

## Xevo G2 QTof System: High Resolution and Mass Measurement Accuracy for the Analysis of Complex Peptide Mixtures

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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 utilize the Xevo™ G2 QTof for LC/MS<sup>E</sup> analysis of complex tryptic peptide mixtures, illustrating the mass resolution and accuracy that can be obtained.

### Benefits

The use of Xevo G2 QTof to analyze complex mixtures allows for protein identification and quantification with high resolution and mass accuracy.

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### Introduction

The identification of proteins in complex mixtures by nanoscale LC/MS is well accepted. More recently it has become apparent that high mass accuracy and resolution, particularly of the fragments produced by CID, provide significant specificity for identification purposes. By operating at high resolution and mass accuracy, more confident results can be obtained with a reduction in chimericity. In addition, false

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positive identifications are minimized due to the better mass accuracy and increased specificity. In this technology brief, we describe the analysis of complex peptide mixtures using the benchtop Xevo G2 QToF Mass Spectrometer, which operates at greater than 20,000 resolution (FWHM) with high sensitivity and mass accuracy.

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## Results and Discussion

An 800 ng sample of digested E. coli cell lysate was prepared and analyzed using a nanoACQUITY UPLC® System coupled to a Xevo G2 QToF operating in resolution mode. The MS method used was LC/MS<sup>E</sup> where the collision cell was switched between low and elevated energy during alternate scans acquiring peptide precursor information in the first function, and fragment ion information in the second function. A reference spray was sampled every thirty seconds to provide a lock mass correction. The low energy chromatogram is shown in Figure 1 and mass spectral resolution for an eluting peptide at m/z 486.27 is shown in Figure 2. Using ProteinLynx Global SERVER™ v. 2.4, data were processed and searched against a non-redundant E. coli database. To visualize the spread of mass accuracies of identified precursor and fragment ions, bar charts were created from search outputs, as shown in Figure 3 and Figure 4. The data shows that approximately 84% of identified precursors had mass errors within 2 ppm.

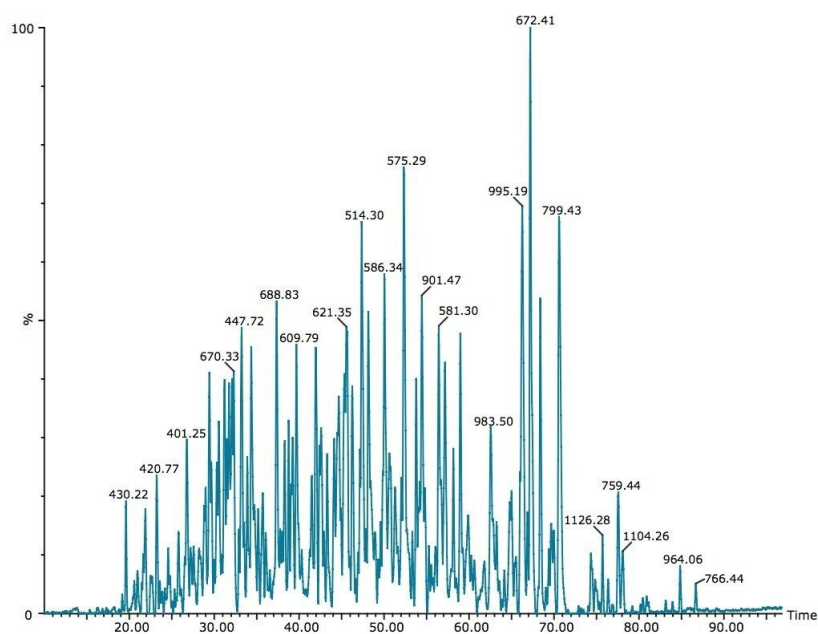
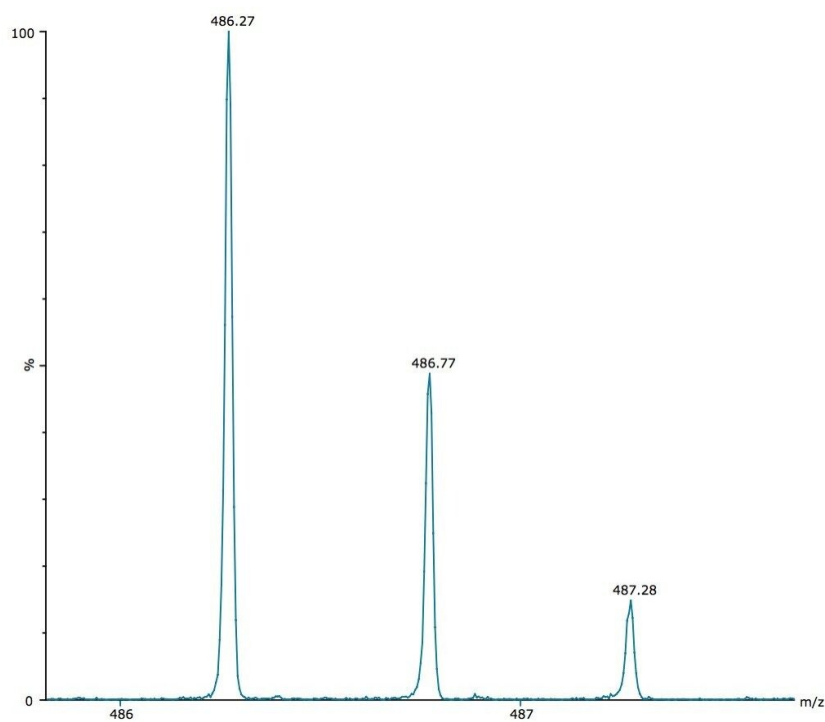


