

Extended automation for on-line or off-line micro-SPE clean-up of QuEChERS extracts for GC-MS/MS analysis of pesticides residues in food

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Abstract

Purpose: Show the suitability and the benefit of an automated sample preparation workflow for pesticide analysis including clean-up by micro-SPE (μ -SPE), automated calibration standards preparation and sample dilution.

Methods: A robotic autosampler is used to execute an automated sample preparation workflow for GC-MS analysis. 172 pesticides in 5 different cereal matrices have been considered in a validation study, with the scope to possibly scale up the methods to all raw cereal products. The samples were extracted using the citrate-buffered QuEChERS according to the EN method, starting with 5 g of sample.

Results: The μ -SPE method showed the removal of more than 70% of the observed matrix interferences. The μ -SPE sorbent composition is optimized and offers a more intimate interaction with the extract, providing a better clean-up efficiency compared to the standard d-SPE approach. Spiked recoveries were between 70 and 120% for all pesticides with RSD < 20%. The automated workflow allowed preparation of samples 24/7 with substantial labor savings.

Introduction

As demand for routine pesticide residues testing increases, laboratories are under pressure to analyse many different food commodities for hundreds of different pesticides in compliance with statutory Maximum Residue Levels (MRLs). However, the process is often slowed by the need for manual sample extraction and clean-up before chromatographic separation.

A quicker, simpler, more generic method with minimum clean-up may facilitate higher sample throughput, increased method scope and lower cost per sample, but it might also lead to extracts with high concentrations of matrix co-extractives which would contaminate the analytical system more quickly. A more effective clean-up procedure that can remove more of the co-extractives without impacting on the recovery of pesticides of interest or extending the overall analysis time is required.

This poster details the use of a more efficient automated solid phase extraction using an optimized blend of sorbents contained in miniaturized cartridges (μ -SPE). This system is available as a dedicated tool for the Thermo Scientific™ TriPlus™ RSH robotic autosampler, which supports on-line or off-line automated workflows, for GC-MS/MS and LC-MS/MS.

Automation of sample preparation is essential for laboratories that want to extend unattended operations, reduce labor time and increase efficiency and productivity. To enhance these benefits, an additional automated step in the overall workflow was developed to include the automatic preparation of the calibration standards and automatic dilution of samples. This is further reducing manual operations and exposure to human errors, while combining higher sample throughput with better data quality.

Materials and methods

Sample preparation

172 pesticides in 5 different cereal matrices (barley, oat, rice, rye, and wheat) were extracted using the citrate-buffered QuEChERS according to the EN 15662 method, starting from 5 g of sample. Figure 2 reports the sample preparation workflow steps comparing the manual clean-up using the d-SPE approach and the automated clean-up achieved with the μ -SPE approach.

μ -SPE cartridges (Cart- μ SPE-GC-QUE-0.3 mL) were purchased from CTC-Analytics. Supel™ QuE QuEChERS tubes containing 4 g magnesium sulfate ($MgSO_4$), 1 g sodium chloride (NaCl), 0.5 g sodium citrate sesquihydrate, and 1 g sodium citrate dihydrate were purchased from Thermo Scientific. The d-SPE clean-up sorbent Supel™ QuE (EN) tubes were purchased from Supelco.

Instrumentation

The analyses were performed on a Thermo Scientific™ Exactive™ Orbitrap GC-MS while the automated sample clean-up was performed using a Thermo Scientific™ TriPlus™ RSH autosampler. It can be used as an off-line bench station, where two sets of samples can be prepared, one for GC-MS analysis and one for LC-MS analysis, or it can be mounted on top of the GC-MS for fully unattended on-line operations.

The TriPlus RSH robotic autosampler comprises three tools: the μ -SPE tool that holds a 1000 μ L syringe, the analyte protectant or internal standard tool that holds a 25 μ L syringe, and the injection tool that holds a 10 μ L syringe. The system also contains a standard wash module, a solvent station module, and a fast wash module.

The miniaturized μ -SPE cartridges contain a small amount of sorbent material (45 mg for GC analysis), ensuring a much-reduced dwell volume compared to conventional SPE elution cartridges, to minimize dilution. Sorbent material amount and composition (PSA, C18EC, CarbonX, $MgSO_4$) are optimized for QuEChERS extract clean-up.

