

## Increasing the Sensitivity of MRM Measurements for Tryptic Peptides Using Xevo TQ-S

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Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

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### Abstract

To evaluate the performance of the Xevo™ TQ-S tandem quadrupole mass spectrometer incorporating StepWave™ Technology for tryptic peptide quantification using nanoflow LC-MS/MS in multiple reaction monitoring (MRM) mode.

### Benefits

Xevo TQ-S provides a highly sensitive and easily automated platform for high-throughput verification of candidate biomarkers.

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### Introduction

Tandem quadrupole-based multiple reaction monitoring mass spectrometry is the most sensitive and robust method for the quantification of target analytes, particularly those in complex biological matrices. However, for the quantification of putative protein biomarkers, sensitivity and specificity are paramount because the target analytes are often present at low concentrations in the relevant tissue or biofluid sample. For this reason, increasing the sensitivity of the MRM assay is crucial for providing a more robust analytical method,

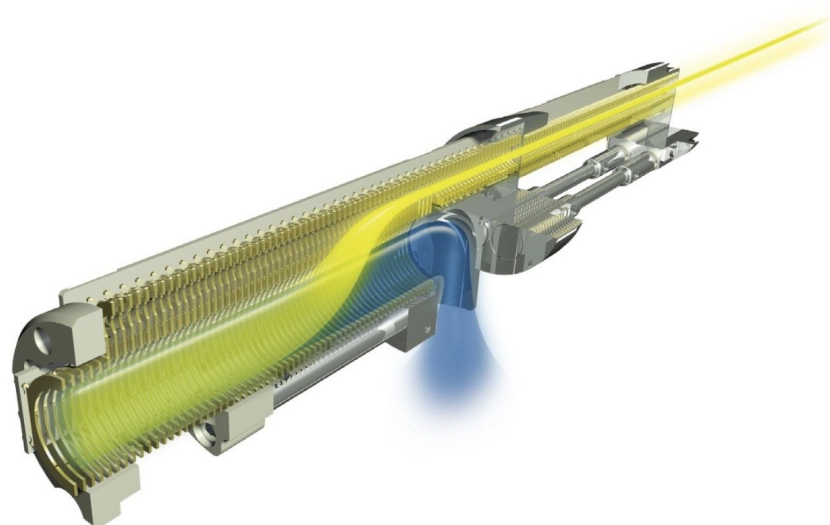
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which requires less biological material or sample pre-fractionation.

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## Results and Discussion

A standard protein digest containing Enolase (P00924), ADH (P00330), BSA (P02769), and Phosphorylase B (P00489) (p/n: 186002865 <<https://www.waters.com/nextgen/global/shop/standards--reagents/186002865-massprep-digestion-standard-mix-1.html>> ) was dissolved to 1 fmol/ $\mu$ L ( $H_2O$  + 0.1%  $HCO_2H$ ). A Waters nanoACQUITY UPLC System was used to separate peptides on a 1.7- $\mu$ m ACQUITY UPLC BEH, Column (130  $C_{18}$ , 75  $\mu$ m x 200 mm 200 mm) utilizing a gradient from 1% to 40% acetonitrile over 90 min at a flow rate of 300 nL/min. The UPLC eluent was passed directly to the NanoFlow™ Ion Source of the mass spectrometer, which incorporates the StepWave device, as shown in Figure 1.



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*Figure 1. StepWave ion optics for enhanced sensitivity.*

Two proteotypic peptides from each protein were selected, with the two most intense MRM transitions per peptide chosen for MRM. A total of four MRM transitions per protein were acquired. MRM acquisitions were performed on the Xevo TQ and Xevo TQ-S mass spectrometers. MRM chromatograms for peptide VLYPNENFDGK,  $m/z$  722 to 856, are shown in Figure 2. It can clearly be seen that the signal intensity was significantly increased on the Xevo TQ-S. As illustrated in Figure 3, the MRM transition from 722 to 1067 saw a 73-time gain in absolute sensitivity with the Xevo TQ-S, which equates to an eight-time improvement in signal-

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to-noise as compared to the Xevo TQ.

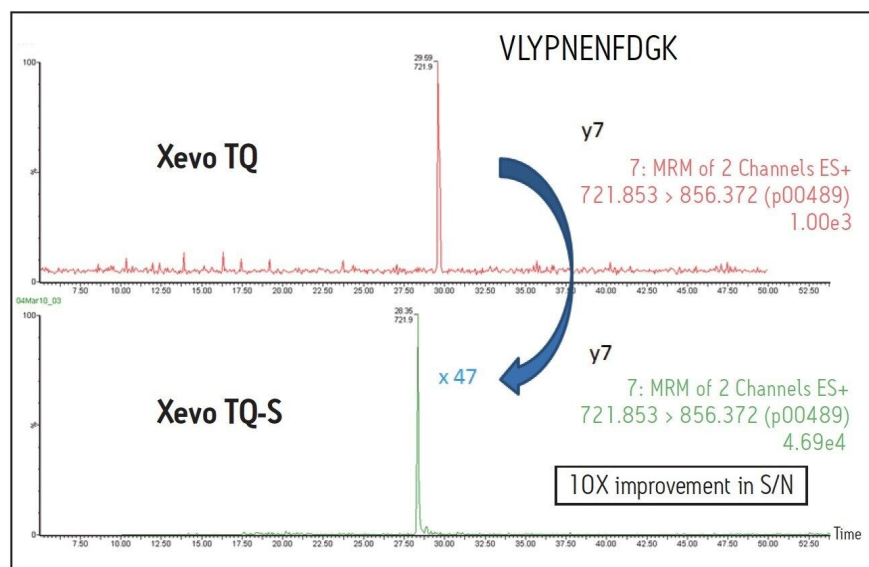


Figure 2. MRM chromatograms for peptide VLYPNENFDGK,  $m/z$  722 > 856 from Xevo TQ-S (lower panel) and Xevo TQ (top panel).

