

OCTOBER 2015

# PESTICIDE RESIDUE ANALYSIS:

TIPS AND TRICKS FOR THE WHOLE WORKFLOW

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# INTRODUCTION

**P**esticide residue analysts are challenged to detect, identify, and quantify hundreds of different pesticides from different compound classes at low ng/g concentrations in a large number of diverse sample types with a fast turnaround, often within 24 hours of receipt. Analyses must be within budget and in compliance with official method validation, analytical quality guidelines, and accreditation body requirements.

Truly comprehensive monitoring requires analysis by both gas chromatography (GC) and liquid chromatography (LC) techniques, using both targeted and non-targeted approaches. The latter is required to detect illegal usage, because targeted approaches will not detect pesticides not programmed into the acquisition method. Optimum method performance may also require a separate sample extraction regimen for different sample types. The analyst's challenge is to select the optimum combination of consumable products, instrumentation, and methods to be able to develop innovative and cost-effective solutions that are fit for purpose.

This e-book, based on a series of four web seminars, provides pesticide residue analysts with valuable information from Thermo Fisher Scientific about the development and optimization of methods and workflows for the analysis of pesticide residues in food. The authors share their knowledge of the critical method control points, assisting analysts in modifying existing methods, optimizing current workflows, and understanding instrumental and software technologies with the goal of developing methods to deal with the most complex analytical problems.

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# SAMPLE PREPARATION TIPS AND TRICKS USING QUECHERS AND ACCELERATED SOLVENT EXTRACTION

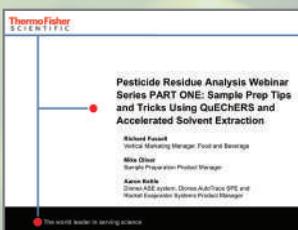
Richard Fussell, Mike Oliver, Jennifer Massi, and Aaron Kettle

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Web Seminar:  
Sample Prep Tips Using  
QuEChERS and ASE

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This article begins a four-part tutorial series on workflow design and optimization for pesticide residues analysis. The authors provide practical information on food sample preparation with commonly used techniques including QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) and Accelerated Solvent Extraction. The authors demonstrate a number of practical steps to improve pesticide recovery and extraction efficiency, provide criteria for selecting the appropriate method based on sample type, and discuss ways to develop or modify methods to meet laboratory-specific challenges.

Effective sample preparation is a critical yet challenging step in pesticide residues analysis workflows involving the use of gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS). Analysts must contend with a diverse array of complex food sample matrices in the effort to determine an equally diverse and complex range of target compounds. Not surprisingly, sample handling can be a source of substantial experimental error. In the interest of maintaining consumer confidence in food safety,

pesticide residue laboratories should develop and routinely use the most efficient and effective sample preparation methods possible.

In general, food sample matrices are highly complex and appear in many different physical forms. Many sample types require a significant amount of time-consuming manual processing, extraction, and clean-up before the extracts are ready to be introduced into a mass spectrometer. To improve qualitative and quantitative performance, the user must try to remove compounds from the sample to minimize matrix effects that might interfere with the accuracy of the measurement itself, such as suppression of the ionization process in LC-MS.

As the demand grows for more sensitive measurements of a larger number of pesticide compounds per analysis, historic large-scale extraction methods that can create a bottleneck in laboratory efficiency are being replaced by smaller-scale extraction methods that are more cost effective and more sustainable. By contrast to the older methods, which often included one or more clean-up steps, some of the newer, faster methods tend to be less selective, and consequently the final extracts contain an increased number and concentration of matrix compounds. Although these modern methods offer productivity benefits, the high amount of co-extractives, and the high number of method variations create a dilemma: Which method is the best for the task at

hand? For laboratories facing this dilemma, we focus on two main approaches with the potential to overcome some of the challenges: the widely used QuEChERS method, and the Accelerated Solvent Extraction technique with and without automated evaporation. Each has been proven to be a practical and effective sample preparation method for certain sample-target compound combinations.

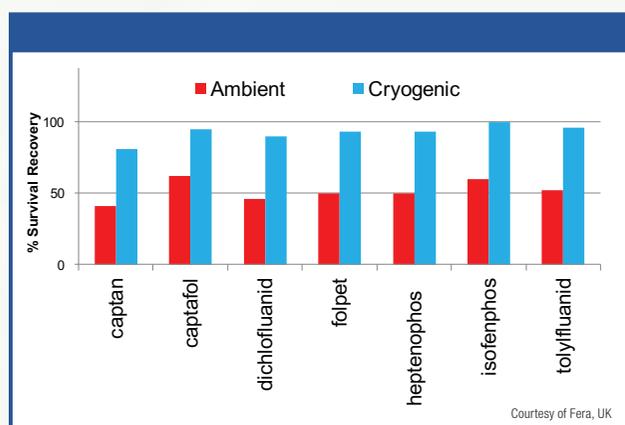
### Sample Processing: The Step Before Prep

Significant errors can be introduced into the analytical process even before the primary sample preparation step. Improperly processed samples can adversely affect the accuracy of the result. Errors introduced at this stage are not corrected by subsequent steps in the measurement process.

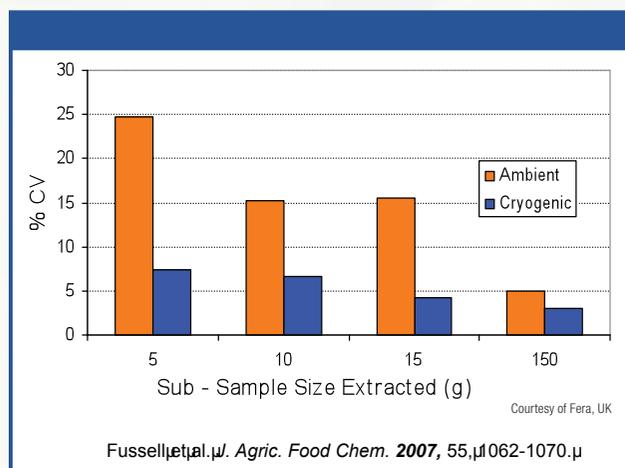
Surprisingly, many analysts go to great lengths to achieve modest 2–3% RSD improvements in measurement repeatability but pay relatively little attention to reducing the much greater errors that can be introduced during sample processing. One common example occurs with the comminution of a sample material at room temperature. Ambient conditions give rise to various chemical and biological reactions including hydrolysis and oxidation, enzymatic activity, and chemical complexation, that can degrade or complex the target pesticide in the sample. Lower-temperature comminution using liquid nitrogen or dry ice can

minimize these losses (1). In the case of dry ice, the sample is usually frozen (for example, placed in a freezer overnight) and then homogenized in a blender with dry ice to form a dry, homogeneous, and flowable powder. Before analysis the powder can be divided into test portions and placed in a freezer (typically overnight) to allow any excess carbon dioxide to dissipate.

**Figures 1** and **2** illustrate how cryogenic processing of sub-samples can improve recovery and homogeneity compared to room-temperature processing. Unfortunately, analysts often ignore



**Figure 1:** Cryogenic milling effect on the stability of pesticides.



**Figure 2:** Cryogenic milling effect on homogeneity.

the errors that can be introduced at the sample homogenization stage. Such errors are often excluded from validation experiments and from routine recovery, as well as from the calculation of measurement uncertainty. Laboratories that don't have on-site cryogenic capabilities can minimize error of room-temperature homogenization by processing the material as quickly as possible and stirring the homogenate continuously during withdrawal of sub-samples or test portions. Laboratories can also improve homogeneity by "double-processing"—in other words, re-processing a large sub-sample obtained after the initial processing—but this step poses the risk of increased degradation of some pesticides.

## Extraction

The goal of the extraction and clean-up stage is to extract analytes with maximum efficiency and with minimal recovery of matrix components. Matching the best technique to a sample type requires consideration of the sample's physical properties, the nature of the target compound, and the correct solvent for the task at hand. Although nearly all of the established multiresidue methods perform equally well for the majority of pesticides, reduced-scale techniques such as QuEChERS are becoming increasingly popular.

**QuEChERS overview.** QuEChERS (an acronym for "Quick, Easy, Cheap, Effective, Rugged, and Safe") is

a streamlined method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction (dSPE) for the determination of multiple residues (2). QuEChERS has gained rapid popularity in pesticide residue analysis because it enables laboratories to meet growing demands for high sample throughput, low detection limits, and cost-effective operation. It removes the need for blending, filtration, and evaporation steps, which also reduces the chance of losing volatile analytes. Its use of acetonitrile in the solvent extraction stage also helps mitigate the loss of polar pesticides. Because the technique is performed at reduced scale using less solvent, less equipment, and less laboratory space, the cost of analysis is reduced and environmental impact is lessened.

The original QuEChERS method is a very simple process (Figure 3). The homogenized sample is added to a centrifuge tube, followed by acetonitrile. If the sample is dry, then water should be added to rehydrate the sample (at least 80% water) before addition of acetonitrile. Magnesium sulfate and sodium chloride are then added (proportion-wise) and mixed in the capped tube by shaking. After centrifugation, the water (lower layer) is separated from the acetonitrile phase, an aliquot of which can then be transferred to a QuEChERS clean-up tube containing the appropriate dSPE sorbents, shaken and centrifuged, and an aliquot of the “cleaned-up” sample

is finally transferred to a vial before instrumental analysis. The dSPE clean-up step is optional. Another time-savings is the fact that the various reagents can be purchased conveniently pre-weighed

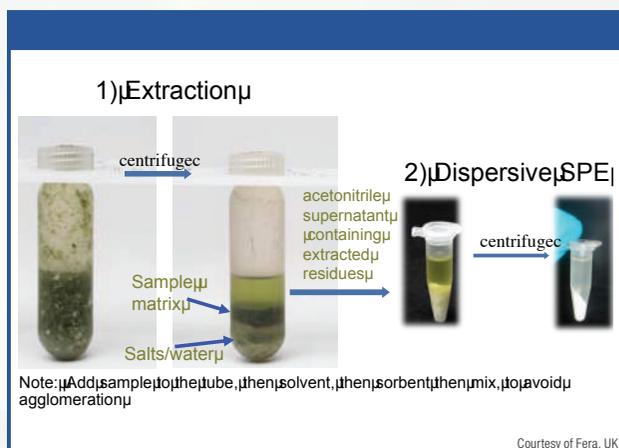


Figure 3: Steps of extraction and sample clean-up in QuEChERS preparation of a lettuce sample.

Matrix Type	Examples	Sorbent Requirements for Clean-Up
General Matrices	Apples, Cucumber, Melon	MgSO <sub>4</sub> , PSA Removal of excess water organic acids, fatty acids, sugars
Fatty Matrices	Milk, Cereals, Fish	MgSO <sub>4</sub> , PSA, C18 Additional removal of lipids & sterols
Pigmented Matrices	Lettuce, Carrot, Wine	MgSO <sub>4</sub> , PSA, C18, GCB Additional removal of pigments & sterols
High Pigmented Matrices	Spinach, Red Peppers	MgSO <sub>4</sub> , PSA, C18, GCB, Chlorofiltr™ Additional removal of chlorophyll

Figure 4: Selecting proper sorbent material for clean-up is determined by the properties of the sample matrix.

in sachets or pouches, for example, the Thermo Scientific™ HyperSep™ Dispersive SPE materials in metalized pouches. (See more at: <http://www.thermoscientific.com/en/products/quenchers-dispersive-spe.html>).

