RESULTS

Elution profile optimization

With a column volume of 4.5 µL, visit volume, sample loading and column equilibrium can have a significant impact on instrument overhead. By using variable flow rate methods with high flow rate (750 nL/min) applied during the first 7 min of the gradient, and low flow rate (250 nL/min) applied as an elution start delay, excellent performance can be achieved at sample throughput. Chromatographic methods reported here are superior to any other reported long gradient separation with pulled-tip packed bed columns, improving peak width by 30% for 180-min gradient separations.

LC-MS method optimization

When varying isolation window size (4, 6, 8 and 10 Th) and combining with different scan ranges to yield at total of respectively 90, 80, 70 or 60 scan events, significantly higher coverage was achieved with the narrowed window size of 4 Th. With 100 scan events between 450 and 800, more than 15,000 protein groups could be successfully identified with high confidence.

Near-full proteome coverage for single species protein digest

As precursor ion mass to charge tends to increase slightly along the course of a reversed phase LC gradient, eight increases in coverage can be achieved by using an optimized DIA method where the position of 450 m/z wide scanning window is varied along the 180 min gradient. Up to 2% more protein groups and 4% more peptide groups were identified as compared a method with a fixed scanning window from 475 to 875 m/z.

Figure 5. Scanning window used for (A) fixed window method, (B) variable window method.

CONCLUSIONS

- 110 cm long µPAC Neo columns are an ideal match for deep-dive proteomics, delivering the sharpest peak width for long gradient separations.

- Flow rate and gradient optimization can reduce instrument overhead significantly and facilitate higher throughput.

- Data-independent acquisition strategies outperform conventional data dependent acquisition, both in absolute identification as well as in quantitation.

- Using narrow window DIA and 180 min LC gradients, near full proteome coverage can be obtained with Orbitrap MS and 110 cm µPAC Neo columns.

TRADEMARKS/LICENSES

© 2023 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is not intended to encourage or promote the use of these products in any manner that might infringe the intellectual property rights of others.