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Note d'application

Using Empower 3 Software for Monitoring Synthetic Peptide Impurities with an ACQUITY QDa Detector for Improved Confidence in Analysis

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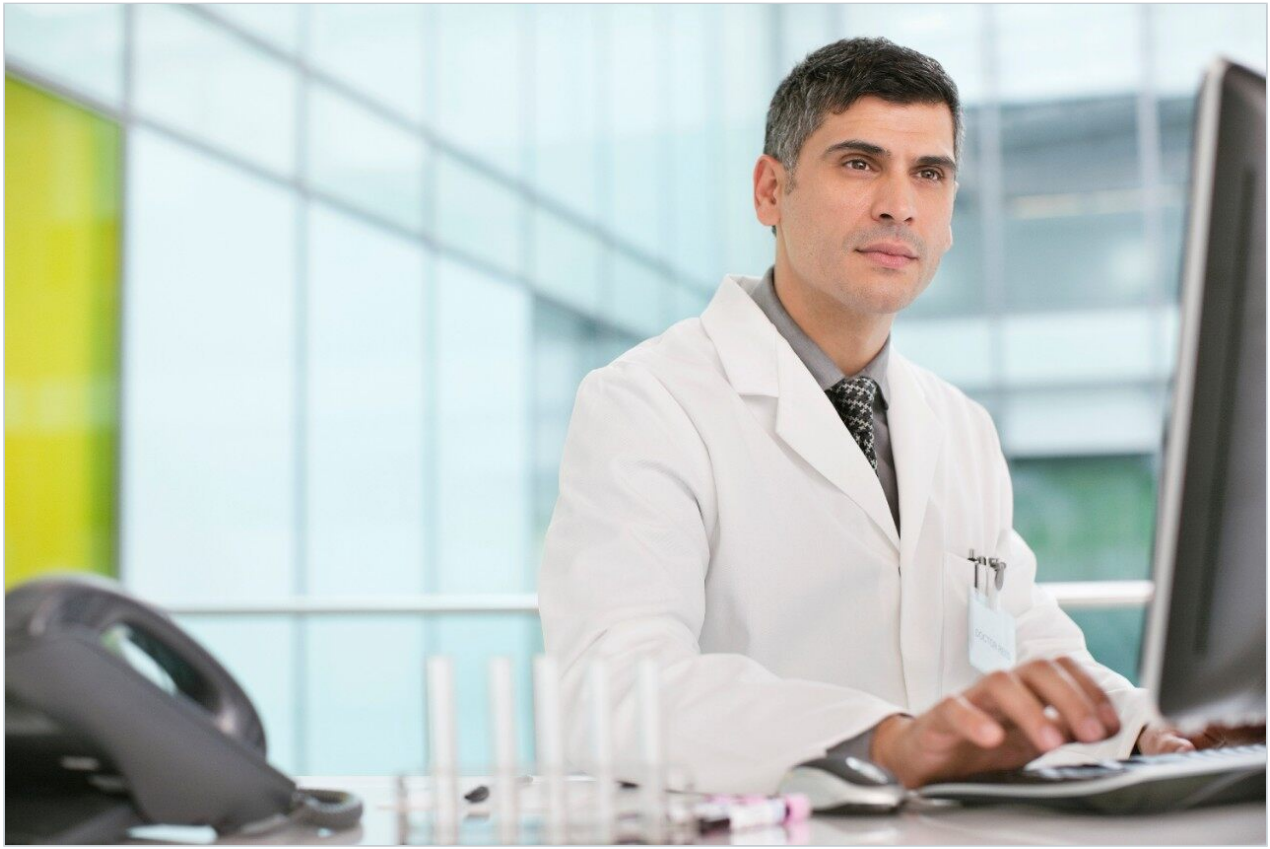

Abstract

In this study, eledoisin, a vasodilator, is used as a clinically relevant sample to demonstrate how the ACQUITY QDa Detector with Empower 3 Software can be used for synthetic peptide impurity monitoring.