**Variations.** There are a number of variations of QuEChERS, and selecting the most appropriate option is a matter

of evaluating the type of matrix and the pesticides being measured. The three primary variations are currently supported by commercial accessories and consumables (Thermo Scientific, Waltham, MA). The original 2003 method (2) uses NaCl to enhance partitioning of the pesticides into the acetonitrile phase. In an effort to address problems caused by base-sensitive compounds in the sample, the AOAC later introduced a method using NaOAc and acetic acid instead of NaCl. The European Union Reference Laboratory for Single Residue Methods has developed a variation using citrate buffer salts.

Choosing between the buffered versions and the original method is straightforward. If base-sensitive compounds such as captan, chlorothalonil, dichofluanid, dicofol, folpet, or tolylfluanid are present, a buffered option will give better recovery. For non-base-sensitive compounds, the recovery differences between the methods are insignificant. In the AOAC method the presence of acetic acid in samples can reduce the removal of matrix co-extractives by PSA, which can cause elevated baselines in subsequent analyses.

#### **QuEChERS troubleshooting.**

Laboratory equipment companies are continuously developing new pesticide applications that demonstrate the optimal use of QuEChERS in challenging applications. Our laboratory has published a number of technical notes

(3–5) demonstrating the method's effectiveness with a diverse range of foods including cucumber, grapes, and lettuce using GC-MS. Although the QuEChERS method is simple, occasional issues can occur in certain applications—notably diminished analyte recovery. Certain sample types require additional procedures. For example, dry-state samples such as tea or cereal require hydration with water before extraction; previously frozen samples must be fully thawed (after addition of solvent) and often require extra agitation with an automated shaker; and unstable compounds prone to breaking down require pH adjustment.

#### **Accelerated Solvent Extraction**

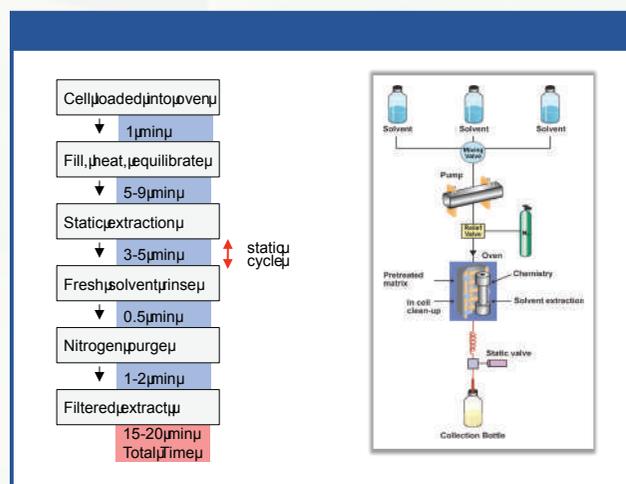
The Accelerated Solvent Extraction technique further expands a laboratory's capabilities to obtain high-quality extractions from difficult samples with a low water content and those with a high fat content. The Accelerated Solvent Extraction method can extract analytes from the sample and, in certain applications, can also employ in-line absorbents to perform clean-up, minimizing the need for an offline gel-permeation chromatography (GPC) step.

The Thermo Scientific™ Dionex™ Accelerated Solvent Extraction system is available with both single-sample (Thermo Scientific™ Dionex™ ASE™ 150 Accelerated Solvent Extraction system) and 24-sample (Dionex ASE®-350 system) processing capacities. Both

platforms decrease run times by elevating the temperature, thereby increasing the analyte's extraction kinetics. The system also utilizes elevated pressure (1500 psi) to enable extraction solvents to stay in the liquid state above their atmospheric boiling point. Since the extraction solvents remain liquid at elevated temperature, the extraction efficiency is greatly improved and the system uses significantly less solvent than techniques such as Soxhlet and sonication.

Depending on the method, Accelerated Solvent Extraction reduces extraction times to 15–30 min and uses 10–15 mL of solvent per sample.

**Figure 5** illustrates the six primary steps used during the Accelerated Solvent Extraction technique. The procedure is characterized by one or more static



**Figure 5:** Overview of the Accelerated Solvent Extraction technique.

extraction cycles in which analytes diffuse from the sample into the extraction solvent. The diffusion process moves target analytes down a concentration

gradient. To allow the user to maximize extraction efficiency, the procedure allows optimization of methods to include multiple static cycles to exhaustively extract samples and produce analyte-rich extracts for analysis.

## Solvent Evaporation

The Accelerated Solvent Extraction technique can perform extraction and cleanup, but isn't capable of evaporation. The Thermo Scientific™ Rocket™ Evaporator is a centrifugal device that evaporates solvent through low-temperature boiling. The system exploits Boyle's Law by reducing the pressure below atmospheric so the boiling point of solvents will be lowered. A vacuum-sealed outer chamber containing deionized water is heated to 40 °C to produce steam for the heat source. Samples are housed in a vacuum-sealed inner chamber that is exposed to the heat source to evaporate the solvent. Solvent vapors are collected and condensed in a cold trap within the instrument so there is no vapor exposure to the analyst. The Rocket™ Evaporator will evaporate large volumes of solvent (up to 400 mL) to complete dryness or concentrate directly into a GC autosampler vial with fixed endpoint detection.

A wide variety of sample holders is available to meet specific requirements of a method, such as drying or concentrating an Accelerated Solvent Extraction sample. For drying, a tool called the Thermo Scientific™ Dionex™

ASE® puck is an adapter that fits into each of the six rotor positions to hold three of the 60-mL ASE® collection vials. The puck is useful for handling Accelerated Solvent Extraction extracts intended to be dried for reconstitution. For concentrations, the flip-flop style vessel is a modified 60-mL vial with caps on either end. When placed in the extractor, it functions as a conventional collection vial, but after the run it can be flipped over, opened, and fitted with an adaptor that accepts a GC autosampler vial. These vials can be placed onto the Dionex™ ASE® puck, enabling the device to concentrate up to 18 accelerated solvent extraction samples into the autosampler vial.

## Conclusion

This overview is intended to help users take into account several factors that could potentially improve analytical results when dealing with complex measurements of pesticide residues in food samples. An understanding of the requirements and challenges unique to analytical food testing laboratories can help laboratories develop workflows that deliver the best possible results in the most cost-effective, time-efficient manner for a wide range of analytical demands.

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- (3) Thermo Fisher Scientific Application Note 20549.
- (4) Thermo Fisher Scientific Application Note ANGSC PESTGRAPES 0700.
- (5) Thermo Fisher Technical Note 10222.

## About the Authors

**Richard Fussell** focuses on food safety, GC-MS and trace elemental applications in his current role. Before joining Thermo Fisher Scientific, he gained more than 35 years of experience working for the UK government, including 21 years at the Food and Environment Research Agency (FERA, formerly called CSL). At FERA, Richard was an advanced research fellow and senior scientist managing research and collaboration projects focused on emerging MS techniques for pesticide residues and veterinary drugs and food.

Since 2010, **Mike Oliver** has been responsible for the development and introduction of new innovative technologies to the marketplace, such as the Thermo Scientific Accucore Vanquish UHPLC column and SOLA SPE plates and cartridges. Previously, Mike worked for nine years for two leading MS vendors, where he was responsible for biotechnology sales in the UK and provided application solutions for proteomic and metabolic workflows, based on high-resolution LC-MS platforms. Mike holds a PhD in mass spectrometry and biochemistry from the MS Research Unit at the University of Wales in Swansea, UK.

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**Aaron Kettle** is a Product Manager for the Thermo Scientific sample prep systems, including Accelerated Solvent Extraction, solid-phase extraction, and the Rocket evaporator. He works for key industry experts to develop improvements in these technologies so that laboratory and regulatory leaders receive the best possible sample preparation solution. Over the last seven years, Aaron has held both sales and marketing positions at Thermo Fisher Scientific (previously Dionex), and before that, he was a Commissioned Officer in the US Navy and served as a Technical Director and Biochemist for the Department of Defense Forensic Laboratory. Aaron holds an MSc in Toxicology from the University of Michigan's School of Public Health and an MBA from the Lake Forest Graduate School of Management.

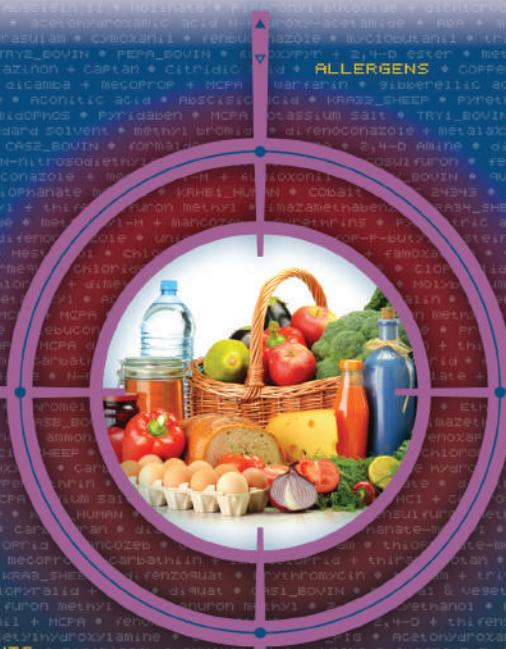
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# OPTIMIZED WORKFLOW FOR THE ANALYSIS OF PESTICIDE RESIDUES USING LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY

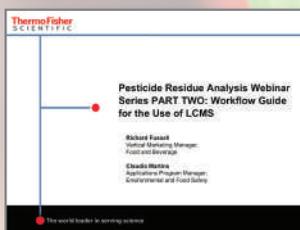
*Richard Fussell, Claudia Martins, and Jennifer Massi*

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Workflow Guide for LC–MS  
in Pesticide Analysis

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This second installment of a four-article series of tutorials on pesticide residue analyses focuses on workflows using liquid chromatography–mass spectrometry (LC–MS). During the past decade, significant technological improvements in both sample preparation and instrumentation have contributed to dramatic growth in the use of LC–MS in pesticide residues analyses. Indeed, the technique is now essential for the determination of modern pesticides, most of which are more amenable to direct analysis by LC–MS compared to GC–MS. To aid pesticide residue laboratories in the selection of the best analytical method for their specific challenges, this article focuses on practical suggestions for optimized chromatographic separations and mass detection for a range of pesticides in a variety of sample matrices.

Rapid technological developments in liquid chromatography mass spectrometry (LC–MS) have brought significant benefit to the analysis of pesticides in food products. With a combined total of around 30 years of involvement in this field, the authors have employed a wide range of LC–MS approaches, beginning

with atmospheric pressure chemical ionization with single-quadrupole mass spectrometers capable of determining only two to three compounds in a single analytical run. By the year 2002, electrospray triple-quadrupole mass spectrometers were readily available with the ability to quantify around 50 pesticides at 10–50  $\mu\text{g}/\text{Kg}$ . Soon thereafter, continuing advances in MS along with the introduction of reduced-scale sample preparation techniques such as QuEChERS opened the door to the analysis of a much larger number of pesticides in a greater range of sample types.

