

Screening and Quantitation in Food Matrices Using Combined SWATH® + IDA Acquisition



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ABSTRACT

Confidence in a compound identification for a screening assay is greatly increased by making use of MS/MS data. It can be used for library searching, or measuring fragment ion ratios. However, obtaining an MS/MS for every compound in a run can be a challenge. SWATH® acquisition can acquire MS/MS that represent every precursor mass of interest at every time point. However, the deconvolution of MS/MS requires that each fragment ion have a different chromatographic profile or elution time. Information dependent acquisition (IDA) will consistently trigger on the most abundant compounds, but can occasionally miss some candidates. Combining SWATH acquisition with IDA improves the identification of compounds in screening assays.

INTRODUCTION

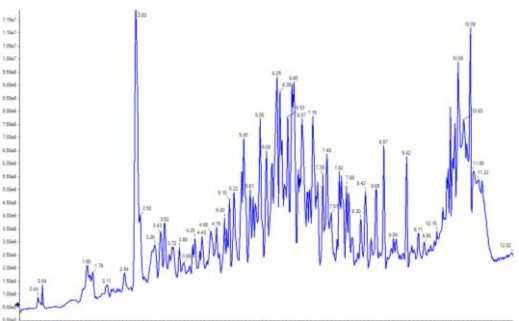
SWATH acquisition uses precursor isolation windows that typically range from 5-50 Da or wider. Even when two or more compounds fall within a SWATH acquisition precursor isolation window, and have similar elution times, deconvolution of the MS/MS is usually possible using a PCA/PCVG based technique¹. This MS/MS is used for library searching and subsequent compound identification. With IDA, the precursor isolation window is 1 Da wide, which greatly reduces, but does not eliminate, the chance of two compounds being in the same window. However, if IDA does not trigger on a candidate, there is no MS/MS data to confirm a compound identification with.

- **SWATH acquisition and IDA methods run separately on tea and orange samples spiked with pesticides**
- **Combining SWATH + IDA in one acquisition method and measuring its impact on confidence in identification using targeted screening**

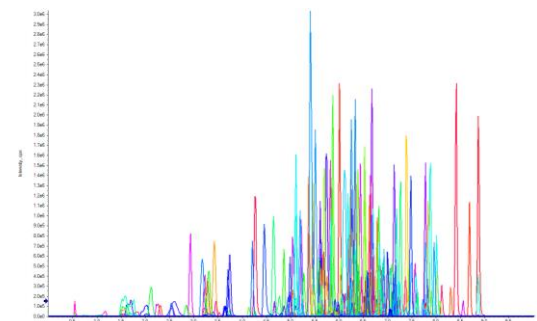
MATERIALS AND METHODS



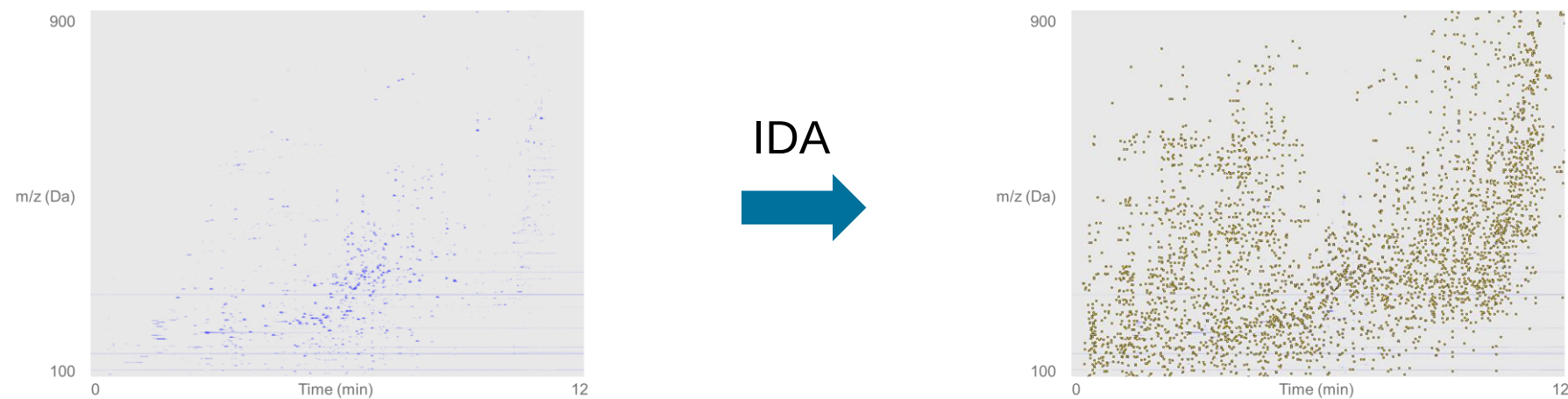
Tea or orange samples were prepared using QuEChERS protocol.
SCIEX Exion LC
Phenomenex Kinetix Polar C18 100x2.1 mm
2% to 95% in 10 minutes + 5 minutes re-equilibration
X500R QTOF
0.25 sec MS1
0.7 sec on either IDA, or SWATH acquisition, or a combination
SCIEX OS 1.4