Figure 1. Schematics of the μ -SPE operation

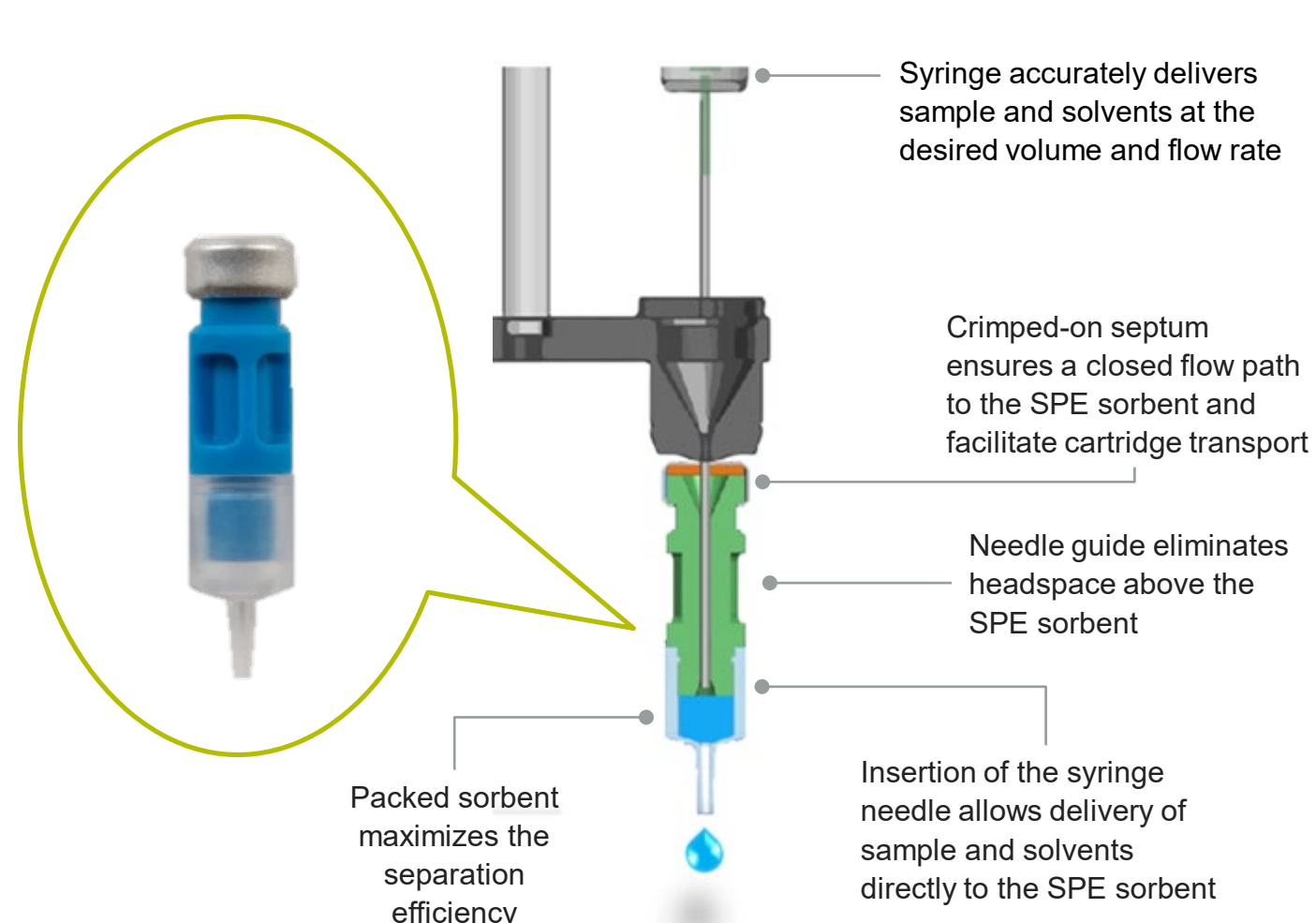
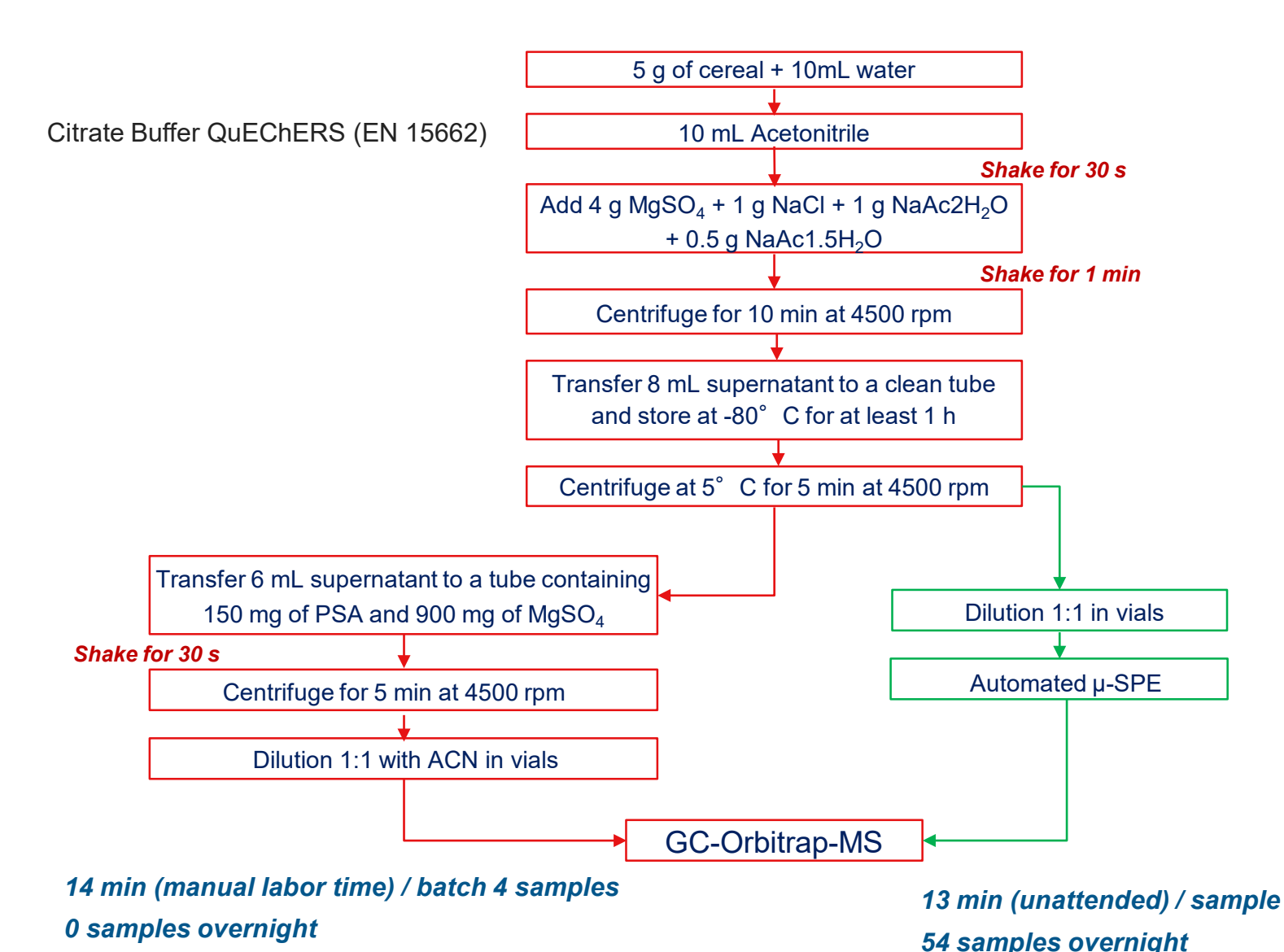


