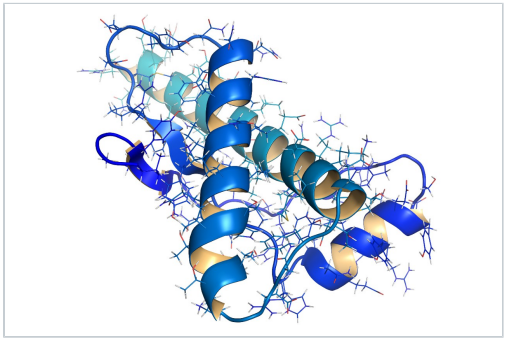
Waters™

Application Note

High Sensitivity Quantitative Analysis of a Therapeutic Peptide in Plasma using UPLC and Xevo TQ-S

Robert S. Plumb, Gareth Booth, Paul D. Rainville

Waters Corporation



Abstract

In this application note, we describe the use of the ACQUITY UPLC System and a new high-sensitivity tandem quadrupole MS, Xevo TQ-S, for high-sensitivity peptide bioanalysis.

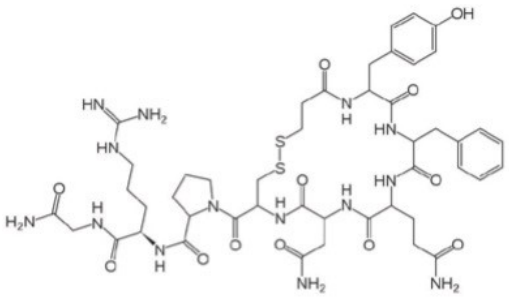
Benefits

- The Xevo TQ-S, a new tandem quadrupole MS system, provides the highest possible sensitivity for peptide bioanalysis.
- Its fast data capture rate and dual scan MRM capability allows for the accurate quantification of the narrow chromatographic peaks produced by UPLC as well as confirmation of the peak identity.

Introduction

The use of peptides as therapeutic agents is increasing due to their high tolerability, target receptor selectivity, and high potency. The ability to accurately quantify these therapeutic peptides in biological fluid requires a selective isolation process, a high-resolution chromatography system, and a high-sensitivity detector. A highresolution chromatography system is required to separate the target analyte from the endogenous peptides in plasma and blood, some of which may be isobaric or form identical fragment ions. As these therapeutic peptides imitate or replace the activity of endogenous peptides, the detection process must be able to differentiate between these endogenous and exogenous compounds.

Despite the fact that they are observed as multiply-charged compounds, the high molecular weight of peptides, 1000 to 4000 amu, the detection of these peptides requires a mass spectrometer with an upper mass range in the region of 2000 m/z for successful analysis. In this application note, we describe the use of the ACQUITY UPLC System and a new high-sensitivity tandem quadrupole MS, Xevo TQ-S, for high-sensitivity peptide bioanalysis.



Chemical structure of desmopressin.

Experimental

Chromatography

LC system:	ACQUITY UPLC (binary solvent manager, sample manager, HT column oven)
LC column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 50 mm
Column temp.:	40 °C
Gradient:	Acetonitrile 0.1%, Formic acid (Aq) 5 to 45% over 1.5 min
Flow rate:	450 µL/min
Injection vol.:	10 µL

Mass spectrometry

MS system:	Xevo TQ-S and Xevo TQ Negative ion electrospray mode MRM data acquisition 535 =>328
Voltages:	Capillary, cone, and collision voltage where optimized for each mass spectrometer as well as cone gas flow
Source temp.:	140 °C
Desolvation temp.:	625 °C
Nebuliser gas flow:	1200 L/Hr

Data management

MassLynx 4.1

TargetLynx Application Manager

Results and Discussion

Peptide therapeutics are extremely potent but are also quickly eliminated from the body either via metabolism or as unchanged drug. In order to accurately characterize the pharmacokinetics (PK) of these medicines, it is necessary to have a high-sensitivity LC-MS/MS system to define the later time points and hence the elimination phase of the PK curve. The Xevo TQ-S is a new tandem quadrupole instrument equipped with a novel source design. This new design significantly improves the efficiency of the ion sampling process allowing more analyte ions to be transferred to the analyzer. This is achieved by the use of a larger sampling orifice and differentially pumped region, and off-axis stacked ring transfer optics to prevent neutral compounds entering analyzer region of the instrument.

To evaluate sensitivity increase for the analysis of peptides in biological fluids, desmopressin was spiked into plasma and extracted via protein precipitation with acetonitrile (2:1). The data obtained for conventional optics and new StepWave optics are shown in Figure 1. Here we can see that the desmopressin peak is barely visible with the conventional transfer optics instrumentation, whereas there is a significant peak with the Xevo TQ-S. The data in Figure 2 compares the data with the baseline expanded for the conventional source. We can clearly see that there is a peak at 0.73 minutes for the desmopressin in the conventional instrumentation chromatogram. This increase in sensitivity was determined to be 25-fold.

