

Routine Quantitative and Qualitative Methodologies for Food Pesticide Residue Laboratories Using Tandem and High Resolution Accurate Mass (HRAM) LC/MS Instrumentation

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Overview

Purpose: The aim of the poster is to demonstrate complete, routine workflows for pesticides analysis, known as the **Thermo Scientific Pesticide Explorer Collection**, by comparing two MS based techniques: liquid chromatography-triple quadrupole mass spectrometry (MS/MS) and liquid chromatography-high resolution accurate mass spectrometry (HRAM).

Methods: Quantitative methods in three representative matrices are presented on both LC tandem and HRAM instrumentation. Method validation was performed according to the EU guidelines to create a robust, high-throughput triple quadrupole method for targeted analysis as a 'ready-to-go' solution for pesticide residue laboratories. In addition, a comparison of the same residue method was made to HRAM analysis with data- dependent MS/MS acquisition. The use of accurate mass compound databases along with web-enabled searches via easy-to-use processing software allow the user to look beyond target lists and screen for other contaminants.

Results: Both the tandem and HRAM instrument platforms provide robust data for QuEChERS matrices prepared without dSPE cleanup with excellent precision and accuracy using a 1 μ L injection volume. Both platforms have comparable limits of quantitation, important for labs meeting MRLs in a regulated environment. Screening for unknowns with HRAM is greatly simplified for the user with built-in workflows and data processing that allow easy data review.

Introduction

Food pesticide residue laboratories face significant analytical challenges. Growing target compound lists, large numbers of samples, wide varieties of matrices, and decreasing limits of detection are pressuring labs to become more efficient than ever before. In addition, customers often require more information on contaminants that are not on any target lists that may be a threat. An integrated solution using both HRAM and tandem MS is needed to address this ever-changing landscape.

Methods

Sample Preparation

Samples were homogenized with reduced temperature to prevent losses of target analytes. The QuEChERS extraction described below was based upon the European EN 15662 Method (Use of sodium chloride, magnesium sulfate and citrate salts). However, the dispersive solid phase extraction (dSPE) step was omitted to prevent potential loss of analytes in the multi-class method.

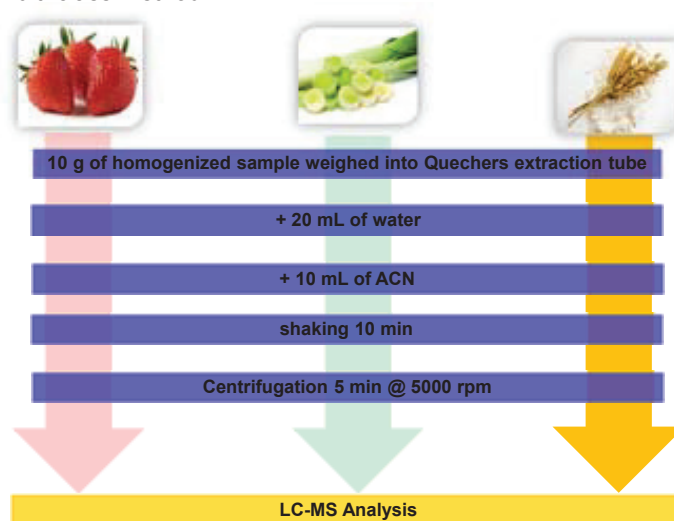


Figure 1: QuEChERS extraction for the three matrices

Thermo Scientific™ UltiMate® 3000 RSLC :

• **Mobile phase:**

A: Water:MeOH (98:2) + 5mM Ammonium formate & 0.1% FA

B: MeOH:Water (98:2) + 5mM Ammonium formate & 0.1% FA

• **Injection volume:** 1 µl

• **Column:** Accucore column aQ 100 mm x 2.1 mm x 2.6 µm

• **Column temperature:** 25°C

• **Flow rate:** 300 µl/min

• **Run time:** 15 min

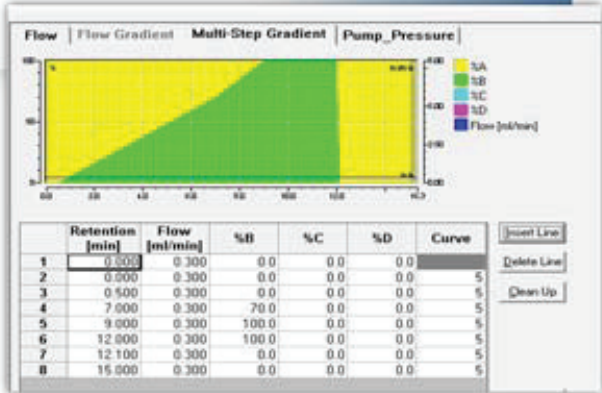


Figure 2: HPLC conditions used on both instruments platforms. The 1 µL injection volume of the pure acetonitrile extracts prevented peak distortion of early eluting pesticides.

