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# Use of New Full Scan Fragmentation Options in the Detection of Pesticides by LC-Q-Orbitrap MS

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## Background

The use of LC with full scan high resolution MS as a replacement of targeted triple quadrupole MS/MS is gaining in popularity in pesticide analysis. The SANCO guideline on pesticide residue analysis (12571/2013) requires the detection of two accurate mass ions, of which at least one fragment. Today's instruments offer different options to obtain the required fragment ion while still maintaining a fully untargeted measurement.

## Objective

The aim of this work is to compare different scan options of a quadrupole-Orbitrap system to optimise performance in terms of sensitivity and selectivity while still obtaining enough data points over the peaks for quantitative purposes.

## Full scan fragmentation

To maintain the advantage of full scan measurements (non targeted acquisition), and at the same time comply with the identification criteria set in 12571/2013, fragmentation needs to be 'full scan' as well. One option is All Ion Fragmentation (AIF) where all ions in the used scan range are sent to the collision cell, fragmented, and the resulting fragments are measured in the orbitrap mass analyzer. Another option is so-called Data Independent Acquisition (DIA): By 'splitting' the mass range for the precursor ions into multiple events, both sensitivity and selectivity can be improved:

Sensitivity: higher number of analyte precursor ions in the C-trap
Selectivity: fragments originate from a smaller range of precursors

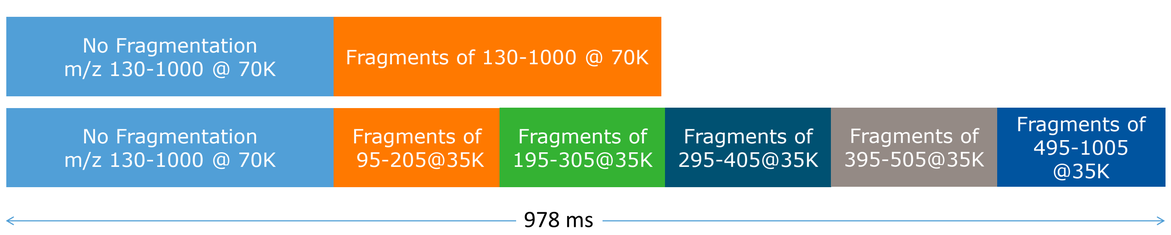


**Figure 1.** Schematic representation of measured scan event cycles:
Option 1: FS+AIF (top bar), Option 2: FS+5 DIA events (lower bar).

## Method

| | |
|---|---|
| Matrices tested: | see Table 1 |
| Extraction: | modified QuEChERS, final extract concentration: 1 g/mL (apple, liver); 0.5 g/mL (wheat, compound feed); 0.1 g/mL (food supplement) |
| LC system: | Thermo Scientific Ultimate 3000 |
| Column: | Waters Atlantis C18 (3 µm, 3x100 mm) |
| LC Eluents: | $H_20$/MeOH with formic acid and ammonium formate |
| Injection volume: | 5 µL |
| Mass Spec: | Thermo Scientific Q-Exactive with HESI-II source |
| Scan Events: | See Figure 1. AIF and DIA: 30 and 80 NCE |
| Data processing: | TraceFinder 3.2. Analyte detection requirements: 1 precursor+1 fragment at $t_r$±0.5 min; m/z±5 ppm |

## Results

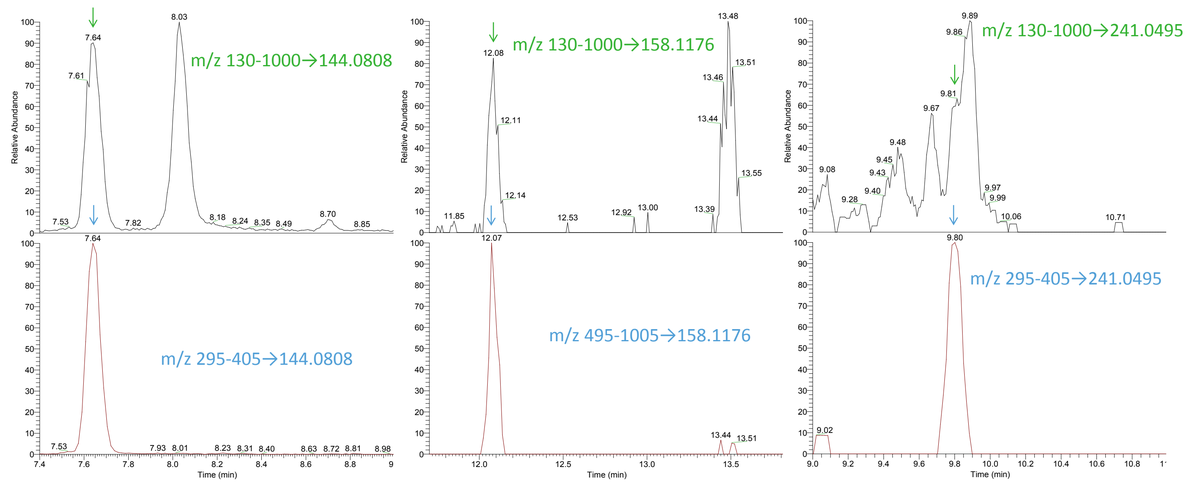


**Figure 2.** Comparison of XICs of fragment ions, fragmented with AIF (top) and DIA. From left to right: yohimbine in wheat, emamectin in wheat and aflatoxin B1 in compound feed. Spike level: 10 ng/g.

## Detectability

Fig. 2 shows the XICs of selected compounds measured both with AIF and DIA. The DIA data clearly shows the benefits in sensitivity and selectivity compared with AIF. The usability of the DIA method was tested by analysing a mixture of 37 compounds (pesticides, natural toxins, veterinary drugs), in solvent and five matrices at four levels (see Method section for details). Table 1 shows the number of detected compounds based on precursor+fragment.

**Table 1.** # of compounds automatically detected by TraceFinder at different levels in five matrices.

| Matrix | 1 ng/g | 10 ng/g | 50 ng/g | 200 ng/g |
|---|---|---|---|---|
| Solvent Standard | 36 | 37 | 37 | 37 |
| Apple | 33 | 36 | 37 | 37 |
| Chicken liver | 30 | 37 | 37 | 37 |
| Food Supplement* | 22 | 35 | 37 | 37 |
| Wheat | 27 | 35 | 37 | 37 |
| Compound Feed | 2 | 20 | 31 | 35 |

* Spiking levels in Food Supplement 10x higher.

## False Detects

Another important parameter to asses the suitability of a method is the number of false detects.
To check this a database containing 170 pesticides was used to process the blank samples. Fully automated analyte detection resulted in 4-12 detects/sample. With the software used, manual verification of these potential detects was quick and straightforward.
For none of the software-detects, coinciding peaks for precursor and fragment were present → no (false) detects found in any of the blanks.

## Conclusions

- New fragmentation options improve sensitivity, selectivity and the ability to identify target analytes without compromising the full scan measurement.
- The sensitivity, the limited number of false detects obtained by software-based detection, and the ease to review and discard them make LC-full scan HRMS suited for routine application.

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## Acknowledgements

Thermo Scientific is acknowledged for providing the Q-Exactive instrument. Markus Kellman, Olaf Scheibner and Frans Schoutsen are acknowledged for technical and software support.

The work described in this poster was funded by the Dutch Ministry of Economic Affairs.