

A Comparative Qualitative and Quantitative Assessment of SONAR for High-Throughput Proteomic Applications

Christopher Hughes, Lee A. Gethings, Robert S. Plumb, James I. Langridge

Waters Corporation

For research use only. Not for use in diagnostic procedures.

Abstract

This application note describes a qualitative and quantitative protein identifications using SONAR mode of acquisition.

A comparative study of DIA methodologies show qualitative improvements with increased protein identification numbers for data acquired using SONAR when compared against SWATH. Results based on varying gradient length showed SONAR to provide significantly more protein identifications than SWATH, particularly when adopting a shorter chromatographic timescale, i.e. the utilization of capillary LC for improved throughput and robustness. Reducing the gradient from 60 to 30 min resulted in only a marginal difference in protein identification rates for SONAR, whereas comparative SWATH data showed a decrease of 23%. The rapidly scanning nature of the quadrupole for SONAR also provides quantitative benefits, with precision being maintained for high throughput analysis.

Benefits

Qualitative improvements with increased protein identification numbers for data acquired using SONAR

when compared against SWATH. SONAR provides significantly more protein identifications than SWATH, particularly when adopting a shorter chromatographic timescale, i.e. the utilization of capillary LC for improved throughput and robustness.

Introduction

The continued requirement to analyze larger sample cohorts to detect quantitative biologically significant differences is becoming of greater importance and placing greater demands on instrument time. Traditionally, proteomic LC-MS analyses have been conducted using nanoscale chromatography in combination with data dependent analysis (DDA). However, the adoption of faster chromatography to increase sample throughput and data independent approaches (DIA) are proving increasingly popular. A number of DIA strategies with enhanced specificity exist, such as SWATH, whereby the quadrupole is stepped across a mass range of interest to increase specificity. However, this approach can have drawbacks when utilizing faster chromatographic methods since the duty cycle of the instrument is challenged. An alternate DIA method, which also uses a quadrupole analyzer for additional selectivity, is SONAR, whereby the quadrupole is scanned as opposed to being stepped over the mass range of interest. The fast scanning nature of the method makes the technique particularly suited for fast chromatographic, high throughput workflows. Here, we present results from a comparative DIA experiment set using a tryptic digest of the K562 cell line, separated using capillary scale chromatography and MS data acquired using SWATH (stepped quadrupole) and SONAR (scanning quadrupole) modes of acquisition.

Experimental

Sample preparation

K562 cell line tryptic digest standard (Sigma-Aldrich, St. Louis, MI)

LC conditions

LC system:

M-Class ACQUITY UPLC

Column(s):	1.8 μm HSS T3 C ₁₈ 300 μm x 100 mm NanoEase analytical
Column temperature:	35 °C
Flow rate:	7 $\mu\text{L}/\text{min}$
Mobile phase:	Water (0.1% formic acid) (A) and acetonitrile (0.1% formic acid) (B)
Gradient:	3% to 40% B in 30 or 60 min
Injection volume:	1 μL (1, 5 or 10 μg)

MS conditions (SONAR)

MS system:	Xevo G2-XS QTof
Ionization mode:	ESI (+) at 3.2 kV
Cone voltage:	30 V
Acquisition mode:	SONAR
Acquisition range:	50 to 2000 m/z both functions (low and elevated energy)
Acquisition rate:	0.5 s both functions (low and elevated energy)
Quadrupole scan range:	400 to 900 m/z
Isolation window:	24 Da
Collision energy:	5 eV (low energy function) and from 19 eV to 45 eV (elevated energy function)

Resolution: 35,000 FWHM

MS conditions (SWATH)

MS system: Sciex TripleTOF 5600

Acquisition mode: SWATH 50 m/z to 2000 m/z both functions

Quadrupole window: 60 target windows (variable) stepping
quadrupole with 1 Da overlap

Informatics

The LC-MS data were processed with Mascot Distiller (Matrix Science, London, United Kingdom) and Spectronaut Pulsar (Biognosys AG, Schlieren, Switzerland). Data were searched against a study specific K562 library.

