# LC GC

New Methods to Increase Productivity in the Analysis of Pesticide Residues in Food

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# Automated Online $\mu\text{SPE}$ Clean-up of QuEChERS Extracts before LC-MS/MS and GC-MS/MS Analysis of Pesticides in Foods

Daniela Cavagnino and Cristina Jacob



# FAQs about Automated Online µSPE Clean-up of QuEChERS Extracts for Pesticides Analysis

Interview with Daniela Cavagnino and Cristina Jacob



Implementation of Dual Channel Chromatography to Improve Productivity in the Analysis of Pesticide Residues in Food Łukasz Rajski



#### FAQs: Dual Channel Chromatography for Pesticide Residues Analysis in Food

Interview with Łukasz Rajski

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# Introduction

Laboratories analyzing large numbers of samples for the presence of pesticide residues in foods are increasingly interested in new approaches enabling higher sample throughput and productivity. This e-book on *New Methods to Increase Productivity in the Analysis of Pesticide Residues in Food* highlights two validated options. First is an automated online solid phase extraction (SPE) solution using miniaturized cartridges for more effective clean-up of QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) acetonitrile extracts before analysis by GC-MS/MS or LC-MS/MS. The second is an approach using a dual-channel ultrahigh performance liquid chromatography (UHPLC) system that can operate two analytical columns in parallel, independently and simultaneously.

The most commonly cited approach for pesticide analysis is the QuEChERS acetonitrile extraction with dispersive SPE clean-up. As Daniela Cavagnino and Cristina Jacob at Thermo Fisher Scientific, explain, however, the QuEChERS approach can result in extracts with high levels of matrix co-extractives that inevitably lead to faster contamination of instrument systems. Although SPE in the traditional cartridge format is more effective than dSPE, it requires more analyst intervention and hence time.

Therefore, Cavagnino and Jacob describe an optimized, online, miniaturized cartridge approach that provides effective clean-up of QuEChERS extracts through unattended automated workflows. The fully integrated system is built around the Thermo Scientific<sup>™</sup> TriPlus<sup>™</sup> RSH<sup>™</sup> multipurpose robotic autosampler.

In the second half of the ebook, Łukasz Rajski, PhD, of the University of Almería, Almería, Spain, explains how using an UHPLC system that operates two analytical columns independently and simultaneously can increase sample throughput and reduce the co-elution of compounds to improve data quality without increasing analysis time.

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A simple, robust, automated workflow for most food matrices.

#### **Overview**

The trend for multi-residue pesticides analysis is toward faster, simpler, more generic methods of extraction with minimal, if any, clean-up to facilitate higher sample throughput. The most commonly cited approach is the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) acetonitrile extraction with dispersive solid-phase extraction (dSPE) clean-up. Despite its many benefits, this approach can result in extracts with high levels of matrix coextractives that inevitably lead to faster contamination of gas chromatographytandem mass spectrometry (GC-MS/ MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) systems. The reluctance to use a more effective clean-up, such as SPE in the

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traditional cartridge format, is most likely a consequence of the time required for manual procedures as well as for optimizing the removal of co-extractives without the loss of analytes.

This article, therefore, describes an automated online SPE solution using miniaturized cartridges for effective clean-up of QuEChERS extracts for pesticide residues analysis. The fully integrated system is built around the Thermo Scientific<sup>™</sup> TriPlus<sup>™</sup> RSH<sup>™</sup> multi-purpose robotic autosampler, which supports completely automated workflows for GC-MS/MS and LC-MS/ MS analyses. This pre-optimized solution enables laboratories to implement rapid and robust quantitation of multi-pesticide residues in complex food matrices.

#### **SPE Methods**

Pesticides laboratories strive to meet the growing demand for high throughput

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analytical methodology while achieving the required level of sensitivity, precision, and accuracy of the results. Typically, hundreds of pesticides are monitored simultaneously in numerous food matrices, in strict compliance with official regulations, and with an effort to maintain low operational costs. These demands underscore the need for simplified sample preparation procedures (extraction and clean-up), fast chromatographic cycle times, and efficient processing and review of data and reporting of results.

Manual sample preparation can be laborious and inconsistent, and streamlining such procedures is particularly challenging. The end goal of sample preparation for pesticides analysis is to maximize the recovery of analytes while minimizing the coextraction of matrix interferences that contaminate analytical instrumentation.

Therefore, the QuEChERS approach often incorporates a simple dSPE cleanup step whereby a small amount of bulk SPE packing sorbent(s), in loose form, is added to the extract to be purified. The mixture is then shaken for a few minutes to let the sorbent interact with the sample and then centrifuged to provide a cleaned-up supernatant extract for further analysis.

Although this approach is fast and low-cost, it is not always effective in the removal of certain types of coextractives. Hence, the clean-up step is often considered the Achilles heel of the QuEChERS approach because extracts containing relatively high levels of coextractives can quickly contaminate the instruments, which then require pro-active maintenance to avoid costly instrument downtime.

SPE in a traditional cartridge format is more effective at removing coextractives compared to the dispersive mode, but it can also prove time consuming to optimize the experimental conditions to achieve a satisfactory balance between the removal of coextractives and the recovery of analytes.

The procedure is based on the principle of partitioning between a liquid and a solid sorbent phase, where the liquid can be a solvent or sample extract containing the analytes. The cartridge SPE clean-up process typically consists of four steps: conditioning, sample loading, washing, and elution. First, the sorbent material is conditioned with a suitable solvent. Next. the sample extract is loaded onto the sorbent bed and percolates through the sorbent. The analytes, together with some matrix components, are retained and concentrated on the SPE packing material. Ideally, the washing step removes the majority of interfering co-extractives, while the final elution step elutes the target compounds in a cleaned-up extract. Depending on the dilution factors and

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sensitivity required, a further concentration step may also be necessary.