Today, state-of-the-art triple-quadrupole MS systems such as the Thermo Scientific™ TSQ Endura™ triple-quadrupole mass spectrometer and the Thermo Scientific™ TSQ Quantiva™ triple-quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA), enable analysts to detect, identify, and quantify several hundred targeted pesticides at low concentrations (10  $\mu\text{g}/\text{Kg}$  and below) in complex matrices in a single run. Alternatively, mass spectrometers offering high resolution and mass accuracy, such as the Thermo Scientific™ Orbitrap™ -based systems, enable laboratories to combine screening of unexpected residues with the identification and quantification of targeted analytes into a single analysis, dramatically increasing the capabilities of LC-MS. The high-resolution approach meets today's demand for increased

scope (more analytes), screening for non-targeted compounds and the capability for retrospective analysis should new information emerge in the future. When evaluating an analytical technique, laboratories take into account a number of key factors, including method sensitivity, robustness, and sample throughput. The challenge is to meet the established maximum residue levels, or tolerance values, in the most efficient and cost-effective manner possible. Selecting the most appropriate technique from the array of sophisticated technologies can be challenging. This article provides practical insights to help analysts make better-informed decisions on method development and instrument selection.

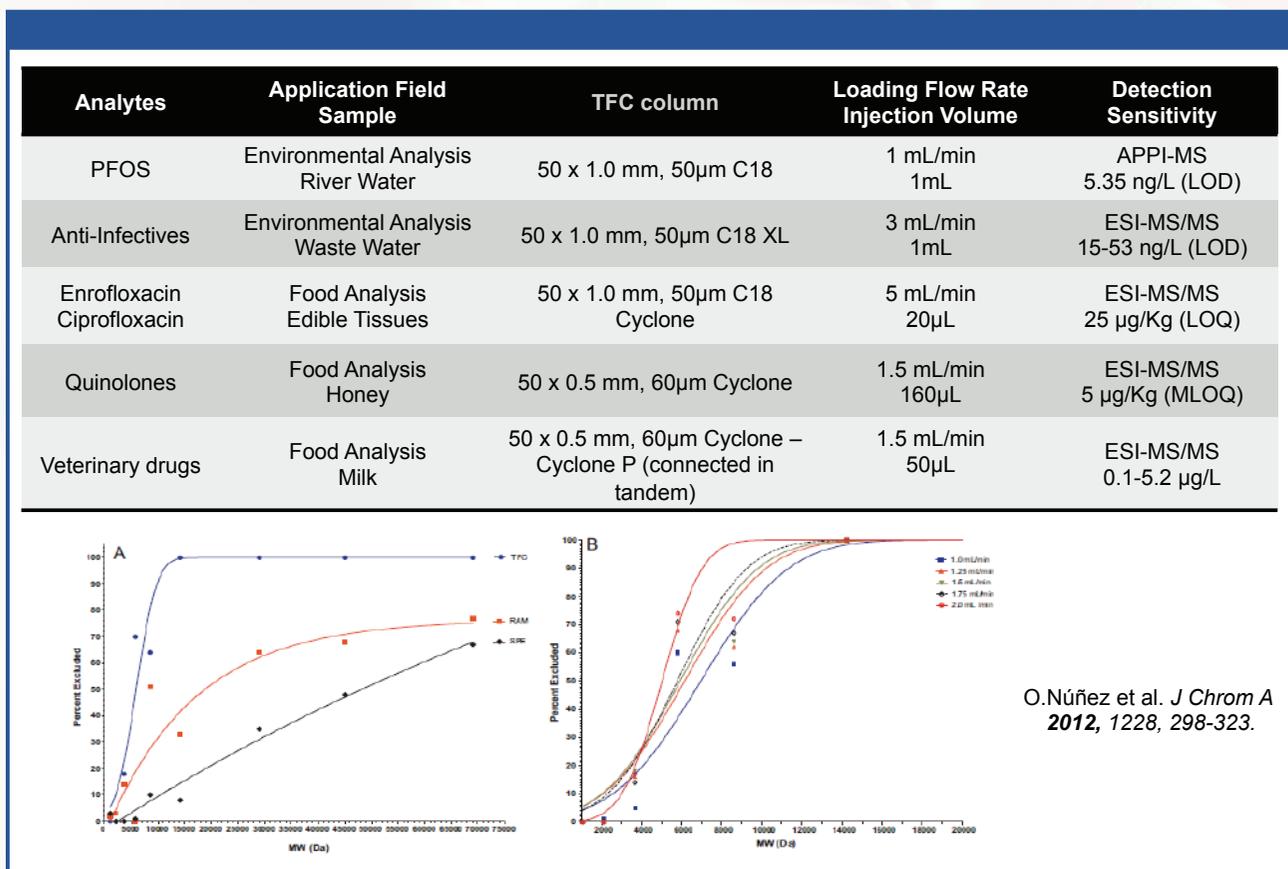
### Sample Preparation and Separation

The first installment of this series focused in depth on the QuEChERS approach, which is a generic sample preparation protocol that has been successfully validated for a large number of pesticides in a wide range of sample matrices. Laboratory efficiency gains come from higher sample throughput with less demand for manual labor, laboratory space, consumables, and, as a result, less waste. QuEChERS has become a popular method for pesticide residue analysis. One disadvantage of QuEChERS can be the high concentration of matrix components remaining in the extract after the crude clean-up provided by dispersive solid-phase extraction (SPE). Alternatively, in-line automated

cleanup approaches such as turbulent flow chromatography (TFC) have been reported in the literature (1) (**Figure 1**).

TFC works by combining a column (0.5 or 1.0 mm, packed with large particles, typically 50–60  $\mu\text{m}$ ) with high flow rates (higher than 1 mL/min), creating a very high linear velocity inside the turbulent flow column. Molecules with low molecular weight will diffuse faster than molecules with high molecular weight, and therefore a separation between matrix components and analytes can be achieved. TFC seems to be more efficient at removing proteins based on their size than restricted-access media (RAM) or SPE.

The optimization of the various on-line extraction steps is crucial. Parameters such as mobile phase composition, flow rates, and extraction time windows will affect recovery and extraction efficiency in general. In pesticide analysis TFC may require some minimal preparation for certain sample types. A liquid sample such as orange juice may require that the chemist adjust the pH, dilute with methanol, vortex mix, and centrifuge before TFC separation and MS analysis. Solid samples require homogenization before the dilution, vortex, and centrifugation steps. Using TFC-LC-MS/MS, researchers have observed recovery



**Figure 1:** Turbulent flow chromatography has recently emerged as a choice for environmental and food samples.

rates of 80 to 110% for more than 48 pesticides of different classes, mostly at 10 µg/Kg (2). The main benefit of more effective removal of matrix components is increased analyte response due to reduced ion suppression and decreased contamination of the MS system, resulting in a decrease in the required frequency of system maintenance. Changing and cleaning the TSQ Endura and TSQ Quantiva ion transfer systems when required is simple and doesn't require the analyst to break the vacuum, thus increasing system up-time.

### Mass Spectrometer Selection

Triple-quadrupole MS can be used to screen, identify, and quantify a wide range of pesticide compounds of current interest. The use of a triple-quadrupole mass detector offers several advantages, including high sensitivity, compatibility with ultrahigh-performance LC, and direct compatibility with extracts prepared by streamlined methods such as QuEChERS.

In selecting a triple quadrupole system, laboratories should take the nature and volume of their pesticide residue workload into account. For laboratories in need of a workhorse system engineered for continuous operation, the TSQ Endura is a good choice. By contrast, the TSQ Quantiva offers laboratories a higher level of sensitivity and overall performance for challenging, complex samples. **Figure 2** compares the features and specifications of these systems.

Triple-quadrupole technology is

the established gold standard and is used routinely on a daily basis in many pesticide residue laboratories around the world. One drawback is the need for time-consuming optimization of the conditions to optimize the response for each pesticide included in the acquisition method. An alternative to manual optimization is the use of a comprehensive database of SRM transitions included in Thermo Scientific™ TraceFinder™ software.

A nominal mass triple-quadrupole system configuration is essentially a series of mass filters. In selected reaction monitoring mode (SRM), the conditions of the first quadrupole (Q1) are set to allow precursor ions at a selected  $m/z$  ratio to pass through into a collision cell (Q2), where the ions are collided with gas molecules and hence fragmented to form product ions. The product ions of selected  $m/z$  pass through the third quadrupole (Q3) to the detector. If the first mass selective quadrupole in the series is not selective enough, ions of masses similar to that of the target analyte can be fragmented too, resulting in isobaric interference and uncertainty regarding the correct identification of the analyte. In SRM experiments of heavily contaminated matrices, the analyst can increase selectivity in a way only possible when using hyperbolic quadrupole rods. An important feature of both the TSQ Endura and TSQ Quantiva is hyperbolic quadrupole rods. The use of highly-selective reaction monitoring mode



	TSQ Quantiva	TSQ Endura
Mass Range	10-1850	10-3400
Max SRM Number	30,000 SRMs	30,000 SRMs
SRM/Sec	500 SRMs/sec	500 SRMs/sec
Ion Optics	<b>Active Ion Management (AIM)</b> <ul style="list-style-type: none"> <li>• Ion Max NG source</li> <li>• Electrodynamic ion funnel</li> <li>• ion beam guide with neutral blocker</li> <li>• 6 mm HyperQuad quadrupoles with asymmetric RF drive</li> </ul>	S-LENS with Beam Blocker Technology
Quadrupole Design		4mm Quadrupoles with Asymmetric RF
Reserpine Specification (RFQ)	100,000 : 1 S/N for 1 pg Reserpine	10,000 : 1 S/N for 1 pg Reserpine

## TSQ Endura™ Triple Quadrupole Mass Spectrometer

### Extreme Quantitative Value

- Designed for non-stop operation.
- For scientists who need to run routine samples day in and day out.

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### Extreme Quantitative Performance

- Designed for the most challenging assays.
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Figure 2: A technical comparison of the TSQ Endura and TSQ Quantiva systems.

(H-SRM) increases the selectivity of the analysis even in highly complex samples, as shown in **Figure 3**.

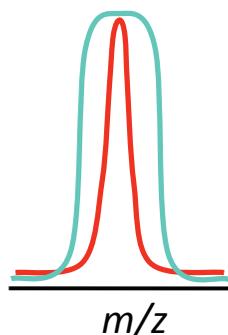
Even with H-SRM the triple quadrupole only detects those compounds included in the acquisition method. It is almost inevitable that some pesticides will be present in some samples but not included in the acquisition method and hence not detected. For this reason there is an increasing interest in screening approaches using “full scan” high resolution accurate mass (HRAM) technologies.

Again, mass resolving power is a key selection criterion. In general, the higher the mass resolving power used, the better the mass accuracy and the possibility

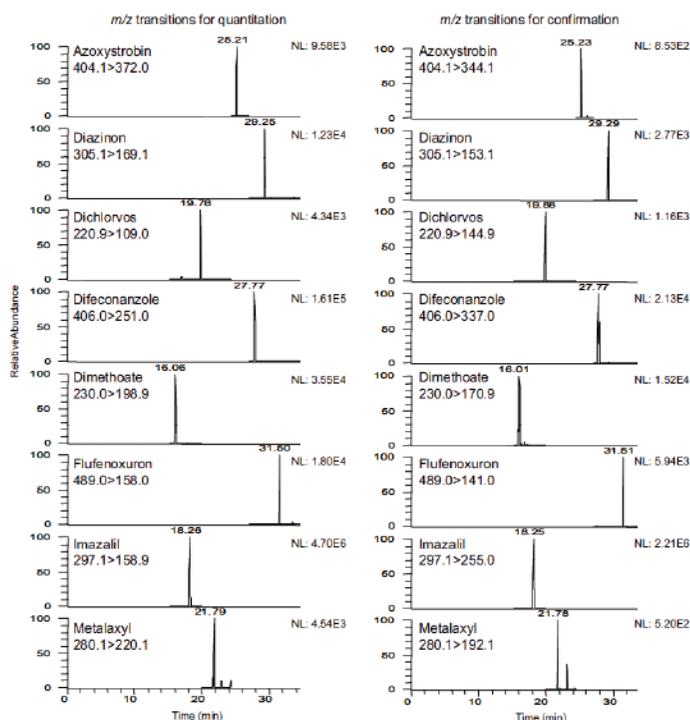
to differentiate between the analyte of interest and matrix compounds with masses close to that of the analyte of interest. Laboratories requiring systems for both targeted and non-targeted analysis can choose from a wide range of MS systems including the Orbitrap line, the Q Exactive Plus, and the Q Exactive HF systems. Most recently, the Q Exactive Focus was introduced for pesticide residue analysis. This system has the same sub-1-ppm mass accuracy of other Q Exactive systems across a range of 50 to 2000  $m/z$ , with resolution of 70,000 FWHM at 200  $m/z$ .