TIC



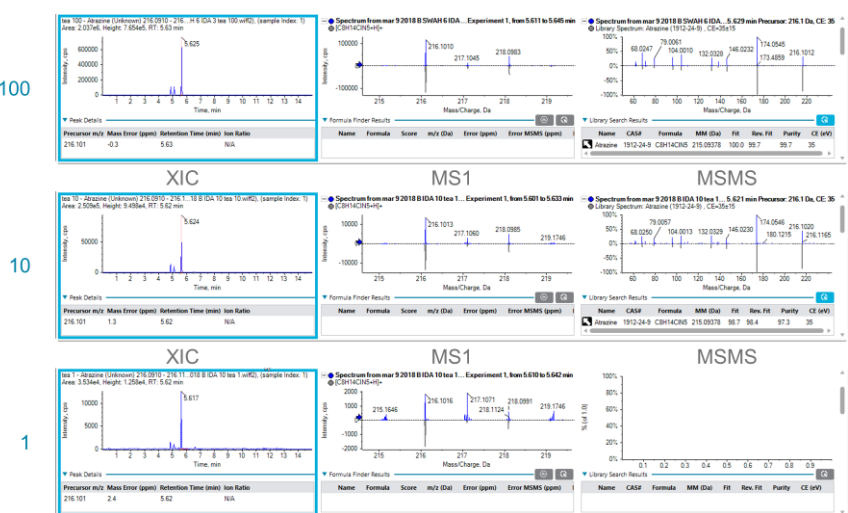
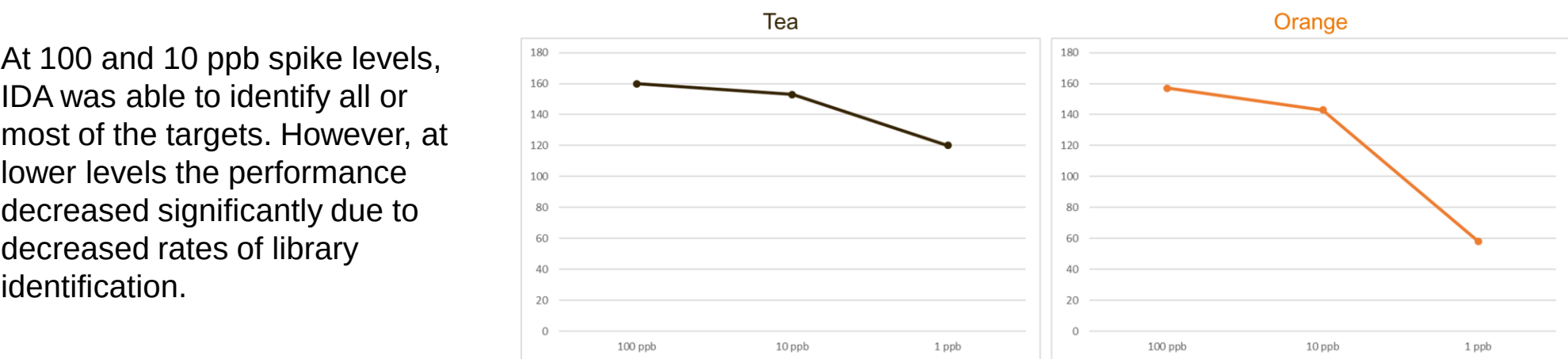
XIC of 160 pesticides at 100 ppb