Methods

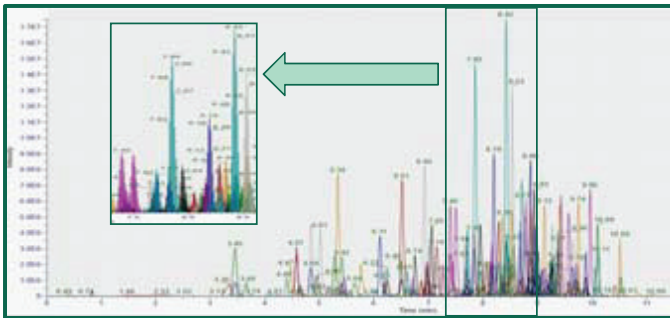


Figure 3: Example chromatogram for the optimized HPLC method for 276 compounds in 15 minute run time.

Typical instrumental workflow for pesticide residue analysis is described in Figure 4 below.

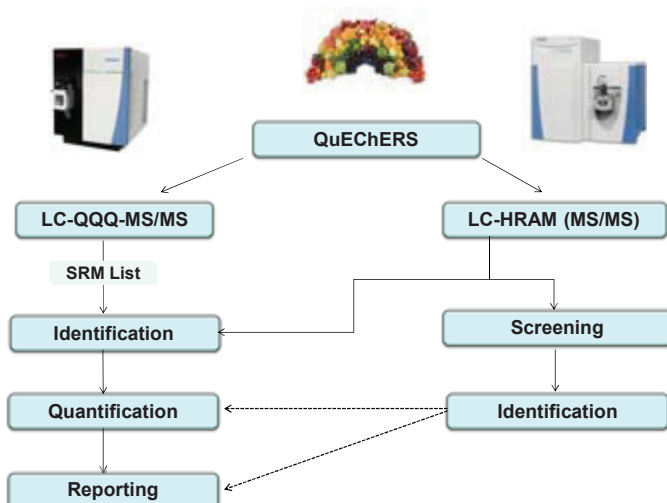


Figure 4: Tandem MS workflow only allows for target compounds typically with timed SRMs. The HRAM instrument can be set up with an inclusion list for target quantitation and MS/MS confirmation / identification with a compound spectral library and database. Screening for unknowns is possible using the acquired full scan data and/or acquisition with data dependent or independent MS/MS modes without Inclusion lists.

Results

Method validation protocol included the following: Matrix-matched calibrations (linearity), recovery and reproducibility at two levels (10 and 100 ppb), injection precision, determination of LODs/LOQs, and accuracy using certified reference material (FAPAs samples).

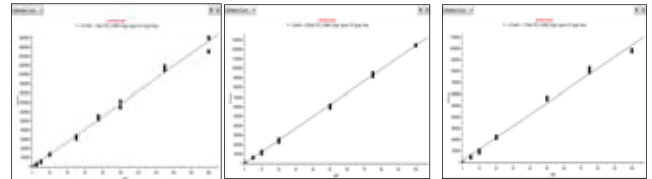


Figure 5: Example of matrix matched calibration curves for the pesticide Dimethomorph by tandem MS. From left to right: Strawberry, leek, and flour. Most curves had r^2 values greater than 0.995 over the calibration range – 7 levels (0, 5, 10, 20, 50, 75, 100, and 200 ppb)

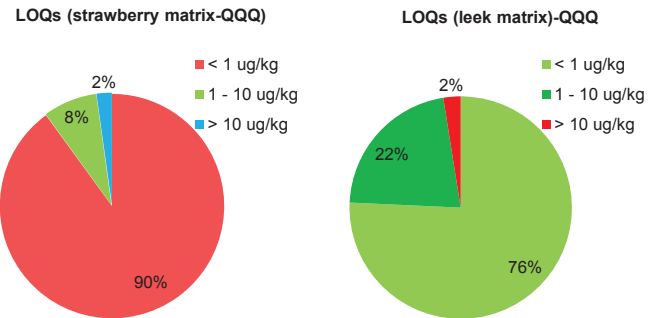
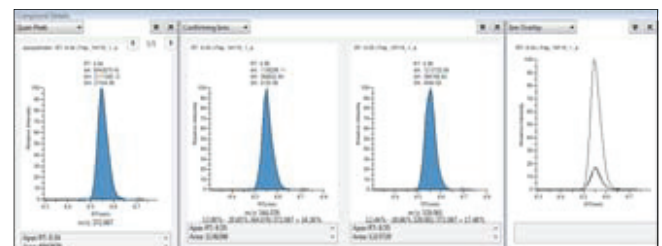


Figure 6: Summary of LOQs obtained in strawberry and leek matrices by tandem MS as percentages of the total number of pesticides (276). Flour matrix had very similar distribution to leek.