The European Reference Laboratory for Pesticides in Fruit and Vegetables recently

O.Núñez et al. *J Chrom A.*, **2012**, *1249*, 164-180.



- Remove background interferences
- Smooth baselines
- Improve % CV
- **Increase signal-to-noise**

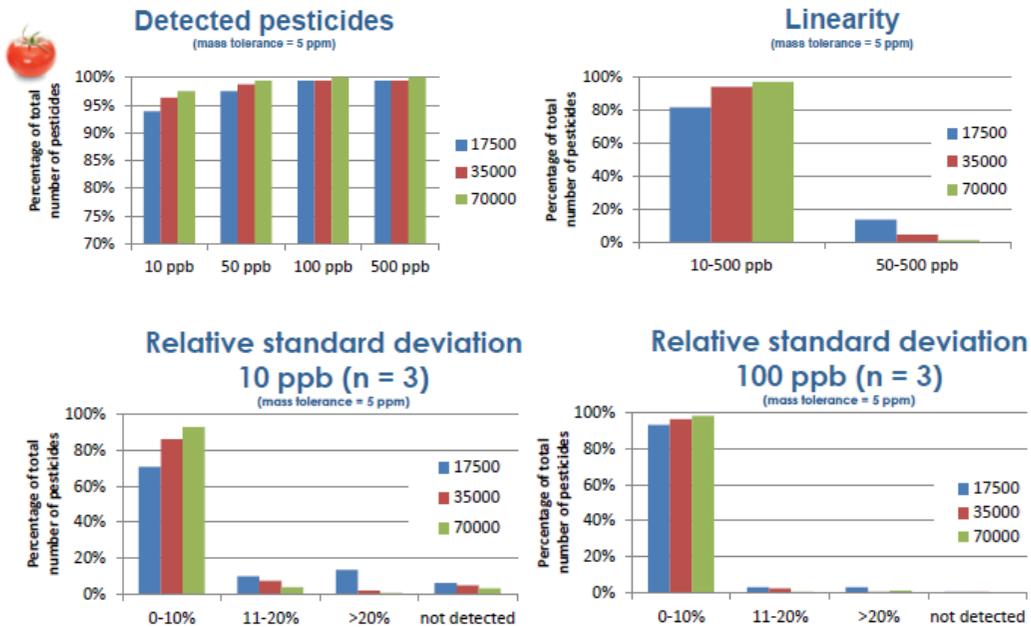


**Figure 3:** Results from a 2012 study published in the *Journal of Chromatography A* showing background-free peaks even in highly complex samples. (1)

demonstrated the use of the Q Exactive system for the analysis of pesticide residues in fruits and vegetables. An assessment of the effect of mass resolving power on the number of pesticides that could be detected at different analyte concentrations with a mass tolerance of 5 ppm showed that higher resolving power becomes increasingly important as the concentration of the pesticide decreases toward the 10-ng/g level (3). As the resolving power was increased from 17,500 and 70,000 the percentage of total number of identified pesticides also increased. The increased resolution helps in terms of linearity, both at low and high

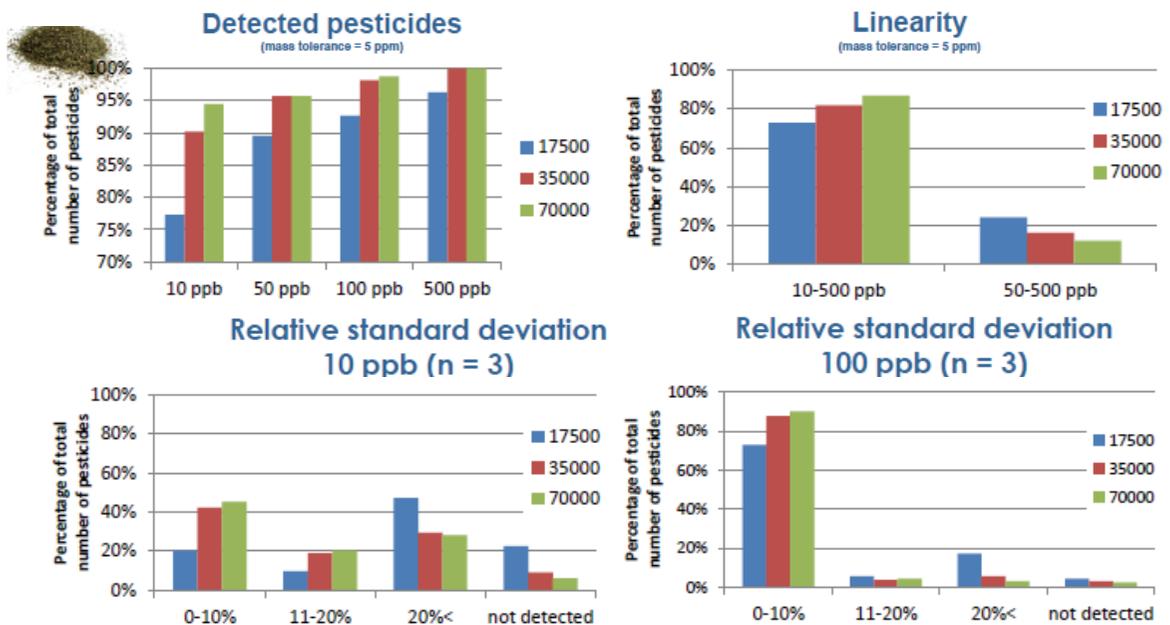
concentration levels, and also improves the method reproducibility (**Figure 4a**). In tea, a more complex matrix compared to tomato, this difference is more evident (**Figure 4b**). A resolving power of 70,000 FWHM was required to detect 95% of the target pesticides at the lowest (10-ppb) concentration. This underlines some of the tradeoffs associated with method selection. A targeted analysis might offer extra sensitivity, but at the expense of the ability to conduct non-targeted screening. According to the European guidelines for Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food & Feed (4), high-

a)



Data presented at EPRW 2014

b)



Data presented at EPRW 2014

Figure 4: The importance of high mass resolving powers used in conventional and complex sample types (tomato and tea).

resolution accurate mass identification criteria should include at least two ions, one of which should ideally be the quasi-molecular ion. Mass accuracy should be below 5 ppm, and at least one fragment ion should be detected. Resolution of at least 20,000 FWHM is specified, although no specific corresponding mass has been specified. The data obtained using the Orbitrap technology is compliant with these criteria, and typically exceeds the requirements, providing increased confidence in the identification and quantification of residues.

## Conclusion

This article has discussed the critical importance of adapting an analytical methodology for maximum performance in the demanding applications of pesticide residue analysis. We have emphasized that a wide range of instrumental technologies and configurations can complement one another in laboratories faced with a growing demand to perform efficient, cost-effective analytical measurements of an ever-growing range of pesticide compounds.

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**Richard Fussell** focuses on food safety, GC-MS and trace elemental applications in his current role. Before joining Thermo Fisher Scientific, he gained more than 35 years of experience working for the UK government, including 21 years at the Food and Environment Research Agency (FERA, formerly called CSL). At FERA, Richard was an advanced research fellow and senior scientist managing research and collaboration projects focused on emerging MS techniques for pesticide residues and veterinary drugs and food.

**Claudia Martins** is the environmental and food safety applications program manager with Thermo Fisher Scientific. She has a diploma in biochemistry and a PhD in analytical chemistry from the University of Manchester in the United Kingdom. During her many years at Thermo Fisher Scientific she has held various marketing and sale support roles in which she has focused on assisting customers in method development for environmental and food analysis.

**Jennifer Massi** is a Market Development Manager for the food and beverage market in the Chromatography and Mass Spectrometry group at Thermo Fisher Scientific Inc. A former genomics scientist who contributed to the sequencing of the human genome through the Human Genome Project, she now works on connecting laboratories with food safety testing workflows. Jennifer completed her B.Sc., Honors, in Biochemistry and Molecular Biology at the University of California at Santa Cruz and received an M.B.A., Honors, from Saint Mary's College of California.

# A new chapter

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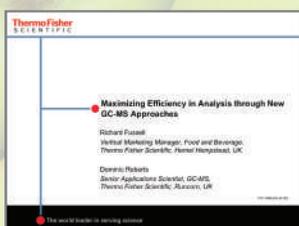
Richard Fussell, Dominic Roberts, and Jennifer Massi

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This article reviews the limitations and advantages of different approaches to the development of effective and complete gas chromatography-mass spectrometry (GC-MS) workflows for pesticide residues in food. It addresses some of the generic-but-critical aspects of GC methods, including injector parameters and column configurations, and also discusses the latest developments in GC triple-quadrupole MS and Thermo Scientific GC Orbitrap MS technologies.

One of the many challenges in the analysis of pesticide residues in food is the determination of a large number of different pesticides in a wide range of diverse matrices. In pesticide applications, chromatographers encounter simple matrices with relatively high water content such as fruits, but they must also be prepared to analyze more complex matrices such as herbs, spices, and tea. Regardless of the matrix, analytical methods must accurately detect and quantify pesticides at low concentrations while also offering high throughput, fast sample turnaround, and low analysis costs.

Liquid chromatography-MS (LC-MS) and LC-MS/MS technologies have progressed rapidly in recent years, prompting some people to question whether there is still a need for GC-MS in pesticide residues applications. However, some compounds do not give a good response

using LC-MS with atmospheric ionization. In fact, GC-MS offers better detectability than LC-MS for a number of compounds. Therefore, GC-MS capability remains essential to achieve a comprehensive multianalyte method for pesticide residue analysis.