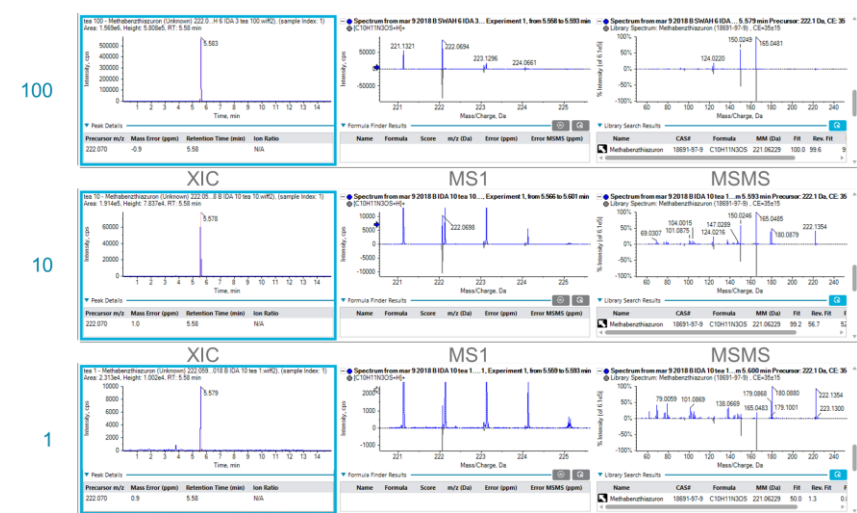
Tea sample spiked with 100 ppb of pesticides displayed as a contour map. Using ImageJ (Fiji), every IDA trigger was overlaid on this contour plot. The vast majority of features are covered by an IDA event. Also, features that do not display any chromatographic resolution (smears across the entire run) are not triggered on.

Mass Error Confidence	Mass Error (ppm)	RT Confidence	Retention Time Delta (min)	Library Confidence	Library Score	Library Hit
✓	0.4	✓	0.01	✓	95.5	3-Hydroxyprothion
✓	0.5	✓	0.00	✓	90.9	Atrazine
✓	-2.1	✓	0.00	✓	100.0	Atrazine
✓	0.7	✓	0.01	✓	95.4	Atrazine
✓	0.2	✓	0.01	✓	95.1	Atrazine
✓	-2.3	✓	0.00	✓	100.0	Atrazine
✓	-1.7	✓	0.01	✓	100.0	Atrazine
✓	-0.9	✓	0.00	✓	100.0	Atrazine
✓	-2.7	✓	0.01	✓	100.0	Atrazine
✓	2.3	✓	0.04	✓	94.7	Atrazine
✓	0.1	✓	0.00	✓	100.0	Atrazine