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Elution through the sorbent material is usually achieved by applying vacuum at the bottom of the SPE cartridge via the vacuum manifold. Although this technique is effective, it is difficult to maintain precise control of the flow through the cartridge, an issue that can lead to poor reproducibility.

Compared with the conventional cartridge SPE clean-up method, dSPE is less effective but the compromise is still generally accepted due to the productivity benefits of dSPE.

Thermo Fisher Scientific has introduced a new, more effective way to clean-up QuEChERS acetonitrile extracts by using a miniaturized SPE cartridge containing a small amount of sorbent material (45 mg for GC workflows and 30 mg for LC workflows). This technique, named µSPE, offers several advantages over classical and dSPE approaches. The µSPE procedure involves the elution of the sample through the sorbent exactly like classical SPE, but instead features a much smaller particle size in the sorbent bed. As in every chromatographic process, a smaller particle size provides higher separation efficiency, thus increasing selectivity and creating a sharper elution profile (1).

Another important factor making the  $\mu$ SPE cartridge more efficient than the

classical SPE is that it is a closed system, which works using positive pressure as opposed to negative pressure using a vacuum. This enables very precise control of the flow rate through the sorbent, ensuring optimum conditions for separation, thus maximizing the selectivity, recovery, and repeatability of the process. Moreover, the sorbent material amount and composition have been optimized specifically for the cleanup of QuEChERS pesticides extracts. For those reasons, the µSPE method results in a more effective clean-up, but assuring a higher recovery of the target analytes. By covering a variety of food matrices, including many difficult ones, µSPE offers a streamlined method for the analysis of most pesticide-commodity combinations.

#### Automated µSPE

Miniaturized SPE is ideal for automated and unattended workflows online with direct injection into a chromatographic system. For maximized productivity, automation of the µSPE clean-up is fully integrated with the TriPlus RSH Autosampler. The system features an automatic tool change (ATC) to automatically swap syringes with different volumes, a fast washing station for syringe cleaning, and a solvent station. The TriPlus RSH is compatible with the Thermo Scientific<sup>TM</sup> TSQ<sup>TM</sup>, LC- and GC- triple quadrupole, Orbitrap Exploris<sup>TM</sup>, and Q-Exactive<sup>TM</sup> mass

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#### **Figure 1:** Automated workflow for µSPE clean-up QuEChERs extracts.



spectrometer product lines. A dedicated script for automated QuEChERS cleanup is available for fully integrated GC/ MS or LC/MS analysis. The methods have been validated and comprehensive details for LC-MS/MS are included in the Thermo Fisher Scientific application note AN 65684, available on the Thermo Fisher Scientific website.

The  $\mu$ SPE cartridge takes only microliters of sample extract and does not require the dilution and evaporation steps involved in typical manual SPE operations (**Figure 1**). The syringe accurately delivers sample extract and solvents at the desired volume and flow for excellent reproducibility. The  $\mu$ SPE cartridge is sealed by a septum, thus allowing the syringe to push the QuEChERS extract through the sorbent bed when the plunger is depressed. In this way, the syringe acts like an LC pump as it fully controls the flow rate through the cartridge. A needle guide eliminates headspace above the SPE sorbent and ensures that the insertion of the syringe needle delivers sample and solvents directly to the SPE sorbent. The clean sample extract is collected in the vial positioned at the bottom of the cartridge and then is injected into the chromatographic system for analysis. A completely automated workflow.

The TriPlus RSH autosampler is configured with a dedicated sample tray for the µSPE cartridges, as seen

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"The timed SRM acquisition mode automatically sets the scan time for each transition, thus avoiding the need for the timeconsuming process of building a segmented multi-reaction monitoring (MRM) method."

in **Figure 1**. The autosampler can accommodate two 54-vial trays, which are positioned above a draining rack for discharging the solvent used for cartridge conditioning. Although conditioning is not a mandatory step, it has been reported to improve the recovery for most pesticides (2).

After the conditioning step, an aliquot of the QuEChERS extract (300 µL) is loaded onto the µSPE cartridge and pushed through the sorbent bed. Clean vials in the eluate tray, located underneath the elution tray, collect the cleaned extract as it exits the cartridge. The autosampler then retrieves and injects the cleaned extract directly into a GC or LC system, and the exhausted cartridge is automatically discarded into a waste container. Since the clean-up steps are performed concurrently with the ongoing analysis of the previous sample, the instrument cycle time is not increased. Therefore, productivity is boosted by

eliminating the manual steps of SPE or dSPE while maintaining the original run time of the analysis. Furthermore, the automated approach provides welldocumented processing that complies with 21 CFR Part 11 rules.

# Application of µSPE for GC-MS/MS Analysis of Pesticides

GC-MS/MS was employed for the analysis of 209 pesticides in rice and wheat using a Thermo Scientific™ TraceGOLD<sup>™</sup>TG-5SilMS GC Column and 1 µL splitless injection at 250°C. The initial oven temperature was 90°C, and the analytes were eluted using a temperature gradient up to 300 °C, for a total run time of 33 min. The TriPlus RSH autosampler with µSPE was interfaced with a Thermo Scientific<sup>™</sup> TSQ<sup>™</sup> 9000 GC-MS/MS equipped with an Advanced Electron Ionization (AEI) source to enhance the sensitivity of the system. Method set-up was accomplished in time-selected reaction monitoring (t-SRM) with a minimum of two or more transitions per compound. The timed SRM acquisition mode automatically sets the scan time for each transition, thus avoiding the need for the time-consuming process of building a segmented multi-reaction monitoring (MRM) method.