Results and Discussion

The principle of SONAR, a scanning quadrupole based data independent acquisition (DIA) method, is illustrated in the left hand side image of Figure 1. In short, alternate datasets are acquired in low (MS1) and elevated (MS2) collision energy mode.^{1,2} During each low and elevated energy segment, the quadrupole isolation window is scanned linearly between two user-selected positions and 200 TOF spectra are acquired. The quadrupole scan duration is application/chromatographic peak width dependent and typically varies from 0.1 s to 1 s. In the elevated energy mode, the collision energy can be ramped between two values, which are selected to optimize fragmentation efficiency at each quadrupole position. The selectivity of the acquisition method is illustrated by the middle image where, dependent on the position of the quadrupole, i.e. transmission m/z window, precursor (even those close in mass) and product ions can be exclusively isolated.

The requirement for acquisition speed is clearly demonstrated in Figure 2, showing a 50 Da wide mass extracted chromatogram for a 30 min reversed phase gradient separation of non-fractionated K562 tryptic digest. Shown inset is a 10 mDa wide mass extracted chromatogram over a narrow chromatographic window of 30 s. Typical peak widths at half height were 3 s; hence, to retain a sufficient number of points across the

peaks for precise quantitation while maintaining optimum S/N, the scan speed was set to 0.5 s, providing between six and eight data points across a peak. The peak capacity for 30 min high throughput proteomic separations was estimated to be ~ 450 . The importance of peak sampling frequency and its effect on quantitative precision is described in more detail elsewhere¹

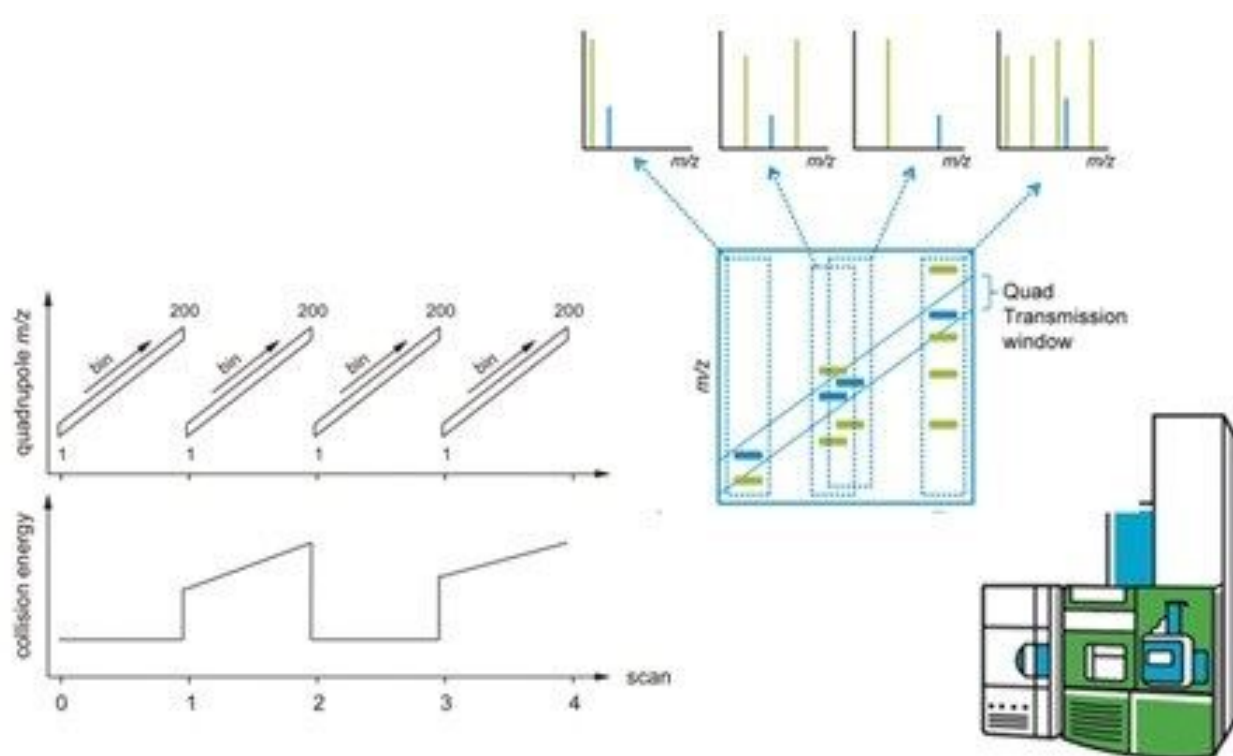


Figure 1. A schematic of the SONAR acquisition method showing the behavior of the collision energy, which alternates between a constant low value and a linear ramp to generate informative precursor and fragmentation data, respectively, for as many peptide precursor ions as possible. The quadrupole scans with the same parameters in both the low and high energy CID scans.