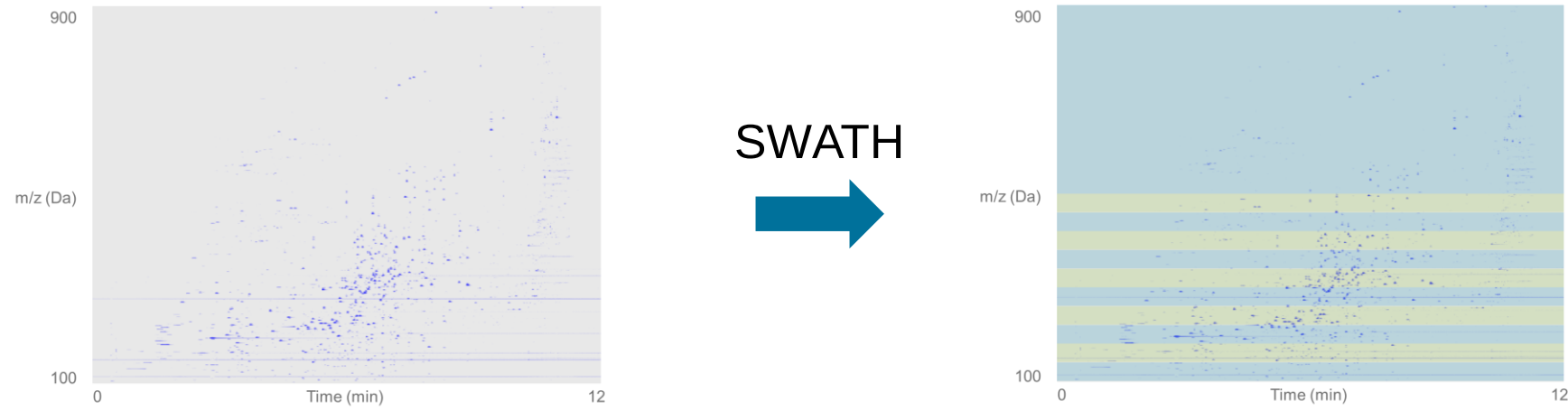
SCIEX OS 1.4 Analytics module was used for screening the samples using a targeted method. A positive identification was called when the precursor mass accuracy was less than 5 ppm, the retention time was within 0.05 minutes of expected, and the library score was > 70%.



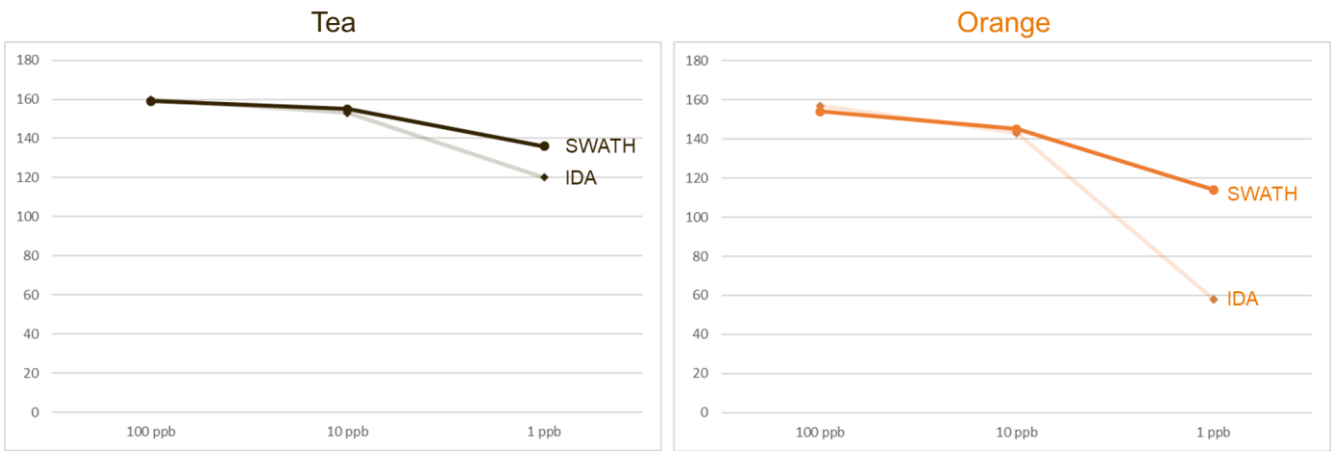
Atrazine. At high spike levels, IDA triggers and atrazine is confidently identified by library search. At lower levels IDA did not trigger and there is no MS/MS to score.