The recovery and precision of the automated  $\mu$ SPE clean-up workflow were evaluated in both matrices after

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# **Figure 2:** Pesticides in wheat: recovery and relative standard deviation (RSD).



spiking at 0.01 mg/kg (n=6 for each). The rice extracts showed excellent recoveries of 70–120% for 204 of the 209 pesticides with the majority of the compounds yielding relative standard deviations (RSD %) below 10%.

Similar results were obtained for the wheat samples (**Figure 2**). The recoveries were within 70–120% for 200 pesticides, while the RSD was less than 10% for almost all the compounds. A direct comparison of the recovery using  $\mu$ SPE and dSPE with the same sorbent blend has been reported for spice samples by Goon et al. (2). Using dSPE, most of the compounds showed acceptable recovery (70–100%) except a few late eluting pesticides including cypermethrin, deltamethrin, etofenprox, which showed poor recovery of less than 70%. This effect was not observed with the  $\mu$ SPE clean up, possibly indicating that the removal of late eluting nonpolar matrix compounds, such as oil and pigments was more effective.

Better sample clean-up equates to reduced contamination of the instrument and increased instrument uptime.

#### Application of µSPE for LC-MS/MS Analysis of Pesticides

For LC-MS/MS, three different food matrices were chosen to assess the  $\mu$ SPE clean-up procedure: grapes as a high-water content matrix, rice as a dry commodity, and tea as a difficult matrix. Importantly, only one  $\mu$ SPE cartridge type was used for all three matrices. The

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Z-Sep/C18/CarbonX sorbent (QuEChERS blend for LC, Thermo Scientific<sup>™</sup> P/N 60101-30LC) provided one unified procedure for all of the microextractions. As with GC-MS, the parallel functioning of the clean-up step alongside the LC-MS/MS analysis of previous sample did not compromise the sample throughput.

The TriPlus RSH µSPE autosampler was coupled to a Thermo Scientific™ Vanguish<sup>™</sup> binary pump and the Thermo Scientific<sup>TM</sup> TSQ Fortis<sup>TM</sup> triple quadrupole mass spectrometer, which used heatedelectrospray ionization (H-ESI). The chromatography separation was carried out with the reversed-phase Thermo Scientific<sup>™</sup> Accucore<sup>™</sup> aQ column (P/N 17326-102130) at room temperature, while the mass spectrometer was operated with positive and negative switching. A timed SRM method was developed with approximately 390 SRM transitions for the 195 selected LCamenable pesticides, including isomers and metabolites.

Recoveries were evaluated by spiking the pesticides at 10  $\mu$ g/kg, 50  $\mu$ g/kg, and 100  $\mu$ g/kg in each of five sub-samples of each of the matrices. The recoveries were then calculated using matrix-matched standards, spiked after the clean-up. For comparison purposes, both an automated  $\mu$ SPE clean-up procedure and a manual dSPE clean-up were performed on the QuEChERS acetonitrile extractions. The spiking levels of 10 µg/kg are equivalent to the EU default maximum residue level (MRL) of 0.01 mg/kg. The results for the majority pesticidecommodity combinations were in compliance with the EU SANTE method validation criteria. After µSPE clean-up, the recoveries for 80% of the target pesticides at 10 µg/kg in tea were in the range of 70-120% compared to 78% for dSPE. For rice, it was 91% compared to 80% for dSPE. For both µSPE and dSPE, 94% of the analytes in grape gave recoveries in the range of 70-120%. The µSPE clean-up procedure led to more accurate and precise results when compared with dSPE clean-up for rice and tea matrices, with a more dramatic improvement for rice.

The robustness of automated  $\mu$ SPE-LC-MS/MS was evaluated by injecting more than 200 consecutive extracts of dry tea, without any instrument maintenance. The responses for fenpyroximate, tolfenpyrad, imazaquin, and diuron were within the expected range of ±20% for

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#### Figure 3: Analyte responses in tea with the µSPE-LC-MS/MS method.



#### Figure 4: µSPE clean-up effectiveness and matrix effects.



- Grape and Tea sample matrix effects were less than 20% for 90% of target pesticides
- All monitored pesticides showed matrix effects <20% in µSPE cleaned rice extracts
- · Reduced matrix effects when compared with d-SPE
- µSPE clean-up was more effective in removing pigments

all injections, and RSDs were well below 10% as shown in **Figure 3**.

Matrix effects were evaluated by comparing the responses of pesticide





standards prepared in neat solvent to those prepared from the different sample matrices. The left side of **Figure 4** illustrates the reduced matrix effects of µSPE compared to dSPE. In A

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# **Figure 5:** High efficiency in removing matrix co-extractives from fatty samples (3).



addition, the chromatograms show that the µSPE procedure achieved higher efficiency in removing adverse components from the crude rice extracts.

The µSPE clean-up was also more effective than dSPE at removing pigments from the matrix, as shown in **Figure 4**. The photo in the lower right corner of the figure clearly shows the outstanding





- LC-HRAM MS profile of crude avocado extracts using a Thermo Scientific<sup>™</sup> Q Exactive<sup>™</sup> Hybrid Quadrupole-Orbitrap<sup>™</sup> Mass Spectrometer
- Removal of >90% of the TAGs and DAGs (\*) by automated µSPE (QuEChERS Blend for LC)

performance of the µSPE cartridges in removing chlorophyll from the leek matrix.