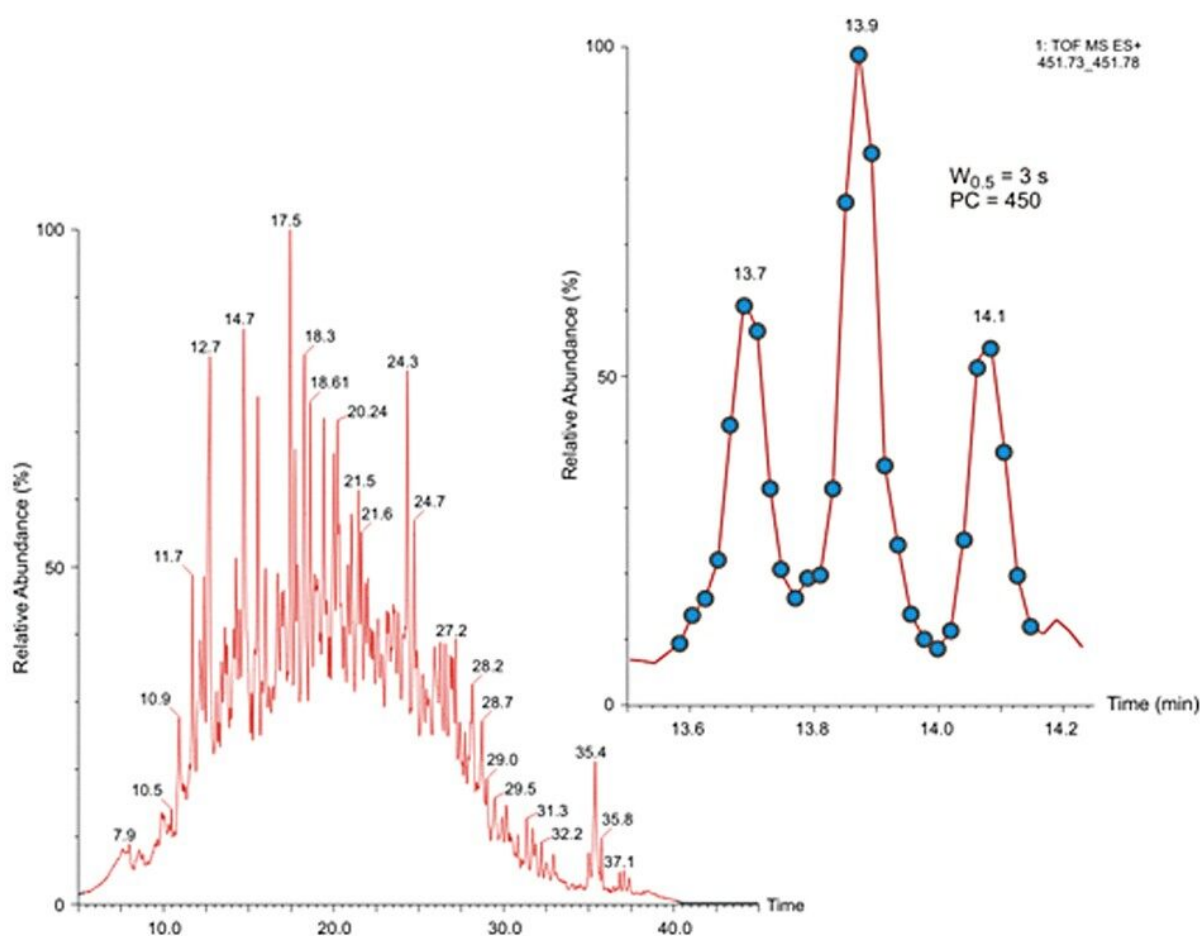


Figure 2. Mass extracted chromatogram from m/z 700-750 illustrating the complexity and chromatographic density for 5 μ g of K562 tryptic cell line digest analyzed on a 300 μ m i.d. reversed phase column and eluted at 7 μ L/min (see Experimental section for more detailed information). Shown inset is a small portion, ~ 0.6 min, of a narrow range XIC from m/z 451.73-451.78 that shows the average peak width at half height is approximately 3 seconds from which a peak capacity (PC) of 450 can be derived.

