High Resolution Mass Spectrometry Quantitation Explained: Adding Sensitivity and Selectivity Through Instrument Design and Practice

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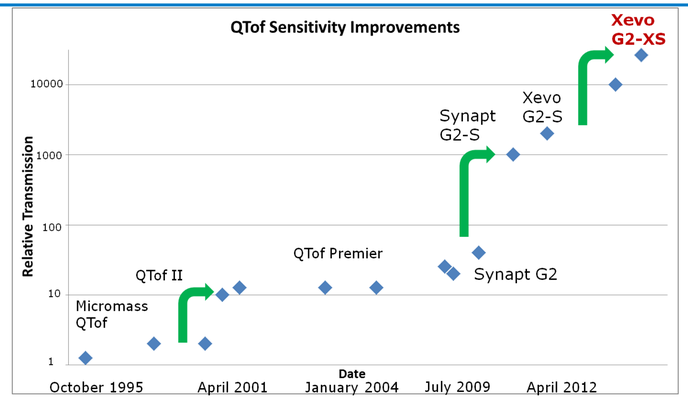
Abstract

Introduction: Performing bioanalysis using an accurate mass platform is gaining wider acceptance in a field that has traditionally relied on nominal mass using tandem quadrupole mass spectrometry (QqQ). This acceptance is partly due to the performance characteristics of newer generation QTof instruments having high mass resolution, and with sensitivity approaching those for QqQ instrument. In this poster, the fundamentals of high resolution mass spectrometry (HRMS) quantitation based on time-of-flight instruments (Tof) is explained, including scan speed, spectra quality, and the effect of mass selectivity on sensitivity and peak's signal to noise. The newly introduced Tof MRM mode of data acquisition on the Tof platform enables parent or product ion monitoring similar to that for QqQ MS. To illustrate this evolution in sensitivity, examples include quantitation of small drug-like compounds in human plasma, as well as both peptides and 150 kDa proteins.

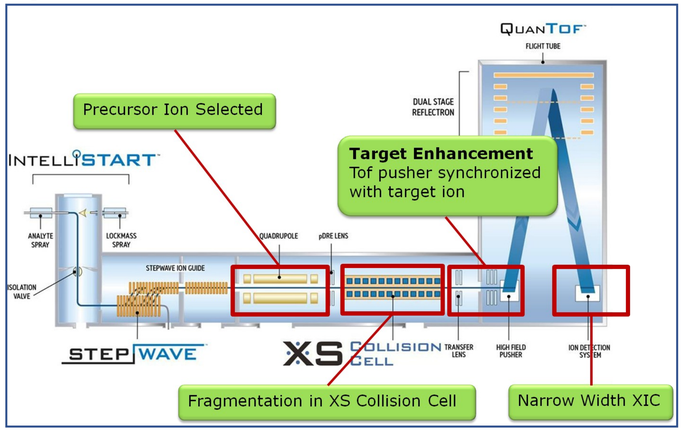
Methods: Various test compounds were analyzed using two generations of QTof instruments including Xevo G2-S and Xevo G2-XS. The LC front end employed was either the Acquity I-Class or Acquity M-Class with the ionKey/MS source. Full scan and Tof MRM mode of data acquisition were used. Data were processed using either MassLynx or UNIFI.

Results: Benchmark studies of the Xevo G2-XS QTof suggests it is >1000 times more sensitive than those introduced just 10 years ago. This step jump improvement can be attributed to both StepWave ion guide technology and a new collision cell design that minimizes background noise, improves ion focusing, and reduces losses in ion transfer. Reducing the nominal mass range window from 0.5 – 1.0 Da to an accurate mass range of 0.01 Da, one can realize a 10x improvement in the signal:noise ratio. Datasets produced using Tof MRM mode of data acquisition show that the Xevo G2-XS QTof can routinely reach femtogram on- column sensitivity for small drug-like compounds in human plasma, and sub nanogram on-column sensitivity for peptides and proteins. Adding a microflow LC delivery system to the Tof platform leads to another 10 fold increase in sensitivity through increased mass sampling efficiency and reduced matrix interference.

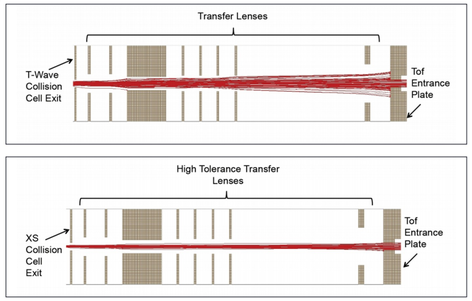
Novel aspect: The novel aspect of the work includes the employment of HRMS and state of the art microflow LC for the bioanalysis of small molecules, peptide and proteins.