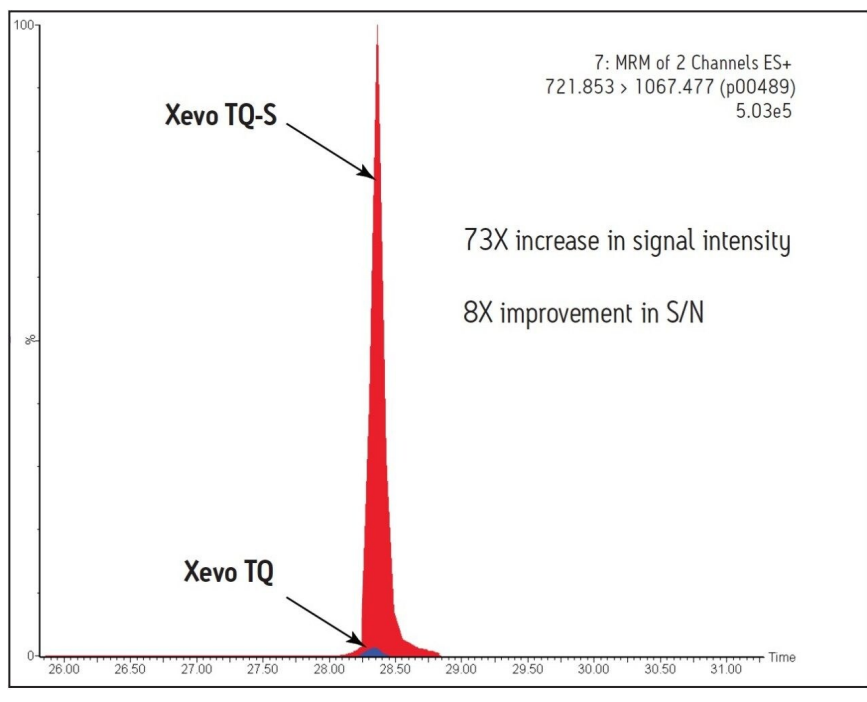


Figure 3. MRM chromatograms for peptide VLYPNENFDGK, from Glycogen Phosphorylase B,  $m/z$  722 > 1067, from Xevo TQ-S (red), and Xevo TQ (blue) vertical axes are linked to show relative intensities.

Protein	Peptide Sequence	Peptide transition and ion type	Signal intensity improvement
<b>ADH (P00330)</b>	IGDYAGIK	419 → 666 (y <sub>6</sub> )	11
		419 → 723 (y <sub>7</sub> )	13
		724 → 778 (y <sub>6</sub> )	74
	VVGLSTLPEIYEK	724 → 1080 (y <sub>9</sub> )	62
<b>BSA (P02769)</b>	AEFVEVTK	462 → 476 (y <sub>4</sub> )	52
		462 → 722 (y <sub>6</sub> )	29
		582 → 595 (y <sub>5</sub> )	37
	LVNELTEFAK	582 → 951 (y <sub>8</sub> )	47
<b>Phos B (P00489)</b>	VIFLENYR	527 → 581 (y <sub>4</sub> )	15
		527 → 841 (y <sub>6</sub> )	42
		722 → 856 (y <sub>7</sub> )	47
	VLYPNDNFFEGK	722 → 1067 (y <sub>9</sub> )	73
<b>Enolase (P00924)</b>	NVNDVIAPAFVK	644 → 745 (y <sub>7</sub> )	47
		644 → 1074 (y <sub>10</sub> )	59
		645 → 834 (y <sub>8</sub> )	51
	VNQIGTLSESIK	645 → 947 (y <sub>9</sub> )	35

Table 1. The sensitivity gain for the eight peptides monitored using 16 MRM channels is presented in Table 1.

## Conclusion

Xevo TQ-S shows excellent sensitivity for tryptic peptides at nano-scale flow rates. Significant improvements in absolute signal intensity and in signal-to-noise ratios for the tryptic peptides were observed in comparison to the Xevo TQ MS. In combination with Verify<sup>E</sup> bioinformatics, the nano ACQUITY UPLC System coupled with the Xevo TQ-S provides a highly sensitive and easily automated platform for high-throughput verification of candidate biomarkers.

## Featured Products

Xevo TQ-S <<https://www.waters.com/10160596>>

StepWave <<https://www.waters.com/nextgen/global/products/mass-spectrometry/mass-spectrometry-technologies/stepwave.html>>

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