Scanning SWATH® Acquisition Method for Improved Compound Screening

Gordana Ivosev, David M. Cox, Nic Bloomfield, Fras Wasim, Yves LeBlanc
SCIEX, 71 Four Valley Drive, Concord, ON, L4K 4V8 Canada

ABSTRACT

Ideally, a generic screening method would work for any compound. Targeted methods are useful, but they must be maintained: new compounds must be added to them, and retention times need adjustment occasionally. SWATH® acquisition is close to ideal; it acquires MS/MS that represent every precursor mass of interest at every time point. However, there are some challenges. The deconvolution of MS/MS (for library searching), or calculation of ion ratios from fragments, requires that fragments from different compounds have a different chromatographic profile or elution time. Internal standards often have similar fragmentation and retention time with the compound they are based on, which can make identification difficult. A new acquisition technique, Scanning SWATH acquisition enables measurement of both precursor mass and fragmentation for all precursors during an LC run.

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 by techniques based on LC profile correlation.

However, when two or more compounds fall within the same precursor isolation window and have identical elution times, the deconvolution of the fragmentation signals is not possible. One solution was to design the SWATH acquisition precursor isolation windows such that each internal standard had its own narrow isolation window. However, this made the method very specific to the compounds being measured. Adding new compounds to this method would require additional work, which negates one of the key benefits of SWATH acquisition, in that it is a generic method.

Scanning SWATH acquisition technique continuously scans the quadrupole along the mass range. Ion events are recorded along each TOF pulse in a synchronized fashion as Q1 is ramped. This way, every ion event is characterized by 3 independent coordinates: mass, LC time, and Q1. Quadrupole dimension is providing information used to identify the fragmentation signal originating from internal standard and separate it from the matching compound fragmentation signal.

Additionally, this quadrupole dimension from scanning SWATH acquisition was used to distinguish precursor signal from any interfering signal at the same m/z that come from internal fragments, adducts or losses occurring at low collision energy. Thus, resulting in a cleaner spectrum than a simple MS1 scan.