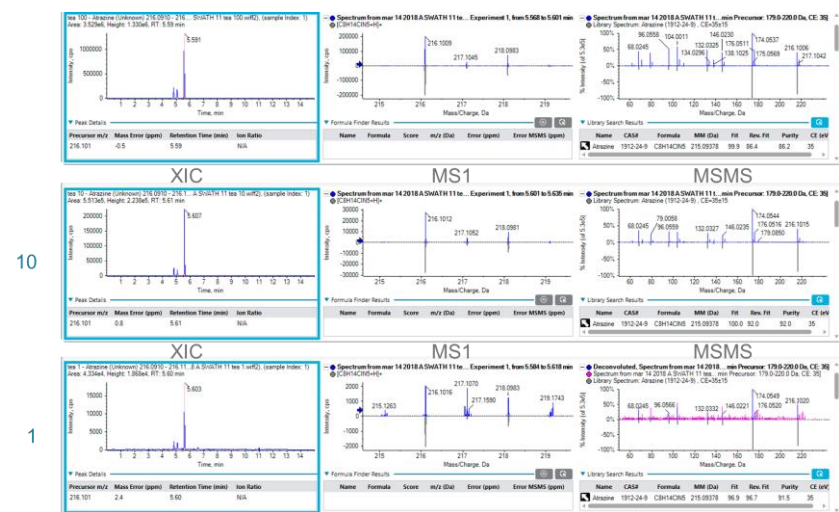
Methabenzthiazuron. At high spike levels, the IDA spectra confidently identifies methabenzthiazuron. At lower levels the IDA isolation window contains an interfering compound, the MS/MS is overwhelmed with fragments from this other compound, and the library score is low.



Tea sample spiked with 100 ppb of pesticides displayed as a contour map. The SWATH acquisition isolation windows that were run on every cycle of the acquisition are displayed as coloured bands. Most mass regions are covered by 40 Da SWATH acquisition windows, while the higher mass range is covered by one large 400 Da SWATH acquisition window.



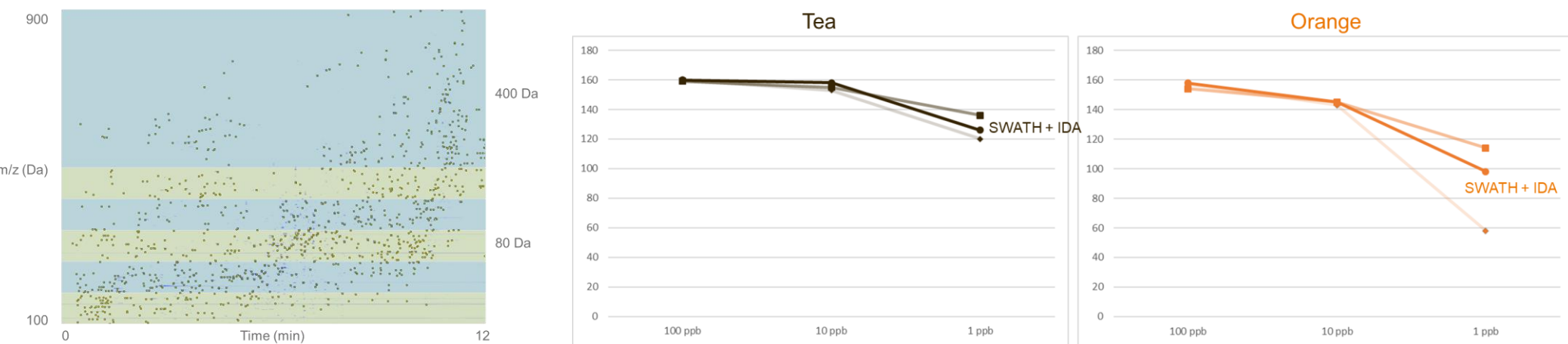
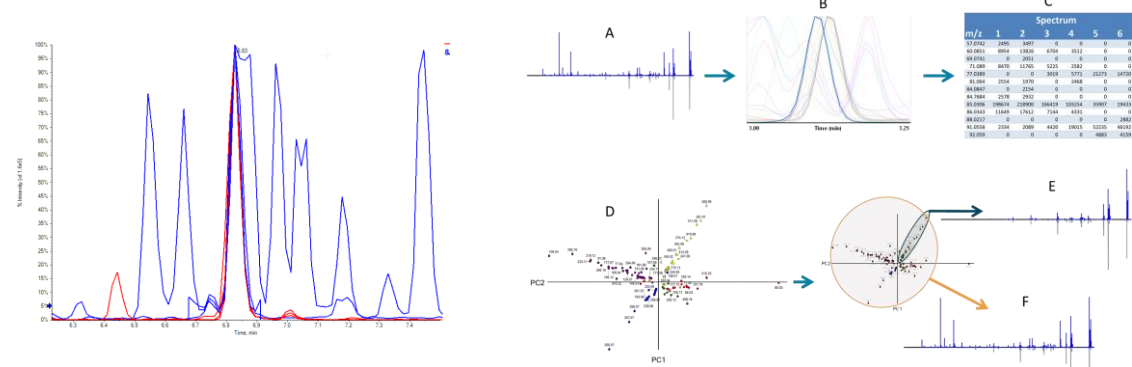
SWATH acquisition performs similar to IDA at high concentrations and considerably outperforms IDA at low concentrations.



