Confident Multi-Residue (> 400) Pesticides Analysis
Using High Resolution Orbitrap Mass Spectrometry with Optimized Chromatography and Verified Library

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Overview

Purpose: To develop a fast and sensitive method and evaluate its performance for multi-residue (>400) pesticides analysis in food matrices using ultra-high performance liquid chromatography and high resolution accurate mass (UHPLC-MS^{HRAM}) platform.

Methods: Chromatographic separation was achieved on a Thermo Scientific Dionex UltiMate 3000 RSLC UHPLC system with a Thermo Scientific Accucore aQ column. A Thermo Scientific Exactive Plus orbitrap mass spectrometer was operated in full scan/all ion fragmentation (FS/AIF) scan mode for data acquisition.

Results: More than 400 pesticides were sufficiently retained and separated within 16 minutes with improved chromatographic performance with respect to retention, chromatographic resolution, peak capacity and peak shape. Screening for target pesticides was performed using Thermo Scientific ExactFinder data processing software with retention time used for identification, isotopic pattern and/or fragment ions used for confirmation. A verified library was established with each library entry confirmed with identity, retention time, quantitation ion and fragmentation ions.

Introduction

While advanced HRAM mass spectrometers are accepted as suitable instruments for large number (>100) multi-residue pesticide analysis, efforts are in urgent need to develop and optimize fit-for-purpose chromatographic separation to improve peak capacity, reduce peak overlapping which consequently reduce false positive results. Meanwhile, better chromatography, with symmetric peak shape and enhanced run to run reproducibility, improves method performance to reduce false negative results.

With an optimized and standardized chromatographic separation, establishing a verified library becomes feasible. Here a verified comprehensive library is presented with each individual entry confirmed with its identity (retention time, empirical formula, chemical structure, and common name), as well as verified and annotated fragments.

This study demonstrates a total solution for multi-residue pesticides analysis using the optimized and standardized UHPLC-MS^{HRAM} method with verified pesticide library for confident screening and quantitation results.

Methods

Sample Preparation

Blank food matrices were prepared using QuEChERS (details not included) then spiked with pesticides standard mixture solution. Liquid samples with particulates were diluted in water (10X), centrifuged for 15 minutes at 5000 rpm and supernatant was injected for LCMS analysis. Clear liquid samples were injected directly for UHPLC-MS^{HRAM} after dilution.

Liquid Chromatography

System:	UltiMate® 3000 RSLC HPG binary with 35 µL gradient mixing kit
Column:	Accucore aQ (2.1 × 150 mm, 2.6 µm)
Flow Rate:	0.4 mL/min
Temp.:	25 ºC
Injection:	5 µL
Mobile Phase:	A) H_2O/CH_3OH (98:2) B) H_2O/CH_3OH (2:98) buffered with ammonium formate (5mM) and formic acid (0.1%)
Gradient:	Optimized gradient profile

Mass Spectrometry

System:	Exactive™ Plus or Q Exactive
Ionization:	ESI with HESI II probe
Vaporizer Temperature:	220 ºC
Capillary Temperature:	300 ºC
Spray Voltage:	2.2 kV
Sheath Gas:	32 arbitrary unit
Auxiliary Gas:	7 arbitrary unit
S-lens value:	60
Resolution:	70,000 (FWHM)
Data Acquisition:	Full Scan / All Ion Fragmentation (80 – 1200 m/z)

Results

Extracted ion chromatograms (XIC) for randomly selected pesticides are shown in Figure 1. Under the optimized conditions, most analytes were sufficiently retained with the minimum retention factor (k') greater than 2. With the combined selectivity from chromatographic separation and HRAM measurement, all targeted pesticides can be identified. Figure 1 also shows the optimized standard UHPLC method features:

- Sufficient retention for hydrophilic compounds
- Excellent observed peak capacity
- Necessary chromatographic resolution for isomers
- Robust performance in matrices
- UHPLC optimized Viper connection for better performance

Targeted/Unknown Pesticides Screening in Smoothies

In this study, targeted and unknown screening was performed using ExactFinder™ software. To evaluate the screening performance of this methodology. Two smoothie samples were prepared (1 to 10 diluted in water/acetonitrile (9/1), centrifuged for 15 mintutes at 5000 rpm) and analyzed in duplicates. As shown in Figure 2, 12 pesticides were identified and confirmed with isotopic pattern and fragment match in sample 1, and 13 pesticides in sample 2. The details of isotopic pattern match and fragments match are demonstrated in Figure 2.

Figure 2 also demonstrates that due to the complexity of the sample matrices, a good chromatographic separation as well as high resolution mass spectrometry are necessary to differentiate interferences thus to achieve high scores in isotope pattern match and fragment ion match, which lead to highly confident results.