Figure 2. Extraction and clean-up workflow, comparing manual d-SPE and automated μ -SPE



Data analysis

The instrument method was developed on Thermo Scientific™ XCalibur™ Software, while the full scan MS data were processed on the Thermo Scientific™ TraceFinder™ 4.1 software.

Results

Clean-up efficiency

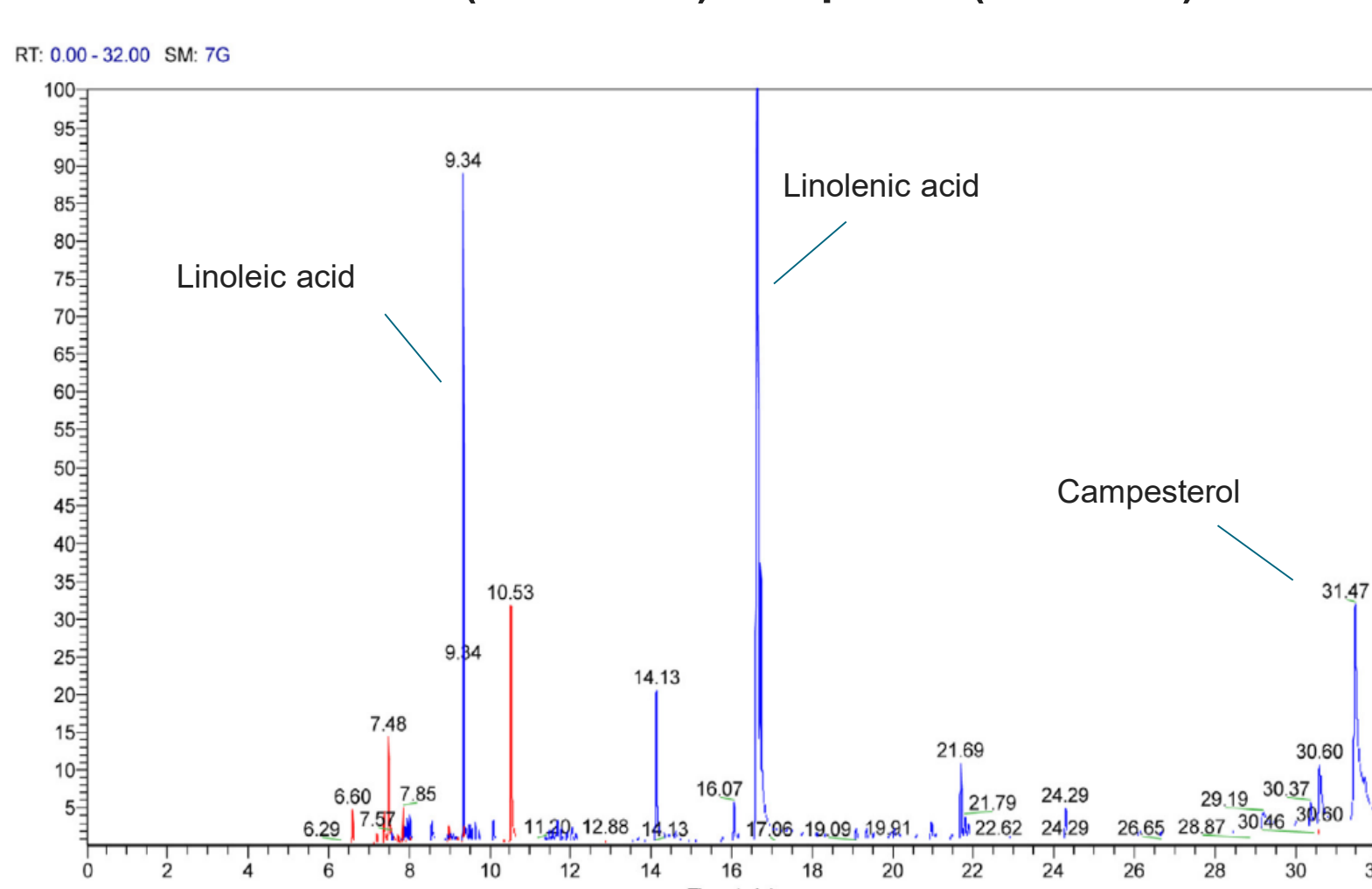
Elution SPE is typically more efficient than dispersive SPE (d-SPE). The μ -SPE technology offer the efficiency of the elution approach with additional benefits. μ -SPE uses much smaller particle size of the sorbent for a higher separation efficiency, thus increasing selectivity and creating a sharper elution profile. Additionally, the μ -SPE cartridge is a closed system which works with positive pressure and highly controlled flow rate through the sorbent at optimum separation conditions, thus maximizing matrix removal, analyte recovery and repeatability of the process¹.

Due to the reduced amount of sorbent and low dwell volume, a limited extract amount of 300 μ L is loaded on the cartridge.

To assess and compare the clean-up efficiency of d-SPE and μ -SPE, the TIC chromatograms from the two procedures were overlaid. Figure 3 reports the example of wheat blank extract (0.5 g/mL) showing high concentration of interfering matrix components like fatty acids and campesterol more effectively removed by the μ -SPE clean-up.

The TIC intensity of the two chromatograms demonstrates that the μ -SPE method resulted in the removal of approximately 70% more matrix interferences than the d-SPE method. A more efficient clean-up of the μ -SPE was also observed for the other cereal samples.

