

IMPROVEMENTS IN SENSITIVITY, QUANTITATIVE LINEARITY, AND ROBUSTNESS USING A MODIFIED BENCH-TOP QUADRUPOLE ORTHOGONAL TIME-OF-FLIGHT MASS SPECTROMETER (Q-TOF MS).

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OVERVIEW

PURPOSE: Increases in Sensitivity, quantitative dynamic range and robustness on a bench-top Q-ToF instrument

METHOD: A commercial Q-ToF was modified to include optimized source transfer optics (StepWave™ XS ion guide), 10 bit acquisition electronics, adaptive intensity control, and modified transfer optics for improved robustness

RESULTS:

- 10x increase in sensitivity for thermally labile compounds.
- ~4x increase in 'in-spectrum' dynamic range
- ~10x increase in dynamic range for targeted analysis
- Significant reduction in surface charging using modified optics.

INTRODUCTION

Q-ToF mass spectrometers are a well-established analytical tool utilized in a wide range of application areas. However, for routine targeted quantification, Tandem Quadrupole (TQ) instruments dominate. In this paper we describe modifications to a commercial Q-ToF MS to extend the dynamic range, improve the robustness and sensitivity, particularly for thermally labile compounds. These improvements result in a platform capable of bridging the gap between the qualitative performance of the Q-ToF and quantitative performance of the TQ instruments.

METHODS

All data were acquired using a modified Xevo™ G2-XS Q-ToF instrument (Waters Corporation) (Figure 1). The instrument was fitted with a StepWave XS, co-joined source ion guide, a 10bit ADC acquisition system as well as adaptive intensity control (AIC) software to extend dynamic range for non-targeted and targeted analysis. In addition the beam conditioning optics were modified, to increase robustness. ACQUITY™ Premier UPLC™ system conditions:

Column: ACQUITY UPLC BEH C18 1.7µm
 Mobile Phase: A. Water + 0.1% FA
 B. Acetonitrile + 0.1% FA
 Gradient: 5%B with a linear ramp to 95%B at 3minutes
 Column temp: 40°C
 Flow rate: 0.2mL/min

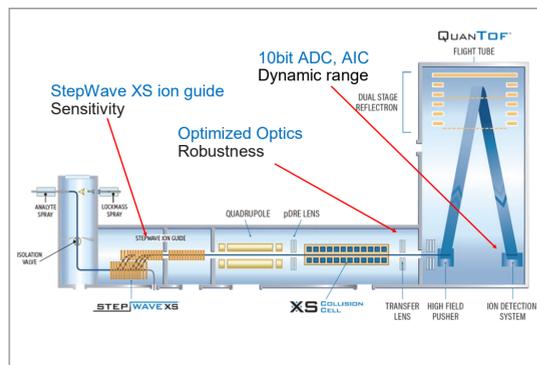


Figure 1. Schematic of modified Q-ToF instrument.

SENSITIVITY

Figure 3 shows the StepWave XS¹ conjoined ion guide. The large diameter entrance efficiently captures ions from the API source. A DC potential is applied to move the ions from the larger ring region into the smaller ring region while neutrals entrained in the gas flow are pumped away. In contrast to the previous design, the horizontal electrodes result in a lower electric field between the conjoined sections, reducing ion heating and increasing sensitivity particularly for fragile, thermally labile compounds. The focussing quadrupole ion guide in the second, differentially pumped stage improves transfer into the downstream analyser.

