

Effect of SONAR Quadrupole Window Size on LC-MS Sensitivity and Selectivity

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GOAL

To evaluate the effect of SONAR™ quadrupole window size on sensitivity when narrowing the window to gain higher selectivity in discovery omics analysis.

BACKGROUND

The accurate identification of peptides, proteins, lipids, and small molecule metabolites in omics studies requires the generation of high quality precursor MS and product ion MS/MS data. Accurate mass data independent acquisition (DIA) LC-MS is frequently employed in metabolite identification and omics studies, providing a broad coverage of MS and MS² information simultaneously during an analytical batch.¹⁻²

DIA strategies implemented on QToF platforms have been demonstrated previously. For example, a highly specific DIA approach with use of a rapidly scanning quadrupole (SONAR) has shown to provide high specificity that is compatible with fast chromatographic separation.³ Quadrupole separation prior to fragmentation provides specificity allowing fragment ions to be readily correlated with their associated

SONAR DIA is for highly selective fragmentation spectra resulting in more efficient database searching.

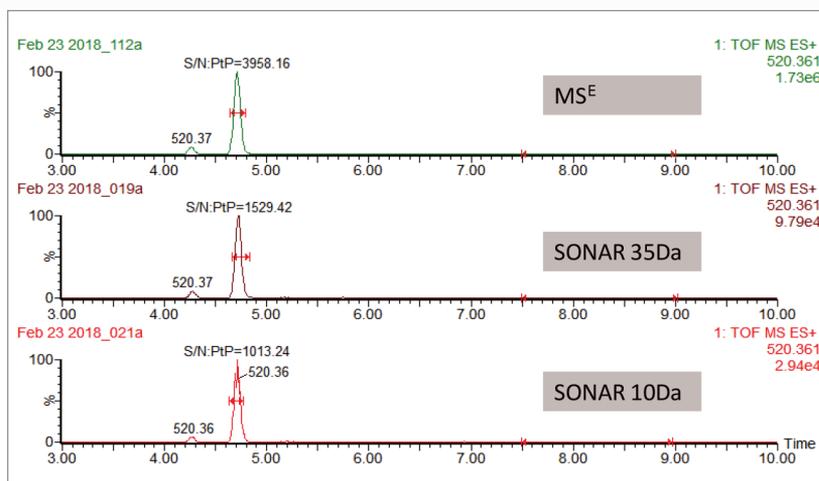


Figure 1. Lipidomic analysis of human serum using 10, 35 Da SONAR quadrupole window, and MS^E.

precursors, highlighting the advantages over techniques which do not use a quadrupole or alternative means of MS¹ isolation. SONAR is shown to provide increased MS² spectral quality and ultimately improved database search results.

Narrowing the quadrupole spectral window, provides cleaner spectra and therefore more efficient compound library searches ([720006036EN](#)). A narrow quadrupole window requires a fast scanning quadrupole to provide a sufficient number of points across an LC peak, to retain quantitative accuracy; however, this increased duty cycle can give rise to a reduction in sensitivity.

THE SOLUTION

SONAR is a novel DIA acquisition method that employs a narrow pass filter and rapid scanning quadrupole. The SONAR acquisition window can be varied from 5–35 Da allowing for a smaller or larger selection of ions prior to entering the collision cell. The effect of the quadrupole window on analytical system sensitivity was evaluated by analyzing the lipid profile of human serum using a reversed phase UPLC-MS method over 15 minutes with the ACQUITY™ UPLC™ I-Class System and Xevo™ G2-XS QTof.

Data were collected in positive ion mode using a SONAR quadrupole window which varied between 10 and 35 Da as well as an unbiased DIA acquisition (MS^E) and full scan MS over the TOF mass range of 100–1000 Da. The resulting data were processed and database searched using Progenesis™ QI Software. Results from the analysis allowed for the low energy peak count of each sample, average low energy peak count and total ion current intensity to be determined.

The data displayed in Figure 1 illustrates changes in the lipid assay sensitivity for SONAR based quadrupole window sizes of 10 and 35 Da in addition to MS^E. From this data, it is clear that the MS^E mode of analysis provided the highest response (based on overall total ion count (TIC)) with a maximum intensity of 1.73e6. The SONAR analysis using a 10 Da window produced a peak response of 2.94e4, while the 35 Da window produced a peak response of 9.79e4 (Table 1). The signal-to-noise for each peak was then compared to evaluate the usable sensitivity of each acquisition mode. The data showed that while full scan MS and MS^E acquisition modes were virtually equivalent, SONAR was shown to provide a signal response that was only 2.4 times lower than full scan MS mode for equivalent sample loading. Observing only a small reduction in signal response can be attributed to the substantial noise reduction observed with SONAR data, due to the enhanced specificity provided by the scanning quadrupole.

