thermoscientific

Fast analysis of multi-class pesticides panel in garlic and cumin extracts using a Single Run LC-High Resolution Mass Spectrometry

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ABSTRACT

Purpose: To present LC-MS HRAM multi-class pesticide residue analysis methods in cumin and garlic that are robust, rapid, easy to use, and have the sensitivity, accuracy, and precision that is required in order to meet regulatory guidelines. All aspects of these methods represent a 'workflow' from sample to final report for food safety laboratories. Also have the capability to expand to looking for the unknowns utilizing a new software feature AcquireX.

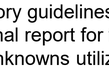
Methods: Cumin and garlic were processed and analyzed to test the core methodology- from sample preparation using a modified QuEChERS protocol (Quick, Easy, Cheap, Effect, Rugged, and Safe) to analysis, data processing, and reporting with LC-MS HRAM and comprehensive data handling software. A compound database of over 700 compounds with Fragments, Retention Time, Spectral library from mzCloud and liquid chromatography conditions was created, along with a single screening and quantitative method. Two MS methods was tested Full Scan-DIA and AcquireX workflow.

Results: Results demonstrate that the methods are fit-for-purpose for both quantitative and broad-spectrum of pesticide residue for quantitation and screening for unknown that can be easily implemented in food safety testing labs. Calibrations with matrix matched standards (MMS) and matrix extracted standards (MES) were performed. Acceptable results were obtained for the key figures of merit: Limit of Quantitation (LOQs), calibration range/linearity, fragmentation matching scoring and spectra library matching.

INTRODUCTION

The demand for quick and simple analysis for a multi-class list of pesticides in large numbers of diverse food samples in agricultural applications is growing year by year. Throughout the world, pesticides are used to control pests that are harmful to crops, humans and animals. These substances can pose a significant health threat and therefore need to be accurately detected at the lowest levels. Government agencies typically set maximum residue levels for pesticides in different products of plant and animal origin at low part per billion (ppb or µg/kg) levels. The regulations present significant analytical challenges with respect to the low limits of quantification and high number of target analytes. Sample preparation is also a critical part of the workflow. The use of QuEChERS (Quick Easy Cheap, Effective, Rugged, and Safe) methods have been widely adopted for the extraction of pesticide residues from a wide range of food matrices including spices. This work describes the method performance parameters using the latest benchtop LC - Orbitrap Exploris 120 mass spectrometer for the quantitation of a targeted list of pesticides at or below maximum residue levels in both cumin and garlic matrices. The optimized method was verified according to SANTE/12682/20191 guidelines and evaluated for compliance with the EU MRL requirements. Due to the acquisition modes, we can also look for unknown compounds not being targeted in the samples.

MATERIALS AND METHODS



Sample Preparation

Sample preparation involves a protocol that was optimized to be easy to implement and reduce matrix co-extractives, resulting in enhanced sensitivity and robustness in electrospray ionization LC-MS HRAM. The basic elements of the preparation procedures are described below using QuEChERS Extraction with no dSPE clean up. Weigh 2 g of each powder spice into a 50 mL conical tube. (For MES) Spike samples of pesticide mega mix @ 1 µg/mL for the levels required at 0.5, 1, 5, 10, 50 and 100 ng/mL final. Let spike sample sit at room temperature for 30 mins. Add 15 mL of water with 1% Acetic Acid; mix for 5 mins and stand at room temperature for 10min soaking. Then add 15 mL of Acetonitrile to the mixture and mix vigorously for 1 min on a benchtop vortexer. Add QuEChERS salt (6G MgSO4, 1.5G Sodium Acetate) to tube and shake vigorously for 1min. Then place in benchtop vortexer for 5 min. Aspirate or pour top layer into 15mL vial. Aspirate 2 mL from vial and filter through a 0.45 µm filter into a HPLC vial. Aliquot 1mL into individual HPLC vials and make calibration levels at 0.5, 1, 5, 10, 50, and 100 ng/mL using mega mix stock.

Instrument Method

Analytical condition use for the analysis are described here below in Figure 2

Figure 2: LC gradient, mobile phase, column, and API source conditions for the Vanquish Flex Binary UHPLC pump with Orbitrap Exploris™ 120 mass spectrometer.