Figure 1. Low energy chromatogram for the injection of 800 ng *E.coli* cell lysate.



*Figure 2. Approximately 24 k (FWHM) resolution for a doubly-charged peptide at m/z 486.27.*

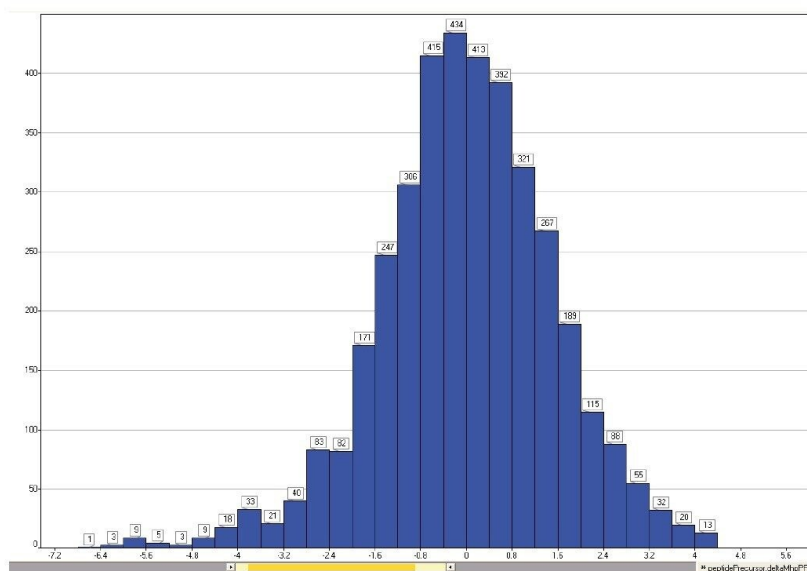


Figure 3. Mass accuracy for identified peptide precursors.

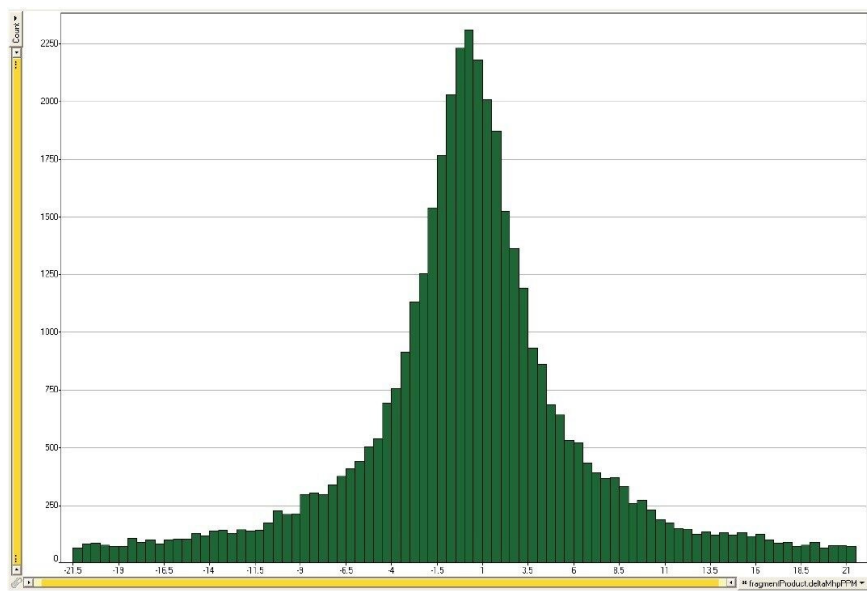


Figure 4. Mass accuracy for identified fragment ions.

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## Conclusion

The Xevo G2 QTof System provides high resolution and mass accuracy for the analysis of complex peptide mixtures, delivering a highly specific platform for protein identification and quantification.

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## Featured Products

[Xevo G2-XS QTof Quadrupole Time-of-Flight Mass Spectrometry <https://www.waters.com/134798222>](https://www.waters.com/134798222)

[MassLynx High-Resolution Applications <https://www.waters.com/513821>](https://www.waters.com/513821)

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