Full-Scan Fragmentation Options for the Detection of Food Contaminants by an Affordable LC-Q-Orbitrap MS

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Key Words
Q Exactive Focus, pesticides analysis, mycotoxin analysis, veterinary drugs analysis, high resolution, data independent acquisition, sensitivity, selectivity

Goal
To compare two different scan options of a quadrupole-Orbitrap™ system, both offering full mass range fragmentation techniques, and to optimize performance in terms of sensitivity and selectivity.

Introduction
The analysis of food toxicants is a challenging task because of the high number of substances that needs to be analyzed. Pesticides alone account for over 800 analytes and many food commodities may contain other types of toxicants such as mycotoxins, plant toxins and/or veterinary drugs. Such a great number of analytes can be difficult to handle in a single run by targeted, triple quadrupole MS/MS measurements since the instrument will reach its limits with respect to scan speed. The use of liquid chromatography with full-scan, high-resolution accurate mass spectrometry (HRAM) as an alternative is therefore gaining in popularity, especially in pesticide analysis. HRAM enables simultaneous screening, quantitative determination, and identification of multiple analytes in one run. For identification, the SANCO guideline on pesticide residue analysis (12571/2013) requires the detection of two accurate mass ions, at least one of which is a fragment. Today's instruments offer different options to obtain the required fragment ion while still maintaining a fully non-targeted measurement.

On a Thermo Scientific™ Q Exactive™ Focus™ instrument, besides fragmentation modes with precursor ion selection, full mass range fragmentation modes are available. With these, all possible fragments are recorded over the full chromatographic time range, which offers the advantages of full scan measurements for non-targeted screening and retrospective data analysis, while still complying with the identification criteria set in 12571/2013 (Figure 1). These criteria are the detection of at least two diagnostic ions, including the quasi molecular ion and at least one fragment. One option is all-ion fragmentation (AIF) where all precursor ions are sent to the collision cell and fragmented; then, the resulting fragments are measured in the Orbitrap mass analyzer. Another option is variable data-independent acquisition (vDIA) where the mass range for the precursor ions is split into multiple events[1]. This way, sensitivity is improved through the higher number of analyte precursor ions in the C-trap, and selectivity is improved because fragments originate from a smaller range of precursors.

vDIA method is not available in the United States of America.

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Experimental

Sample Preparation

Samples were prepared using a modified QuEChERS method. The final concentrations were as follows: 1 g/mL (apple, chicken liver); 0.5 g/mL (wheat, compound feed); 0.1 g/mL (food supplement). Final extracts were diluted 1:1 with water prior injection.

LC-MS/MS

The analyses were conducted on a Thermo Scientific™ UltiMate™ 3000 LC system interfaced via a heated electrospray ionization (HESI-II) source to a Q Exactive Focus mass spectrometer. The LC was equipped with a C18 analytical column (100 x 3 mm, particle size 3 µm). A gradient based on water/methanol containing 0.1% formic acid and 2 mM ammonium formate (Fisher Chemical brand) was used. The injection volume was 5 µL.

Figure 1 describes the scan events. Fragmentation was done at normalized collision energy (NCE) settings of 30 and 80 (stepped collision energy) in both modes.

Data Analysis

Thermo Scientific™ TraceFinder™ software was used for data analysis. The analyte detection requirements were one precursor plus one fragment ion at t_r ± 0.5 min with *m/z* ± 5 ppm.

Results and Discussion

Figure 2 shows the extracted ion chromatograms (XIC) of selected compounds measured both with AIF and vDIA. The vDIA data clearly shows the improvements in sensitivity and selectivity compared with AIF. Although the vDIA method includes more scans per scan cycle, the number of data points per chromatographic peak is still more than sufficient. The usability of the vDIA method was tested by analyzing a mixture of 37 compounds (pesticides, natural toxins, veterinary drugs) in solvent and five matrices at four levels. Table 1 shows the number of detected compounds based on precursor plus fragment.

Another important parameter to assess the suitability of a method is the number of false positives. To check this, an internally developed database containing 170 pesticides was used to process samples of the blank matrices used for spiking. Fully automated analyte detection resulted in 4–12 primary detects/sample. With the software used, manual verification of these potential detects was quick and straightforward and for none of the software-detects coinciding peaks for precursor and fragment were observed. Hence, no false positives were found in any of the blanks.

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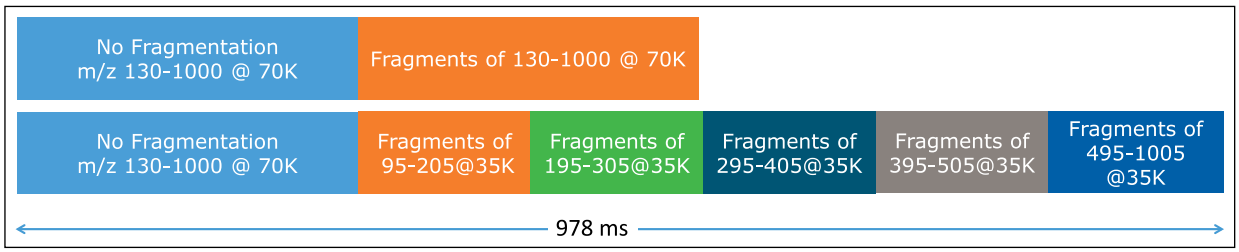

Figure 1. Schematic representation of measured scan event cycles. Option 1: FS+AIF (top bar), Option 2: FS+5 vDIA events (lower bar).