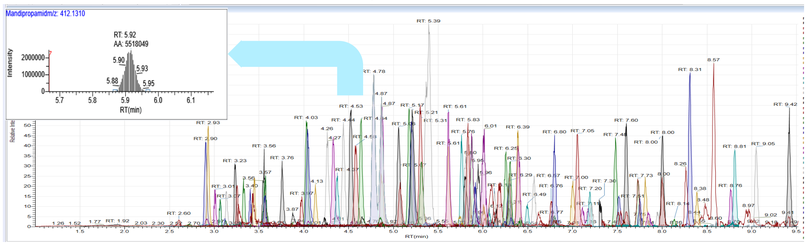
Thermo Scientific™ Vanquish™ Flex Binary UHPLC system:	Thermo Scientific™ Orbitrap Exploris™ 120 MS:
Mobile phase:	Positive Voltage: 3500V
A: Water + 5mM Ammonium formate & 0.1% Formic Acid	Sheath Gas 30 units
B: MeOH + 5mM Ammonium formate & 0.1% Formic Acid	Auxiliary Gas 6 units
Injection volume: 1 µl	Sweep Gas 1 units
Column: Thermo Scientific™ Accucore™ aQ 100 x 2.1mm x 2.6µm	Ion transfer tube Temp: 290° C
Column temperature: 25° C	Vaporizer Temp: 350° C
Flow rate: 300 µl/min	Resolution : Full Scan 60,000, DIA 15,000
Run time: 15 min	AcquireX : Full Scan 60,000, ddMS2 15,000

Data Analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ software to ensure full automation from instrument setup to raw data collection, processing, and reporting. Experiment 1: Data acquired from FS-DIA were analyzed with an extraction mass tolerance of ±5 ppm for both precursor and product ions. Analytes were quantified based on full scan precursor accurate mass. In addition, confirmation of target pesticides was performed by DIA fragment matching using a curated high-resolution spectral library. Experiment 2: The samples were then analyzed for other contaminants, using a new 'data-mining' software function called AcquireX intelligent data acquisition workflow. This functionality has several workflows. One such workflow is called Background Subtraction and uses a blank matrix to automatically generate an exclusion list of matrix co-extractives prior to acquisition, while using a targeted MS2 inclusion list with retention times for added specificity for the targeted pesticides. Data were extracted with a mass tolerance of 5 ppm for both precursor and product ions of targeted pesticides. Analytes were first quantified using the full scan precursor mass trace and then identified using a targeted list of pesticides from a compound database and matched with a spectral library. All data were evaluated against SANTE Guidelines criteria using EC SANTE/12682/2019.1

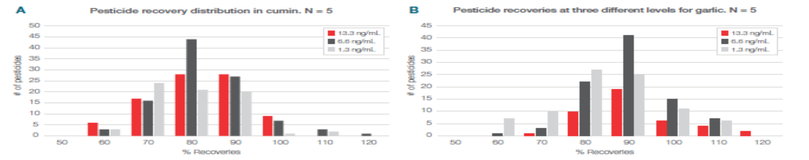
RESULTS

Experiment 1: Simplified in-house validation for screening and quantitative methods was carried out for targeted pesticides. The linearity of the calibration curves for MMS was assessed over the range from 0.5 to 100 ng/mL to demonstrate the potential of the method for quantitative analysis. The evaluation was based on accurate mass of the analyte at the specified retention time window (±0.1 min). Full MS scan acquisition-based quantitation using mono-isotopic match, presence of fragment ions (FI), and a high resolution curated pesticide spectral library match (LS) were additionally applied for identification according to References 1 and 2. Acceptance values were set ≤5 ppm for mass accuracy (FS, DIA anddMS2), ±0.1 min for retention time, reproducibility at limit of quantitation (LOQ) RSD ≤15% and limit of detection (LOD) between 15–20% RSD with at least one fragment ion (FI) present and ≥50% for LS matching, however reporting standards were set at ≥60% and a R2 ≥ 0.9800. Figure 3 shows some select pesticides across the retention time range of the method (1–10 min) as well as demonstrates sufficient scans across each peak for accurate quantitation.