Atrazine. SWATH acquires an MS/MS at every cycle. At high levels a simple background subtraction results in confident library match. At low levels, deconvolution of the MS/MS is used, resulting in a confident library match.

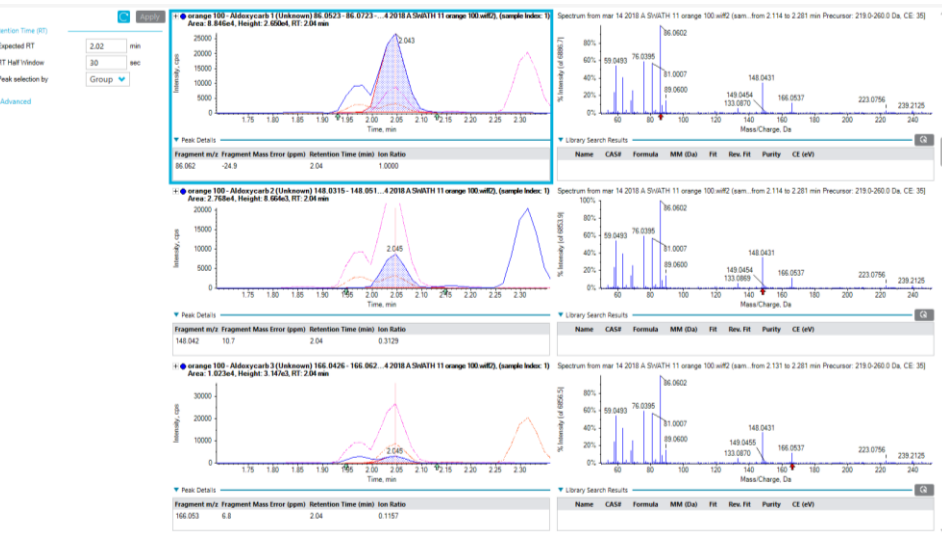


However, IDA did outperform SWATH acquisition in a small number of interesting examples. When two compounds perfectly co-elute, no amount of deconvolution can help improve the library identification. Deconvolution of the MS/MS is accomplished by performing PCA on all XICs and then grouping features using PCVG¹. Each group of features represents the MS/MS for a specific compound.



SWATH + IDA

Combining SWATH acquisition and IDA acquisition in a single injection. Half of the cycle time was spent on SWATH acquisition (resulting in larger isolation windows than pure SWATH acquisition) and half of the time on IDA (resulting in fewer triggers). At low levels the performance is somewhere between pure SWATH acquisition and pure IDA. At high levels, the combined method works better than either technique alone.



Identification by library searching is one level of confidence, but ion ratios between two XICs from the same compound is another means. IDA does not allow for this, but SWATH acquisition can. In this example, three fragment ions from a compound have been extracted. The largest intensity ion has some interference from a closely eluting compound, but is easily integrated correctly using the AutoPeak integration algorithm. The middle ion is cleanly resolved. The lowest intensity ion has some interference and is deconvolved using AutoPeak.

All three ions give accurate ion ratio information. Using SWATH acquisition, confidence in an identification can be achieved using any or all of: precursor mass accuracy, isotope pattern, retention time, multiple ion ratios, and library spectral matching.

CONCLUSION

- **SWATH acquisition and IDA can be run in the same acquisition method. It combines the best performance characteristics of each technique in one injection and analysis.**

REFERENCES

1. Gordana Ivosev, Lyle Burton and Ron Bonner. Dimensionality Reduction and Visualization in Principal Component Analysis. *Anal. Chem.*, **2008**, 80 (13), pp 4933–4944.

TRADEMARKS/LICENSING

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