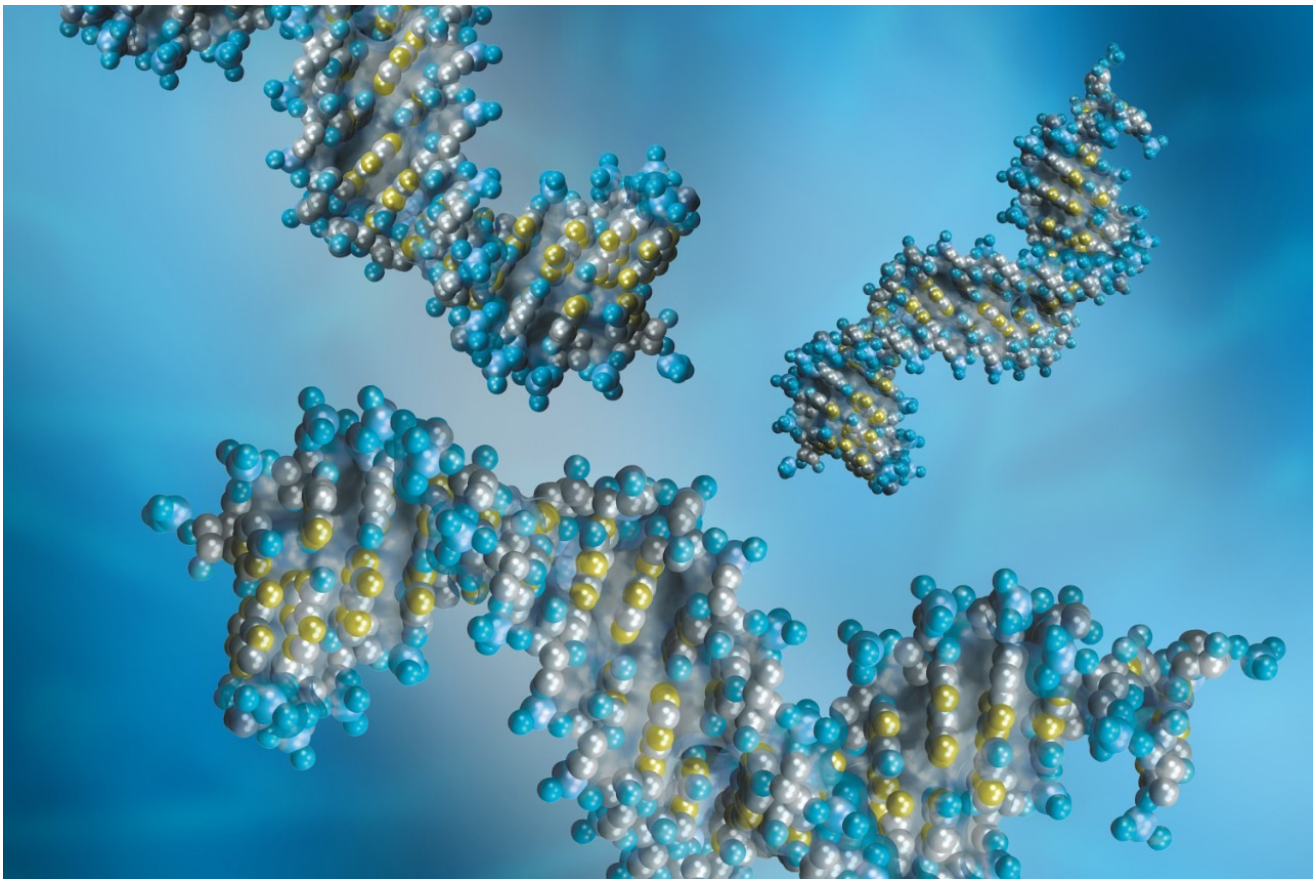
Waters™

應用手冊

Impressive Sensitivity and Linearity of Oligonucleotides on a Xevo QTof MS

Sean M. McCarthy, Vera B. Ivleva, StJohn Skilton, Martin Gilar

Waters Corporation



Abstract

The sensitivity demonstrated in this application note illustrates that Waters has achieved approximately three to

six times higher sensitivity when compared to previous-generation MS instrumentation. This will potentially prove useful for those laboratories that wish to push the levels of sensitivity further. The ability to obtain a linear calibration curve over multiple orders of magnitude at this low level is useful for those who need to quantify oligonucleotides at low levels.

