

## Characteristics of Proteomics Experiments Performed on the SYNAPT XS Q-ToF Mass Spectrometer

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Christopher J. Hughes, Lee A. Gethings

Waters Corporation

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

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### Abstract

We examine the performance characteristics of the SYNAPT XS Mass Spectrometer for conducting proteomic experiments. The SYNAPT XS Mass Spectrometer is the latest iteration of ion mobility enabled Q-ToF systems, possessing enhanced resolution and sensitivity compared with previous versions.

### Benefits

SYNAPT XS and ACQUITY UPLC M-Class with nanoscale chromatography enhances resolution and sensitivity, as compared to previous platform versions.

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### Introduction

Quadrupole time-of-flight (Q-ToF) MS is a well-established tool for both discovery and quantitative proteomic

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applications. These instruments display sensitivity, speed and high mass resolution, which are all important characteristics of instrument performance required to perform these challenging types of experiments. The SYNAPT XS Mass Spectrometer is the latest in the SYNAPT family of research grade Q-ToF mass spectrometers and performance over previous iterations is enhanced with new technologies that provide enhanced sensitivity and superior mass resolution. SYNAPT XS and ACQUITY UPLC M-Class with nanoscale chromatography enhances resolution and sensitivity, as compared to previous platform versions.

For analyses where sample amounts are limited, coupling the SYNAPT XS Mass Spectrometer with nanoscale chromatography provides a very powerful combination to generate high quality, information rich datasets. In this technology brief, we describe experiments where we have analyzed tryptically digested samples of *E. coli* and a K562 human cell line, covering samples of medium to high complexity. The results show that the enhanced performance characteristics of the SYNAPT XS Mass Spectrometer provide identification increases at both the protein and peptide level, when compared with the previous SYNAPT Mass Spectrometer iteration.

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## Results and Discussion

An ACQUITY UPLC M-Class System was configured with trap (ACQUITY UPLC M-Class, Symmetry C<sub>18</sub> Trap Column, 100Å, 5 µm, 180 µm × 20 mm, 2G, V/M, p/n 186007496) and analytical (nanoEase MZ HSS T3 Column, 100Å, 1.8 µm, 75 µm × 250 mm, p/n 186008818) columns. Analytical column eluent was coupled with a PicoTip emitter attached to a NanoFlow ESI source housed upon the SYNAPT XS Mass Spectrometer. A reversed phase gradient was performed whereby acetonitrile containing 0.1% formic acid was raised from 5% to 40% over a 90-minute period. Data were acquired in HDMS<sup>E</sup> mode, an ion mobility enabled data independent acquisition (DIA), in which alternate low and elevated transfer region collision energy scans were performed. Samples injected were MPDS *E. coli* (MassPREP *E.Coli* Digest Standard, p/n 186003196) and a K562 cell line (Promega Corporation) at loadings of 25 ng, 50 ng, 100 ng, and 150 ng. The SYNAPT XS Mass Spectrometer was operated using either sensitivity or resolution optic modes.

Extracting peptide information within different regions of the chromatogram allows mass resolution and signal intensities for the different optic modes of operation to be compared with those typically observed when using the SYNAPT G2-*Si* Mass Spectrometer. These are summarized in the table, Figure 1. The data suggests that for the two instruments operating at the same mass resolution, signal increases of approximately ten-fold were

observed. Data were processed and searched against species-specific reviewed entry databases using ProteinLynx Global SERVER and Progenesis Q1 for Proteomics. The effect of column loading and optic mode for injections of *E.coli* is shown in Figure 2, where that data represents the average number of proteins from duplicate injections.