The µSPE cartridges are also highly effective at removing lipids from fatty samples. It is well known that triglycerides (TAGs) and diglycerides (DAGs) can accumulate in LC columns and can cause significant ion suppression. Therefore, a potent extract clean-up is of great benefit when

> analyzing fatty samples. The work shown in **Figure 5** was conducted at Hill Laboratories in New Zealand and demonstrates a 90% removal of DAGs and TAGs from avocado samples (3). Note the significant amount of

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DAGs and TAGs remaining in the dSPE extract in the bottom chromatogram, which is in stark contrast to the cleaner extracts from  $\mu$ SPE. This underscores the benefit of using the  $\mu$ SPE cartridge for removing lipids from fatty samples.

#### Conclusion

The advantages of automated  $\mu$ SPE with the TriPlus RSH robotic autosampler have been demonstrated for both GC-MS/MS and LC-MS/MS multi-residue pesticide analysis in food matrices.

- Automated workflows fully integrated with analytical instrumentation and supplied with dedicated methods for QuEChERS clean-up, offer simplicity, reproducibility, accuracy, and savings of analyst time.
- Optimized sorbent amounts and composition contained in the minaturized SPE cartridges allows one workflow for many different matrices, greatly simplifying the method set-up.
- Optimized cycle time is achieved with the clean-up step performed concurrently with analysis of the previous sample so the overall cycle time is unaffected.
- Importantly, the cleaner extracts resulting from µSPE enable longer instrument uptime and increased productivity.

- Automation decreases the possibility of human error and improves traceability of results.
- The productivity and performance improvements delivered by the automated µSPE workflows make it the ideal protocol for reliable pesticide analysis.

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aboratories are increasingly interested in doing faster, simpler multi-residue pesticides analysis with minimal clean-up in an effort to achieve higher sample throughput. The limitation of this approach is that matrix coextractives can interfere with the chromatographic analysis and contaminate liquid chromatographytandem mass spectrometry (LC-MS/MS) or gas chromatography-tandem mass spectrometry (GC-MS/MS) systems. The addition of a solid-phase extraction clean-up step can help reduce these issues but laboratories are reluctant to do so because of the time required for such manual procedures.

In a recent *LCGC* webcast, Daniela Cavagnino and Cristina Jacob at Thermo Fisher Scientific, discussed this issue. They explored an optimized, online, miniaturized cartridge approach that FAQs about Automated Online µSPE Clean-up of QuEChERS Extracts for Pesticides Analysis

Interview with Daniela Cavagnino and Cristina Jacob

provides effective clean-up of QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extracts through unattended automated workflows. Here, they answer questions posed by the audience during the webcast.

Your presentation explored µSPE cleanup of QuEChERS extracts for online GC-MS/MS and LC-MS/MS analysis of pesticides. You included data for pesticides analysis in dry food matrices (e.g., rice, wheat), tea, grapes, and avocado. Have you also evaluated other classes of analytes like polar pesticides, veterinary drugs, or mycotoxins?

**Jacob:** We have not tested polar pesticides because we prefer to use a workflow based on metal-free ion chromatography systems rather than liquid chromatography. The clean-up of polar pesticides is extremely difficult. Many publications in the literature have reported most clean-up options

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evaluated are ineffective. Either the clean-up results in losses of polar analytes or if the analytes are recovered, there is minimal removal of the matrix. It is the classic clean-up conundrum. However, one off-line clean-up that is effective when used in combination with ion chromatography is the use of Thermo Scientific<sup>™</sup> Dionex<sup>™</sup> OnGuard<sup>™</sup> II RP cartridges as described in Application Note 73204. Regarding veterinary drugs, our colleagues have been testing this application and the results are very promising. Hopefully, we will have more information to share in the near future. We did not evaluate mycotoxins, but I agree it is an interesting possibility and has potential.

#### For GC analysis, is it possible to automatically add an analyte protectant before injection?

**Cavagnino:** Yes, the automated workflow includes the optional step for the addition of internal standards or analyte protectants. In fact, analyte protectants are particularly useful in GC

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analysis of pesticides because they help to mask the active sites in the GC inlet and in the column, improving the peak shape, signal response and repeatability for target analytes, as well as reducing possible decomposition of more labile compounds. Typical analyte protectants are those compounds like sorbitol or ethyl glycerol that are capable of interacting with the active sites with their hydroxyl groups. This effect is known as "matrix-induced signal enhancement."

#### Can you explain how you prepared the standards? Did you clean up the matrix for preparation of the standards offline or online?

**Jacob:** In the case of GC analysis, the standards were prepared off-line and then the individual standards were cleaned up automatically, a kind of procedural standard approach, thus any losses in the cartridge are automatically corrected by the standards. The recovery and precision were excellent

using this approach, which is permitted by the EU SANTE Guideline Document N° SANTE/12682/2019 for Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed.

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The matrix-match approach was used in the case of the LC, where the blank matrix extract was cleaned up using  $\mu$ SPE and aliquots of the cleaned-up extract were spiked with the standard mixture at different concentration levels.

#### Is it possible to reuse the µSPE cartridge?

**Cavagnino:** This question comes up quite often, but it is not recommended to reuse the same cartridge for another sample. One key benefit of the  $\mu$ SPE cartridges is they are disposable, which avoids any risk of cross contamination between samples. In addition, the disposability ensures the maximum repeatability of the cleanup process. For all these reasons, it is not recommended to reuse the  $\mu$ SPE cartridge.

#### Can the system automatically prepare the standards from a bulk volume of blank sample extract and a standard solution if placed somewhere on the tray? That would save a lot of time and also reduce errors.

