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

Poster Note 64786

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ABSTRACT

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. Clearly, an integrated solution using both HRAM and tandem MS is needed to address this ever-changing landscape.

The aim of the poster is to demonstrate a complete package 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 were validated according to the European SANCO guidelines 12495/2011.

INTRODUCTION

Routine 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 designed 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. In this mode, full scan accurate mass information is stored for each sample, and the isolated precursor/product ion pairs serve as a means of confirmation. 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.

MATERIALS AND METHODS

Three matrices were evaluated for the comparison: strawberry (high sugar and water content), leek (complex green vegetable with high pigment content), and flour (dry matrix with low water content).

Samples were homogenized with reduced temperature to prevent losses of target analytes. The QueChERs[2] 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.

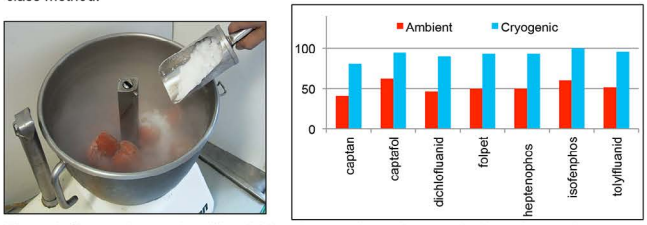

Figure1: Cryogenic sample milling (left) and comparison of recoveries for select analytes (right)

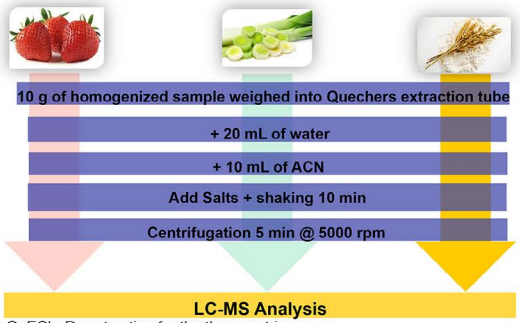

Figure 2: QuECheRs extraction for the three matrices

MATERIALS AND METHODS (Cont.)

A high-throughput 15 minute LC method was optimized for 276 target compounds that were used to spike the matrices in the study.

Thermo Scientific™ UltiMate™ 3000 RSLC Nano Systems:
- 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: Thermo Scientific™ Accucore™ aQ 100 mm x 2.1 mm x 2.6 µm
- Column temperature: 25°C
- Flow rate: 300 µl/min
- Run time: 15 min

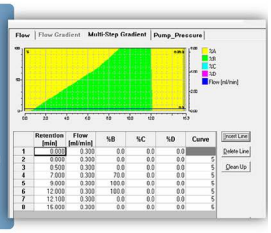

Figure 3: HPLC conditions used on both instruments platforms. The low 1 µL injection volume of the pure acetonitrile extracts prevented peak distortion of early eluting pesticides.

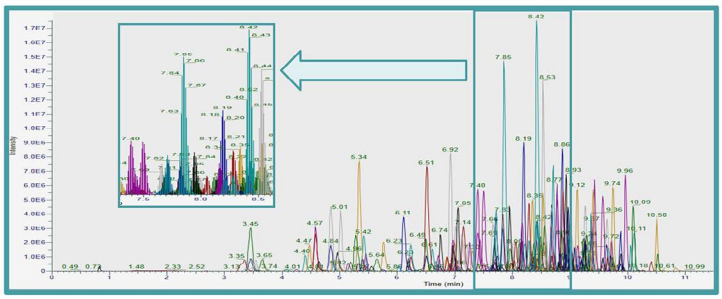

Figure 4: Example chromatogram for the optimized HPLC method for 276 compounds

The typical instrumental workflow for pesticide residue analysis is described below. Targeted quantitation can be performed with confirmation using either HRAM or traditional tandem mass spectrometry. The ability to expand beyond target lists and look for unknown contaminants allow labs to expand their scope.

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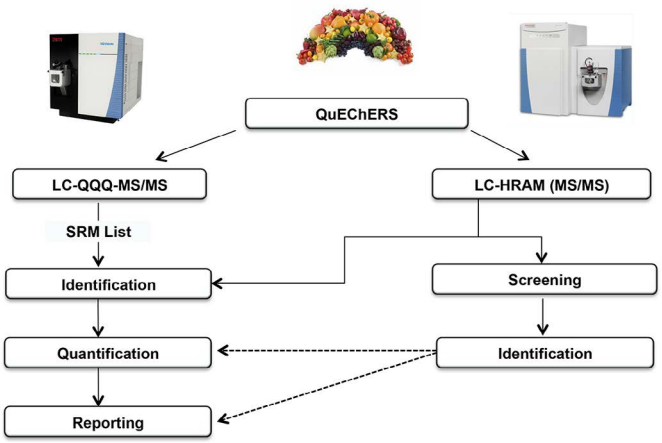


Figure 5: Triple Quadrupole MS and HRAM Workflows. The Triple Quadrupole workflow only allows for target compounds typically with timed SRMs. The HRAM instrument can be set up with an inclusion list for target quantitation, typically at full scan resolution of 70,000 FWHM, along with MS/MS confirmation and identification with a compound spectral library and data base. Screening for unknowns is possible using the acquired full scan data and/or acquisition with data independent 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).