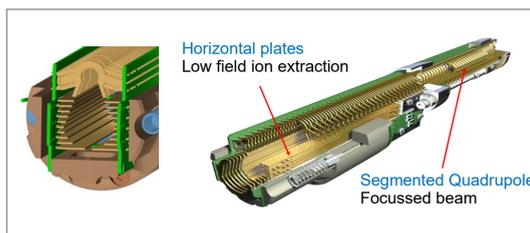


Figure 3. StepWave XS ion guide. Reduced ion heating, efficient ion

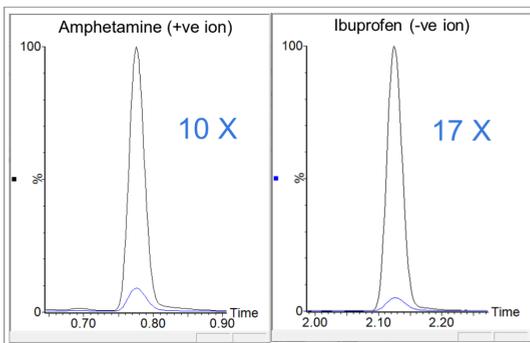


Figure 4. StepWave XS ion guide sensitivity increase Amphetamine positive and Ibuprofen negative ion mode.

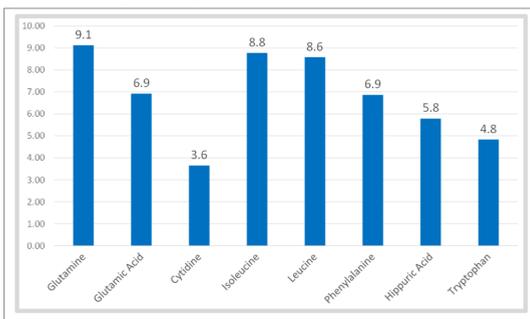


Figure 5. Observed increase in absolute signal for a range of common metabolites

The chromatogram in Figure 4 shows the increase in sensitivity between the original ion guide and the StepWave XS ion guide for the molecular cation of Amphetamine [M+H]⁺ = 136.1126, and the molecular anion [M-H]⁻ = 205.1129 of Ibuprofen. Figure 5 shows the absolute increase in intensity observed for a selection of common metabolites using the StepWave XS ion guide.

IN-SPECTRUM DYNAMIC RANGE

The dynamic range within a single time of flight spectrum is, in part, dictated by the vertical 'resolution' of the digitizer or analog-to-digital converter. The number of vertical intensity steps is expressed in powers of 2. For an 8 bit digitizer there are 256 values and for a 10 bit digitizer there are 1024 values. With correct signal amplification the 10bit ADC results in approximately a 4x increase in dynamic range for all peaks within a single spectrum compared to the 8bit ADC.

Figure 5 (A) shows intensity vs concentration for a series dilution of the tripeptide Val-Tyr-Val (VYV) in 8 bit mode. Figure 5 (B) shows the same dilution series in 10 bit mode demonstrating an increase in linear dynamic range. Figure 5 illustrates the difference in vertical resolution between the 8 and 10 bit digitizer modes.

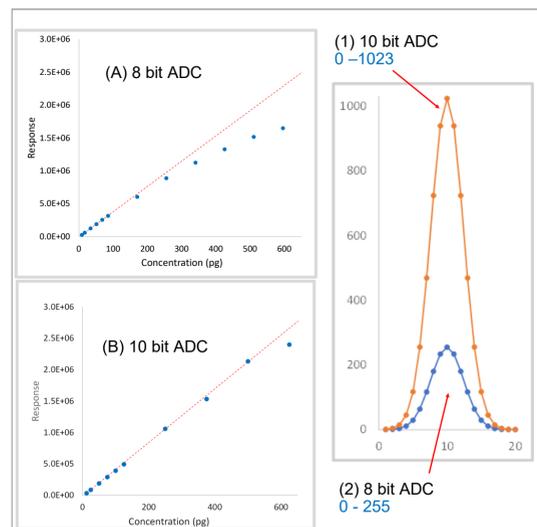


Figure 5. Quantification calibration curve VYV for 8 bit (A) and 10 bit (B) ADC. (C) Representation of signal from ion detector digitized with 8 bits (1) and 10 bits (2) illustrating 4x increase in dynamic range.

ADAPTIVE INTENSITY CONTROL

In ToF MRM mode, precursor ions selected by the analytical quadrupole, are subjected to collisionally induced dissociation (CID) to form product ions. The duty cycle of the ToF is optimized for a particular product ion using Enhanced Duty Cycle (EDC) mode by synchronizing orthogonal extraction with release of ions from the collision cell. High resolution extracted ion chromatograms (XIC) for the product ion are monitored during chromatography. In this mode the dynamic range of the instrument may be optimized for this specific product ion alone by dynamically adjusting the transmission of the instrument as the target compound elutes.

Figure 6 shows the simplified flow diagram representing the Adaptive Intensity Control method. For each target ion, the required transmission is calculated using a predicted ion arrival rate based on the rate-of-change of signal at each retention time. The transmission is adjusted using an attenuation lens between the quadrupole and the CID cell. This lens is capable of deterministically adjusting transmission from 100%-1%. The displayed spectrum is rescaled to account for the attenuation factor to maintain quantitative performance.

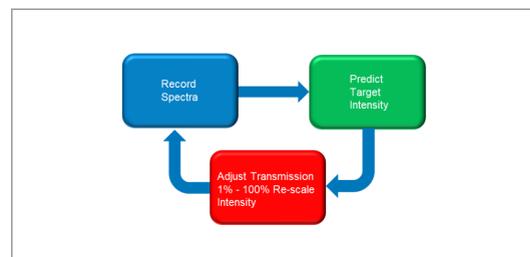


Figure 6. Simplified flow diagram illustrating the Adaptive Intensity Control (AIC) method.

Figure 7 shows the results from a series dilution of the tripeptide VYV, (30fg—3ng on column) monitoring the transition m/z 380.2 to 136.1. Figure 7 (A) without AIC. Figure 7 (B) with AIC. The spectral acquisition rate was 30 spectra/sec. AIC improves the linear dynamic range by ~10X from 4 to 5 orders of magnitude.

Figure 8 shows ToF-MRM for a selection of reference compounds acquired in three functions. Plots of Transmission vs time are shown for each function. The attenuation applied for each analyte is independently calculated and controlled.

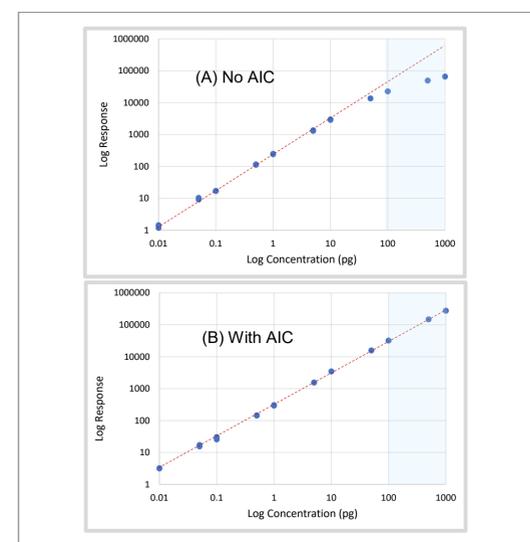


Figure 7. Quantification calibration curve 30fg—3ng VYV (A) With AIC off showing 4 orders of dynamic range (B) with AIC on showing 5 orders of dynamic range

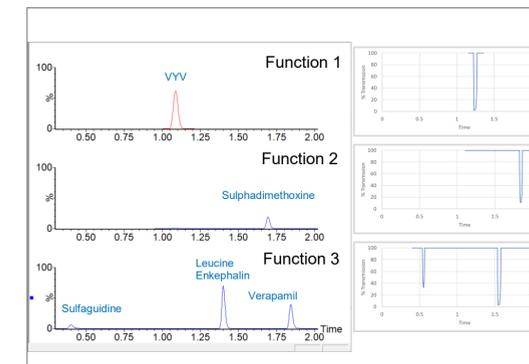


Figure 8. ToF MRM chromatograms for a range of reference compounds over three functions. Transmission vs time independent for each function and each analyte within each function.

ROBUSTNESS

Control of the spatial and energy spread of the ion beam as it enters the orthogonal pushout region of the instrument is critical to obtain high mass resolution. Contamination of the surfaces of the transfer optics (Figure 1) can lead to charge build up and subtle changes in focussing degrading performance.

To alleviate this issue the transfer optics were modified to allow compensation for mechanical misalignments and the tuning solution optimized to minimize ion impact without compromising mass resolution.

Figure 9 (A) and (B) Show SIMION™ ion optical modelling of the transfer region. Figure 9 (A) shows a previous tuning solution illustrating the region of ion impact. Figure 9 (B) shows the optimized tuning solution.

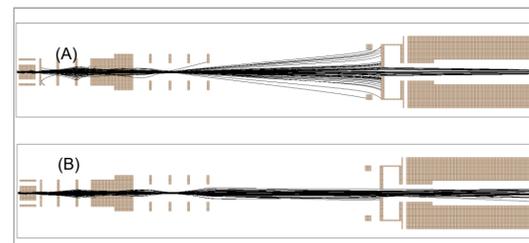


Figure 9. (A) SIMION™ ion optics model, original tuning solution. (B) optimized tuning solution with modified transfer optics.

To examine the effect of the new transfer ion optics and tuning solution the instrument was subjected to a 'rapid aging' protocol specifically designed to highlight the effect of surface charging on mass resolution. Repeated injections of a 6 component protein mixture were performed using a fast chromatographic gradient. Figure 10 shows a table of the mixture used. 409ng total protein was introduced in each injection.

ACQUITY UPLC Conditions: Accelerated Aging

Column: ACQUITY UPLC Protein BEH C4
 Mobile Phase: A. Water + 0.1% FA
 B. Acetonitrile + 0.1% FA
 Gradient: 19%B with a linear ramp to 60%B at 15 minutes
 Column temp: 80°C
 Flow rate: 0.2mL/min

Protein Sample	Molecular Weight, MW	SwissProt Sequence Information	Part Number (Sigma)
Ribonuclease A, bovine pancreas	13,700 K	P61823*	R5500
Cytochrome c, horse heart, 96%	12,384 K	P00004*	C7752
Albumin, bovine serum, 96-99%	66,430 K	P02769*	A8022
Myoglobin, horse heart >90%	16,950 K plus the heme group 616 K Total:17,600 K	P68082	M1882
Enolase, from baker's yeast (S. cerevisiae)	46,671 K	P00924	E6126
Phosphorylase b, rabbit muscle	97,200 K	P00489	P6635

Figure 10. Six Component Protein mixtures used for rapid aging

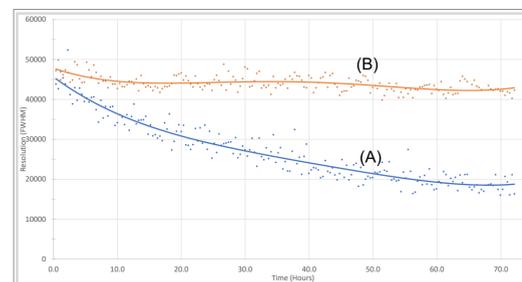


Figure 11. Resolution (FWHM) vs time, accelerated aging test (A) original transfer optics, (B) modified transfer optics.

Figure 11 shows a plot of Resolution FWHM vs time during the 'rapid aging' experiment for original tuning solution (A) and optimized tuning solution (B). This test suggests a marked improvement in robustness of the instrument employing the modified transfer optics and new tuning solution.

CONCLUSION

- StepWave XS gives 10 X improvement in sensitivity for thermally labile compounds.
- 10Bit ADC increases in-spectrum dynamic range by~4X for all ions within spectrum.
- AIC increases dynamic range for targeted analysis by ~10X.
- Modified transfer optics increase robustness and system up-time.
- This combination of technologies represents a significant enhancement in performance.

References

1. Proceedings of the 64th ASMS conference on mass spectrometry, San Antonio, 2016
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