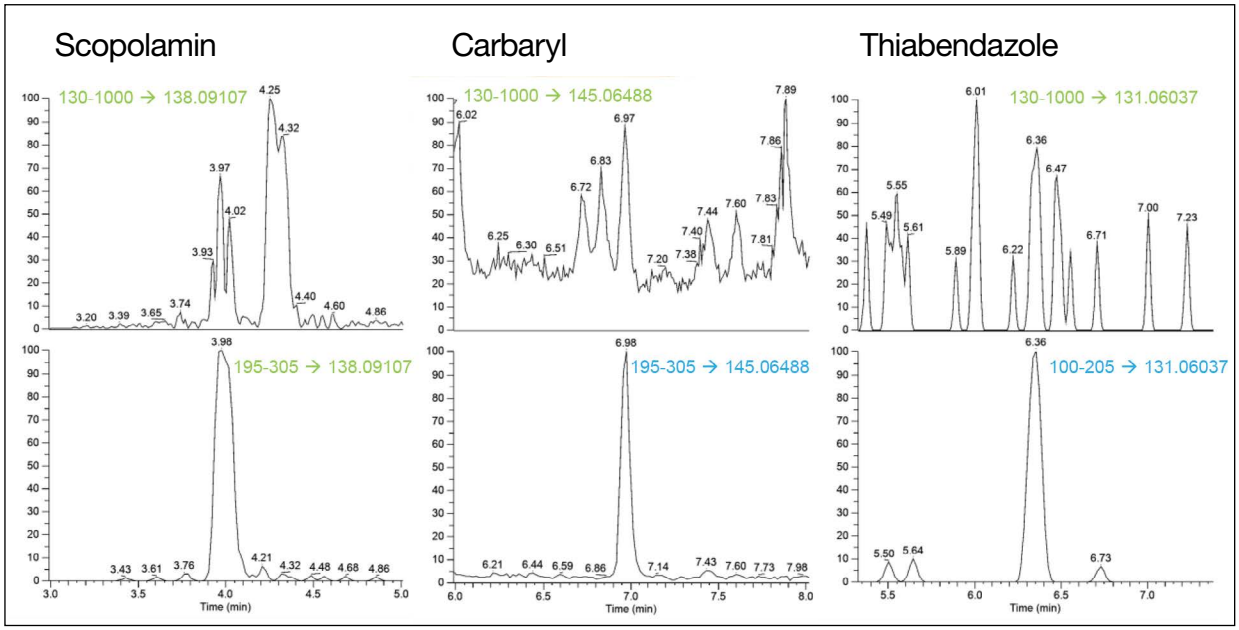

Figure 2. Comparison of XICs of fragment ions, fragmented with AIF (top) and vDIA (bottom). From left to right: scopolamin in wheat, carbaryl in wheat and thiabendazole in compound feed. Spike level: 10 ng/g.

Table 1. Number of compounds out of a total number of 37 automatically detected by TraceFinder software at different levels in five matrices, comparing vDIA mode (left) with AIF mode (right).

vDIA				
Matrix	**1 ng/g**	**10 ng/g**	**50 ng/g**	**200 ng/g**
Solvent	33	37	37	37
Apple	31	37	37	37
Liver	28	35	37	37
Food Supplement*	26	32	37	37
Wheat	21	33	37	37
Compound Feed	9	13	24	34

AIF				
Matrix	**1 ng/g**	**10 ng/g**	**50 ng/g**	**200 ng/g**
Solvent	32	37	37	37
Apple	26	35	37	37
Liver	24	35	37	37
Food Supplement*	14	23	37	37
Wheat	11	30	36	37
Compound Feed	1	19	21	31

*Spiking levels in food supplement 10x higher.

Conclusion

- Variable data-independent data acquisition improves sensitivity, selectivity, and the ability to identify target analytes adding extended non-target screening capabilities.
- The sensitivity, as well as the limited number of false detects obtained by software-based detection and the ease with which to review and discard them, make LC-full-scan analysis with vDIA in high resolution mass spectrometry (HRMS) suited for routine applications.

Reference

1. Scheibner, O.; Kellmann, M.; Yang, C.; Bromirski, M. Thermo Scientific Technical Note 64283; Variable Data-Independent Acquisition (vDIA) Delivers High Selectivity and Sensitivity in Combined Targeted and Untargeted Analyses for Small Molecules, 2014.

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