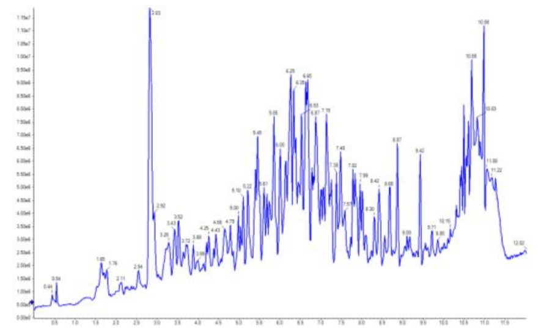
MATERIALS AND METHODS

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
6600 TripleTOF® system

Analyst® 1.7.1
0.25 sec MS1
0.7 sec on either IDA, or SWATH acquisition

Research version of Analyst 1.7.1
1 sec / cycle for Scanning SWATH acquisition

Processed using MATLAB and PeakView® software



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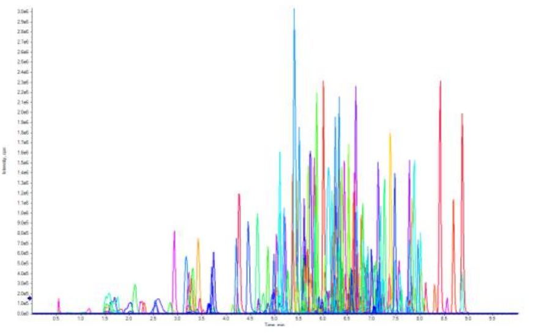
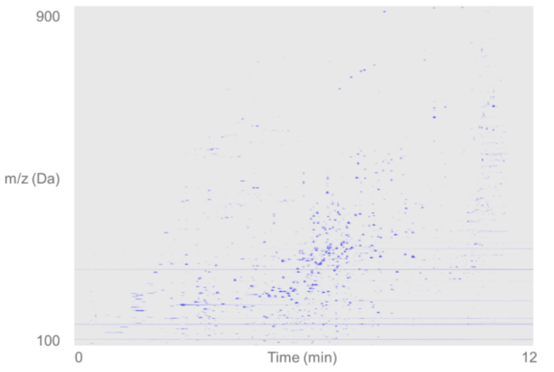
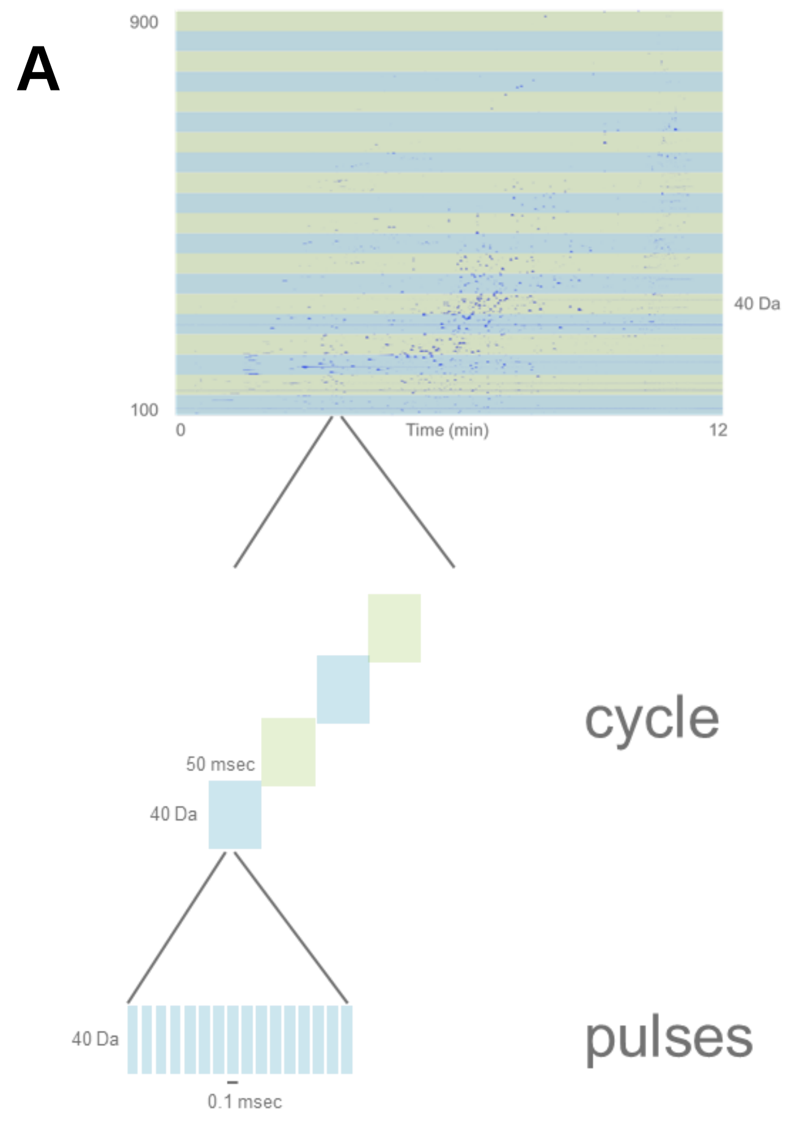