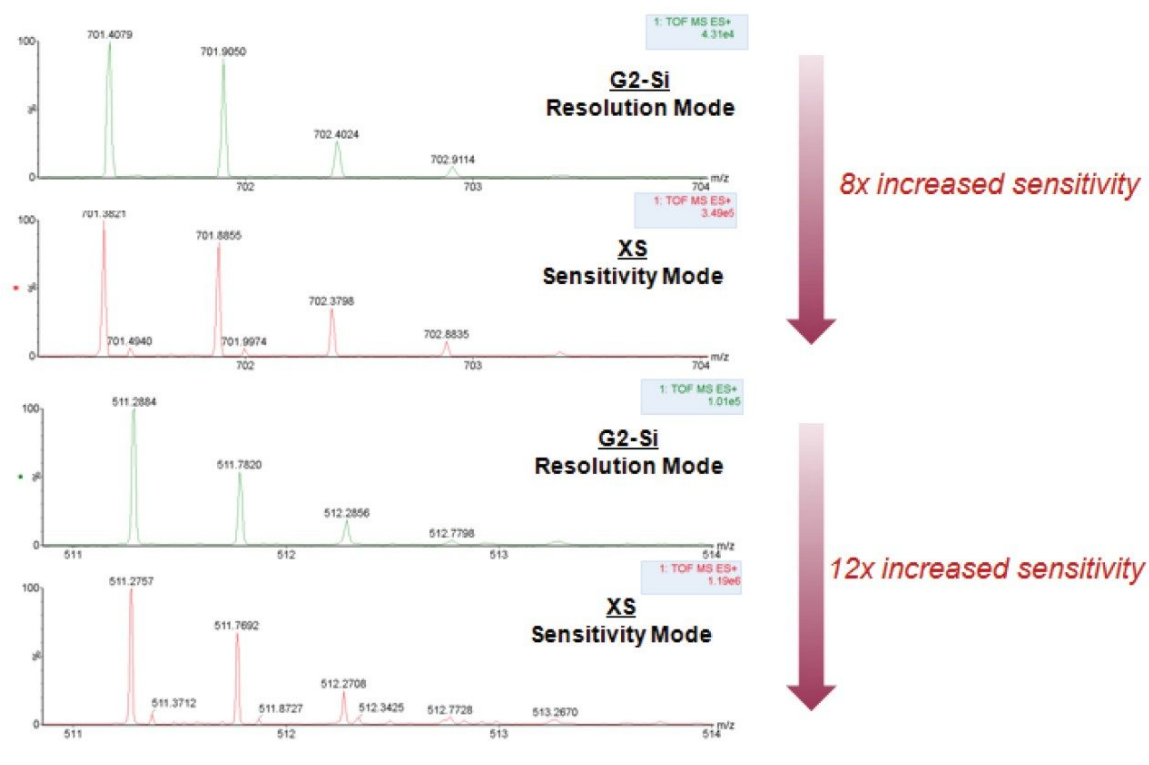


Figure 1. Signal intensities at different optic modes of operation compared between the SYNAPT XS Mass Spectrometer and the SYNAPT G2-Si Mass Spectrometer.

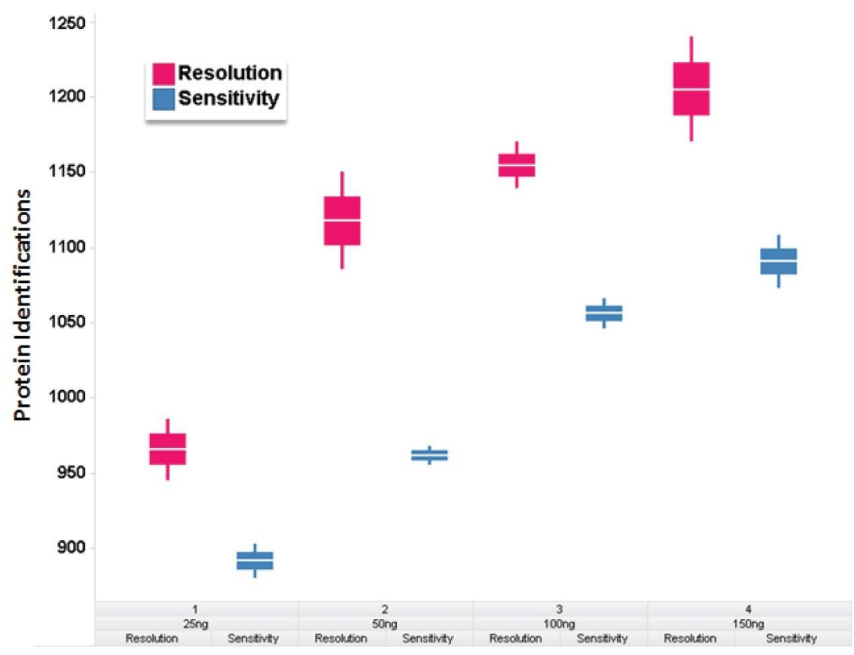


Figure 2. *E. coli* tryptic digest protein identifications at increasing column loading and different mass resolution settings.

