

## SYNAPT G2: A Next-Generation, High-Resolution Time-of-Flight Mass Spectrometry Platform

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### Introduction

We introduce the SYNAPT™ G2 System, a new high-resolution quadrupole, time-of-flight MS platform providing a new level of performance for the most challenging MS and UPLC®/MS-based applications.

We demonstrated the use of orthogonal acceleration time-of-flight (oa-ToF) MS to generate resolution greater than 40,000 (FWHM), which can be used to identify and mass-resolve two isobaric peptides that differ by only 18.2 mDa.

We also show how full isotopic envelope resolution can be obtained on proteins that range in mass from 5730 kDa to 16,951 kDa.

Waters® SYNAPT G2 platform is an innovative hybrid quadrupole time-of-flight (ToF) instrument that employs QuanTof Technology. QuanTof combines innovative high field pusher and dual-stage reflectron designs with a novel ion detection system in an optimised, folded, TOF geometry (Figure 1) to provide resolution of greater than 40,000 (FWHM), mass accuracy of typically less than 1 ppm RMS, and dynamic range of up to 10<sup>5</sup>. The resolution performance is available across the mass range, particularly at high mass where it provides the greatest analytical benefit.

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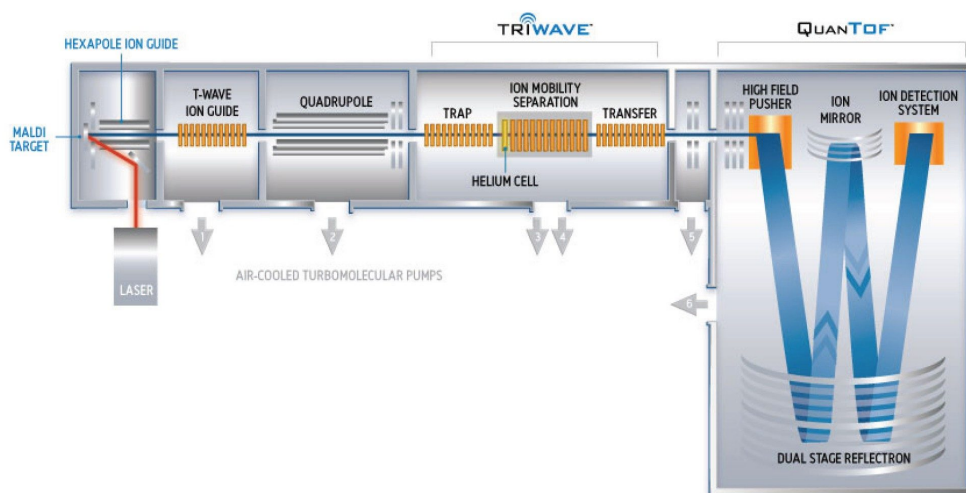


Figure 1. QuanTof Technology, the enabling high-resolution ToF technology of the SYNAPT G2 System: 1. QuanTof's high field pusher and dual-stage reflectron, incorporating high transmission parallel wire grids, reduce ion turnaround times due to pre-push kinetic energy spread and improve focusing of high energy ions respectively. These innovative technologies combine to provide the highest levels of TOF performance. 2. The novel ion detection system combines an ultra-fast electron multiplier and hybrid ADC detector electronics to provide outstanding sensitivity and quantitative performance for both MS and the elevated data acquisition rates of IMS/MS (HDMS™) analysis.

The ToF analyzer is capable of acquiring spectra at up to 20 Hz without loss of resolution, which makes it ideal to take full advantage of the speed, sensitivity, and resolution of UPLC separations on a Waters ACQUITY UPLC® System.

## Results and Discussion

Figure 2 shows the ability of the SYNAPT G2, operating at over 40,000 FWHM resolution, to mass resolve two doubly-charged peptide species that differ by only 18.2 mDa. This resolution is not achievable on traditional oa-ToF instrumentation, which typically provides resolution of up to 20,000 FWHM.