Eledoisin impurities can be documented via traditional optical-based methods, but by incorporating the ACQUITY QDa Detector, co-elutions can be realized. This strategy demonstrates the ability to improve confidence in a manufacturing environment by combining optical detection and orthogonal mass detection into a single workflow.

Benefits

- Incorporating mass detection of synthetic peptide impurities using compliant-ready Empower 3 Software
- Integrated peak purity functionality for readily assessing product quality

Introduction

Peptide-based therapeutics have recently seen a renewed interest due in part to overcoming some of the early challenges imparted by their physicochemical properties.[1,2] Therapeutic peptides can be produced through chemical synthesis, recombinant strategies, or through extraction from a natural source.[1] Although each route can offer advantages, an increasing number of these drugs are now being made synthetically.

When following a synthetic approach, the raw materials as well as the manufacturing process can both contribute to process-related impurities, where many of the modifications or deletions can be pre-determined,[3] and are generally considered to be safe at low levels.[4]

Most often, determining synthetic peptide purity is an LC-UV-based assay.[5,6] Recent work by Zeng and colleagues reported that their LC-HRMS method could identify additional impurities not reported through the USP HPLC-UV method for a currently marketed product.[7] The notion that manufacturers should continually strive to improve product knowledge with the interest of public health in mind has global implications. The International Council for Harmonisation (ICH), which brings together regulators and the pharmaceutical industry from Europe, Japan, and the United States, addresses this need with ICH Q10, a quality document that supports innovation and continual improvement throughout product lifecycle.[8] In the current study, we

show that by incorporating the ACQUITY QDa Detector, a cost effective mass detector, impurities can be identified without the need for expensive instrumentation. Because impurities that are not qualified generally have tighter acceptance criteria,[6] added mass detection can more readily confirm impurity identification than relying on relative retention time from an optical-based assay.

In this study, eledoisin, a vasodilator, is used as a clinically relevant sample to demonstrate how the ACQUITY QDa Detector with Empower 3 Software can be used for synthetic peptide impurity monitoring. Eledoisin impurities can be documented via traditional optical-based methods, but by incorporating the ACQUITY QDa Detector, co-elutions can be realized. This strategy demonstrates the ability to improve confidence in a manufacturing environment by combining optical detection and orthogonal mass detection into a single workflow.

Experimental

Sample description

The synthetic peptide eledoisin (pE-PSKDAFIGLM-amide) was purchased from New England Peptide Inc. (Gardner, MA) at ≥95% purity by HPLC percent area. A stock solution of 2 mg/mL eledoisin in water was further diluted to a working concentration of 0.4 mg/mL.

LC conditions

LC system:	ACQUITY UPLC H-Class Bio System
Detector:	ACQUITY UPLC Tunable Ultra-Violet (TUV) Detector
Wavelength:	215 nm
Vials:	LCMS Certified Clear Glass 12 x 32 mm Screw Neck Total Recovery Vial (p/n 600000750cv)
Column:	ACQUITY UPLC Peptide CSH C_{18} 130 Å 1.7 µm, 2.1 mm x 100 mm (p/n 186006937)

Column temp.:	60 °C
Sample temp.:	10 °C
Injection vol.:	5 µL
Mobile Phase A:	H_2O with 0.1% (v/v) FA
Mobile Phase B:	Acetonitrile with 0.1% (v/v) FA
Gradient:	Original: 15–45% B over 20 minutes Optimized: 16–24% B over 30 minutes

MS conditions

MS system:	ACQUITY QDa Detector (Performance Model)
Ionization mode:	ES+, centroid
Mass range:	350–1250 Da
Capillary voltage:	1.5 kV
Cone voltage:	10 V
Probe temp:	500 °C