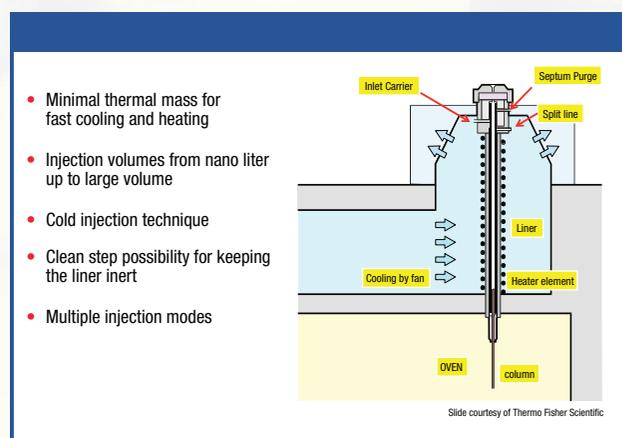
One of the most important recent developments in GC-MS technology was the introduction of GC triple-quadrupole MS, which provides better selectivity and higher signal-to-noise (S/N) performance than is possible with single-quadrupole MS. A quadrupole is a nominal mass filter that allows ions of a specified mass-to-charge ratio ( $m/z$ ) to pass through. In a triple-quadrupole system, ions produced by ionization in the source initially pass into the first quadrupole, Q1. System parameters are set to allow the precursor ions of preselected  $m/z$  to pass through into the second quadrupole, Q2. In Q2, the precursor ions are fragmented by collision with neutral gas molecules and with collision energy applied. This is the process underpinning selected reaction monitoring (SRM), which is the basis of GC-MS/MS analysis. Some extracts contain thousands of different compounds and it is often the case that a matrix peak and the pesticide of interest will share the same nominal mass and co-elute at the same retention time, passing through Q1 together. Using selective ion monitoring (SIM), or single-quadrupole MS, all of the co-eluting ions are monitored effectively at the end of Q1, and that results in chemical interference with the pesticide signal.

By contrast, in triple-quadrupole MS, we use SRM. The pesticide and the matrix will have different chemical structures, so they will typically produce different product ions when fragmented in Q2 under controlled conditions. Only the unique product ions from the pesticide of interest pass through Q3 and reach the detector. The result is a cleaner signal, which leads to improved selectivity and S/N compared to single-quadrupole MS.

## Injection Methods

Although fundamental to any GC-MS method, sample injection is the step where most problems with analysis by GC-MS originate. The two most commonly used injection techniques are splitless, and programmed temperature vaporization (PTV) (**Figure 1**). Each has advantages and disadvantages in pesticide residue analysis, and careful optimization of the selected method is required for best results.

Splitless injection is the simplest and probably most widely used method,



**Figure 1:** Schematic of a PTV injector system.

offering excellent sensitivity and repeatability for low sample volumes. During sample injection, the split line is closed so the entire sample is transferred to the head of the column. In the solvent focusing mode the initial oven temperature is set 10–20 °C below the solvent boiling point. The solvent and analytes are refocused into a tighter band at the head of the column. At a predetermined time, set to allow transfer of analytes to the column, the split vent is opened to “clean” the injector. The use of a pressure purge during injection helps to compress the solvent vapor in the liner, and can allow slightly increased sample injection volumes and help to protect pesticides prone to thermal degradation, especially in the “hot” splitless mode.

Although less commonly used in pesticide residue analysis due to limited sensitivity, the technique of a split injection can be used to overcome matrix problems. During split injection, a proportion of the injected sample is split or diverted to waste. In split injections, the sample is swept by the carrier gas through the liner and split between the column and split-line in the ratio of at least 1:5 and often much higher. This “shoot-and-dilute” approach helps keep the inlet and GC liner clean for a longer period of time and increases column lifetime. Split injections yield very reproducible results and good, sustainable peak shapes. The residence time of the analyte in the inlet is reduced, and that can lead to less thermal

degradation of certain problematic pesticides. The main disadvantage is that less analyte is injected into the column so the detector response is decreased proportionately.

In PTV injections the sample is injected into the liner at a relatively low temperature with precisely time-controlled evaporation of solvent (to waste); this allows large volume injection (typically 5–10 µL). The liner design is selected to help with retention of the injected sample in the liner and to increase the surface area for evaporation. After the evaporation step, a small volume of sample (typically less than 1 µL) is left in the liner. The injector port temperature is then increased rapidly up to 300 °C to transfer the analytes to the head of the analytical column. Because of the precise control of the PTV temperature profile, the PTV technique can help minimize analyte discrimination during injection. This results in improved recovery of thermally labile pesticides and fewer adverse effects from non-volatiles present in the sample during injection. These features make PTV especially well-suited for trace pesticide analysis.

### GC Liner Selection

Liner selection is an important facet of optimizing a robust GC–MS method. In pesticide residue analysis, the widely used QuEChERS extraction method typically yields extracts in acetonitrile solvent. Using acetonitrile is not ideal for GC as it often involves injecting a polar

solvent onto a mid-polar column, which will often result in poor peak shapes. Acetonitrile also has a high solvent expansion coefficient, which therefore limits the volume that can be injected. When selecting a GC liner for such an application, it is important to consider factors such as: the type of injection to be used; the internal diameter and volume of the liner, the liner construction, the liner packing (if required), deactivation, and other features. Acetonitrile has a large expansion volume, and the liner volume must therefore be sufficient to accommodate the sample in gaseous form. If the internal diameter is too narrow, the sample will expand beyond the liner's capacity, resulting in sample losses, peak tailing, and poor peak-area reproducibility. If the diameter is too wide, however, a large dead volume slows down sample transfer time and, again, contributes to peak tailing.

Various liner types are suitable for pesticide residue analysis by GC-MS. In split injection, liners that are open-ended enable the split flow to pass across the bottom of the liner. By contrast, splitless injection requires a tapered line to funnel the sample onto the GC column.

The use of baffled liners is recommended for PTV as they create a turbulent flow in the liner, which aids sample mixing; this improves reproducibility. Liners are available empty (no packing), with internal protrusions (e.g., asymmetric or baffled), or packed with materials such as Carbofrit® or deactivated wool (glass

or quartz for example). The advantage of injecting a sample onto the wool in a packed liner is that it increases the surface area for complete vaporization of the sample before it reaches the column. This improved vaporization improves reproducibility and lower boiling point discrimination. The wool can also clean the injection needle and reduce the chance of any particulate material reaching the column. However, the wool is also a potential source of active sites and regular injector maintenance should be performed. For the wide range of available Thermo Scientific consumables see [www.thermoscientific.com/gconsumables](http://www.thermoscientific.com/gconsumables)

### Avoiding System Contamination

The analysis of dirty extracts will eventually give rise to a build-up of matrix which will cause counter-productive shifts in peak shape and retention time. This situation highlights the need for regular injector maintenance to ensure good system performance.

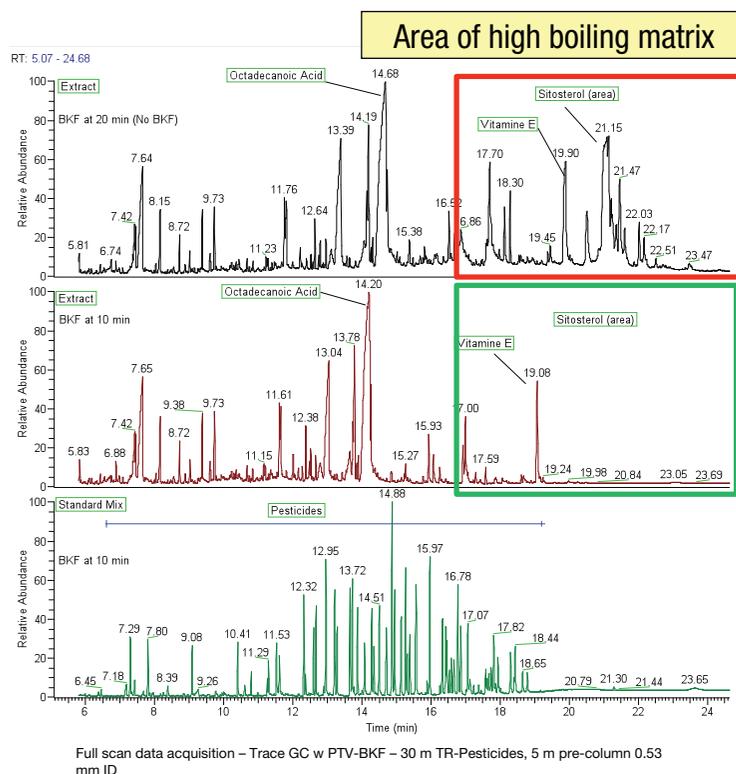
The use of column backflushing is an option to improve method robustness. Co-injected matrix components can cause matrix enhancement by protecting analytes from absorption or degradation on active sites. Some analysts perform "equilibration" injections of matrix to deactivate the inlet system prior to analysis.

However, sample matrix components can also create new active sites in the inlet and column head, compromising

- No BKFL

- BKFL ON 10 min after injection of sample

- BKFL ON 10 min after injection of standard



**Figure 2:** Effect of backflushing during analysis of a pear extract in ethyl acetate. Without backflushing (top), peaks from several high-volume components are present. Those peaks disappear with backflushing 10 min post injection (middle) and when a standard is injected (bottom).

chromatographic performance. Backflushing, a technique that involves reversing the carrier gas flow at a specified time after injection to backflush the retention gap and liner, can help to prevent less volatile matrix components from reaching the GC column. It is important not to reverse the gas flow before the last analyte of interest has entered the column. A T-junction, which connects the analytical column and the retention gap, and a pressure valve, can be operated to reverse the gas flow through the retention gap. Gas flow through the analytical column is

maintained at all times. Chromatograms in **Figure 2** illustrate the retention of analytes and removal of high-boiling point components when the column was backflushed 10 min after sample injection. For challenging samples, backflushing will provide better retention time precision and better spectrum quality and quantification accuracy. It will also reduce analysis time, increase column lifetime, and minimize the need for system maintenance.

### Increasing Productivity

State-of-the-art detection technology,

Figure 3a: Decreased Analysis Time

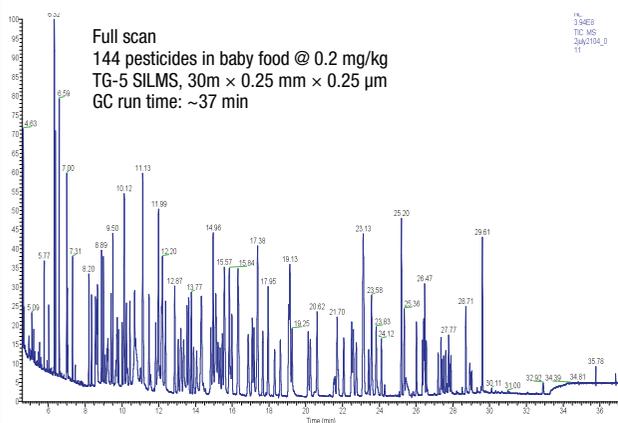


Figure 3b: Decrease the Analysis Time

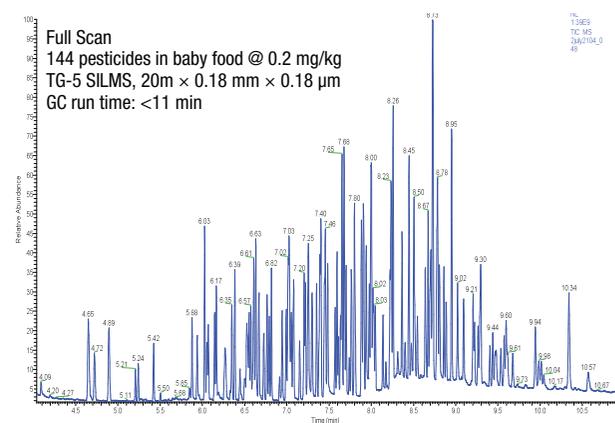


Figure 3: Column length and diameter can be reduced to decrease analysis times without impacting analytical data.

available in systems such as the Thermo Scientific™ TSQ™ 8000 Evo GC-MS/MS, can play an important role in productivity for the routine laboratory with high sample throughput. Shorter GC run times, greater selectivity, and the ability to capture more data in a single run help laboratories to run more samples in less time. The TSQ 8000 Evo features enhanced technologies that can reduce analysis times without compromising data quality. In particular, a fast collision cell provides high speed SRM transitions and rapid data acquisition; this enables acquisition and analysis of more pesticides in a single analytical run. Such features make it possible to take full advantage of fast triple quadrupole GC-MS in pesticide residue analysis.