For comparative purposes the SYNAPT XS Mass Spectrometer provides 966 protein identifications for a 25 ng load, while the SYNAPT G2-*Si* Mass Spectrometer requires a 100 ng load to provide an equivalent number. When analyzing the more complex K562 human cell lysate, a 24% increase in protein identifications is observed for the SYNAPT XS Mass Spectrometer compared with the SYNAPT G2-*Si* Mass Spectrometer, Figure 3.

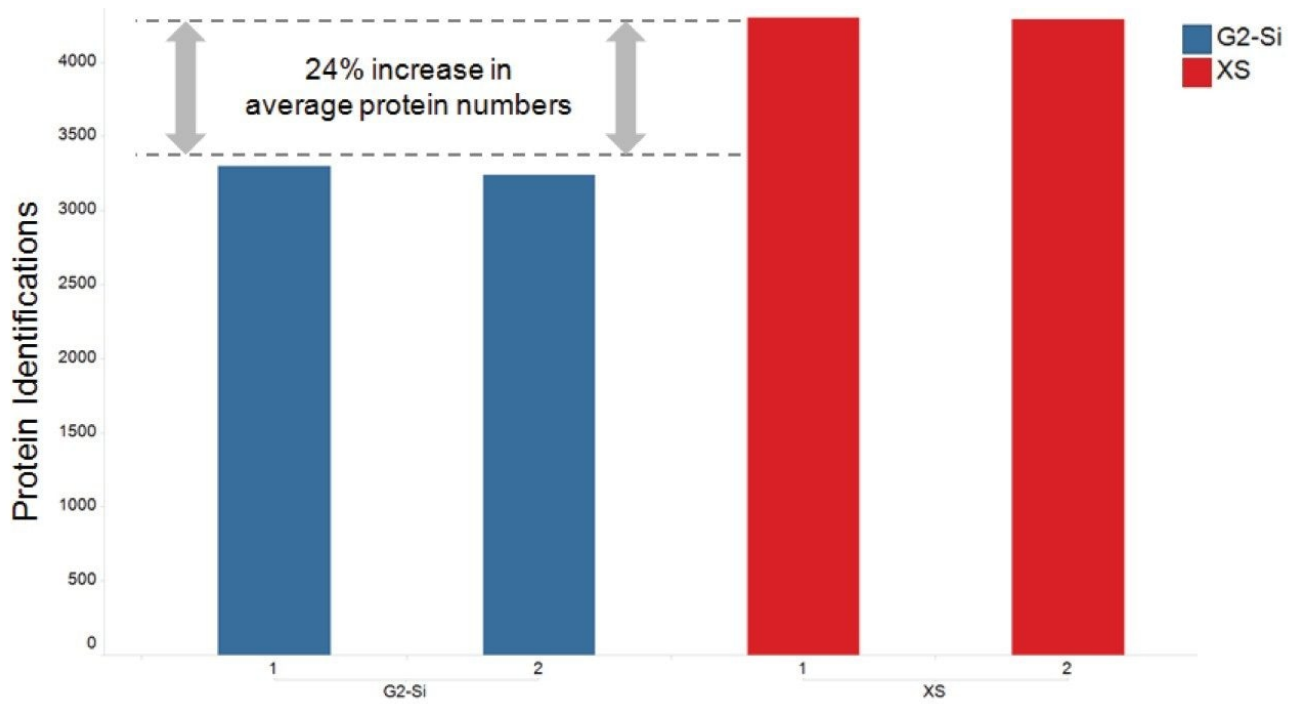


Figure 3. Human cell lysate, K562, protein identifications comparison for the SYNAPT XS Mass Spectrometer vs. the SYNAPT G2-Si Mass Spectrometer.