Data management

Empower 3 CDS, SR2

Results and Discussion

Monitoring individual impurities to meet quality standards

Our previous work has shown how Empower 3 Software can be used for monitoring and reporting eledoisin impurities in accordance with ICH and USP guidelines with functionalities that are integrated into the software.[9] In brief, a 20 minute gradient from 15–45% acetonitrile in 0.1% (v/v) formic acid using CSH C_{18} column chemistry was used to separate eledoisin from its process-related impurities (Figure 1A). To investigate the added benefit of mass detection, an ACQUITY QDa Detector was incorporated as an inline orthogonal detection method. The profile of the total ion chromatogram (TIC) (Figure 1B) shows a strong correlation with the optical data, suggesting that the ACQUITY QDa Detector is an appropriate detector for analysis.

The following criteria were used to determine if an eledoisin sample met acceptance criteria:

Any individual impurity: Not more than (NMT) 1.5%Total impurities: NMT 5.0%

From Figure 1A, Impurity 3 was determined to be 1.6%, and thus is outside of specification. With added mass detection, we can further interrogate this impurity. Figure 2A shows a screen capture of the Mass Analysis Window within the Empower Software. The MS spectrum of Impurity 3 can be displayed for both the peak apex and the combined or average scan. From the combined spectrum, 638.5 and 595.0 are the most dominant *m/z* values. Having previous knowledge of what impurities are present in the eledoisin sample, these *m/z* values are known to represent impurities. Using these *m/z* values, extracted ion chromatograms (XICs) can be used to determine relative area percent of each of the two individual impurities that contribute to Impurity 3.

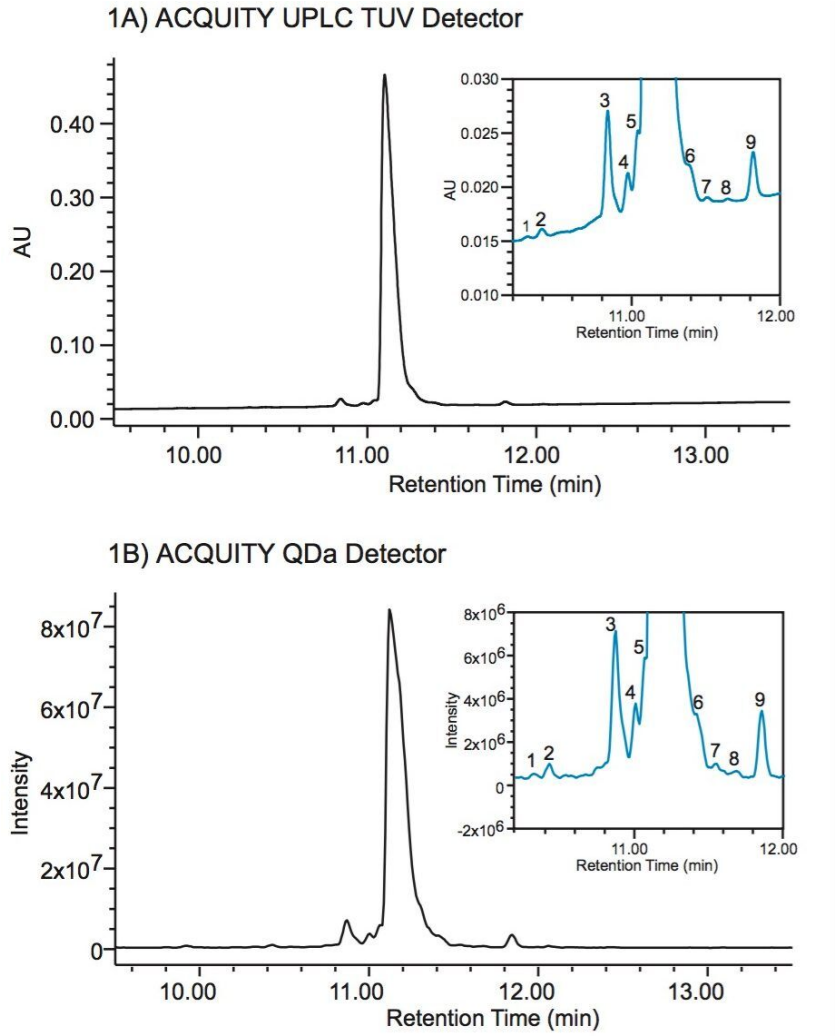

Figure 1. Synthetic peptide separation of eledoisin from its process-related impurities.