Introduction

This work highlights the improved performance characteristics of Waters biopharmaceutical system solutions for oligonucleotides analysis. The ability to detect at better sensitivity and to provide a linear range for quantitation is demonstrated using UPLC Technology with the Xevo QTof MS. Organizations that are able to simultaneously quantify and identify therapeutic oligonucleotides will realize business advantages because they will have a better characterization of their product, and will be able to get products to customers more rapidly.

Oligonucleotides are generally quantified by UV methods because the absorbance measured at 260 nM is not dependent on sequence or structure. Although quantitation by UV may be preferable, Waters' new generation of benchtop mass spectrometer, the Xevo QTof MS, makes comparisons of quantitative and qualitative data more achievable.

In this technical note, we demonstrate that Waters can detect LC-MS oligonucleotides peaks to the low-femtomolar level. The example illustrated shows linear detection in the range of 5 picomolar to 25 femtomolar on-column of a multi-T oligomer.

A standard mix made by Waters that contains a range of oligomers of 15-mer, 20-mer, 25-mer, 30-mer and 35-mer length (186004135 <https://www.waters.com/nextgen/us/en/shop/standards--reagents/186004135-massprep-oligonucleotide-standard.html>) was used for the analysis. This mixture is used to demonstrate the separation capability of the ACQUITY UPLC System with a UPLC Oligonucleotide Separation Technology (OST) Column and to demonstrate the ability of the Xevo QTof MS to detect a mixture of oligomers in 15 minutes. This separation and MS detection is challenging to achieve in HPLC mode.

Experimental

LC Conditions

LC system:	ACQUITY UPLC System
Column:	UPLC Oligonucleotide Separation Technology (OST) Column
Mobile phase A:	15mM TEA in 400 mM HFIP (pH 7.9)
Mobile phase B:	50% MeOH in A
Gradient:	38 to 48% B in 10 min

MS Conditions

MS System:	Xevo QTof MS
Ionization mode:	ESI negative
Capillary voltage:	2.5 kV
Cone voltage:	20 V
Extraction Cone:	4 V

Results and Discussion

Figure 1 shows the calibration curve obtained for the 20-mer oligomer in the range 25 to 500 femtomolar using 12 acquisitions (scans) across the chromatographic peak apex. The signals of the other oligomers in the standard mixture are normalized to that of the 20-mer oligomer and therefore quantities can be extrapolated for the other oligomers.

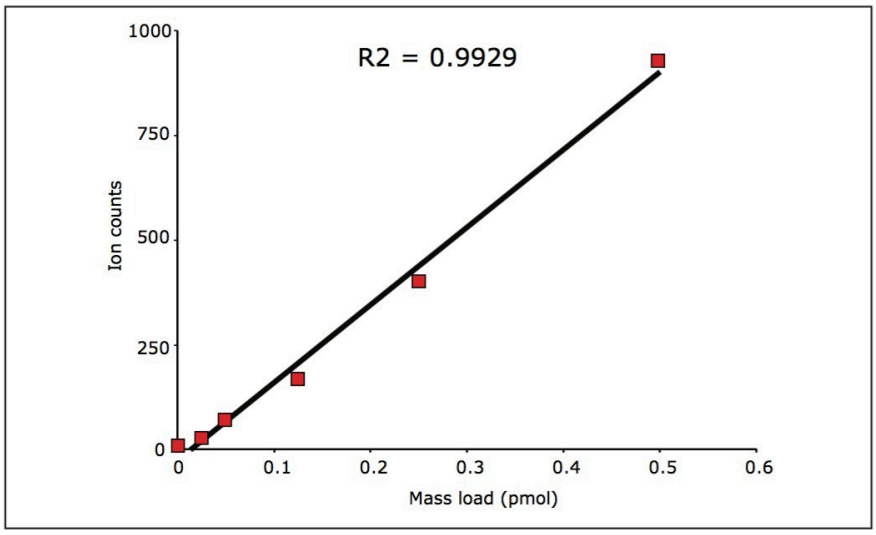


Figure 1. Linearity curve for the 20-mer oligomer at different total column loadings using chromatographic peak areas at the apex.