Recently, the use of the TSQ 8000 Evo GC-MS/MS (coupled to a Thermo Scientific™ TRACE™ 1310 GC and Thermo Scientific™ TriPlus RSH™ autosampler configured for liquid

injection) with shorter, narrow bore columns (20 m × 0.18 mm × 0.18 μm column) allowed faster GC gradients while shortening the GC run time from 37 min to 11 min—more than a 3-fold improvement in speed for the determination of 144 pesticide compounds in baby food, as shown in **Figure 3**.

This system not only is able to achieve faster analysis times and measure more pesticides; it also offers more transitions for better selectivity (especially for complex matrices) and enables full-scan and SRM acquisition in the same experiment. In the analysis of baby foods the narrow-bore column provided narrow peaks and reduced column bleed, both of which improved the S/N performance. The fast acquisition rates provided sufficient data points, even for narrow peaks, to give excellent quantification for a large number of compounds in a short analytical run time.

Dedicated SRM method development

software, Thermo Scientific™ AutoSRM, reduces the time and effort required to add new compounds into the acquisition method. The software guides the user through the steps from precursor ion selection to product ion selection and to collision energy optimization, resulting in an optimized method with minimum operator input. The software also automates the creation of sample sequences and data layouts.

Timed SRM is an alternative to classical segmented SRM for laboratories seeking consistent and rapid acquisition of large amounts of data. Timed SRM can reduce complexity through automated, optimized targeting of a particular compound. Once the user has entered the retention time and the time required to acquire the peak, timed SRM automatically optimizes the instrument duty cycle and sensitivity without the need for spreadsheets, dwell time calculations or segment breaks. This ensures that compound detection is optimized for maximum sensitivity and allows more compounds to be added to the method without compromising the response for analytes. Also, it increases resilience to small retention time shifts and prevents wasteful, unnecessary MS scanning for compounds during times when they are not eluting.

The TSQ 8000 Evo GC-MS/MS is adept at analyzing an increased number of pesticides in a single run, thereby reducing the number of injections required to cover all of the target pesticides with the required number of

SRM transitions for identification. This increases the instrument's availability for other work and reduces maintenance requirements. In the analysis referenced earlier, the system also showed improved selectivity for different matrices with no loss in peak area response when increasing the number of SRM transitions from two to six. Linearity was also consistent whether 2 or 6 transitions were used. With this capability, a chemist can select the most intense interference-free transition for quantitation without having to perform a second injection to monitor different transitions.

Many of these productivity-enhancing performance benefits are embedded in the Thermo Scientific™ TSQ™ 8000 Evo Pesticide Analyzer solution. This system is preloaded with pesticide-specific GC-MS/MS acquisition and processing methods, analytical column consumables, tutorials for method development, and a compound database of more than 600 compounds with retention times and pre-optimized SRMs. The automated Pesticide Analyzer system is suitable for less experienced users but can be customized to meet a wide range of advanced user needs.

### GC and High-Resolution MS

Recent advances in high-resolution accurate mass spectrometry coupled to GC create potential for new approaches in the analysis of GC-amenable pesticides. For example, the Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/

MS system, operating in full-scan mode, allows the detection, identification, and quantification of an unlimited number of target pesticides and screening for non-targeted compounds in a single run. The system covers a broader analytical scope by acquiring and automatically processing full-scan data, enabling the user to substantially increase the scope of the method without a corresponding increase in analysis time. Another major advantage compared to triple-quadrupole systems is the possibility of retrospective data evaluation. The user can revisit older data to search for new compounds that were not on the original target compound list or known or recognized at the time of acquisition. The Q Exactive GC is capable of selective detection of targeted analytes in complex matrices with high resolving power up to 120,000 (FWHM  $m/z$  200) and excellent mass accuracy (typically less than 1 ppm). The sensitivity and specificity of the

Q Exactive GC for routine screening of pesticides in complex matrices has been demonstrated by the analysis of acetonitrile QuEChERS extracts of wheat, horse feed, and leeks containing 55 different pesticides at concentrations between 0.5 and 10 ppb. The goal was to establish the lowest level at which each pesticide could be detected in such complex matrices.

Automated sample injection was achieved using the TriPlus RSH autosampler. Separation was achieved using a TRACE 1310 GC with a Thermo Scientific™ TraceGOLD™ TG-5SiIMS 15 m x 0.25 mm, 0.25  $\mu$ m column with a fast GC method to achieve 11 min analysis time. For detection, a Q Exactive GC Orbitrap mass spectrometer in electron impact full scan mode was used. Data was acquired at 60 K resolving power (FWHM  $m/z$  200) and at 15, 30, and 120 K. Data processing was performed using Thermo

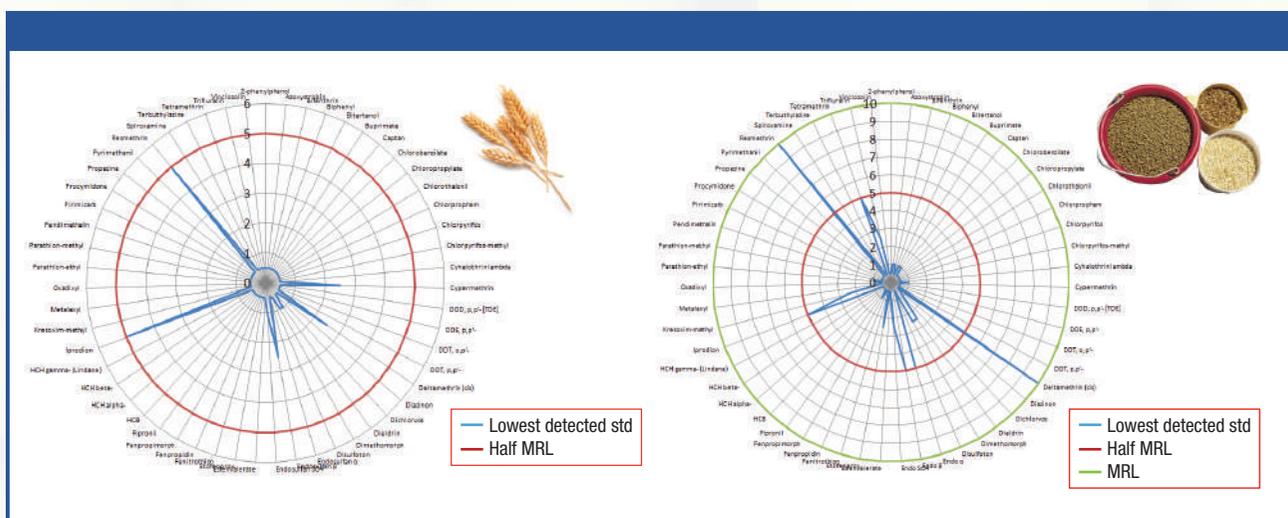


Figure 4: Lowest detected concentrations of our 55 pesticides in (a) wheat and (b) horse feed.

Scientific™ TraceFinder™ 3.3 software.

The selected identification points for a positive screen were a retention time match within 20 s of the pesticide standard and accurate mass with less than 2 ppm mass error. The presence of fragment ions, isotopic pattern, a NIST match, and ion ratios was used for identification and to minimize both false detects (to reduce the need for operator intervention) and false negatives (increased confidence in results). Results demonstrating increased selectivity and sensitivity in wheat and horse feed are summarized in **Figure 4**, which shows the lowest detected concentrations for each of the 55 pesticides in both of these complex samples.

## Conclusion

Chemists using state-of-the-art GC-MS and GC-MS/MS systems can perform efficient and robust pesticide analysis. Careful method optimization is essential, especially with respect to injection parameters. Routine pesticide analysis with the TSQ 8000 Evo system reduces analytical costs while increasing sensitivity, analysis speed, and the ease of database management. By maximizing the use of available dwell time through timed SRM, users can maximize sensitivity in data acquisition to increase the range of pesticides measured. High

resolution MS offers efficiency gains by allowing simultaneous targeted analysis with the quantitative, sensitivity, and selectivity performance of triple-quadrupole MS. It also provides unrivalled capabilities for screening a higher number of pesticides with excellent selectivity provided by unparalleled resolving power and mass accuracy.

## About the Authors and Contributors

**Richard Fussell** focuses on food safety, GC-MS and trace elemental applications in his current role. Before joining Thermo Fisher Scientific, he gained more than 35 years of experience working for the UK government, including 21 years at the Food and Environment Research Agency (FERA, formerly called CSL). At FERA, Richard was an advanced research fellow and senior scientist managing research and collaboration projects focused on emerging MS techniques for pesticide residues and veterinary drugs and food.

**Dominic Roberts** is a senior applications specialist in chromatography and mass spectrometry at Thermo Fisher Scientific. Dominic is an experienced analytical chemist and mass spectrometrist, with more than 15 years of experience using state-of-the-art analytical systems. Dominic has worked in both regulatory and industrial environments gaining strong hands-on experience with GC-MS and LC-MS systems, using them to investigate a variety of chemicals in food, biological, and environmental samples. The majority of his experience comes from 10 years working at FERA as an analytical chemist, focusing on method development using LC-MS and GC-MS systems to detect food contaminants. Dominic currently works with GC-MS technologies, including high resolution accurate mass MS to develop and implement analytical methodologies for the detection and quantification of trace chemicals, including pesticides in food and environmental samples.

**Jennifer Massi** is a Market Development Manager for the food and beverage market in the Chromatography and Mass Spectrometry group at Thermo Fisher Scientific Inc. A former genomics scientist who contributed to the sequencing of the human genome through the Human Genome Project, she now works on connecting laboratories with food safety testing workflows. Jennifer completed her B.Sc., Honors, in Biochemistry and Molecular Biology at the University of California at Santa Cruz and received an M.B.A., Honors, from Saint Mary's College of California.

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# PROCESSING AND ANALYSIS SOFTWARE FOR LC-MS/MS AND GC-MS/MS

Katerina Bousova and Richard Fussell

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Web Seminar: Data Processing and Analysis Software for LC-MS/MS and GC-MS/MS



In LC-MS/MS and GC-MS/MS analysis, processing and evaluation of the raw mass spectrometry data can be the most time-consuming part of the analytical process. These steps also create opportunities to achieve substantial improvements in sample turnaround times. This article reviews enhanced software capabilities now available to increase the productivity and efficiency of pesticide residues analysis workflows.

State-of-the-art instruments combining liquid chromatography (LC) or gas chromatography (GC) with tandem mass spectrometry (MS/MS) are powerful and widely used tools for the analysis of pesticide residues in food. In highly regulated, high-throughput laboratory environments, scientists are under pressure to process a growing number of more diverse samples as rapidly and economically as possible. One of the major time commitments with LC-MS/MS or GC-MS/MS is the task of processing and evaluating the large volume of raw MS data produced. Fortunately, automated solutions are now available to streamline this process for gains in efficiency, productivity, data security, and regulatory compliance.