**Jacob:** Yes, this is possible and is something that we are evaluating. Again, initial results look extremely promising, and we hope to share more information very soon.

# What is the cost of the cartridge compared to the cost of d-SPE sorbent?

**Cavagnino:** This is an important point for the user. We need to consider that the cost of the reagents for the dispersive SPE is variable according to the numbers and types of sorbents that are included in the tube or pouch. Cost also varies depending on the different countries, so it is difficult to make a direct cost comparison. On average, the cost of the  $\mu$ SPE cartridge and the additional vials required in the workflow is likely to be slightly higher. If you take into account the labor cost savings, however,  $\mu$ SPE can be very competitive and convenient. Also,  $\mu$ SPE has been demonstrated to be more effective, delivering high-quality results.

#### If required, is it possible to reinject a cleaned-up extract if there is a reason for reanalysis? In other words, is the seal in the vial good enough to prevent evaporation and or could the system automatically dilute the sample and rerun if the response in the first injection is higher than the calibration range?

Jacob: Yes, it is possible to rerun the sample. The eluate vials are well sealed, which prevents evaporation. In addition, in the case of the LC, the eluate tray can be kept at a controlled temperature. Also, it is possible to automate a dilution step by the system, if required before re-analysis of the sample. I would also like to add that although the workflow presented in this webcast does not include the dilution steps, we are currently working on another QuEChERS workflow where the dilution is included. We will share more information about it in the near future.

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#### How long does the cleanup take?

**Cavagnino:** It is quite quick; it takes 10–12 minutes to complete the cleanup process. But what is more important is that the cleanup workflows are executed during the chromatographic separation of the previous sample. Thus, users will benefit from the best sample throughput because both sample preparation and analysis times are optimized.

## Is it a requirement to add an internal standard in the case of GC-MS/MS?

**Cavagnino:** It is a good practice in pesticide residues analysis to use isotope-labeled internal standards along with matrix-matched calibration standards to compensate for possible matrix effects. In our testing of rice and wheat samples, however, we just added the triphenyl phosphate after the cleanup for GC analysis to check for the injection errors. Even though we did not use any isotopically labeled internal standards, we still achieved excellent results.

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A new approach to boosting sample throughput without sacrificing data quality

#### Introduction

The productivity of a pesticide residues laboratory is often limited by the chromatographic timespan. In a typical liquid chromatography-tandem mass spectrometry (LC-MS/MS) multi-residue method, the run time is a compromise between the number of target analytes, an acceptable number of coelutions, the desired chromatographic peak width, and the minimum dwell time of the MS detector needed to achieve acceptable data quality. A fast-chromatographic run that produces very narrow peaks can have a negative impact on sensitivity, selectivity, and data quality. An effective solution is the use of a novel dualchannel ultrahigh performance liquid chromatography (UHPLC) system with

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two independent flow paths capable of operating two analytical columns simultaneously. Sample extracts are injected alternately on the two columns using optimized gradient elution conditions to either increase sample throughput or reduce the coelution of compounds to improve data quality. The chromatographic separation of the two columns is synchronized so the flow is directed to the mass spectrometer only during the method data window that includes the elution of the first analyte up to the elution of the last analyte of interest. By overlapping the chromatography, the mass spectrometer is continuously acquiring data, so the utilization of the MS is increased substantially, thus enabling more samples to be analyzed in a defined period, or improved chromatographic resolution without increasing the time required for analysis.

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#### **Dual-Channel LC-MS/MS**

Today, a typical LC-MS/MS method for pesticides analysis will use a column that is 100 mm in length, containing particles of 2-3 µm in diameter, and with gradient elution at a flow rate of 300-400 µL/ min. The total chromatographic analysis time is around 15–20 min and the number of target pesticides is 150-400. For compliance with the EU guidelines (SANTE/12682/2019) for identification, at least two precursor to product ion transitions should be monitored for each pesticide, meaning a total of 300-800 ion transitions should be included in the acquisition method. The question for laboratories is how to decrease the analysis time in order to improve productivity without compromising the quality of the data? The simple answer to achieve a shorter method is to use a shorter column, a steeper gradient, or a higher flow rate, but the number of co-elutions will increase, and the data quality will be compromised.

Implementing a faster, shorter method on a short column and with a triple quadrupole mass spectrometer has several considerations and drawbacks. If the duty cycle is maintained, dwell times become shorter and sensitivity is reduced. If dwell times are maintained, the duty cycle is longer, which leads to fewer data points per peak and reduced peak area precision. In addition, the coelution of pesticides with common transitions makes quantitation impossible while coeluting matrix may cause ion suppression and lower sensitivity. Since the elution of matrix compounds and pesticides is not evenly distributed, the most crowded portions of a chromatogram are particularly affected by speeding up the chromatography with an increased probability of interferences from coeluting matrix with the same ion transitions as the analytes. Thus, more time is spent optimizing different transitions, which are more selective and therefore less affected, but generally less sensitive.

A different approach for decreasing the analysis time and increasing throughput, without further compromise to the separation, is the application of dualchannel chromatography. With this methodology, the total analysis time is decreased by minimizing unused mass spectrometer time while maintaining efficient chromatographic separations.

"Implementing a faster, shorter method on a short column and with a triple quadrupole mass spectrometer has several considerations and drawbacks."

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#### Figure 1: Time segments in dual-channel chromatography.