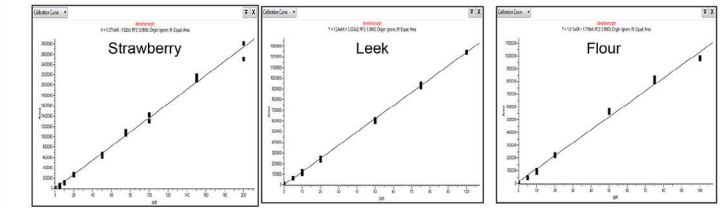


Figure 6: Example of matrix matched calibration curves for the pesticide Dimethomorph by tandem MS. From left to right: Strawberry, leek, and flour. Most curves had r^2 values greater that 0.995 over the calibration range – 7 levels (0, 5, 10, 20, 50, 75, 100, and 200 ppb)

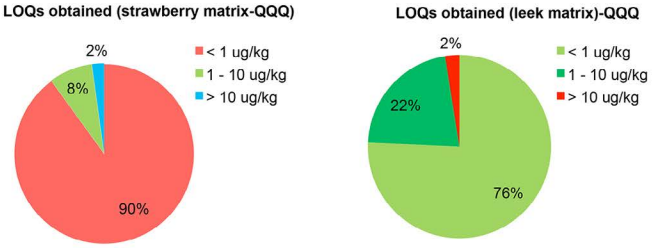
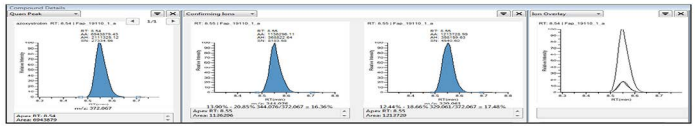


Figure 7: Summary of LOQs obtained by QQQ in strawberry and leek matrices as percentages of the total number of pesticides (276). Flour matrix had very similar distribution to leek.

			Strawberry				Leek				Flour			
Analyte	Sp. Level 1 (µg/kg)	Sp. Level 2 (µg/kg)	RSD 1 (%)	RSD 2 (%)	Rec 1 (%)	Rec 2 (%)	RSD 1 (%)	RSD 2 (%)	Rec 1 (%)	Rec 2 (%)	RSD 1 (%)	RSD 2 (%)	Rec 1 (%)	Rec 2 (%)
Acephate	10	100	16	11	81	89	9	8	106	92	8	16	90	79
Azoxystrobin	10	100	6	3	95	99	8	8	93	93	17	2	89	112
Carbaryl	10	100	5	3	93	95	7	11	103	101	6	10	102	105
Cymoxanil	10	100	11	2	99	96	7	4	103	106	13	3	86	102
Dimetomorph (sum of isomers)	10	100	7	3	94	95	5	3	110	100	13	4	73	109
Diniconazol	20	200	12	3	90	94	12	6	84	98	16	4	88	106
Etrimfos	10	100	6	2	92	91	8	3	94	102	7	3	87	106
Oxamyl	10	100	11	14	91	94	13	18	99	84	15	7	94	106
Pencycurone	10	100	8	3	101	93	3	3	103	100	10	6	86	99
Pyraclostrobin	10	100	9	2	101	102	8	4	102	106	9	1	76	109
Spinosad A	10	100	11	6	99	95	6	6	103	101	17	4	108	121
Zoxamide	10	100	9	1	99	95	6	3	104	100	9	4	78	107

Table 1: Recovery and reproducibility are shown for some representative pesticides in all three matrices. For seven replicate injections, recoveries ranged from 78-106 % at spike levels of 10ppb and 100ppb with % RSD values ranging from 1-17%.

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 sown 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 Thermo Scientific™ Orbitrap™ 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 was a very significant finding, to ensure the HRAM had enough sensitivity and quantitative ability to pass current regulations for maximum residue limits (MRLs).

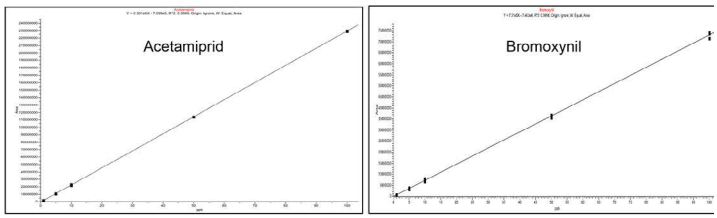


Figure 8: Calibration curves for Acetamiprid (left) and Bromoxynil (right) in strawberry matrix by HRAM LC/MS, from 1ppb to 100 ppb. Each calibration point is an overlay of 7 replicates. The observed accuracy was excellent for both analytes, with % RSDs less than 10% for seven replicates at 1 ppb.