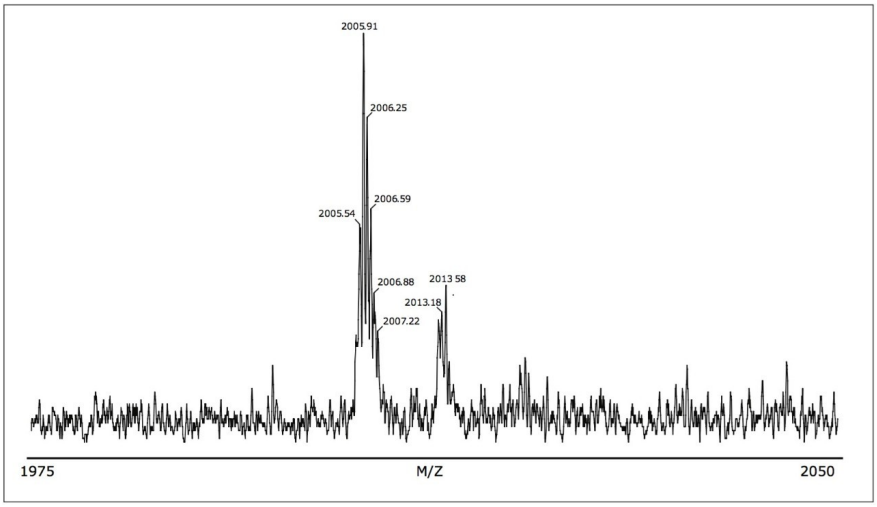


Figure 2. A single spectrum of the multiply-charged (3-) 20-mer oligomer (2005 m/z) with a sodium adduct at 2013 m/z. The resolution of the Xevo QTof MS allows partial isotopic separation at 10,000 RP FWHM.

At the lowest loading level, shown in Figure 3, the most abundant charge state (3-) of the 20-mer is shown to indicate the good signalto-noise ratio of approximately 10:1 (Raw data with no smoothing in continuum mode).

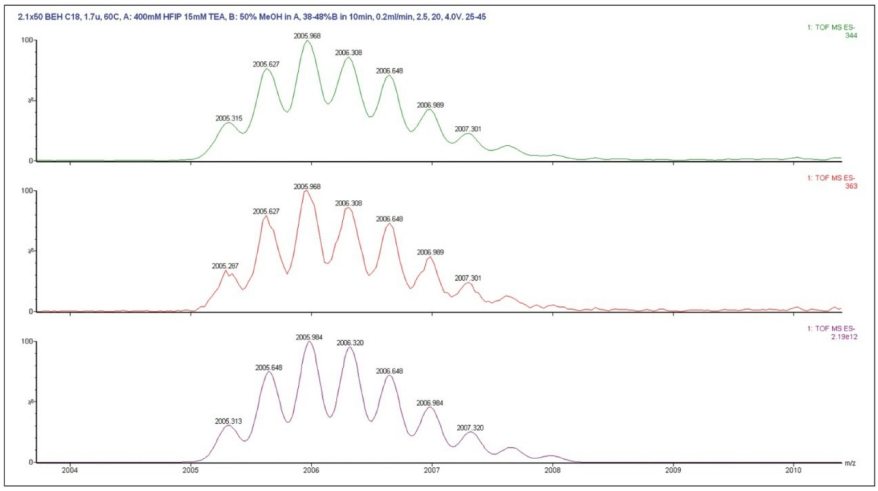


Figure 3. Comparison of actual versus theoretical traces of the 3- charge state of the 20-mer oligomer at 10,000 resolution showing the match to the data obtained on Waters Xevo QTof MS. The top trace shows the smoothed data (1,3 Savitsky- Golay), the middle trace shows the raw continuum data for the 250 femtomol sample and the bottom trace shows the theoretical isotope model for that elemental composition available in MassLynx 4.1. Note the excellent agreement in masses at peak top between theoretical, and raw and smoothed data.

The raw spectrum of the 3- charge state can be compared to the theoretical data at 10,000 RP. This is shown in Figure 3 where 12 scans at the chromatographic peak apex were averaged to obtain the spectrum. The agreement with the theoretical spectrum for that charge state is 0.016 Da and the deconvoluted data shows agreement with the theoretical isotopic masses. Figure 4 shows the raw and smoothed data compared to the MaxEnt1 deconvoluted data.