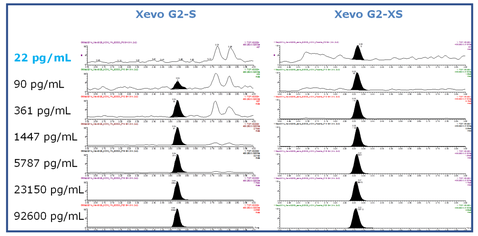
(Above) History of QTof sensitivity improvement. The graph suggests that the recent QTof is 10,000 times more sensitive than the first generation QTof instrument. The two recent step jumps in sensitivity are due to incorporation of StepWave ion guide technology and inclusion of segmented collision cell in Xevo G2-XS.



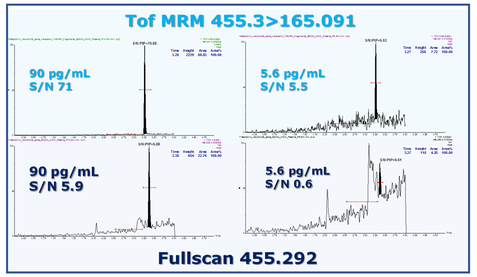
(Above) System diagram of Xevo G2-XS QTof showing Tof MRM detection. After precursor ion selection in the quadruple, the ion is fragmented in the collision cell. The Tof pusher is then synchronized with the target ion, resulting in more detector count for the selected ion compared to full scan, hence an overall enhanced sensitivity.



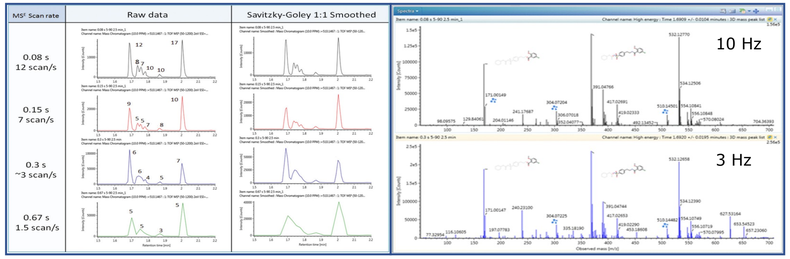
Collision cell comparison of Xevo G2-S vs. G2-XS. The segmented collision cell in XS produces more focused ion beam compared to T-Wave design.



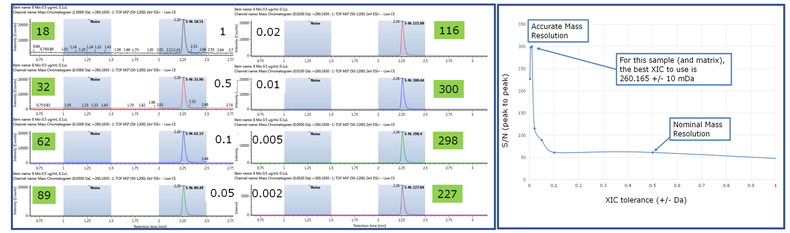
Comparison of verapamil full scan data using Xevo G2-S vs G2-XS. There is 10x sensitivity improvement using G2-XS.



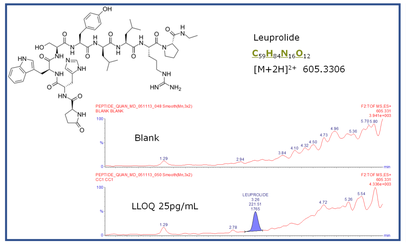
Comparison of full scan vs Tof MRM mode of data acquisition. A 10x sensitivity increase is observed for verapamil using Tof MRM acquisition.



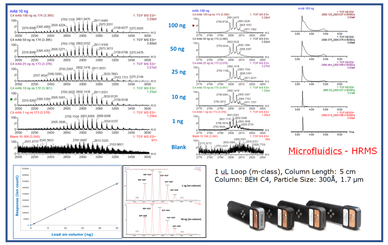
(Above left) Scan speed is important to mitigate false negatives. The overlaid chromatograms show that as scan speed decreases, the isobaric metabolite peaks merge and collapse. Since these metabolites have identical masses, the slower scan speed would result in missed metabolites being detected. (Above right) Time of flight detector runs at a very high scan rate. The MS data collected from faster scan times (top spectrum) is indistinguishable from slower scan rates (bottom spectrum).



The above figure shows effect of mass extraction window on data signal/noise ratio. As the extraction window decreases from nominal mass to accurate mass range, there is exponential increase in detector signal/noise. This figure explains the advantages of using QTof for quantitation especially in the presence of complex matrix.



Example of peptide quantitation using Acquity I-class/Xevo G2-XS



Example of mAb quantitation using ionKey/Xevo G2-XS.

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