One such software solution is the Thermo Scientific™ TraceFinder™ software package (Thermo Fisher Scientific, Waltham, MA), which offers enhanced capabilities that address those needs in both targeted quantitation and screening of non-targeted compounds. Using this platform as an example of current capabilities, this article reviews key software and data processing requirements in LC-MS/MS and GC-MS/MS pesticide residue workflows, emphasizing new functionalities that transform a once time-consuming process into an opportunity to improve overall laboratory productivity and performance.

In addition to automated data processing, analysts rely on the system software to control instruments, to streamline the development and optimization of methods, to alert them to analytical failures, and to create custom reports that help them deliver on the expectations of their customers and auditors. In LC-MS/MS or GC-MS/MS work, chemists find it especially advantageous when software gives them the flexibility to work with multiple instrument platforms and to control how they manage and display the complex data sets involved. Designed for routine quantitative analysis of large sample batches as well as for screening for non-target compounds, the TraceFinder software features new security, reporting, and compliance features with potential to significantly increase efficiency in environmental and food safety laboratories.

## Data Acquisition Mode

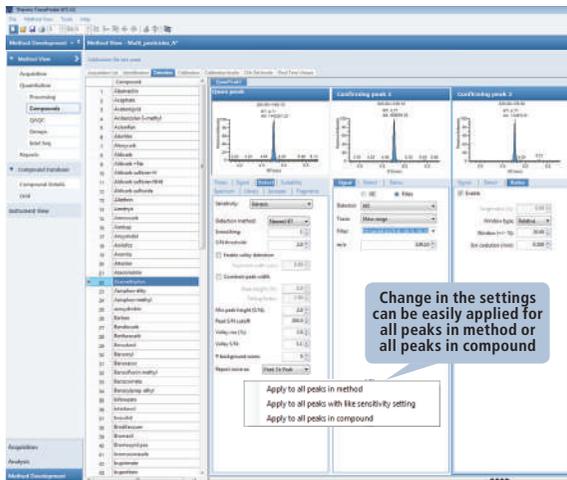
As stated, TraceFinder is ideal for processing large batches of sample data in routine quantitative analysis. The system uses batch templates to simplify set-up and control of the data acquisition mode. Analysts can populate these templates with sample lists built either from scratch or using a batch acquisition wizard tool within the software. Also, the system automatically populates the template with all required quality assurance (QA) and quality control (QC) samples and calibration standards.

TraceFinder's new security features create an unambiguous audit trail. If activated, the security system allows managers to assign very specific roles and access rights to specific users from an extensive list of preset permissions. The system helps regulate and document who has access to start the batch, who has access to the results, or who can change the method. Controls can be set to require users to input the reasons for any changes and to acquire their electronic signatures. Although only one aspect of a total laboratory compliance program, these new security and audit features offer substantial support for regulated laboratories.

## Method Development

**Figures 1a–c** present TraceFinder's display of key method development functions such as method selection, detection settings, calibration options, and QA/QC. TraceFinder is available in

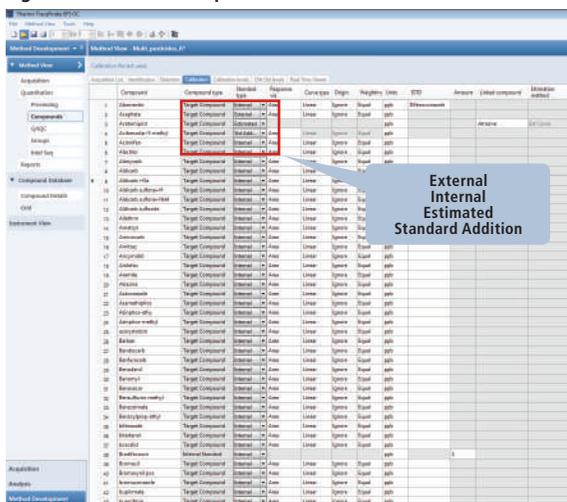
Figure 1a: Detection Settings



Change in the settings can be easily applied for all peaks in method or all peaks in compound

Apply to all peaks in method  
Apply to all peaks with like sensitivity setting  
Apply to all peaks in compound

Figure 1b: Calibration Options



External Internal Estimated Standard Addition

Figure 1c: Criteria for Quality Assurance and Quality Control

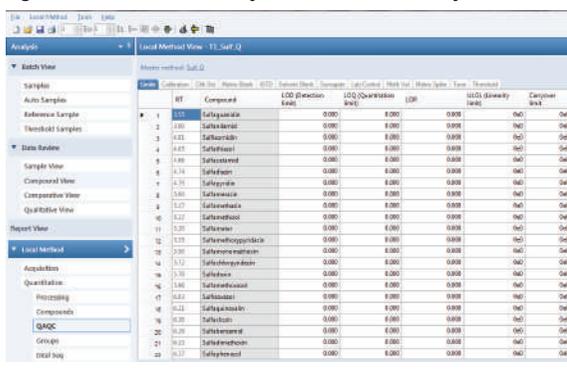
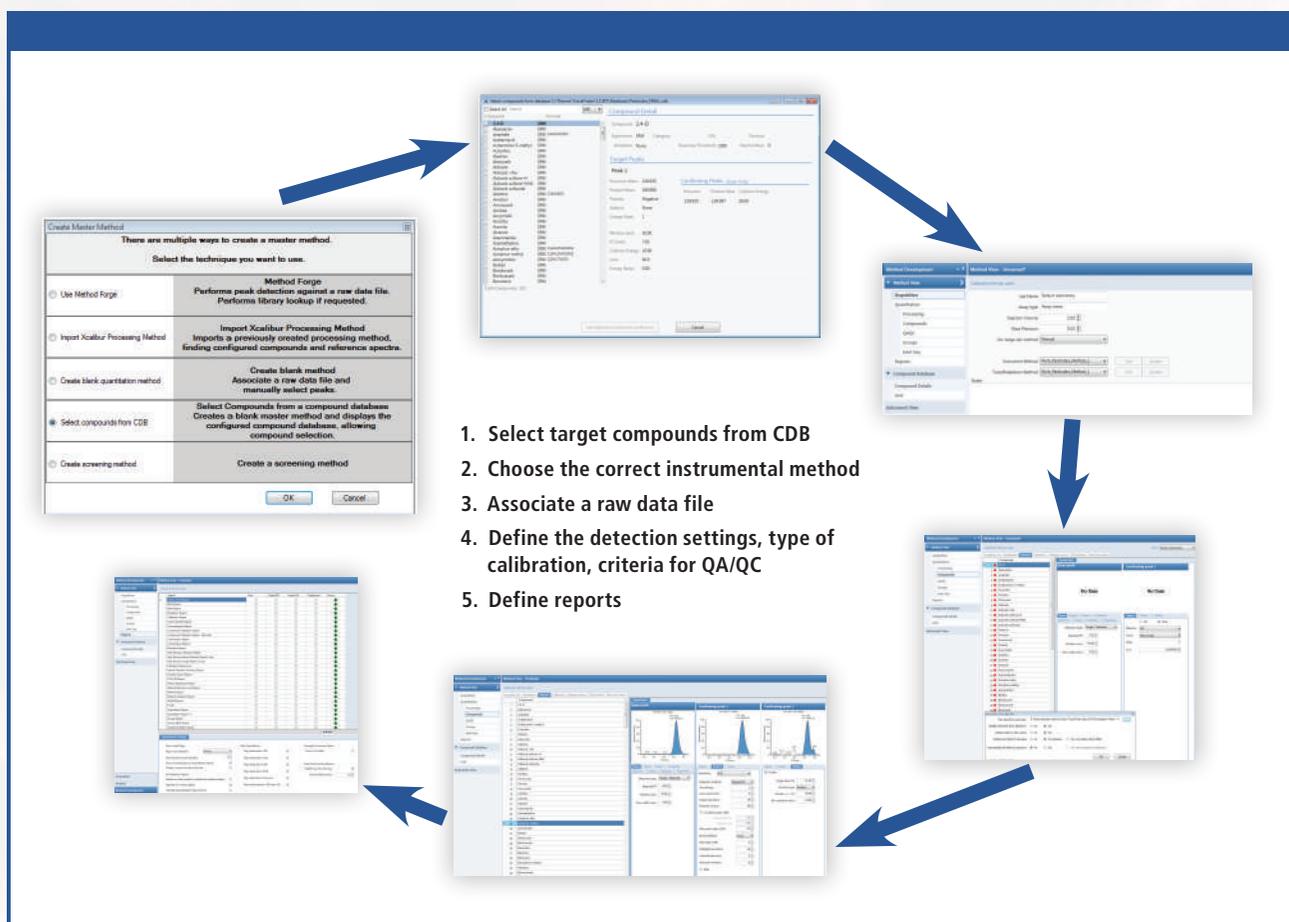


Figure 1a-1c: TraceFinder's tools for method selection, calibration, and QA/QC.

four industry-specific variants, including an environmental and food safety version with many specialized features for pesticide residues work. The platform includes several databases of compounds with data acquired in selective reaction monitoring (SRM) or with high resolution accurate mass (HRAM) mass spectrometry (extracted ion chromatogram, or XIC). The GC-MS/MS database contains more than 1000 compounds (SRM experiments) including pesticides, environmental, contaminants and PCBs. The LC-MS/MS database contains more than 600 compounds (SRM experiments) covering mainly pesticides, mycotoxins, and veterinary medicines while the HRAM database contains around 1700 compounds (XIC experiments) including fragments. To create a new method, the user selects one of five different method procedures from a Create Master Method dialogue box. For explanatory purposes, the most convenient of these tools is the option to Select Compounds from the Compound Database (Figure 2). In the latter case, the user selects target compounds from the database, selects the correct instrumental method, and provides a raw data file of previously measured standards. Next, the analyst defines the desired detection settings, type of calibration level, QA/QC criteria, and other parameters. The last step is to define the report to be created automatically after data processing. In all, the entire process takes only a few minutes.



**Figure 2:** Compound database view of data acquired in SRM mode, including precursor mass, product mass, polarity, and parameters for confirming peaks.

For calibration calculation, the software allows users to select external, internal standard calibration, estimated, or standard addition. Each target compound can have its own initial calibration setting. Modifications are possible during set-up or in acquisition mode after viewing results from an actual calibration batch. Changing is also possible during data review, creating options to compare and choose between calibration options.

For QA/QC, the system allows the operator to set limits and ranges that

enable automated review of data and results. Copy-and-paste functions and other shortcuts are useful for copying grid values from column to column or from one master method to another.