1A) Optical detection of eledoisin with inset showing impurities.

1B) Corresponding QDa data. Inset shows strong visual correlation to optical data.

From Figure 2B, Peak 3 is composed of 69.6% Impurity 3a and 30.4% Impurity 3b. With this in mind, if we now consider Impurity 3 as being composed of Impurity 3a and Impurity 3b, neither of these individual impurities exceeds the acceptance criteria of NMT 1.5% for any individual impurity. Being able to readily identify qualified peaks that are not critical attributes can avoid any further investigation, which can ultimately save both time and money by allowing operations to run more efficiently.

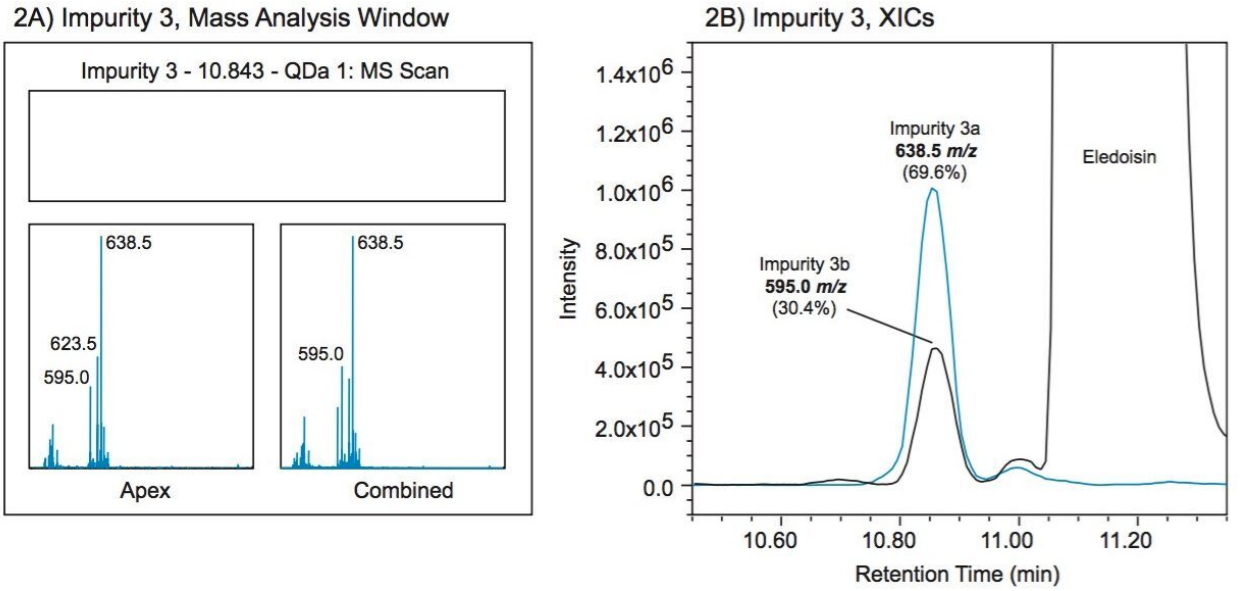

Figure 2. Determining the composition of the Peak 3 impurity. 2A) Screen capture from Empower Software of the Mass Analysis Window. Mass data is displayed for both the highest point (Apex) and the average (Combined) spectrum of Impurity 3. XICs of the two most dominant m/z values from the combined scan were used to determine the composition of Impurity 3, as a combined scan is more representative of the overall peak composition. (Note that the window above the MS spectra is blank because the TUV detector was used.) 2B) 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. By treating each of the impurities separately, the sample no longer contains peaks that exceed the criteria for the maximum allowable value for any individual impurity.