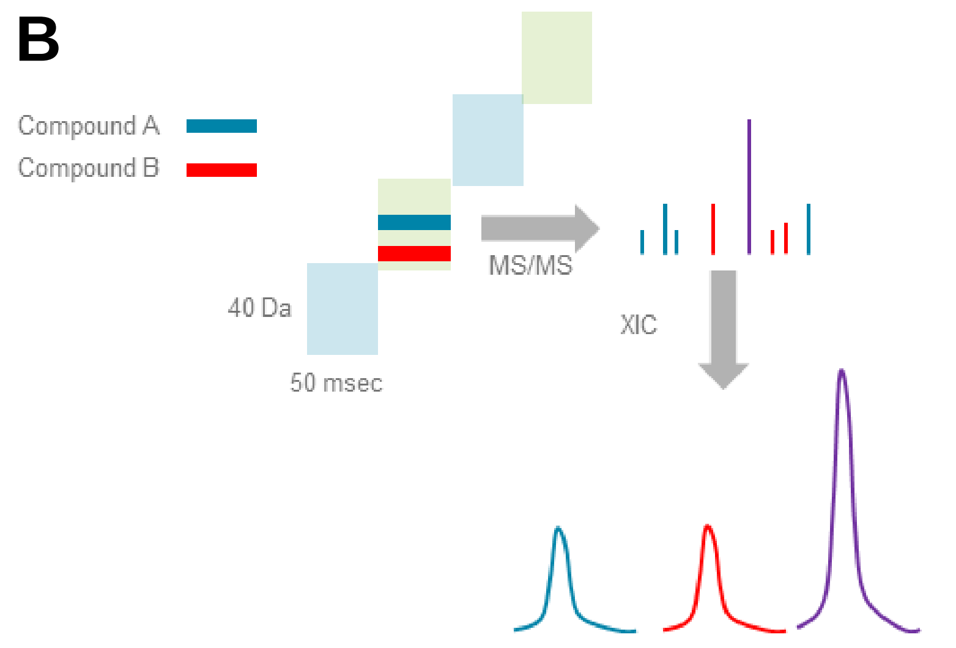
XIC of 160 pesticides at 100 ppb



Contour plot of all features

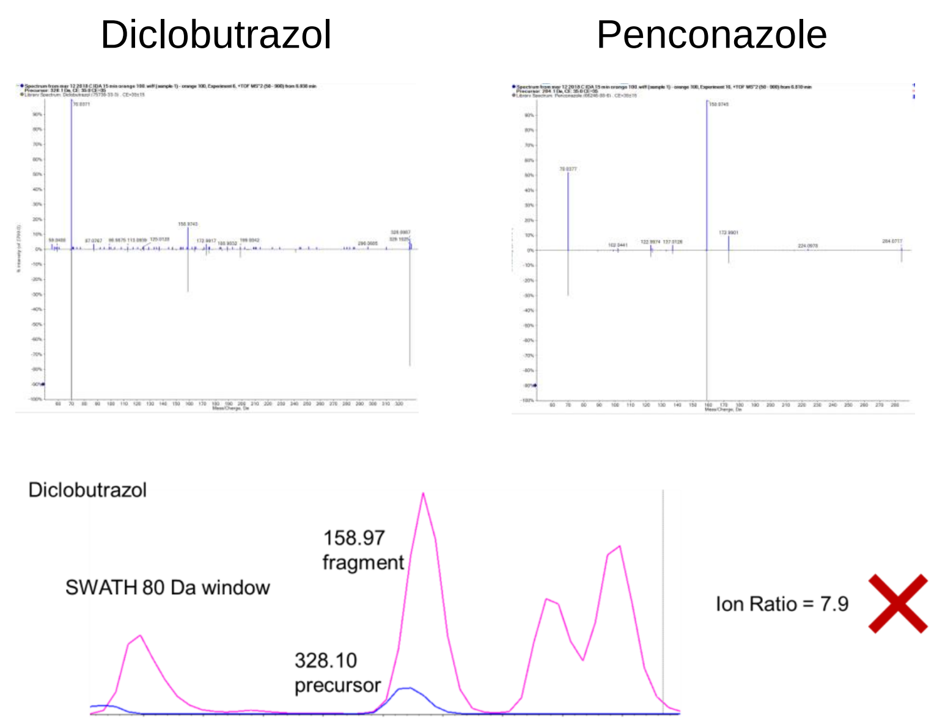
SWATH





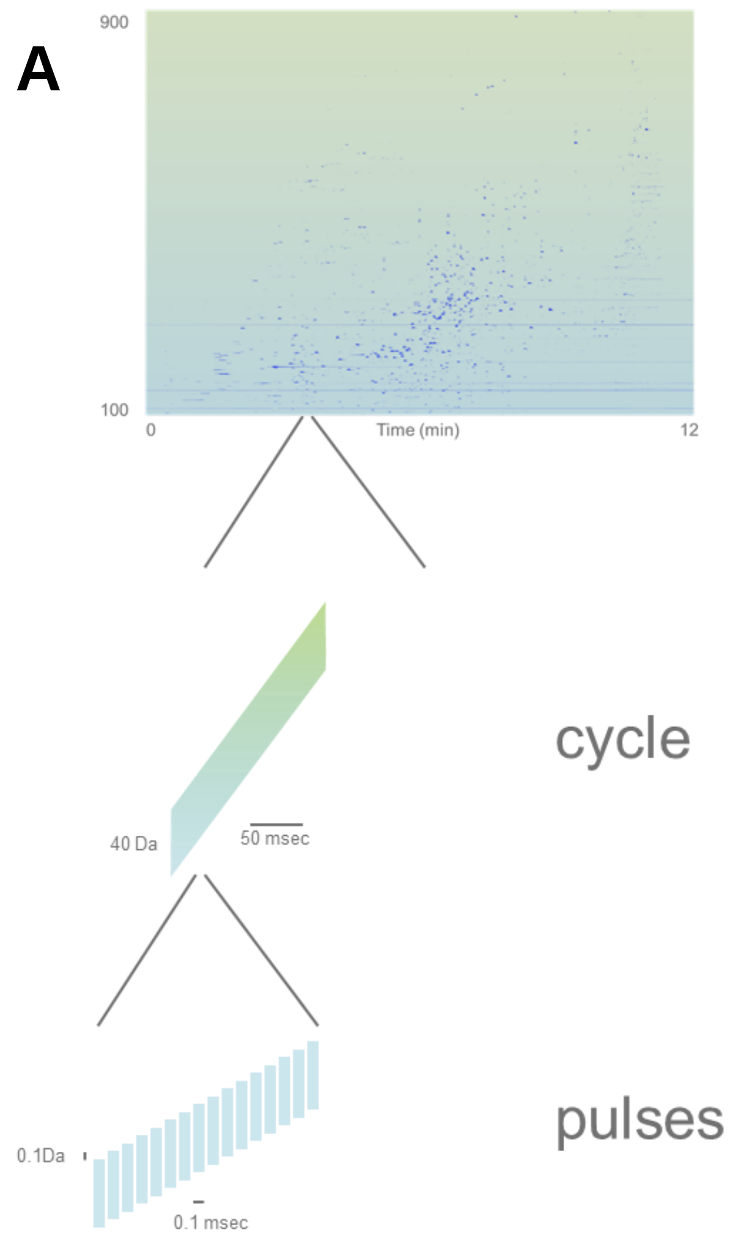
Traditional SWATH acquisition. During each cycle, the mass range of interest is divided between a number of SWATH acquisition window experiments. Each window is accumulated for a set amount of time (for example, 50 msec). During this accumulation time, a number of pulses / detection events occurs. The