The benefit of high speed quadrupole scanning vs. quadrupole stepping with respect to selectivity is presented in Figure 3, where the same sample and amount were analyzed under identical chromatographic conditions. Five mass extracted product ion chromatograms corresponding to fragment ions of the same peptide are shown. The upper pane, representing a stepped quadrupole SWATH acquisition, identified all five product ions of interest; however, it can also be seen that both y_3 and y_4 were interfered, affecting quantitative precision. In contrast, the SONAR method afforded non-interfered detection and extraction of the chromatographic of the same set of fragment ions.

Increased acquisition speed also has a profound effect on qualitative performance, especially in the instance of limited amount(s) and/or increased throughput experiments. The results shown in Figure 4 and Table 1 summarize the number of K562 protein groups (based on a 1 µg loading) identified by Spectronaut informatics. Interpretation of 30 and 60 min gradient separations for three technical replicates indicates a significant increase in protein identifications for SONAR when comparing the two techniques. For the 60 min gradient, approximately 300 additional proteins are identified with SONAR; however, greater gains are observed with the shorter 30 min gradient, with approximately 600 more identifications being achieved when implementing SONAR. The confidence of the identifications in both cases is further exemplified with the peptide retention alignment precision between the observed and library entries.

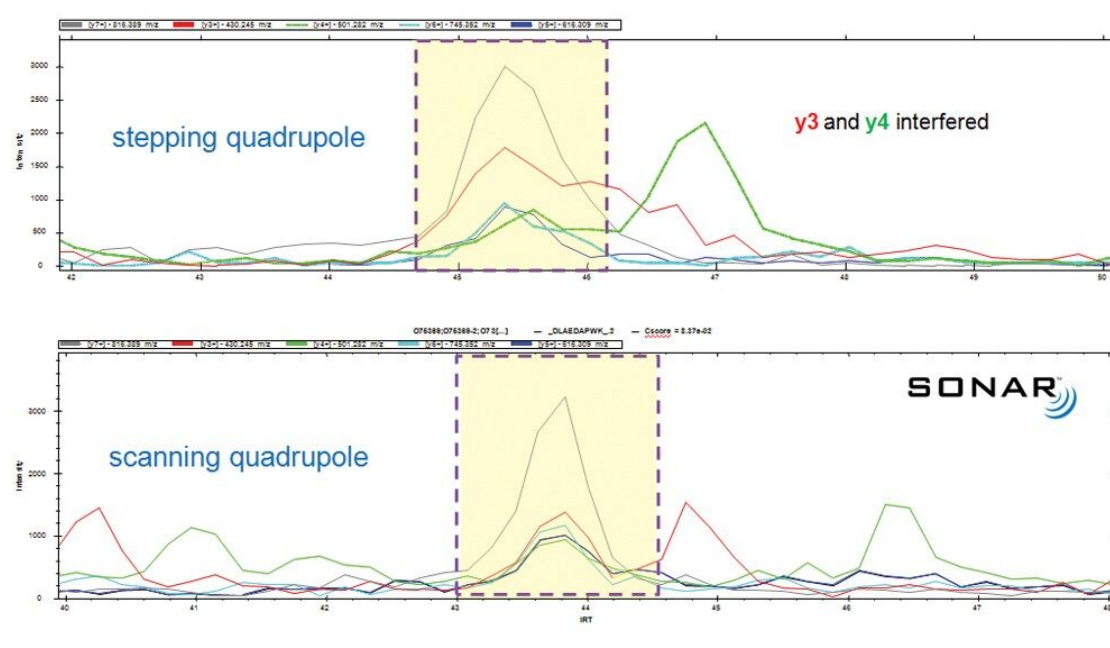


Figure 3. Targeted search identification of a peptide showing the selectivity difference between SWATH (stepping quadrupole DIA) and SONAR (scanning quadrupole DIA). In the instance of the SWATH experiment, both y3 and y4 are shown to be interfered.