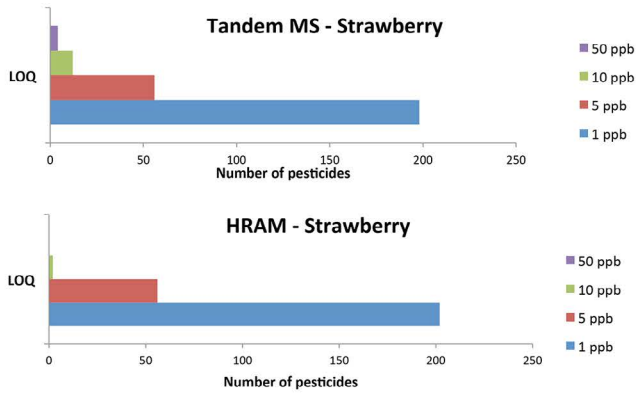


Figure 9: The overall observed LOQs for the 276 pesticides on the HRAM instrument were similar to tandem mass spectrometry for all the matrices studied. The above is an example is strawberry matrix.

In addition to just an MS/MS fragment library, a corresponding compound data base has been created with the same compounds based on the LC method. It provides retention times with exact mass fragments along with other supporting meta data. It is a great place for a routine food lab to start using the method described here or build a custom method. MS/MS library search results can be very useful information for compound identification to have in addition to single exact mass fragments and isotope pattern matching. An example of the pesticide Boscalid detected at 1 ppb in strawberry matrix shows excellent match score against the mass spectral library that is provided with the Thermo Pesticide Explorer solutions kit for HRAM.

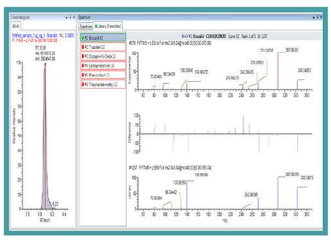


Figure 10: Boscalid identified in strawberry matrix at 1 ppb. Scan the QR Code at right to obtain spectrum and match to *m/z* Cloud using the Mobile App! https://www.mzcloud.org/

RESULTS

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 Scientific™ TraceFinder™ software and Thermo Scientific™ 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 files, typical for food or environmental sample types.

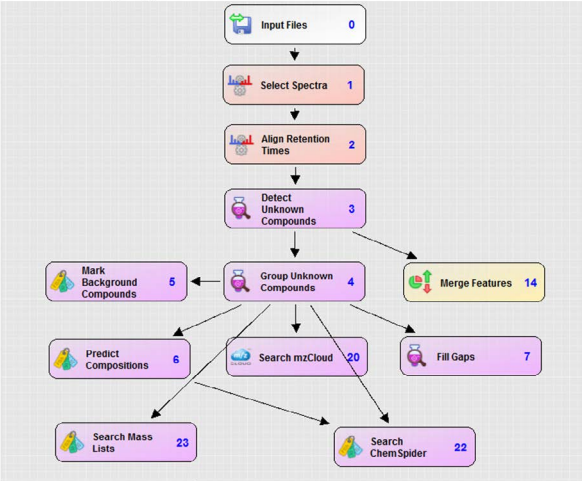


Figure 11: Compound Discoverer has ready-to-go workflows to help laboratories identify unknown compounds and then organize statistically significant findings for easy review and further processing. Databases such as *m/z* Cloud and Chem Spider are readily available, along with any local databases.

CONCLUSIONS

The Thermo Scientific Pesticide Explorer Collection solutions are designed to meet current laboratory needs for both routine targeted quantitation using tandem mass spectrometry and the ability to simultaneously screen for unknown potential threats plus quantitate known pesticides with HRAM. The data presented demonstrates that:

- The solutions were validated against the European SANCO guidelines 12495/201
- Both the tandem and HRAM instrument platforms provide robust data for QuEChERS matrices prepared without dSPE cleanup with excellent precision and accuracy with a 1 µL injection volume
- Both platforms have comparable limits of quantitation, important for labs meeting MRLs in a regulated environment
- The same LC method is available for both, so that it is very easy for labs to begin using HRAM with confidence very rapidly

REFERENCES

1. European SANCO guidelines 12495/201: More information: http://www.eurl-pesticides.eu/docs/public/home.asp?LabID=100&Lang=EN
2. QuEChERS- More information: http://quechers.cvua-stuttgart.de/index.php?nav1o=4&nav2o=0&nav3o=0

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