Assessing individual peak purity for determining product quality

It is not uncommon for synthetic peptide impurities to co-elute with the main peak of interest.[10] These co-eluting impurities may or may not be detrimental to patient safety, but their presence can lead to misinterpretation of overall product quality and purity. In addition to viewing mass data for the peak apex or combined peak, Purity View can be used to display mass data for the leading edge, apex, and trailing edge of the peak of interest. From the Apex and Combined scans of eledoisin (Figure 3A), spectra appear clean and free of *m/z* values of unrelated species. This indicates that any impurities present are likely present at relatively low levels.

However, from the Purity Spectrum View (Figure 3B), spectra from the leading edge (559.5 *m/z*) and trailing edge (630.5 *m/z*) of the main peak show *m/z* values that do not correspond to eledoisin (595.1 *m/z*). This indicates that there are closely eluting peaks present that are not well resolved from the main peak of eledoisin, which is also evidenced in Figure 1.

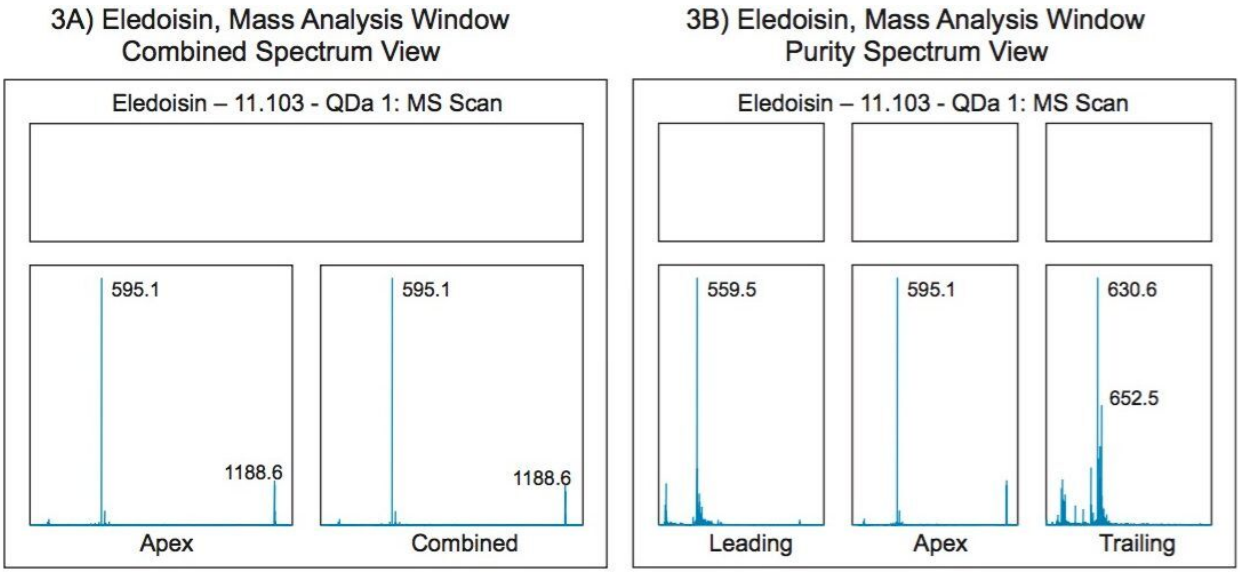

Figure 3. Screen captures from Empower Software Mass Analysis Window. 3A) Mass data is displayed for both the highest point (Apex) and the average (Combined) spectrum of eledoisin. The m/z values shown in the screen captures match the values calculated for the $[M+1H]^{+1}$ and $[M+2H]^{+2}$ charge states. 3B) 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. (Note that the windows above the MS spectra are blank because the TUV detector was used.)

