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Accelerating large scale pesticide screening programs with HR LC–MS/MS using automated non-targeted analysis software

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Overview

- Non-targeted analysis (NTA) was applied retrospectively to HRMS LC-MS/MS analysis of unknown food commodities and known spiked QC sample.
- Component detection was validated using a panel of 280 pesticides at 10 µg/kg maximum reporting limit (MRL) concentration to test sensitivity of the NTA.
- Third party spectral libraries were used in conjunction to an in-house spectral library to identify residues not normally quantified in the routine multi-residue triple quadrupole LC-MS/MS method.

1. Introduction

Residual pesticide analysis has remained a critical part of ensuring food safety. Targeted pesticide quantitation remains the principal vehicle for measuring food commodities by triple quadrupole LC-MS/MS or GC-MS, however, its application is restricted to predefined analyte panels. Expanding coverage requires method development, optimization and validation, a process that can take extensive time and effort. To expand the scope of pesticide detection, analytical strategies are moving towards non-targeted data acquisition methods that can integrate known target lists and unknown suspect screening lists in a single method. In this work a single HRMS LC-MS/MS acquisition method was used for targeted and non-targeted analysis using large scale screening lists using a novel software application, Insight Profiler.

2. Materials and Methods

Analysis included a selection of unknown test samples of various commodities previously quantified by triple quadrupole LC-MS; quality control samples spiked with a panel of compounds were also included. Reversed phase liquid chromatography applied a 17-minute gradient acquired by LC-MS/MS Q-TOF by data independent acquisition (DIA). Data were processed using Insight Profiler software which performed feature detection, alignment and screening using large scale suspect lists for compound identification.

Reversed phase LC Separation: Nexera X2

- Column: Shim-pack Velox™ Biphenyl (2.1x 100mm 2.7µm); 40ºC, flow rate 0.4 mL/min
- A: water + 2 mM ammonium formate + 0.002% formic acid.
- B: methanol + 2 mM ammonium formate + 0.002% formic acid.
- Injection; 4 µL + 40 µL water co-injection

High resolution QTOF analysis: LCMS-9050

- TOF MS mass scan m/z 140-925; 100 msecs
- 31 DIA MS/MS scans with a scan time 29 msecs for each mass scan, collision energy spread 5-55V.
  - m/z 140-540; precursor isolation width of 20 Da
  - m/z 540-925; precursor isolation width of 35 Da
- ESI positive ion mode data was acquired using external mass calibration
- Scan cycle time 1 second (31 mass scans in total)

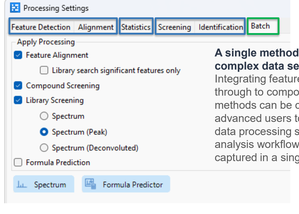
2.1 Non-Targeted Analysis

Insight Profiler non-targeted analysis software was applied to LC/QTOF data acquired with samples from both river systems to identify contaminants of environmental concern (CEC).

Feature Detection and Alignment
Detects and aligns all ion signals that behave as a peak. Default settings applied; threshold set to low.

Statistics and filters
Applied to find ion signals of significance and to remove ions of high variance

Compound identification
Using large scale screening lists and multiple libraries to identify ion signals of interest.



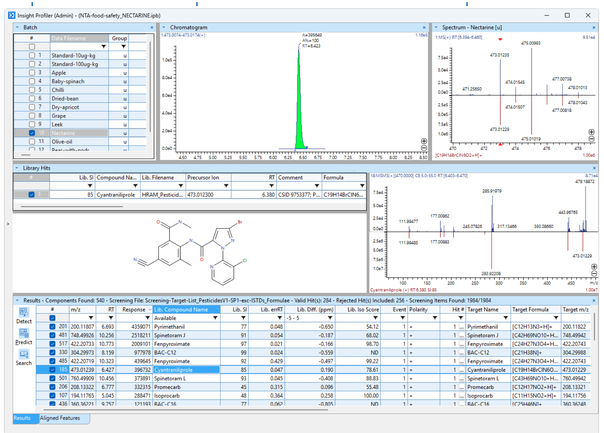
A single method set-up for processing complex data sets
Integrating feature detection and alignment through to compound identification. Processing methods can be configured for both new and advanced users to automate a sequence of data processing steps supporting unknown analysis workflows. All processing steps are captured in a single method for batch analysis.

Figure 1. The Insight Profiler processing application method editor, designed to create a singular workflow for feature detection of unknowns to compound identification.

Sample table
Lists data files for processing. Replicates can be grouped.

Chromatogram
Extracted ion chromatogram of identified features.

Spectrum
Measured spectrum and theoretical spectrum shown.



Results
Individual sample results and aligned results across all samples

Library hit
Top library hit shown in results table

Screening hit
Top screening hit shown in results table

Figure 2. NTA of food commodities screening for pesticide residues.

3.1 Validating non-targeted analysis

Feature detection was tested to determine the sensitivity of compound detection and library identification. This was performed using a spiked matrix QC sample (olive oil) containing 280 pesticides at the maximum reporting limit (MRL), typically 10 µg/kg (Fig. 3).