data recorded from individual pulses is summed and stored as one cycle for that experiment (section A).

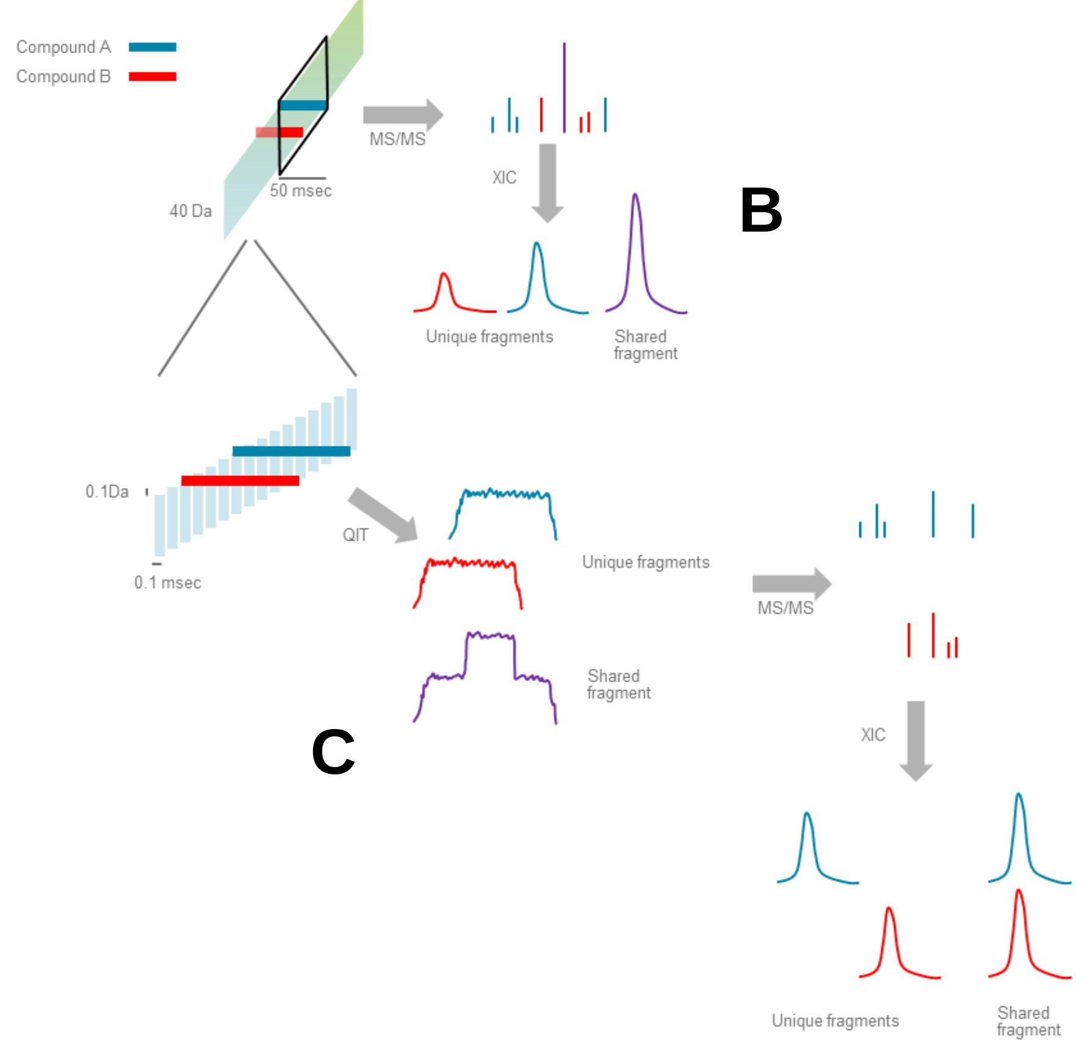
MS/MS at a specific cycle can be a mixture of fragment ions from compounds with different precursor masses (but identical retention times). These fragments are often unique to each compound, but occasionally both compounds share an identical fragment ion m/z. XIC of a shared fragment will lead to an overestimate of the true intensity (section B).