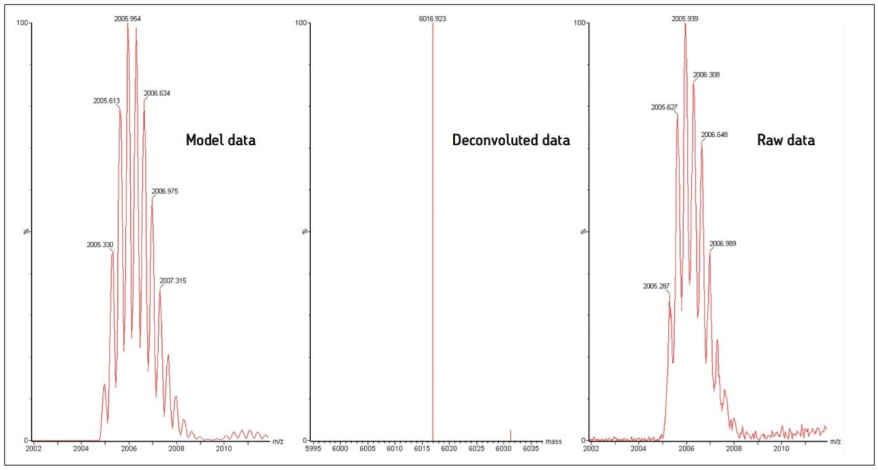


Figure 4. MaxEnt1 deconvoluted spectrum of the 20-mer oligomer at 100 pMol level to confirm the accurate mass of the analyte.

It is important to note that being able to achieve these separations relies on the chromatographic system. UPLC OST Column technology has been specifically designed for the ACQUITY UPLC System and for these types of sample. The lack of carryover is shown in Figure 5, where a blank run and a 25 femtomolar sample are shown.

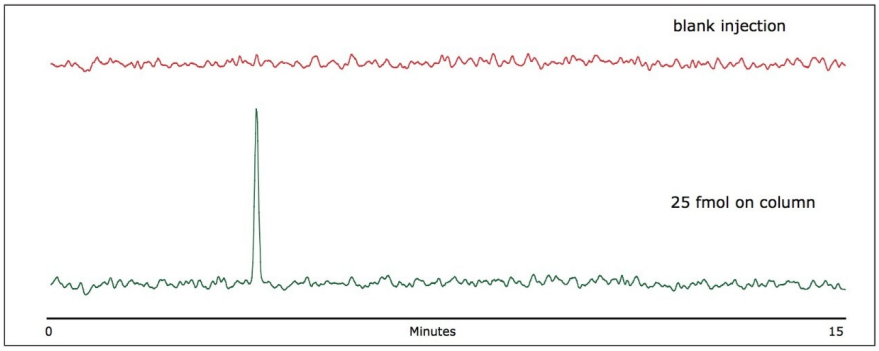


Figure 5. Extracted ion chromatogram of the 20-mer oligomer (bottom trace) showing the lack of carryover in the blank s 0 Minutes 15 ample (top trace).

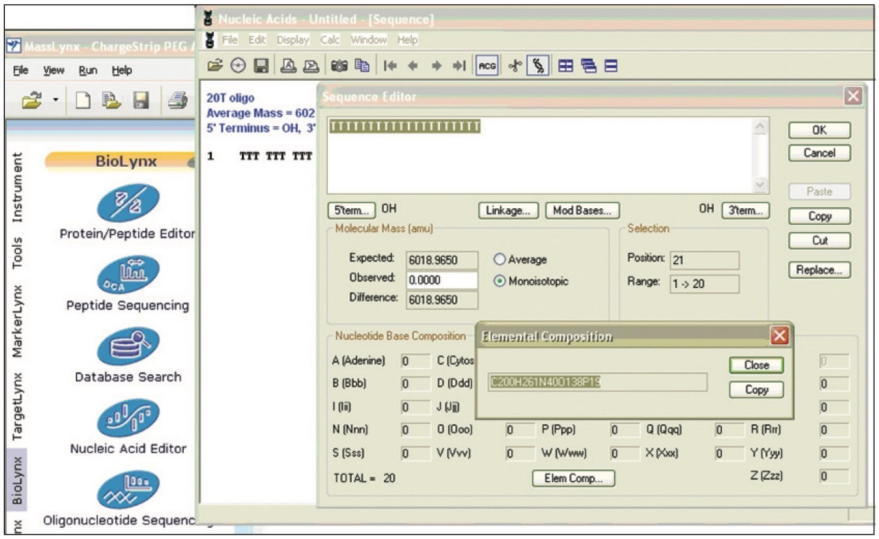


Figure 6. Mass calculations based on the sequence of the 20-mer showing the elemental composition of the oligonucleotides from which an accurate mass is derived.

Conclusion

The sensitivity demonstrated in this application note illustrates that Waters has achieved approximately three to six times higher sensitivity when compared to previous-generation MS instrumentation. This will potentially prove useful for those laboratories that wish to push the levels of sensitivity further. The ability to obtain a linear calibration curve over multiple orders of magnitude at this low level is useful for those who need to quantify oligonucleotides at low levels.

Featured Products

- ACQUITY UPLC System <https://www.waters.com/514207>

720003272, November 2009