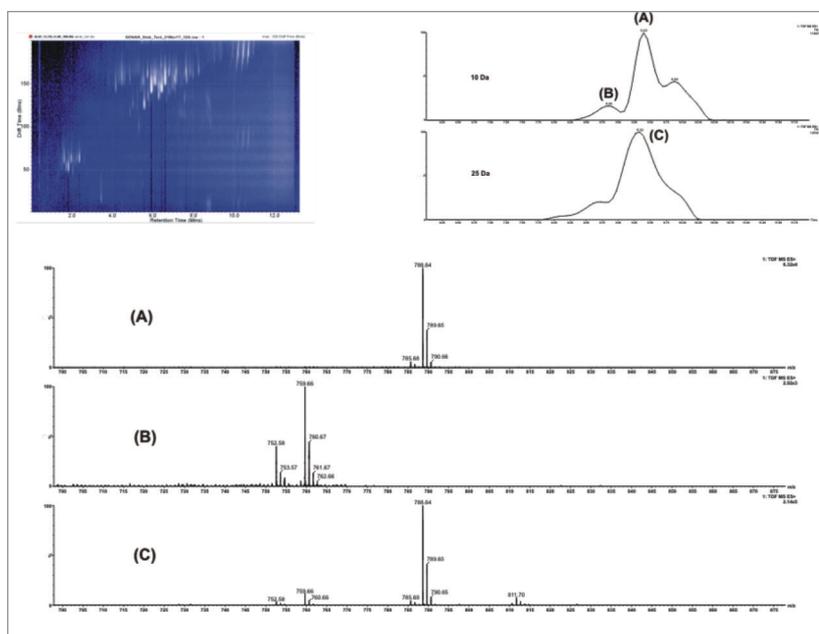


Figure 2. A representative two dimensional peak intensity map demonstrates the SONAR separation for human sera over the course of the gradient (upper left). Extracted profile plots of the peak intensity map (upper right) show increased definition and therefore selectivity for the 10 Da experiment. Extracted peaks of interest (lower spectra) highlight the additional selectivity gained using a 10 Da window (A, B) when compared with 25 Da (C).

Mode of analysis	Peak intensity (TIC)	Signal-to-noise (peak to peak)	Peak intensity compared to MS ^E (signal-to-noise)
MS ^E	1.73e6	3958	1.0
SONAR 10Da Window	2.94e4	1013	0.3
SONAR 35Da window	9.79e4	1529	0.4

Table 1. Comparison of low energy (MS¹) peak count with SONAR 10 and 35 Da quadrupole window size as well as MS^E.

The benefits of the 10 Da window size is illustrated in Figure 2 which shows increased selectivity when using narrower quadrupole settings. Comparing a region of interest highlights the gains in selectivity. Extracted spectra show cleaner spectra with discrete peaks due to enhanced specificity, with lipids of interest clearly distinguished.

SUMMARY

SONAR is a novel DIA acquisition for a hybrid quadrupole time-of-flight mass spectrometer (QToF), which employs a narrow pass, fast scanning quadrupole filter. This mode of operation produces cleaner MS² spectra resulting in more efficient database searching. When using a 35 Da window the SONAR mode of analysis showed a 2.4 fold reduction in detection sensitivity compared to full scan MS based on signal-to-noise (peak to peak). The impact of the quadrupole filter size on MS sensitivity and peak response has been evaluated from 10–35 Da using a lipid profiling method. The results showed that the 10 Da window delivered clear, superior LC-MS data quality while maintaining sufficient sensitivity for quantitative measurements.

References

1. Wrona, M.; Mauriala, T.; Bateman, K. P.; Mortishire-Smith, R.J.; O'Connor, D. *Rapid Commun. Mass Spectrom.* 2005, 19, 2597–2602.
2. Hopfgartner, G.; Tonoli, D.; Varesio, E. *Anal. Bioanal. Chem.* 2012, 402, 2587–2596.
3. Gethings, L.A, Richardson, K, Wildgoose, J., Lennon, S., Jarvis, S., Bevan, C.L., Vissers, J.P.C., Langridge, J.I. *Rapid Commun. Mass Spectrom.* 2017, 31, 1599–1606.

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