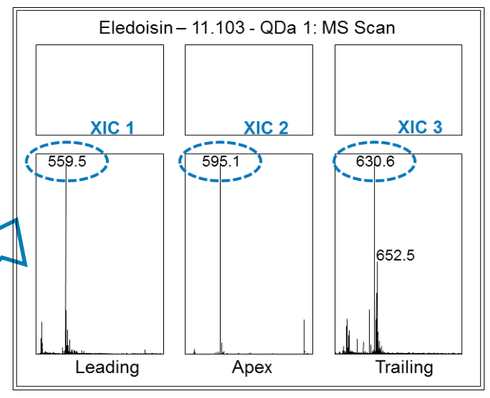
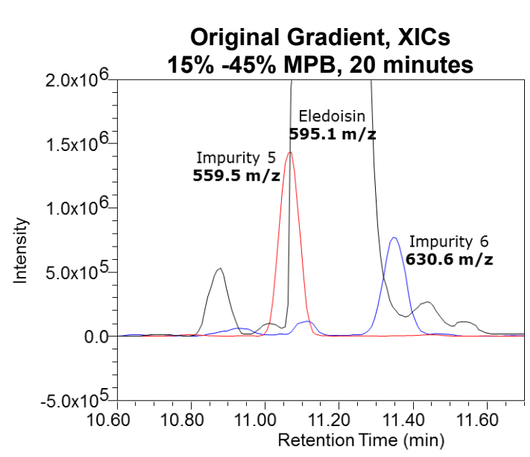


Figure 5 (Above). Screen capture from Empower Software Mass Analysis Window. Mass data is displayed for the leading edge, apex, and trailing edge of eledoisin. The m/z values from the leading edge and trailing edge spectra are not charge states of eledoisin, but instead are impurities that are not resolved from the main peak.

Figure 6 (Right). XICs of 559.5 m/z (Impurity 5), 595.1 m/z (eledoisin), and 630.6 m/z (Impurity 6). 6A) Original gradient. From the chromatogram, Impurity 5 and Impurity 6 are not well resolved from the main peak, which can create inaccuracy in reporting the overall peak purity. 6B) Optimized gradient. A focused gradient further separates closely eluting impurities from the main peak, which can aid in determining product purity more reliably.

Decrease Method Development Time



CONCLUSION

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

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS