[POSTER NOTE]

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

# METHOD OPTIMIZATION OF A NOVEL SCANNING QUADRUPOLE DIA METHOD FOR SYSTEM-LEVEL OMICS

*Chris Hughes, Keith Richardson, Jason Wildgoose, Johannes PC Vissers and James I Langridge*
*Waters Corporation, Wilmslow, United Kingdom*

## OVERVIEW

- **Systematic method and parameters optimization of SONAR™ scanning quadrupole DIA**
- **Qualitative and quantitative performance of this novel DIA acquisition method was characterized as a function of various specificity, and selectivity parameters, including speed of analysis (gradient), on-column amount and scan time**
- **An extension, Multistep SONAR™ (optimized collision energy ramps as a function of precursor *m/z* and retention time) that affords increased coverage across the largest quantitative dynamic range, is described**

## INTRODUCTION

A novel DIA mode of operation, SONAR, which provides both qualitative and quantitative information, has been developed and implemented on a hybrid quadrupole/oa-time-of-flight (Q-ToF) MS. Here,next to the acquisition principle itself, we describe the optimization of analytical parameters such as sample loadings, scanning quadrupole isolation window, scan speed, and *m/z* range dependent collision energy ramps, to obtain the best qualitative and quantitative coverage of complex proteomic samples. The effect of these parameters on the qualitative and quantitative performance in relation to amount of consumed sample was studied in detail. Complex proteins digest samples from *E.coli*, plasma and two human cell lines were analyzed and evaluated using both discovery and targeted informatics tools.

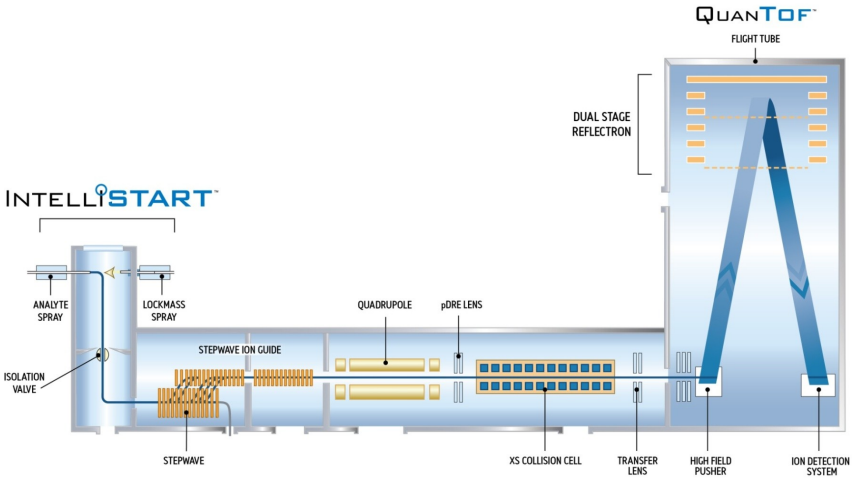



*Figure 1. Xevo G2-XS QTof mass spectrometer instrument schematic.*

# METHODS

### Sample preparation

A standard comprised a four protein digest mixture (Waters Corporation), E. Coli tryptic digest (Waters Corporation), HeLa cell lysate digest (Pierce) and K562 Cell Line (Promega) were used for method optimization and evaluation. Plasma samples were from Innovative Research Inc.

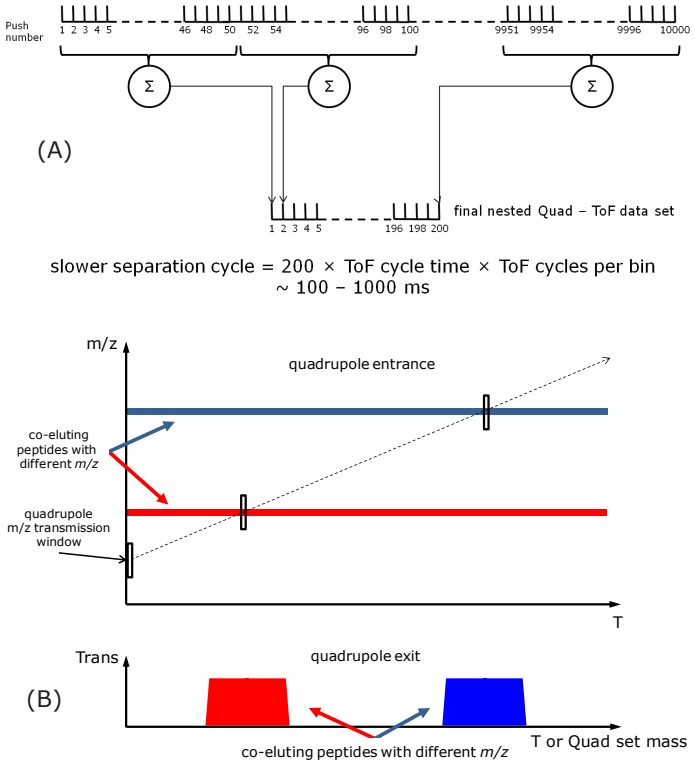
### LC-MS conditions

Label-free LC-MS was used for qualitative and quantitative peptide analyses. Experiments were conducted using various reversed phase gradients from 3 to 40% acetonitrile (0.1% formic acid) at 300 nL/min using a M-Class system (Waters Corporation) and a HSS 1.8 µm 75 µm x 25 cm nanoscale LC column. In addition, a CSH 300 µm x 10 cm column was used at 7µL/min.

A Xevo G2-XS QToF (Waters Corporation), Figure 1, was operated in SONAR mode. The optimized quadrupole window and other parameters employed for peptide analyses are described in Figure 2. MS data were collected using a continuously scanning quadrupole, which isolates specific m/z regions prior to the oa-ToF. Alternate MS scans therefore contain precursor and CID product ions respectively.

### Bioinformatics

SONAR™ DIA data were processed using Scaffold (Proteome Sciences), Progenesis QI for Proteomics (PQIp) Nonlinear Dynamics and ProteinLynx Global*SERVER* (Waters Corporation) using optimized threshold and search parameters. Targeted quantitative analysis was performed by Skyline (University of Washington) using libraries derived from PQIp and PLGS protein database searches. Visualization of the multidimensional data was conducted with DriftScope (Waters Corporation) or development software.



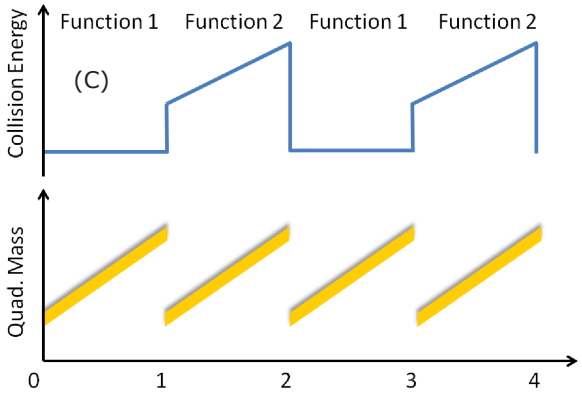




*Figure 2. SONAR™ DIA method and acquisition parameters, illustrating (A) ADC acquisition time scales, (B) quadruple scan, and (C) acquisition methods (CE ramped as a function of quadrupole m/z).*

# RESULTS

### Specificity

The specificity of the method is illustrated in Figure 3 to 5, showing precursor to product ion relationships, and the specificity gains *vs.* wide band DIA ($MS^E$), respectively. Figure 3 shows a 3 dimensional perspective of the data and that product ions are readily identify as they reside in the same m/z bin as their originating precursor. This is also illustrated in Figure 4, where the precursor (top) and product ion (bottom) quadrupole scanning profiles are shown for a tryptic peptide. The spectral clean-up is shown in more detail in Figure 4, where SONAR™ DIA sub-spectra are contrasted with a wide band DIA ($MS^E$) spectrum.

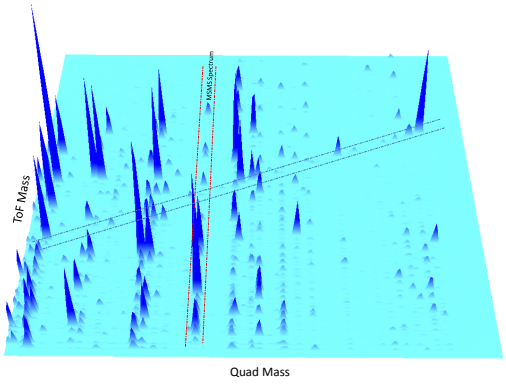



*Figure 3. SONAR™ DIA ToF vs. quadrupole m/z data, showing product ions (vertical bands) from peptides eluting over a 1 min window and the quadrupole sweep (diagonal line).*

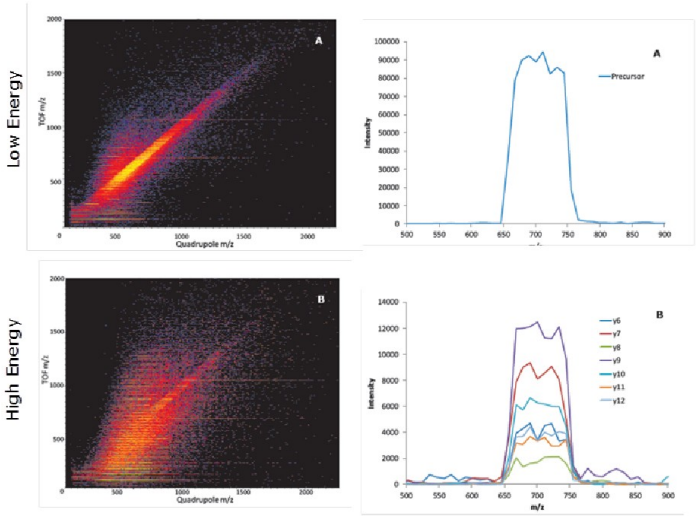



*Figure 4. Two-dimensional SONAR™ DIA ToF vs. quadrupole m/z precursor (A) and product ion (B) data of a 4 protein digest mixture and reconstructed quadrupole profiles of peptide VIELQGIAGTSAAR from RBSB_ECOLI (P02925).*

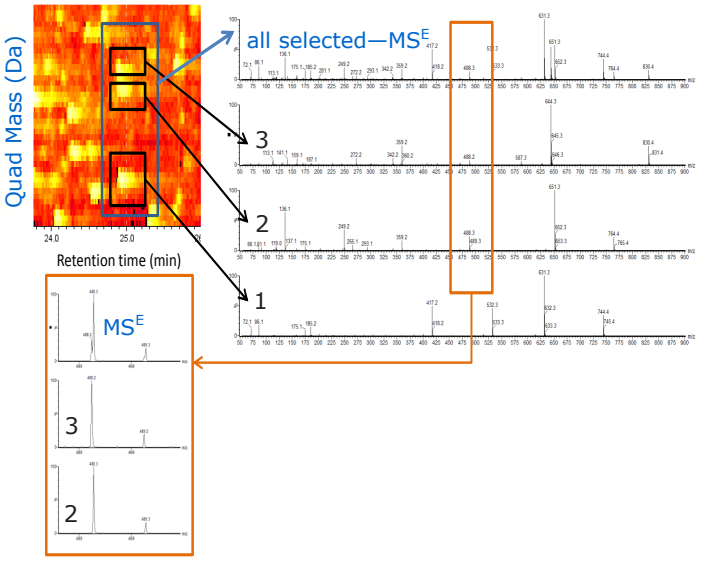



*Figure 5. SONAR™ DIA specificity improvement showing a small product ion region (top left) with 3 co-eluting peptides. Regions can be extracted to produce individual spectra (right, bottom 3 traces), along with DIA ($MS^E$) equivalent spectra (top). Bottom left shows a small region where 2 near isobaric fragments m/z (488 amu) are present, they can be separated and the interference removed using SONAR.*

### Isolation width, gradient, and duty cycly

The analytical parameters investigated and optimized included sample load on-column, quadrupole isolation width, multi m/z step acquisition mode, and applied collision energy. Results from this evaluation are shown in Figures 6 to 8.

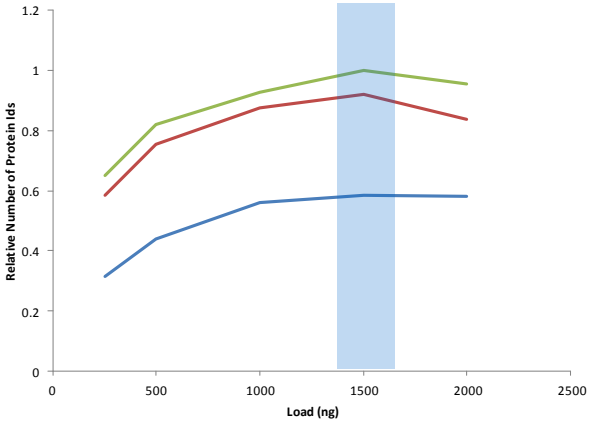



*Figure 6. SONAR™ DIA HeLa protein IDs vs. sample load at different scanning quadrupole transmission widths (blue = 13 Da, red = 23 Da, and green = 28 Da). Similar optima were obtained for samples with a range of complexities, including plasma and E.Coli lysate.*

The results shown in Figures 6 and 7 illustrate the balance between sensitivity and selectivity displaying the qualitative search results and ion current as a function of the protein digest load and quadrupole isolation window. With small isolation windows, sensitivity and duty cycle are challenged and with too wide a quadrupole window, specificity becomes the limiting factor from a database search perspective. In the latter case, a targeted data analysis approach is preferred. When throughput increases, rapid scan times become critical, not only from a precision perspective, but also from a qualitative identifications, as illustrated by the results shown in Figure 7.

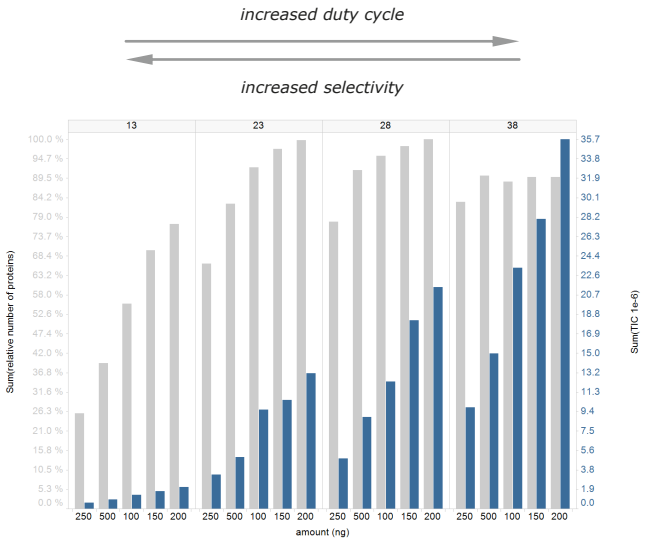



*Figure 8. SONAR™ DIA E. coli protein identification rates (grey) and total ion current values (blue) vs. protein load and quadrupole isolation window (trellis).*

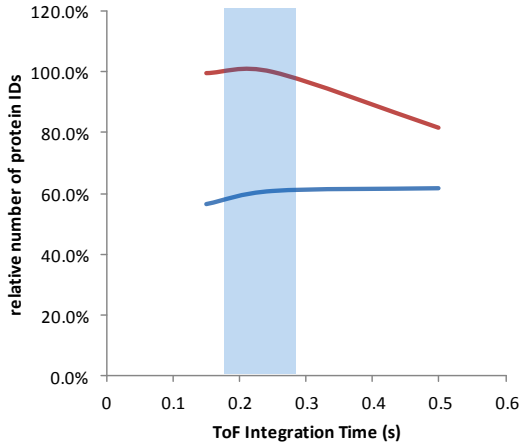



*Figure 7. SONAR™ DIA HeLa protein IDs vs ToF integration time at very fast LC gradients for the complexity of the HeLa sample. A relative reduction in IDs compared with the data shown in Figure 6 is to be expected, but there is an optimum of 0.25 s for the 45 min (red) vs. the 30 min (blue) gradient, suggesting matching the peak width with scan time is important for post processing and quantitative measurements, i.e. under sampling.*

**Multistep SONAR™ DIA**

The potential to optimize collision energy as a function of precursor m/z and retention time, maximizing coverage, is shown in Figures 9 to 11. Figure 9 shows distinct retention time regions, having different m/z ranges and collision energies i.e. hydrophobicity dependent. The evaluated collision energy ramps are summarized in Figure 10, with a results overview shown in Figure 11, suggesting that multistep methods have the potential to increase coverage and subsequent quantitation by > 20%.

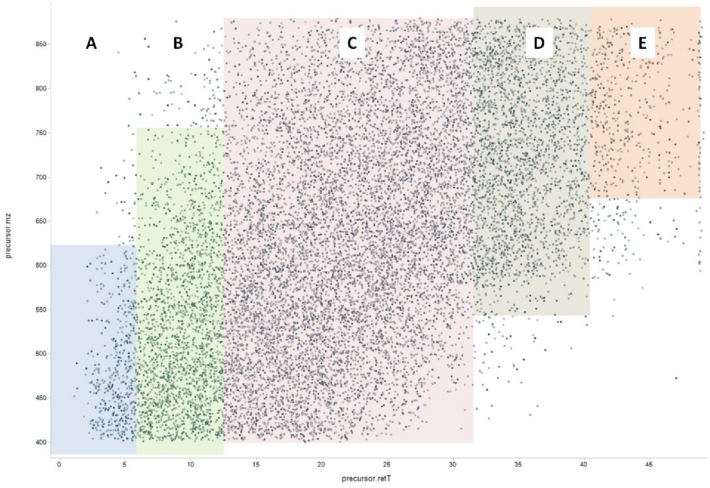



*Figure 9. SONAR™ DIA 6 µg K562 cell line. Identified precursor m/z (2, 3+ only) vs. LC retention time (300 µm i.d. scale, 45 min gradient). The plot shows 5 regions where further m/z and CE optimisation would be feasible.*

| Region | Quad Scan (Da) | Collision Energy (V) |
|---|---|---|
| A | 350 - 620 | 16 -25 |
| B | 350 -750 | 16 - 30 |
| C | 400 - 900 | 18 - 35 |
| D | 550 -950 | 23 - 36 |
| E | 680 - 950 | 27 - 36 |

*Figure 10. SONAR™ DIA multistep experiment design. Multiple steps allow different mass ranges and hence collision energies to be applied. All other parameters, i.e. TOF mass range and integration time, quadrupole isolation width were maintained as in the single step experiments.*

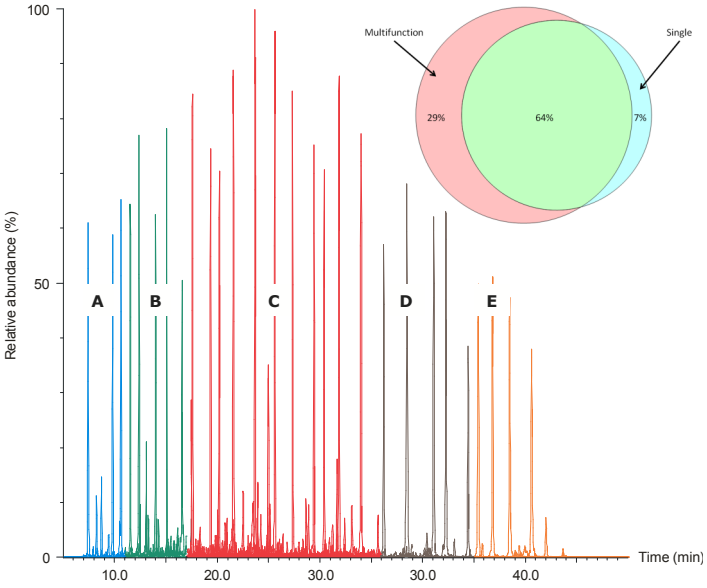



*Figure 11. Multistep SONAR™ DIA (m/z, collision energy) optimised, reconstructed XICs. Shown inset is the relative increase (22%) in the number of identified peptides vs. the single step method.*

### Open source informatics

Displayed in Figure 12 is the quantitative analysis of SONAR™ DIA data using Skyline. Shown clockwise are the product ion XICs, the corresponding library entry of searching the data, multidimensional extraction of the product ions (quadrupole y-axis; ToF *m/z* x-axis), as well as a 5 point calibration curve spanning more than 3 orders of dynamic range, illustrating quantitative performance.

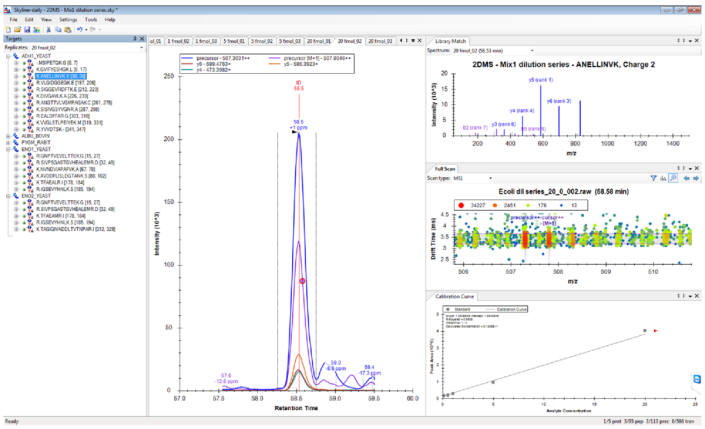



*Figure 12. Targeted analysis — retention time, quadrupole m/z (precursor) and ToF m/z (product ion) ion extraction and detection for a 4 protein digest mixture spiked and serially diluted into E.coli.*

## CONCLUSION

- **SONAR™ DIA acquisition provides multi dimensional data sets exhibiting improved specificity over other (wide band) DIA methods**
- **SONAR™ acquisition parameters for omics experiments, i.e. quadrupole isolation window *vs.* on-column load, scan vs. gradient time, and multi step experiments, can be easily determined for optimal qualitative and quantitative performance**
- **SONAR™ DIA can be applied in discovery and targeted translational omics studies, including metabolomics, lipidomics and proteomics**
- **Further work will include the targeted extraction of ions to investigate maximum coverage, quantitative precision and throughput (as a function of analysis time)**

### References

1. JL Wildgoose, K Richardson and M Green, Two Dimensional MS-MS on a Q-Tof Utilising a Scanning Quadrupole Mass Filter and an Ultra Fast Data Acquisition System, ASMS 2015, poster WOx 476