Diclobutrazol and penconazole almost perfectly co-elute. These compounds have an identical fragment (158.97 Da). In traditional SWATH acquisition, both of these compounds were fragmented in the same SWATH acquisition window. This made it difficult to determine of one, the other, or both compounds were present.

Scanning SWATH

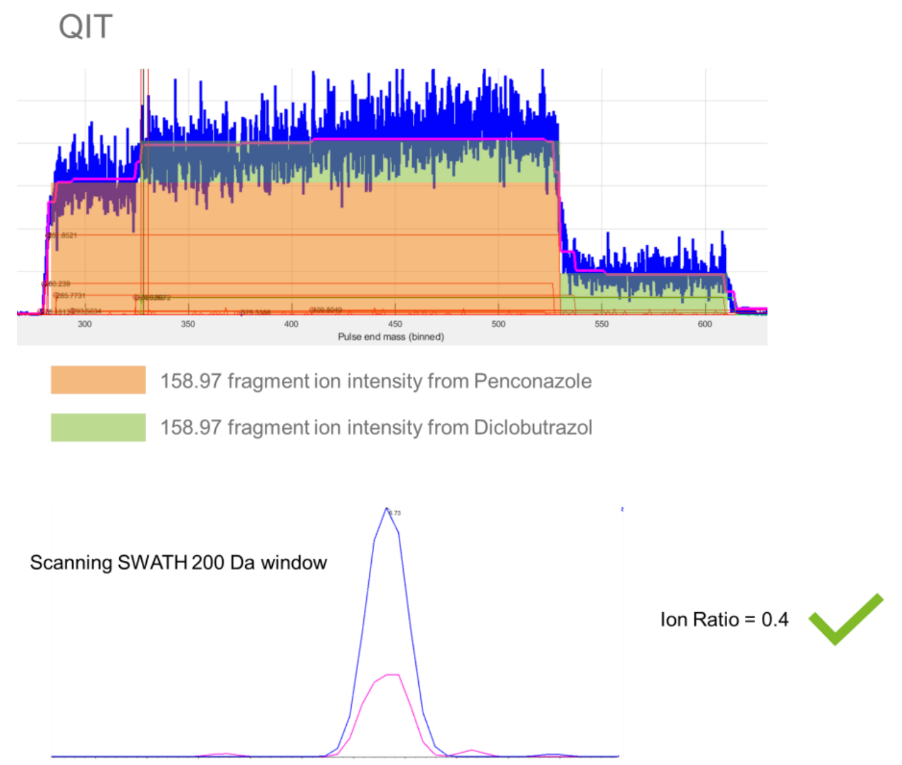




Scanning SWATH acquisition. During each cycle, the isolation window for MS/MS is sliding along the m/z range of interest. At each pulse, the isolation window is shifted slightly higher in m/z. The data from every pulse is stored (section A).