### Data Review and Analysis

The TraceFinder data review screen displays the target compound list, the sample set, and compound details such as quantitative peaks, confirming ions, ion overlay, and calibration data. The data review window is customizable—the size or position of individual display panes can

Figure 3a: Easy data review

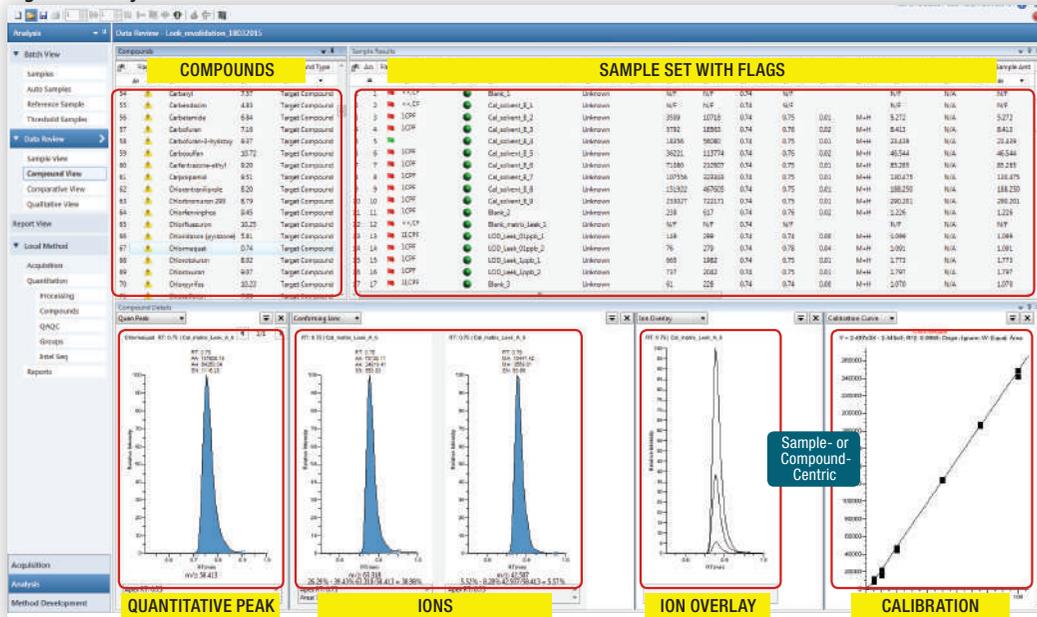
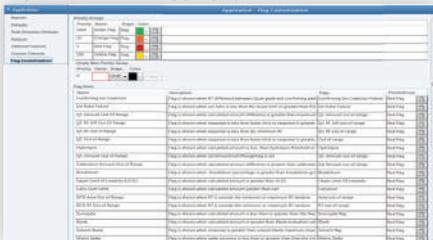


Figure 3b: Data flagging system



Data Flag Customization gives the laboratory the ability to create meaningful visualization of the rules developed for data review

Figure 3c: View by compound—a compound across all samples

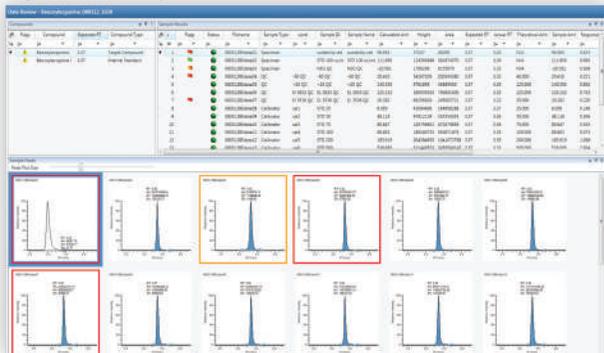


Figure 3d: Data review—ion overlay

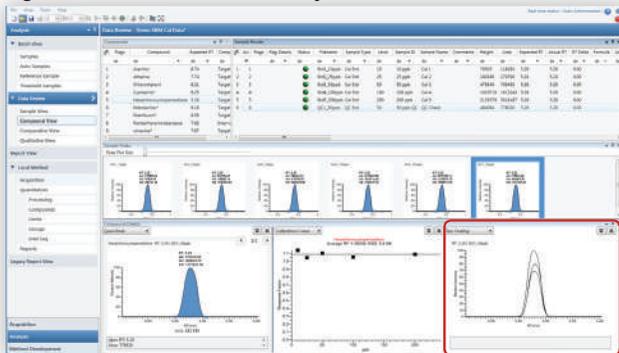


Figure 3e: View by sample—all compounds in a sample

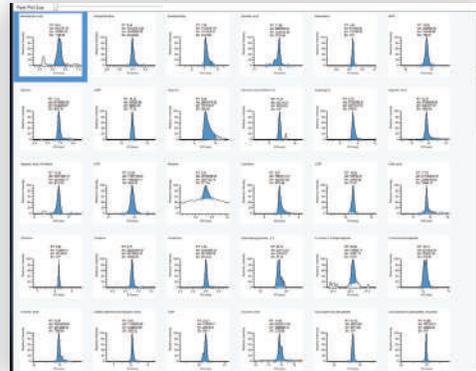


Figure 3a-e: Versatile data review features

be altered, and manual changes can be made to parameters such as integration or calibration. The software automatically recalculates results reflecting these changes.

The user can view a full data overview on one screen, or can customize the display to hide individual fields that aren't of interest. Another option is to display all the peaks in a given sample. Depending on the size of the user's computer monitor, it can require a number of screen pages to display that many peaks. The system uses multiple dockable panels to facilitate a holistic view of multiple peak displays and other larger data sets, in effect creating multiple monitors on one workstation for easier review.

Because many analysts spend so much of their time reviewing data, innovations that add efficiency to the process can translate into significant productivity gains. One such feature in TraceFinder is its novel flagging system, which identifies and highlights batch results that don't match specified requirements. User-customized flags in the data results pane change colors based on criteria set in the master method. Additionally, the sample peaks are enclosed in a red box when ion ratios are not within the defined ranges. Users can edit flag color or shape, change or delete certain error conditions or change their priority, revise flag rules, and modify the color of the error indicator icon. The flagging system links to the user-security feature which, when activated, restricts access to the

ability to edit flag features. **Figures 3a–e** provide representative views of some of the features described above.

### Preparing Methods for Non-Targeted Screening

As stated above, TraceFinder software not only supports routine quantitation of target compounds but also is useful for the automated identification of non-target compounds. The most important step in preparing a screening method is the determination of settings for compound identification and confirmation. The other primary consideration is selection of one or more appropriate compound databases for library searching. TraceFinder searches selected libraries and reports the highest scoring matches back to the user as a percentage value. The unknown's likely identity is narrowed down and further verified through a process of comparing multiple forms of measurement data—retention times, isotopic pattern matching, or library segment matching, for example. High-resolution MS instruments controlled by TraceFinder software can be used to accomplish both screening and quantitation in a single workflow, as shown in **Figures 4a–b**.

There are many high-resolution MS libraries available, including spectral libraries containing data from more than 600 pesticide compounds. These libraries are continuously updated and can be accessed at <http://planetorbitrap.com/compoundlibrary>. In the library

Figure 4a: Quantitation and Screening in a Single Workflow (purple flag indicates detection of a compound for which the standard is not available and thus calibration and quantification is not possible)



Figure 4b: Quantitation and Screening in a Single Workflow (green flag indicates detection of a compound for which the standard is available to permit calibration and quantification)

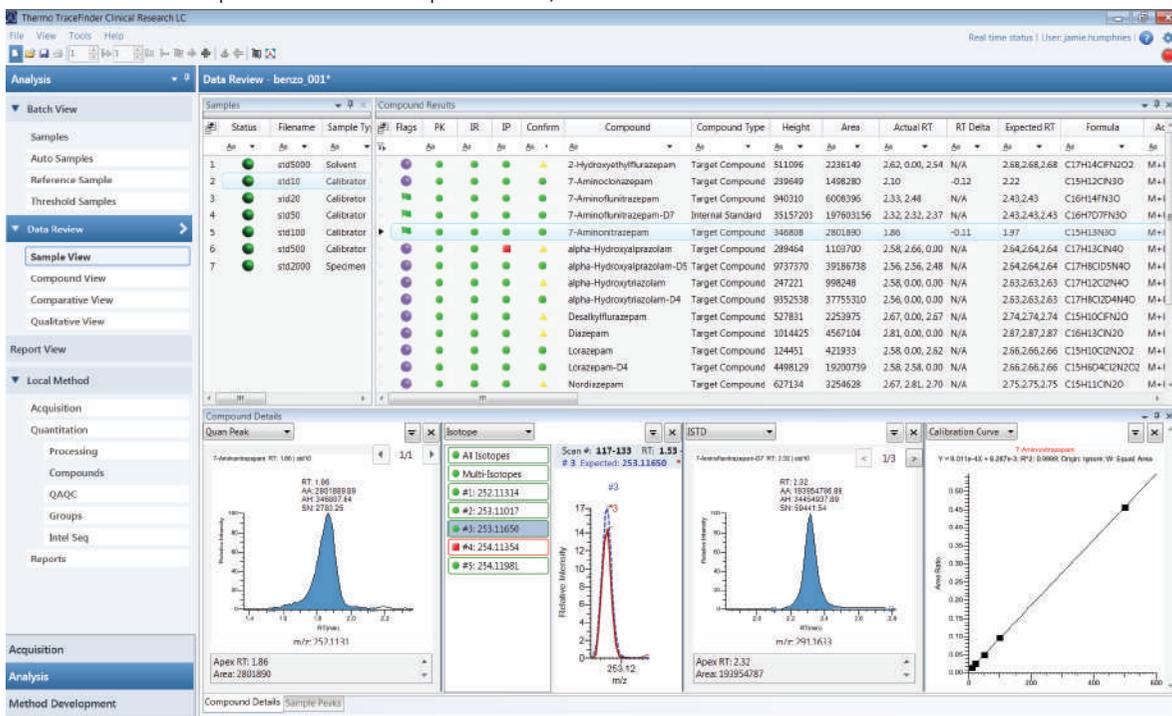


Figure 4a–b: Combined workflow for quantitative analysis and screening

you can find, for each compound, five to six high-resolution MS/MS spectra collected at different collision energies. TraceFinder is also available with an Environmental and Food Safety HRAM MS/MS spectral library that contains over 1500 compounds and 8900 spectra. [<http://www.thermoscientific.com/en/product/high-resolution-3.html>]

## Reporting

TraceFinder includes a wide range of standard reports as well as customizable reporting templates. Using a Microsoft Excel-like report generator, users can edit or add fields and calculations to the standard reports or create original report formats that match specific needs. Among the exhaustive list of templates are tools for batch, breakdown, internal standard summary, and quantitative reports.

## Conclusion

TraceFinder is designed for routine, high-throughput GC-MS/MS, LC-MS/MS, and high-resolution MS qualitative and quantitative workflows. It provides the tools necessary to maximize productivity with quick method development, efficient data review, and custom reporting. It

includes Intelligent Sequencing with an option to specify the actions you want the application to take when there is an acquisition failure, and an audit viewer to view the audit log files in order to track user access and user modifications to the data. There is functionality to exchange the data between TraceFinder and a Laboratory Information Management System (LIMS). Indeed, TraceFinder has the security and auditing features to help meet regulatory requirements.

## About the Authors

**Katerina Bousova** studied food chemistry and chemical analysis at the faculty of food and biochemical technology at the Institute of chemical technology in Prague, finishing her Master's degree there in 2006. From 2006 to 2010, she worked as a chemical analyst at the department of chemistry at the state veterinary Institute in Prague. In 2010, Thermo Fisher Scientific established a new laboratory, the Food Safety Response Center, in Dreieich, Germany, where Katerina started working as an application specialist. She became a member of a three-member team focused on the rapid development of analytical methods to respond to emerging contaminations in food. In 2013, the name of the group changed to the "Special Solutions Centre." Katerina is still responsible for developing new analytical methods, but is also giving presentations about new trends in food safety, providing training for customers, and preparing various workshops in the area of food testing.

**Richard Fussell** focuses on food safety, GC-MS and trace elemental applications in his current role. Before joining Thermo Fisher Scientific, he gained more than 35 years of experience working for the UK government, including 21 years at the Food and Environment Research Agency (FERA, formerly called CSL). At FERA, Richard was an advanced research fellow and senior scientist managing research and collaboration projects focused on emerging MS techniques for pesticide residues and veterinary drugs and food.