An analysis of a certified reference material was performed in three available matrices (lettuce puree, melon puree, and wheat flour.) Twelve pesticides in the reference material were detected using the multi-residue method. The certified reference values from these FAPAs sample ranged from 52 to 383 µg/Kg; all results were within the acceptance ranges. Azoxystrobin in lettuce puree shown below with all qualifier ions detected and passing ion ratio criteria.



Results

The sample extracts were also analyzed by HRAM with the same LC method conditions as described earlier. The orbi-trap instrument was set up in full scan data-dependent MS/MS acquisition mode with the 276 compounds placed in an inclusion list. This mode allows isolation of specific precursor ions cited in the list based upon set threshold values and retention time windows. Calibration and LOD/LOQs were very similar to the results obtained by tandem mass spectrometry. This ensures that HRAM had enough sensitivity and quantitative ability to pass current regulations for maximum residue limits (MRLs).

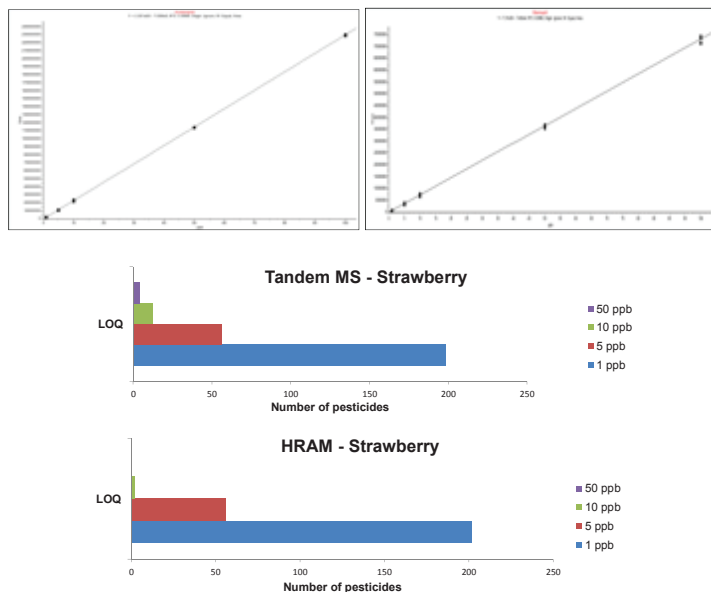


Figure 7: (Top) Calibration curves for Acetamiprid (left) and Bromoxynil (right) in strawberry matrix by HRAM LC/MS, from 1ppb to 100 ppb. The observed accuracy was excellent for both analytes, with % RSDs less than 10% for seven replicates at 1 ppb. **(Bottom)** The overall observed LOQs for the 276 pesticides on the HRAM instrument were similar to tandem mass spectrometry for all the matrices studied. The above is an example is strawberry matrix.

Screening for unknowns against various databases can help labs expand beyond target compound lists and look for other potential contaminants of concern. Software is key to providing an easy-to-use workflow to help the analyst separate significant hits from background matrix and provide statistical analysis. Thermo Fisher TraceFinder and Compound Discoverer software are included in the workflow solution kits that require unknown screening ability. Below is an example of a workflow in Compound Discoverer that can be applied to acquired data sets, typical for food or environmental sample types.

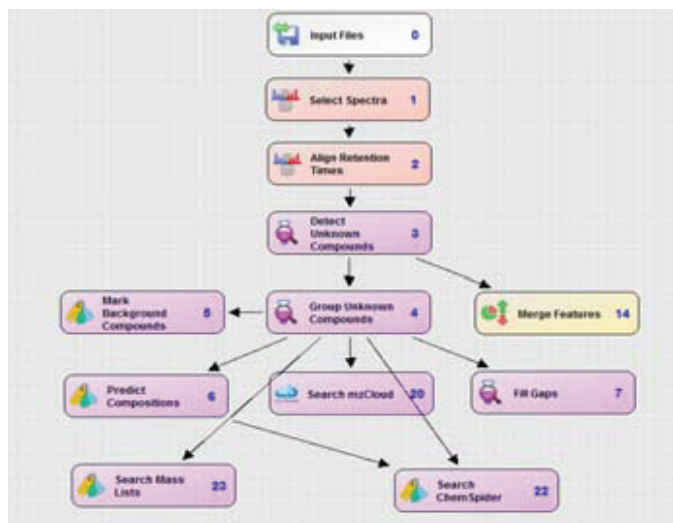


Figure 8: Compound Discoverer has ready-to-go workflows to help laboratories identify unknown compounds and organize statistically significant findings for easy review. Databases such as *m/z* Cloud and Chem Spider are readily available, along with local databases.

Conclusion

The **Thermo Scientific Pesticide Explorer Collection** offers ready-to-go tandem MS and HRAM solutions that meet the challenges in food labs.

- Complete methods available on both platforms for multi-residue analysis
- Common LC methods on each platform for easy cross-over
- Tandem MS-like sensitivity demonstrated on HRAM platform to ensure detection at or below MRLs
- Easy-to-use software for data processing and review in unknown screening workflows

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