Figure 4A shows XICs of 595.1 *m/z* (eledoisin), 559.5 *m/z* (Impurity 5), and 630.6 *m/z* (Impurity 6). From this chromatogram, these two impurities are not well resolved from the main peak. By re-evaluating the original gradient used, a more focused gradient can be created in an effort to tease out additional impurities from the main peak. To generate a new gradient, it was determined what percentage of acetonitrile elutes the main peak under the first set of conditions. A narrower gradient centering on this elution percentage can then be used. Figure 4B shows XICs of these same *m/z* values using the optimized gradient, 16–24% acetonitrile in 30 minutes. From the optimized separation, Impurity 5 and Impurity 6 no longer co-elute with the main peak. The added mass detection in this case helped to determine a more optimal separation, which can lead to a more accurate determination of product purity.

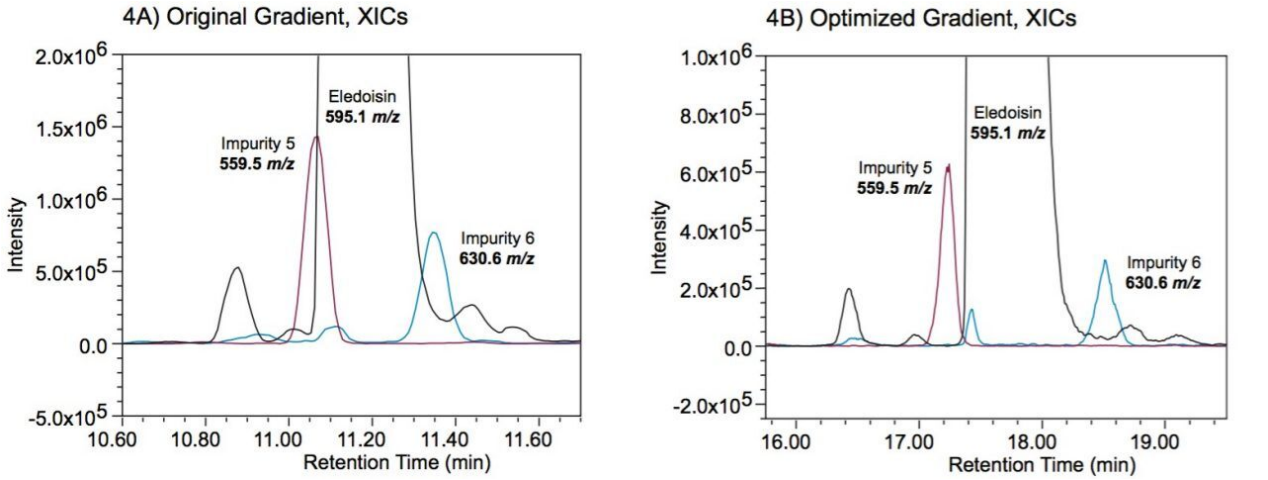

Figure 4. XICs of 559.5 m/z (Impurity 5), 595.1 m/z (eledoisin), and 630.6 m/z (Impurity 6). 4A) Original gradient, 15–45% B in 20 minutes. From the chromatogram, Impurity 5 and Impurity 6 are not well resolved from the main peak. This creates inaccuracy in reporting the overall peak purity. 4B) Optimized gradient, 16–24% B in 30 minutes. The focused gradient further separates closely eluting impurities from the main peak, which can aid in determining product purity more reliably.

Conclusion

This work demonstrates how mass detection can be readily incorporated into an existing optical-based workflow for analysis of synthetic peptide impurities. Our previous work using a traditional LC-UV method was shown to benefit from added mass detection in accordance with ICH Q10, which identifies the need to enhance product quality through innovation and continual improvements in development and manufacturing activities. The ACQUITY QDa Detector was shown to identify the presence of co-eluting impurities and determine product purity for further method optimization. Overall, the added mass detection provides an efficient and cost-effective strategy for improving confidence in analysis.

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