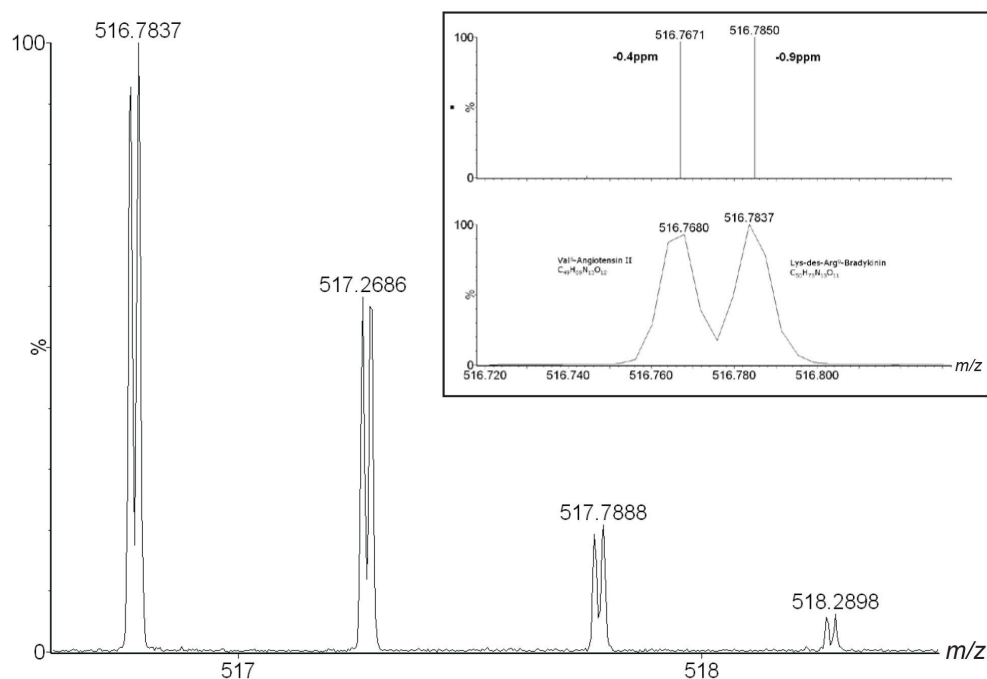
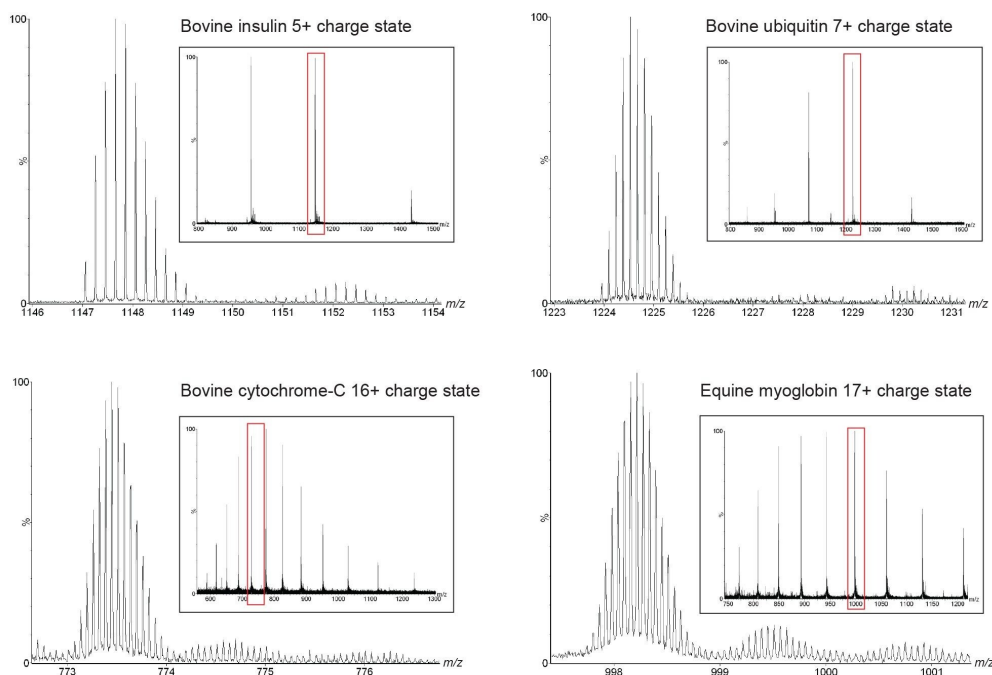


Figure 2. A high resolution oa-ToF spectrum of the isobaric peptides Val5-Angiotensin II and Lys-des-Arg9-Bradykinin.

This increase in mass resolution allows for accurate mass assignment of both doubly-charged and singly-charged (data not shown) precursor ions at better than 1 ppm mass accuracy.

Figure 3 shows high resolution oa-ToF data acquired from the proteins bovine insulin, bovine ubiquitin, bovine cytochrome-C, and equine myoglobin. The insets are the full protein, multiply-charged envelopes. The main spectra are a “zoomed-in” region of the  $m/z$  scale. This data demonstrates that the SYNAPT G2, again with resolution operating in excess of 40,000 (FWHM), is capable of resolving the isotopic envelope for proteins such as equine myoglobin, which has a molecular weight of 16,951 Da. Traditionally, this high-resolution performance has only been demonstrable on Fourier transform-based mass spectrometer systems that operate with extended acquisition periods. With QuanTof, the resolution, mass accuracy, and dynamic range performance is not compromised by the fast scan/acquisition rates required for ACQUITY UPLC, or over the  $m/z$  range of interest.



*Figure 3. A selection of proteins ranging in molecular weight from 5730 Da to 16,951 Da. All data acquired with the QuanTof high resolution oa-ToF analyzer operating at greater than 40,000 (FWHM).*

## Conclusion

- Here we have introduced QuanTof, the enabling ToF technology of SYNAPT G2, and its ability to provide a mass resolution of greater than 40,000 (FWHM) in a convenient, compact laboratory footprint
- The high resolution oa-ToF analyzer is capable of separating two isobaric peptides, which differ by only 18.2 mDa, with exact mass measurement for confident structural identification and characterization. This separation would be impossible at 20,000 FWHM or lower
- Isotopic resolution can be obtained over the entire isotopic envelope for a variety of proteins, including equine myoglobin (M.W. 16,951)
- High resolution in excess of 40,000 (FWHM) can be achieved even at spectral acquisition rates of 20 Hz, to take full advantage of the high speed, sensitivity, and resolution of UPLC separations

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