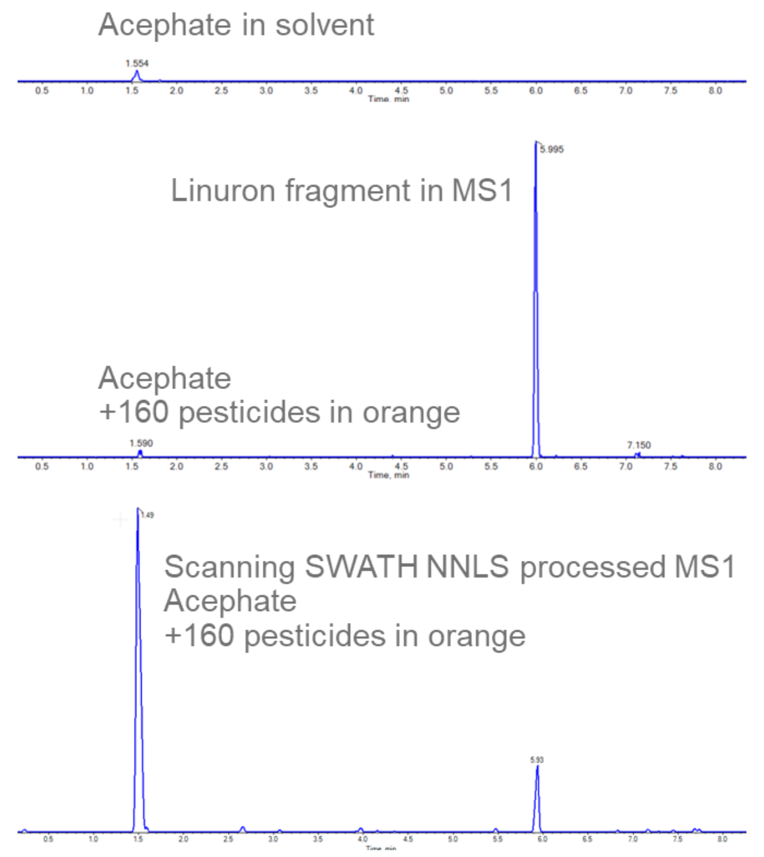
Spectra and XIC can be generated by summing the data from pulses that correspond to isolation windows that encompassed the target m/z of interest. This simple processing reduces the intensity of fragment ions that come from other precursor m/z, but does not entirely eliminate them. Shared fragment ions will still have intensities higher than they should be (section B).

Access to data from individual pulses enables a more sophisticated analysis. An extraction of a unique fragment ion mapped against Q1 m/z (a quadrupole ion trace or QIT) will be a roughly rectangular shaped region. An extraction of a fragment from a different precursor will be a similar shape, but shifted in Q1. An extraction for a shared fragment will be a convolution of these two rectangles. Using non-negative least squares (NNLS) it is possible to determine the most likely set of rectangles that explain the data, and generate separate MS/MS and XICs for each compound (section C).



The QIT trace for the shared fragment (158.97 Da) of diclobutrazol and penconazole at one cycle. The orange rectangle corresponds to the intensity of this fragment ion that belongs to penconazole. The green rectangles are the intensity that belongs to diclobutrazol. This processing is repeated at each cycle to generate an XIC. Scanning SWATH acquisition was able to obtain the correct ion ratio for diclobutrazol.

Scanning SWATH on MS1



Fragmentation during MS1 can also interfere with XICs. Acephate (184.02 Da) elutes at 1.5 minutes. With a pure standard in solvent, it is easy to determine the correct peak. Mixed with many other pesticides and spiked into a orange sample, it is not so clear. A fragment ion from linuron is generated in MS1, even though the collision energy is very low. This fragment ion interference makes it difficult to determine the correct retention time, unless it is known ahead of time.

Processing scanning SWATH acquisition data for MS1 precursor extractions removes the interfering fragment ion from linuron. With no prior knowledge of retention time for acephate, it is now easy to determine the correct peak.

CONCLUSIONS

- **Scanning SWATH acquisition and processing can determine the correct signal intensity for shared fragments, even if they perfectly co-elute.**
- **The correct ion ratio for a diclobutrazol fragment was obtained, even in a high background of penconazole.**
- **Fragmentation of fragile compounds in MS1 causes interreference in traditional acquisition. Scanning SWATH acquisition is able to remove this type of interference.**

REFERENCES

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2. Yves LeBlanc; Gordana Ivosev; Ian Tremble; Jamie Sherman Nic Bloomfield. Scanning-SWATH for Pesticide Analysis and Quantitation. ASMS 2017.

TRADEMARKS/LICENSING

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