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 Phenomonex 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



XIC of 160 pesticides at 100 ppb

Mass Error Confidence ♥	Mas
✓	-0.4
 ✓ 	0.5
✓	-2.1
×	0.7
 	0.2
✓	-2.3
 Image: A set of the set of the	-1.7
×	-0.9
×	-2.7
×	2.3
	0.2

At 100 and 10 ppb spike levels, IDA was able to identify all or most of the targets. However, at lower levels the performance decreased significantly due to decreased rates of library identification.





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

rror (ppm) 🛛	RT Confidence 🖓	Retention Time Delta (min) ♡	Library Confidence	Library Score ⊽	Library Hit 🛛 🖓
	 ✓ 	0.01	✓	99.5	3-Hydroxycarbofuran
	 Image: A set of the set of the	0.00	✓	96.9	Acephate
	 ✓ 	0.00	✓	100.0	Acetamiprid
	 Image: A set of the set of the	0.01	✓	95.4	Acibenzolar-S-methyl
	 Image: A set of the set of the	0.01	✓	86.1	Aldoxycarb
	 ✓ 	0.00	✓	100.0	Ametryn
	 Image: A set of the set of the	0.01	✓	100.0	Aminocarb
	 Image: A set of the set of the	0.00	✓	100.0	Amitraz fragment
	 ✓ 	0.01	✓	100.0	Atrazine
	 Image: A set of the set of the	0.04	✓	94.7	Atrazine d5

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%.



10 - 216H 6 IDA 3 tea 100.wiff2), (sample index: 1) 163 min	Spectrum from mar 9 2018 B SWAH 6 IDA Experiment 1, from 5.611 to 5.645 min © [C8H14CIN5+H]=	Spectrum from mar 9 2018 B SWAH 6 IDA5.629 min Precursor: 216.1 Da, CE: 35 © Library Spectrum: Atrazine (1912-24-9). CE=35±15
3.625	10000 - 216.1010 218.0983	100% 50% 68.0247 79.0061 132.0328 746.0232 773.4659 216.1012 0% 41 104.0010 132.0328 745.0232 773.4659 216.1012
	-100000	-50% -
5 6 7 8 9 10 11 12 13 14 Time, min	Z10 Z16 Z17 Z18 Z19 Mass/Charge, Da	50 80 100 120 140 160 180 200 220 Mass/Charge, Da
Retention Time (min) Ion Ratio	Name Formula Score m/z (Da) Error (ppm) Error MSMS (ppm) I	Name CAS# Formula MM (Da) Fit Rev. Fit Purity CE (eV) Arazone 1912-24-9 CBH14/CIN5 215.08378 100.0 99.7 95.7 35
XIC	MS1	MSMS
0 - 216.118 B IDA 10 tea 10.wift2), (sample Index: 1) 162 min	Spectrum from mar 9 2018 B IDA 10 tea 1 Experiment 1, from 5.601 to 5.633 min © [CBH14CIN5+H]+	Spectrum from mar 9 2018 BIDA 10 tea 1 5.621 min Precursor: 216.1 Da, CE: 35 Ultrary Spectrum: Atrazine (1912-24-9), CE-35a15 100%, 3
3.624	5 10000 - 216.1013 217.1060 216.0855 -10000 -	50% 79.0057 112.0029 146.020 110.1215 296.1165 50% 46.0220 10.0013 132.0329 146.020 180.1215 296.1165 50% 41.0014 41.0013 132.0329 146.020 180.1215 296.1165
5 6 7 8 9 10 11 12 13 14 Time, min	215 216 217 218 219 MassiCharge, Da	-100% 60 80 100 120 140 160 180 200 220 Masa/Charge, Da
Retention Time (min) Ion Ratio 5.62 N/A	Name Formula Score m/z (Da) Error (ppm) Error MSMS (ppm) I	Name CAS# Formula MM (Da) Fit Res. Fit Purity CE (eV) ▲ Atracine 1912-24-9 CBH14CIN5 215.09378 90.7 90.4 97.3 35
XIC	MS1	MSMS
216 11 - 018 100 A 10 tea 1 witt); (sample index 1) 62 min 3 617 4 6 6 2 6 5 10 11 12 13 14	→ 0 5520 m form are 7 2018 BIOA 10 tea 1. Experiment 1, tem 550 b 550 c in (510 HCM CPC) → 100 for 1	90% 27 0% 3 df 27 df 2 df 2 df 2 df 2 df 2 df 2 df 2 df 2
1000, 000	Formula Finder Results	Ultray Search Results
tetention time (min) ion Natio	Name Formula score m/z (Da) Error (ppm) Error MSMS (ppm) 1	Name CAS# Formula MM (Da) Fit Rev. Fit Purity CE (eV)

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.





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



Methabenzthiazuron. Having MS/MS at every cycle enables deconvolution of SWATH acquisition data to able to correctly identify methabenzthiazuron, even in the presence of a large interference.



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.



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

injection and analysis.

REFERENCES

TRADEMARKS/LICENSING

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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.

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

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