Designed to enhance throughput, the Thermo Scientific<sup>™</sup> Transcend<sup>™</sup> Duo LX-2 System is configured with two pumps, and an autosampler with two independent injection units and a column oven. The combination of a pump and its dedicated column is referred to as a channel. Two columns are operated in parallel and sample run times are overlapped, as shown in Figure 1, so the mass spectrometer is not sitting idle during re-equilibration of the column, autosampler needle wash, and other time-consuming steps. Consecutive injections are synchronized such that the first analyte from the second column elutes just after the elution of the last analyte from the first column. The mass

spectrometer collects data during the elution portion— "the data window" for each sample, highlighted green in Figure 1. The mass spectrometer acquisition switches between channels to maximize efficiency. When the data window ends in channel 1, the data

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window starts in channel 2. Then at the end in channel 2, the channel 1 data window starts again, and so on.

Thermo Scientific<sup>™</sup> Aria<sup>™</sup> MX software schedules and controls the staggered but synchronized parallel operations by control of selector and bypass valves. As with a single-channel LC system, the typical method parameters are entered in the software. The dualchannel technique requires only two new parameters to be loaded, namely, the start time for the data collection window and its duration. With this information the software will synchronize the injections on the two channels. The duration of the mass spectrometer data collection is also edited in the MS instrument method.

"If desired, users may override the alternating channel selection to perform consecutive runs on the same channel from the same sample plate with the other channel dedicated to a different sample plate and all data generated will be within the channel."

In typical applications, the Aria MX software will alternate sequence injections between the two channels and the resultant data, if viewed in aggregate, will generate cross-channel results. If desired, users may override the alternating channel selection to perform consecutive runs on the same channel from the same sample plate with the other channel dedicated to a different sample plate and all data generated will be within the channel. The graphical interface is intuitive, making conversion of a single-channel chromatographic method into a dualchannel method rapid and simple.

Although separations are not impacted by switching to a dual-channel method, retention times are altered. Retention times in single-channel systems denote the absolute retention time, reflecting the entire chromatographic run, including the start and end time segments when the mass spectrometer is idle. On sample injection, the autosampler sends a signal to the MS instrument to initiate the start of the retention time measurement. In this traditional mode, the retention time for a compound is a measurement of the time it spends on the column, from injection to elution.

By contrast, data collection in a dualchannel system does not begin on injection, but rather just before elution of the first peak of interest as determined by the data window start time in the LC



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#### Figure 2: Retention time in multi-channel chromatography.



method, and as depicted in Figure 2. Immediately before that, the MS system was busy detecting compounds in the previous sample. In this mode, the start signal for the MS system to begin acquisition occurs at the data window start time configured in the method and typically close to the elution of the last peak of interest in the previous sample. The beginning of the data window marks the 0.0 retention time, resulting in a relative, rather than absolute, retention time. The sample spends the same amount of time on the column, but the MS detection window is shorter in duration to create a narrower data window that is focused only on the elution times of the analytes of interest. This technique optimizes the use of the

MS detector and shortens overall run time without sacrificing the quality of the chromatography.

#### **Applications**

A Transcend DUO LX-2 System was interfaced with a Thermo Scientific<sup>™</sup> TSQ Altis<sup>™</sup> Triple Quadrupole Mass Spectrometer system or a Thermo Scientific<sup>™</sup> Q Exactive<sup>™</sup> Focus Hybrid Quadrupole-Orbitrap<sup>™</sup> Mass Spectrometer system to evaluate the dual-channel approach for the multi-residue analysis of pesticides using columns of different lengths. Using a Thermo Scientific<sup>™</sup> Accucore<sup>™</sup> HPLC column C18, 2.1 x 100 mm, 2.6 µm column with a TSQ Altis, the chromatography method allowed 14 minutes for the elution stage,

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#### Figure 3: Retention time stability (methamidophos). Methamidophos solvent tomato zucchini pear solvent orange 0 20 40 80 100 120 60 Injection number The red lines mark ± 0.1 min 120 injections alternating on column 1 & column 2

three minutes for column re-equilibration, and approximately one minute for all the autosampler operations. **Figure 1** shows the pesticides eluted in the green range, which spanned less than 11 minutes of the 18-minute total run time. By employing the dual-channel method, in which the run time consists of the elution time only, the time of analysis was reduced from 18 minutes to 10:45 minutes.

Compared with the single-channel system, the shortened run times of the dual-channel system amounted to significantly higher throughput. In one hour, three samples could be analyzed using conventional analysis, versus five samples on the new set-up. Accordingly, over a 24-hour period, the single-channel could analyze 80 samples, whereas the dual-channel could run 135 samples, almost a 70% increase in productivity. Thus, the cumulative effect of the productivity enhancement that can be gained with the dual-channel system could substantially improve throughput for laboratories striving to meet demanding analytical turnaround times.

Retention time stability for the dualchannel system was assessed over 120 injections that alternated between column 1 and column 2. Pesticides spiked into samples of varying complexity (e.g., tomato, zucchini, pear, and orange) were analyzed and their retention times plotted as a function of injection number. In the graph for methamidophos, shown in Figure 3, the two red horizontal lines denote the  $\pm 0.1$ retention time tolerance specified in the EU SANTE guidance documents. The retention times for all of the pesticides in every matrix in the study were compliant with the EU SANTE guideline criteria. Two apparent outliers were not outliers at all, but simply the result of a software setup issue of labeling the retention time

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#### Figure 4: Recovery data (apple): single-channel vs dual-channel.



when closely eluting isomers of slightly varying responses were integrated together. This can be easily resolved by integrating the isomers separately.