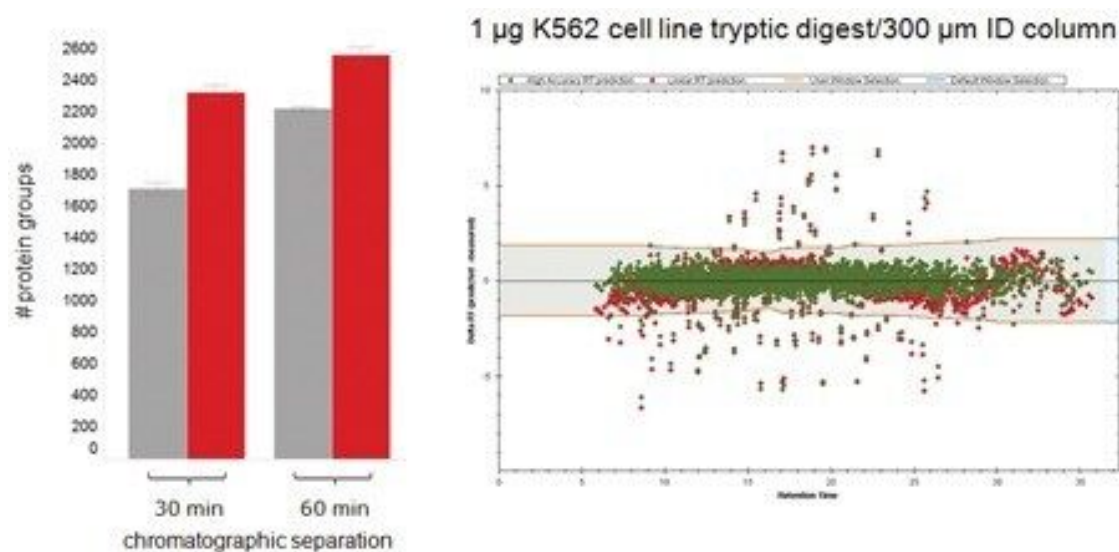


Figure 4. Qualitative performance in terms of identified protein groups SWATH (grey) and SONAR (red) for technical replicates ($n=3$) of 1 μg of K562 tryptic cell line digest analyzed on a 300 μm i.d. reversed phase capillary LC column. Shown right is the retention alignment precision of the observed vs. library entries created from multiple gas phase fractionated DDA experiments.

	30 min RP gradient		60 min RP gradient	
	SWATH	SONAR	SWATH	SONAR
# protein groups	1748	2372	2240	2531
	1725	2323	2213	2531
	1673	2382	2212	2620
# protein groups	1715	2325	2221	2560
(average)				
%CV	2.2	2.0	0.7	2.0

Table 1. Qualitative performance in terms of identified proteins groups for SWATH and SONAR data acquisitions over 30 and 60 min gradients.

Conclusion

A comparative study of DIA methodologies show qualitative improvements with increased protein identification numbers for data acquired using SONAR when compared against SWATH. Results based on varying gradient length showed SONAR to provide significantly more protein identifications than SWATH, particularly when adopting a shorter chromatographic timescale, i.e. the utilization of capillary LC for improved throughput and robustness. Reducing the gradient from 60 to 30 min resulted in only a marginal difference in protein identification rates for SONAR, whereas comparative SWATH data showed a decrease of 23%. The rapidly scanning nature of the quadrupole for SONAR also provides quantitative benefits, with precision being maintained for high throughput analysis.

References

1. Gethings LA et al. Lipid profiling of complex biological mixtures by liquid chromatography/mass spectrometry using a novel scanning quadrupole data-independent acquisition strategy. *Rapid Commun Mass Spectrom*. 2017 Oct 15;31(19): 1599–1606. doi: 10.1002/rcm.7941.
2. Moseley AM et al. Scanning Quadrupole Data Independent Acquisition – Part A. Qualitative and Quantitative Characterization, *J Proteome Res*. 2017 Sep 13. doi: 10.1021/acs.jproteome.7b00464. [Epub ahead of print]
3. Bruderer R et al. High-precision iRT prediction in the targeted analysis of data-independent acquisition and its impact on identification and quantitation. *Proteomics*. 2016 Aug;16(15-16): 2246–56. doi: 10.1002/pmic.201500488.

Featured Products

SONAR for Omics <<https://www.waters.com/134909480>>

Xevo G2-XS QToF Time-of-Flight Mass Spectrometry <<https://www.waters.com/134798183>>

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

Available for purchase online

ACQUITY UPLC M-Class HSS T3 Column, 100Å, 1.8 µm, 300 µm X 100 mm <
<https://www.waters.com/waters/partDetail.htm?partNumber=186007560>>

720006161, January 2018

©2019 Waters Corporation. All Rights Reserved.