In Figure 4, the normalized abundance plot demonstrates that four orders of detected peptide dynamic range were achieved, while Figure 5 shows the excellent mass measurement accuracy for the SYNAPT XS Mass Spectrometer in sensitivity mode.



Figure 4. Human cell lysate, K562, four orders of detected peptide dynamic range were achieved.

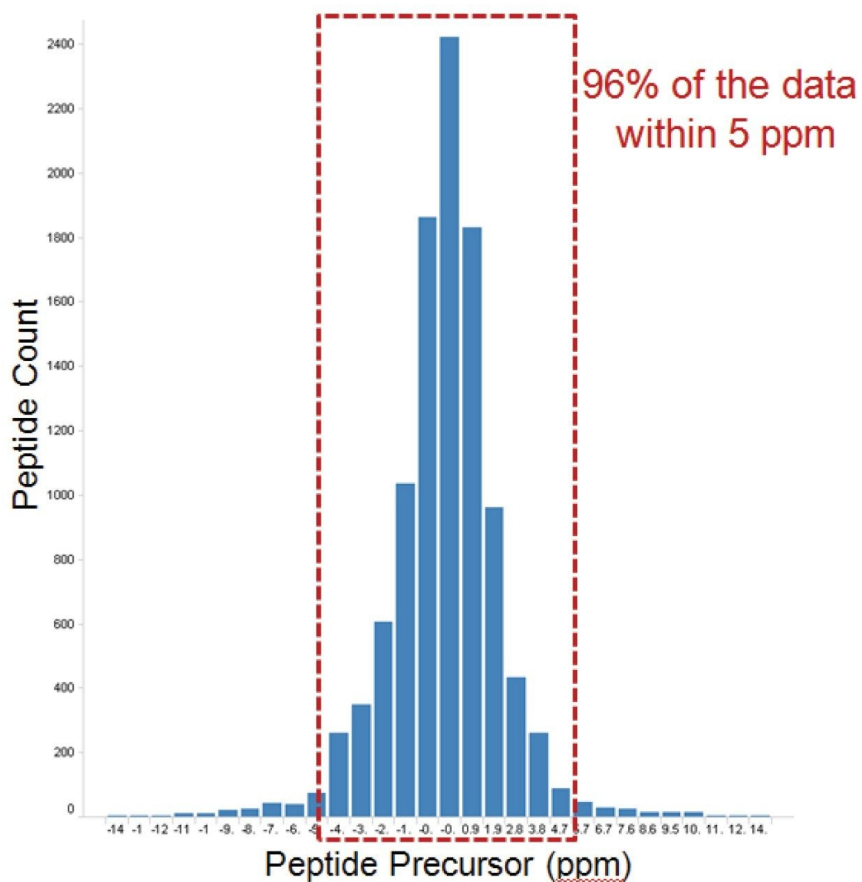


Figure 5. Peptide precursor mass accuracy, sensitivity optic mode.

## Conclusion

This technical brief has demonstrated the performance characteristics of the SYNAPT XS Mass Spectrometer coupled with an ACQUITY UPLC M Class nanoscale chromatography system for the analysis of medium to complex proteomic samples. The mass resolution and sensitivity of the SYNAPT XS Mass Spectrometer increases peptide and protein identification rates for both the *E. coli* and human cell line samples when compared with the previous iteration of the SYNAPT G2-Si Mass Spectrometer. Protein identifications over a wide dynamic range and with excellent mass accuracy also highlight the potential of the new SYNAPT XS Mass Spectrometer performance characteristics.

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[SYNAPT G2-Si Mass Spectrometry <https://www.waters.com/134740653>](https://www.waters.com/134740653)

[SYNAPT XS High Resolution Mass Spectrometer <https://www.waters.com/135020928>](https://www.waters.com/135020928)

[ACQUITY UPLC M-Class System <https://www.waters.com/134776759>](https://www.waters.com/134776759)

[ProteinLynx Global SERVER \(PLGS\) <https://www.waters.com/513821>](https://www.waters.com/513821)

[Progenesis QI for Proteomics <https://www.waters.com/134790665>](https://www.waters.com/134790665)

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