Recoveries were studied in apple, bell pepper, and orange matrices using a QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction protocol with no clean-up applied. The extracts were diluted 5-fold with water prior to injection on the TSQ Altis (2.5  $\mu$ L)

or the Q Exactive Focus (10  $\mu$ L). The recoveries of the dual-channel system were comparable with the single-channel results. **Figure 4** provides the recovery data for apple, which shows equivalence between the two channels. For apple, the recoveries for 269 and 270 of the total 273 pesticides at 0.01 mg/kg were within the 70–120%, in single- and

dual-channel modes of operation, respectively.
The associated %RSDs were all below 20%, with the majority below 5% (Figure 5). It is important to note that the results were equivalent for the single-channel and the dual-channel modes.



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#### Figure 5: Repeatability data (apple): single-channel vs dual-channel.



Cross-channel calibrations for pesticides in apples were performed by using calibration data from both alternating channels in a single calibration curve. Excellent linearity was observed for all calibration curves, in agreement with their single-channel counterparts. Coefficients of determination, R2, of 0.9997+ were achieved in all cases.

#### Increasing Selectivity Without Reducing Throughput

The use of 100 mm columns for pesticide residue analysis is typically a compromise between the quality of separation and the speed of analysis. There are times when separations using a 100 mm column are insufficient, but laboratories would rather not use longer columns because of the increase in the run times. Dual-channel



chromatography is especially useful if laboratories would prefer to improve the quality of the separation without incurring a time penalty. The reduced run time delivered by the dual-channel system can compensate for the extra time involved with substituting a 150 mm column for a 100 mm column. Thus, the separations

Number of compounds: 273

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#### Figure 7: Co-elution of analytes with a 100 mm column.



can be improved without sacrificing productivity because dual-channel chromatography enables the use of longer columns for improved separations but with similar run times to a singlechannel operating with a 100 mm column.

For instance, the same mixture of pesticide residues from Figure 1 that took 18 minutes by the single-channel approach with a 100 mm column required a 26.5-minute run on a 150 mm column. However, the dual-channel system time of analysis only used a data window of 15.5 minutes, which is almost 14% faster than the traditional 100 mm column method, despite the increased column length.

Also, use of a longer column resulted in a fewer number of co-elution's as shown in **Figure 6**. This is important because the coelution of common fragment ions can present serious problems with the analysis (see Figure 6). Using 100 mm columns, researchers must select different fragment ions, which are usually less intense, thereby negatively impacting identification and quantitation limits. Using a 150 mm column can resolve coelution for many compounds, thus providing higher quality data.

The co-elution of bromuconazole, cyproconazole, and paclobutrazole using a 100 mm column is shown in **Figure 7**. Using the high resolution accurate Mass of the Q Exactive Focus, the coeluting pesticides have a common fragment (m/z 70.03997) that cannot be mass resolved, but can be resolved chromatographically using a 150 mm column as shown in **Figure 8**. Thus,

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#### Figure 8: Using a longer column to overcome co-elution of analytes.



sensitivity is maintained with the 150 mm column, as the most intense fragment ion at *m/z* 70.03997 can be measured. Other benefits from the use of a longer LC column are decreased matrix effects as well as increased dwell times, which are helpful with difficult matrices and compounds with low sensitivity. The dual-channel system facilitates the elimination of coelution and offers the other advantages of a longer LC column without adding to the analysis time.

#### Conclusion

The use of the Transcend Duo LX-2 system in dual-channel mode improves sample throughput by increasing the utilization of the mass spectrometer. The use of two 100 mm length columns in parallel can increase sample throughput by approximately 70%, while the use of two 150 mm length columns in parallel can enhance sensitivity and selectivity without any increase in analysis time.

The dual-channel mode produces very stable retention times and results, which are equivalent to the single-channel mode.

Conversion of a single-channel chromatographic method into a dualchannel method is fast and simple as the Aria MX software is user friendly and very easy to use.

For further details, results, and examples, watch the webinar on demand.

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Interview with Łukasz Rajski

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he productivity of a pesticide residues laboratory can be limited by long chromatographic run times. In an LCGC webcast, Łukasz Rajski, PhD, of the University of Almería in Spain, described an effective new solution: using a dual-channel ultrahigh performance liquid chromatography (UHPLC) system that can operate two analytical columns independently and simultaneously. Rajski explained how this method can increase sample throughput and reduce the co-elution of compounds to improve data quality without increasing analysis time. Here, he answers questions posed by the audience.

#### Does the use of relative retention time, rather than absolute retention time, make method transfer more difficult?

**Rajski:** Not at all. The difference between the relative and absolute retention time

is constant, so it's the same for all the analytes in the method. The difference is the retention time when the data window starts. So, if the data window starts at 1.5 min after injection, you must convert the retention time from absolute to relative values. You simply subtract the value, 1.5 in this case, from all the retention times, which can be done in a spreadsheet or the quantitation software. The Thermo Scientific<sup>™</sup> TraceFinder<sup>™</sup> software has an option to easily re-assign the retention time.

#### Why is the gradient for the triple quadrupole instrument different for the Orbitrap?

**Rajski:** The reason why is a combination of factors, the first of which is the difference in sensitivity. The Thermo Scientific<sup>™</sup> TSQ Altis<sup>™</sup> Triple Quadrupole Mass Spectrometer is more sensitive than the Thermo Scientific<sup>™</sup> Q Exactive<sup>™</sup> Focus Hybrid Quadrupole-Orbitrap<sup>™</sup>

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Mass Spectrometer, so we used a higher injection volume on the Q Exactive Focus to compensate. But with a higher injection volume (10 µL), we observed some problems with peak broadening.

This was not unexpected because the extract contained 20% organic composition, while the initial mobile phase used only 2% organic solvent; a mismatch. Because of the very small mixing volume between the injector and the column in our Thermo Scientific™ Transcend<sup>™</sup> Duo LX-2 System, there was insufficient dilution of the extract in the mobile phase before the analytes entered the column.