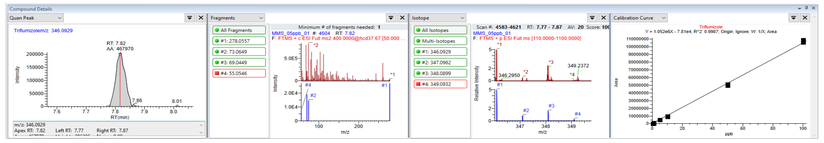


Figures 3: Chromatogram of all pesticides in 15 min spiked cumin MMS at 10 ppb. The peak highlighted at 5.92 min is mandipropamid, showing over 11 scans across the full scan quantitation ion.

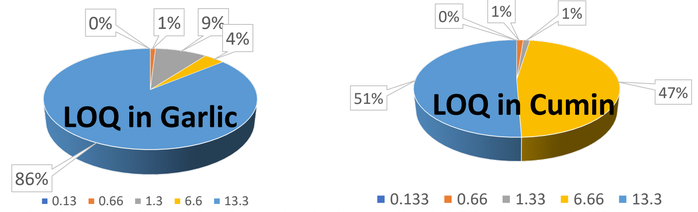
Recoveries were checked for both cumin and garlic to confirm the extraction protocol was universal for both matrices at 3 different concentration levels (1.3, 6.6, and 13.3 µg/kg) and n = 5 replicates/concentration. The results show excellent recoveries between 70–120%. Some compounds in 60% range showed excellent precision between replicates and thus are allowable under SANTE guidance, Figure 4A and 4B (respectively). Figure 5 shows a typical results of calibration curves from 0.5 to 100 ppb. Over 95% of the pesticides studied had calibration curves with r^2> 0.990. Confirmation fragment ions are displayed in the middle of each panel at 0.5 ppb with indicator colors (green) easily visible to show passing isotope criteria. The LOQs for both cumin and garlic were determined as outlined by the EU guidelines, with results shown in Figure 6.



Figures 4: Pesticide recoveries in cumin (A) and garlic (B) at 1.3, 6.6 and 13.3 ng/mL for n = 5 replicates

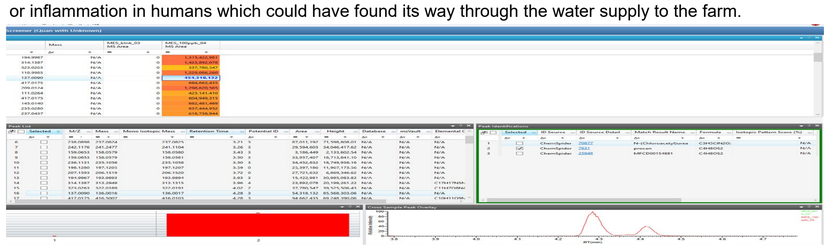


Figures 5: The quantitation and confirmation ions along with calibration range from 0.5 to 100 ppb for Triflumizolem @0.5 ppb



Figures 6: LOQs in Garlic and Cumin obtained following the SANTE guidelines in MES

Experiment 2: The implementation of the AcquireX Background Exclusion workflow also helps in identification of targeted and unknown contaminates using a unique routine to automatically create an exclusion list based on LC-MS analysis of the matrix blank. The instrument method is automatically updated with the exclusion list, so when subsequent samples are analyzed, MS2 experiments are not performed on matrix background signals. As a result, more cycle time is spent on triggering MS2 on the relevant ions of interest. This is groundbreaking for data processing because it minimizes false-positives and -negatives. TraceFinder software can efficiently process these new complex data files and extract results for both targeted quantitation and unknown screening workflows. TraceFinder software can easily go from a targeted quantitation workflow to unknown screening workflow. Example Figure 7, shows an unknown compound found in garlic to be proxan a non-steroidal anti-inflammatory drug used to treat pain or inflammation in humans which could have found its way through the water supply to the farm.



Figures 7: Unknown compound found in garlic and ID as Proxan and anti-inflammatory drug.

CONCLUSIONS

- A large pesticide panels for quantitative analysis and screening at levels below pr at EU MRLs have been shown to provide excellent sensitivity and robustness in a routine laboratory setting for cumin and garlic.
- The QuEChERS extraction procedure demonstrated good recovery and precision, with only 1 µL required for injection to meet EU SANTE validation guidance.
- On-going work is required to evaluate the more unknown detected compounds that did not have an identification but only molecular formula assignment which will require either chemical synthesis, NMR or other techniques to prove the authenticity of the unknown.

TRADEMARKS/LICENSING

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