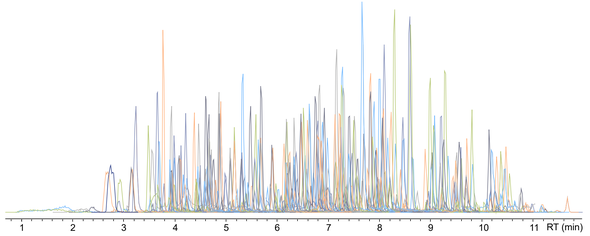


Figure 3. Feature detection of 280 pesticides a the MRL concentration (10 µg/kg) in olive oil.

Insight Profiler software successfully identified all 280 compounds with library identification demonstrating the application was fit for purpose for a broad panel of compounds at meaningful concentration levels.

Unknown samples were processed using Insight Profiler to confirm compounds previously quantified by triple quadrupole LCMS and screen for any additional compounds using an in-house spectral library containing over 500 pesticides. In dry-apricot food commodities dodine, tebuconazole and trifloxystrobin were in agreement with those previously detected; in nectarine, sulfoxaflor (previously quantified at 10 µg/kg) was detected. All compounds identified were in agreement with those detected by triple quadrupole LC/MS/MS targeted results. In the case of nectarine, very high levels of pyrimethanil, a broad-spectrum fungicide were found, a compound outside the scope of the targeted LC-MS/MS method highlighting the advantage of non-targeted screening in food safety.

Table 1. Non-targeted analysis confirmed detection of compounds routinely quantified by triple quadrupole LC-MS/MS (blue) but also detected additional compounds not included in the multiresidue method (green). *Identified using the Food Safety Mass Spectral Library (Wageningen University)[1].

Baby-spinach	Dried-bean	Dry-apricot	Grape	Leek	Nectarine
Abamectin B1a	Acephate	Dodine	Ametoctradin	Azoxystrobin	Acetamiprid
Acetamiprid	Acetamiprid	Acetamiprid	Fenhexamid	Propamocarb	Azoxystrobin
Azoxystrobin	Azoxystrobin	Nicotine	Fluopicolide	Spirotetramat met.	Cyantraniliprole
Chlorantraniliprole	Chlorantraniliprole	Thiacloprid	Spiroxamine	Difenoconazole	Fenpyroximate
Fluopicolide	Clothianidin	Tebuconazole	Boscalid		Spinetoram J
Metolcarb	Cyantraniliprole	Trifloxystrobin	*Dimethomorph		Spinetoram L
Propamocarb	Imibenconazole		Fluopyram		Sulfoxaflor
*Acetamiprid-N-desmethyl	Methamidophos		Penconazole		Pyrimethanil
*Propamocarb N-oxide	Thiamethoxam		Pyrimethanil		

3.2 Unknown compound identification

Spectral library searching was expanded to include third-party repositories, including MassBank and the Food Safety Mass Spectral Library (Wageningen University)[1]. In grape, NTA identified high levels of boscalid, fluopyram, penconazole and pyrimethanil using an in-house pesticide spectral library in addition to dimethomorph using the food safety mass spectral library. Dimethomorph is a systemic fungicide that was banned in the EU in 2024 after it was found to be damaging to human fertility, however, it is still permitted in the UK until 31/1/2030 illustrating divergence between UK and EU residue standards.

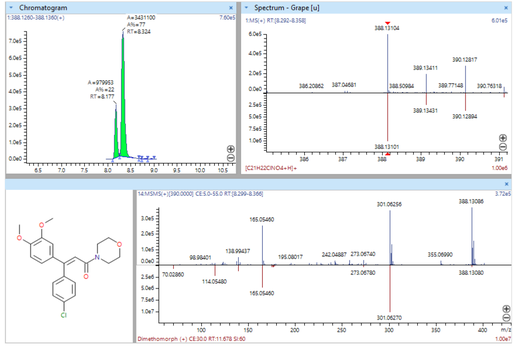


Figure 4. Non-targeted analysis identified an additional residue dimethomorph in a grape sample using the FoodSafetyMassSpectralLibrary (V2.25)[1].

4. Conclusions

- The methodology was successfully applied to detect pesticide residues with differing chromatographic behaviors, across multiple food commodities and in a wide concentration range.
- Detection of acetamiprid-N-desmethyl and propamocarb N-oxide by NTA illustrates the growing trend of measuring pesticide metabolites in food safety analysis.
- Dimethomorph measured in grape highlights divergence between UK and EU pesticide regulations.

5. References

1. Creation of an Open-Access High-Resolution Tandem Mass Spectral Library of 1000 Food Toxicants (2025) Federico Padilla-González, Serena Rizzo, Caroline Dirks, Wout Bergkamp, Sjors Rasker, and Ivan Aloisi, *Analytical Chemistry,* 97 (43), 23822-23830
DOI: 10.1021/acs.analchem.5c03020

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