FIGURE 1. Optimized Chromatography for Multi-Residue Pesticides Analysis: TIC and Extracted Ion Chromatograms

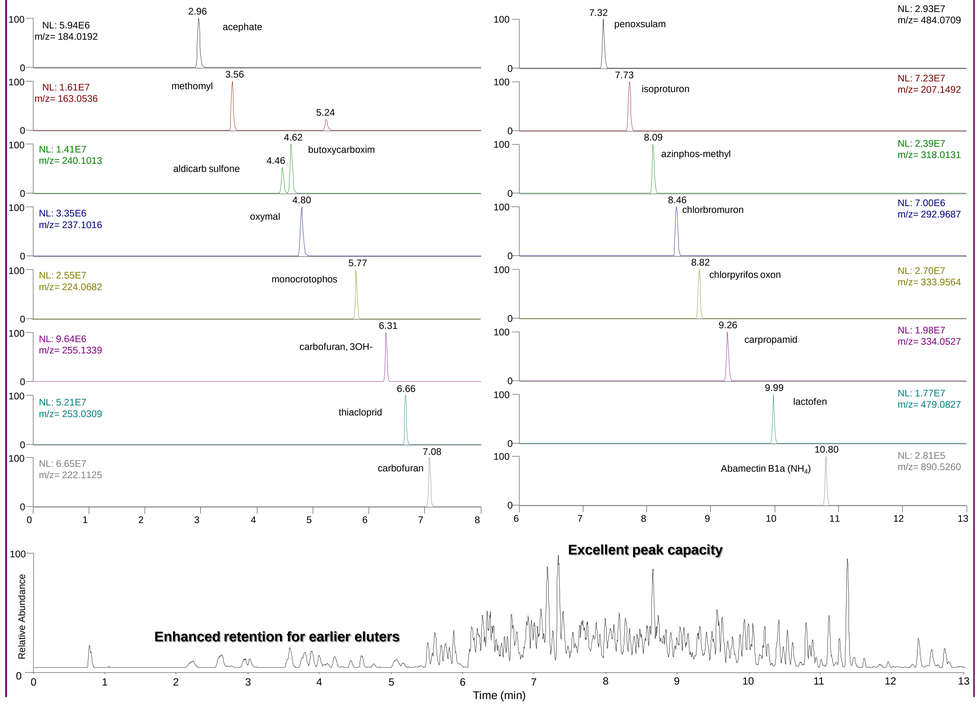
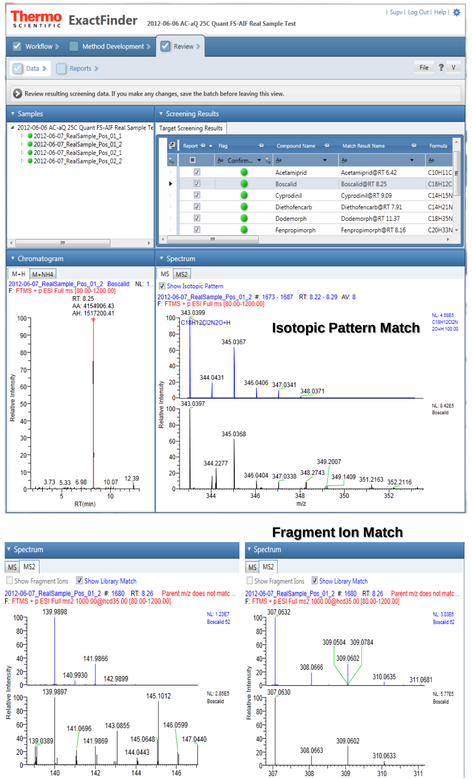


FIGURE 2. Screening Analysis Using ExactFinder



Verified Pesticides Library

To generate highly confident results, each entry in the pesticide library used as reference needs to be individually validated. In this study, more than 400 pesticides were confirmed and verified using individual or mixed reference standards. Figure 3 demonstrates the confirmation of the identity and retention time for each of the library entry. And Figure 4 demonstrates the verification of pesticide fragmentation ions.

FIGURE 3. Identity and Retention Time Confirmation of Library Entries

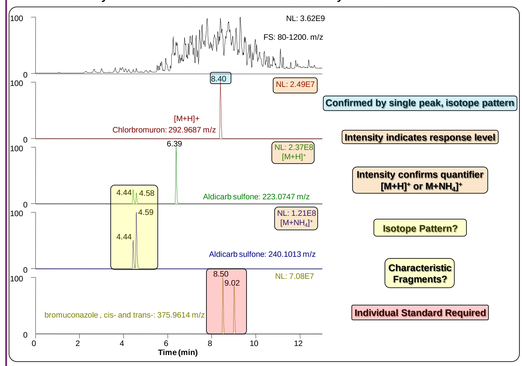
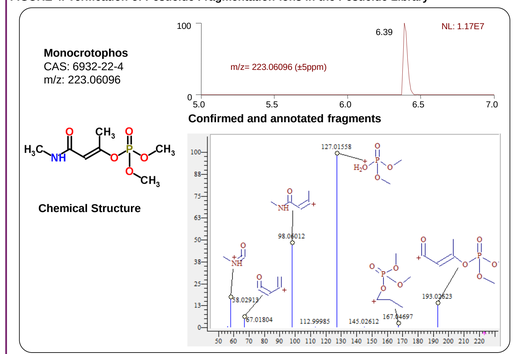


FIGURE 4. Verification of Pesticide Fragmentation Ions in the Pesticide Library



Conclusion

This poster describes an UHPLC-MS^{HRAM} solution for fast and confident analysis of more than 400 pesticides base on optimized and standardized chromatography and verified library.

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