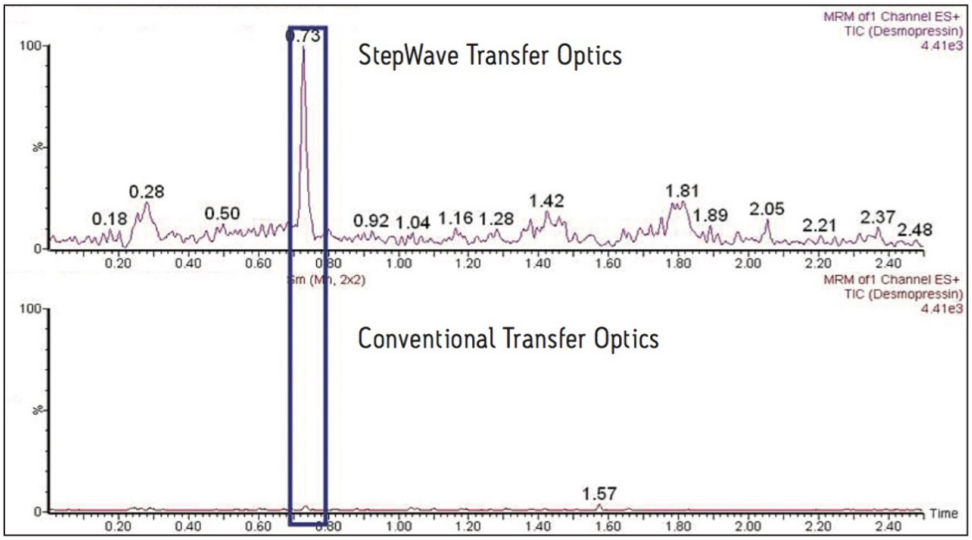


Figure 1. UPLC-MS/MS quantitative analysis of desmopressin in rat plasma extract using MRM mode. The top chromatogram shows the peak response with the new StepWave optics of the Xevo TQ-S; the lower chromatogram shows the peak response with conventional transfer optics.

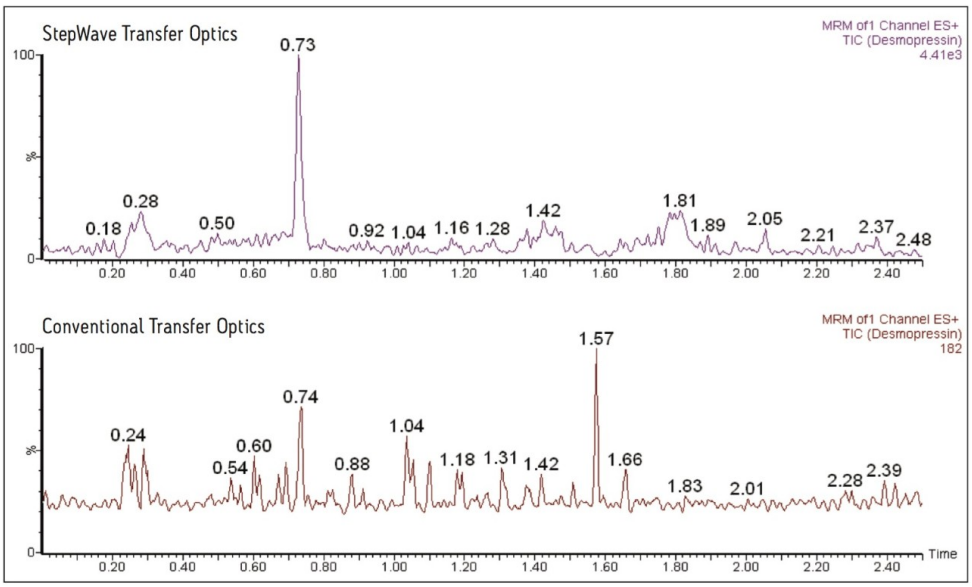


Figure 2. UPLC-MS/MS quantitative analysis of desmopressin in rat plasma extract using MRM mode. The top chromatogram shows the peak response with the new StepWave optics; the lower chromatogram shows the peak response with the conventional transfer optics with the baseline expanded to show the desmopressin peak at 0.74 minutes.

Conclusion

Peptide therapeutics offer the opportunity to treat new diseases with low risk of side effects, drug-drug interactions, or toxicity. The Xevo TQ-S combined with ACQUITY UPLC provides the ideal platform for the analysis of peptides in biological fluids. This combination offers:

- Highest possible levels of sensitivity
- Fast analysis times
- Resolution from endogenous peptides
- Sufficient mass range in the analyzer to quantify large molecular weight peptides

Featured Products

ACQUITY UPLC System

Xevo TQ-S

MassLynx MS Software

TargetLynx

StepWave

720003415, May 2010