Hence, we observed some problems with peak dispersion for the early eluters, so we made two modifications. We changed the gradient to improve the dilution of the organic part of the sample, and we replaced the capillary tubing between the injector and the columns with wider bore tubing to increase the mixing volume. After these simple changes, we obtained an improvement in peak shapes.

#### Can different LC methods be used for Channels 1 and 2 in the same sequence?

Rajski: There is no problem with having different methods for Channels 1 and 2 because each is in a separate column compartment with independent flow paths. You can set up different temperatures for each of the columns and have more than one set of mobile phases.

In the Transcend Duo LX-2 system, we had three bottles with mobile phase A and three bottles with mobile phase B, and they were independent (mobile phases A1, A2, A3, B1, B2, and B3). You can use completely different compositions of the mobile phase for Channels 1 and 2. For example, in Channel 1, you can have reversed-phase chromatography with water in Bottle A1 and an organic modifier in the Bottle B1. Then in Channel 2, you can have another column run with a different eluent pair.

#### How do you deal with compounds requiring negative mode ionization?

**Rajski:** With the TSQ Altis MS, the polarity switching is very fast, so there is absolutely no problem if you want to analyze components that require positive polarity and those that require negative polarity in the same run.

If we are talking about the Q Exactive Focus system, the situation is a little bit more complex. The system has polarity switching capability, but it's not as fast as in the TSQ Altis system. You can do it, but only for full scan screening. If you want to combine full scan MS and some extra MS2 experiments, the cycle time will be too long. In this case, we have found that it's better to analyze positive and negative polarity separately.

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For example, we can analyze the relatively few negative mode compounds in a separate run using a different gradient with a much shorter run time. Then, if you have some problems with the sensitivity with polarity switching, a separate run allows you to use a different mobile phase optimized for the elution of the pesticides that require negative polarity.

Remember that 95% of the pesticides we are analyzing require positive polarity. So, the mobile phase we are using is designed to improve sensitivity in positive polarity. It contains a high percentage of acid, which can suppress the negative ionization and hence the response in the negative mode. Thus, it is a good idea to separate positive and negative polarity compounds.

#### Does a lower injection volume on the **TSQ Altis provide any advantage?**

**Rajski:** It's beneficial to have a very sensitive instrument that permits you to inject small volumes. Sometimes, people think about sensitivity in only one dimension, for instance, "If I buy a new, more sensitive triple quad instrument, I will have lower limits of quantitation (LOQs)" Someone else might say, "I have an older triple quad, but my LOQs are okay because they are below the maximum residue levels, so I don't need a more sensitive instrument."

Having a more sensitive instrument, however, does not just decrease LOQs; it also allows a lower injection volume, which has many benefits.

In the case of electrospray, one problem I described in the webcast was matrix effects. When comparing the results from Q Exactive Focus and TSQ Altis, the matrix effects in TSQ Altis are much lower. And in fact, the matrix effects are often guite problematic in the sense that we are trying to overcome them with tweaks like complex cleanup, matrix-matched calibration, and the use of isotopically labeled standards, all of which are time consuming and add cost. But if you have more sensitive instruments and if you can decrease

the injection volume, you're already decreasing the matrix effects and quantitation is better.

Electrospray has other problems as well. For example, the saturation of the electrospray can be problematic because

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above a certain number of molecules, the ionization is not linear, which creates issues with calibration. If we decrease the injection volume, we are below this limit and we don't have problems with linearity.

Another benefit is less maintenance. If you inject less, the instrument will be less dirty and you don't have to clean it as often. However, something that I really like in instruments from Thermo Fisher Scientific is that the cleaning procedure is quite easy. You can remove the capillary ion transfer tube without breaking the vacuum. You just decrease the temperature, remove the capillary, clean it with water and methanol or methanol with formic acid, dry the capillary, and reinstall it.

#### You obtained very good results with cross-channel calibration, but would you also use this approach for more complex samples such as cereals, soya, or spices?

**Rajski:** I haven't tried to inject cereals, spices, or soya, but for the reliable cross-channel calibration, it is important to have the same peak area on both columns. Two columns are never exactly the same, but a modern column prepared with modern manufacturing processes will be very similar.

We installed two brand new columns and had practically identical results. Once you inject hundreds of samples, however, you may start to observe some differences. There will be some background interferences and the peaks may shift. If you find the peak shape and other parameters to be acceptable, you can still use the column. But if you see differences that can affect the quantitation, it is better to switch from the cross-channel calibration to withinchannel calibration.

#### You used HPLC columns with a particle size of 2.6 µm. If you used 1.7 µm particle columns, might you see a significant improvement in productivity?

**Rajski:** With a smaller particle size, you can get better productivity because you have narrower peaks. You can use steeper gradients, so your run time will be shorter. Remember though, we are not working only with liquid chromatography, but also with mass spectrometry.

In the case of our triple quadrupole instrument, we have a duty cycle fixed for 500 milliseconds. In the case of high resolution, the duty cycle can be even longer for very complex workflows. I don't claim you need 15 data points per chromatographic peak; however, if you have a peak that has a two second-base width and you have 500-millisecond duty cycles, you will get three or four data points, which isn't enough.

The performance of the 2.6  $\mu$ m column fits our needs very well. If you use a 1.7  $\mu$ m column with the optimal conditions for the chromatography, the peaks will be

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too narrow. Changing to a smaller particle size column may also require more time for column re-equilibration. I feel the 2.6 µm Accucore column is a very good choice for pesticide residues analysis.

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