Figure 3. Overlaid TIC chromatograms of wheat blank extract cleaned with d-SPE (blue trace) and μ -SPE (red trace)



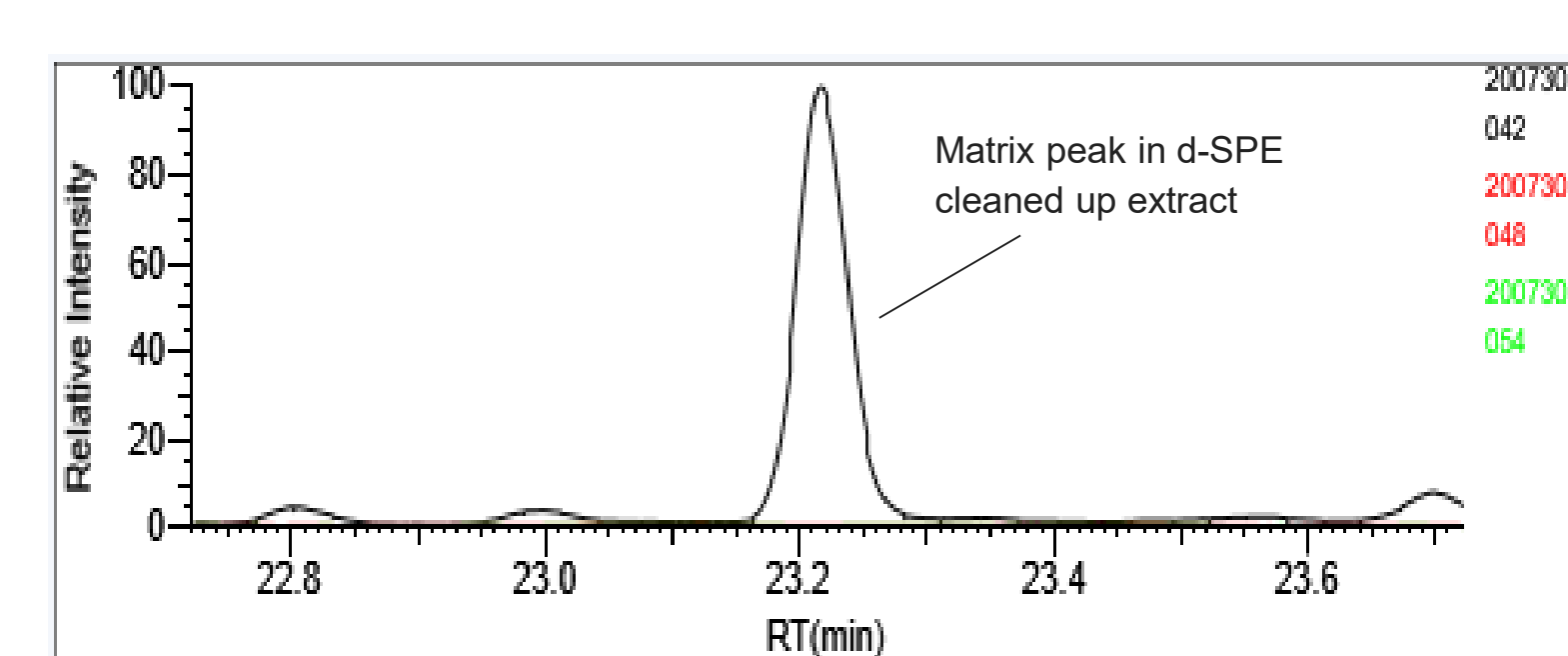
Using the unknown screening and deconvolution plugin with RT alignment of the TraceFinder software, it was possible to compare the total number of peaks detected in the sample extract after clean-up (Table 1).

Table 1. Clean-up efficiency of μ -SPE on blank extract of rye sample, compared to d-SPE

	Rye blank d-SPE + dilution	Rye blank μ -SPE + dilution
Amount of matrix cleaned up	0.5 g/mL	0.5 g/mL
Peaks from deconvolution	172	123
TIC intensity	1.2×10^9	3.34×10^8
		71% of matrix removed compared to d-SPE

The software also automatically performed a peak search and a library search. Combined with the unknown screening functionality of TraceFinder software, the deconvolution software was used to do a cross-sample overlay of analytes (Figure 4).

Figure 4. Cross sample peak overlay of myristic acid phenyl ester in rice (not detectable in μ -SPE cleaned extract)

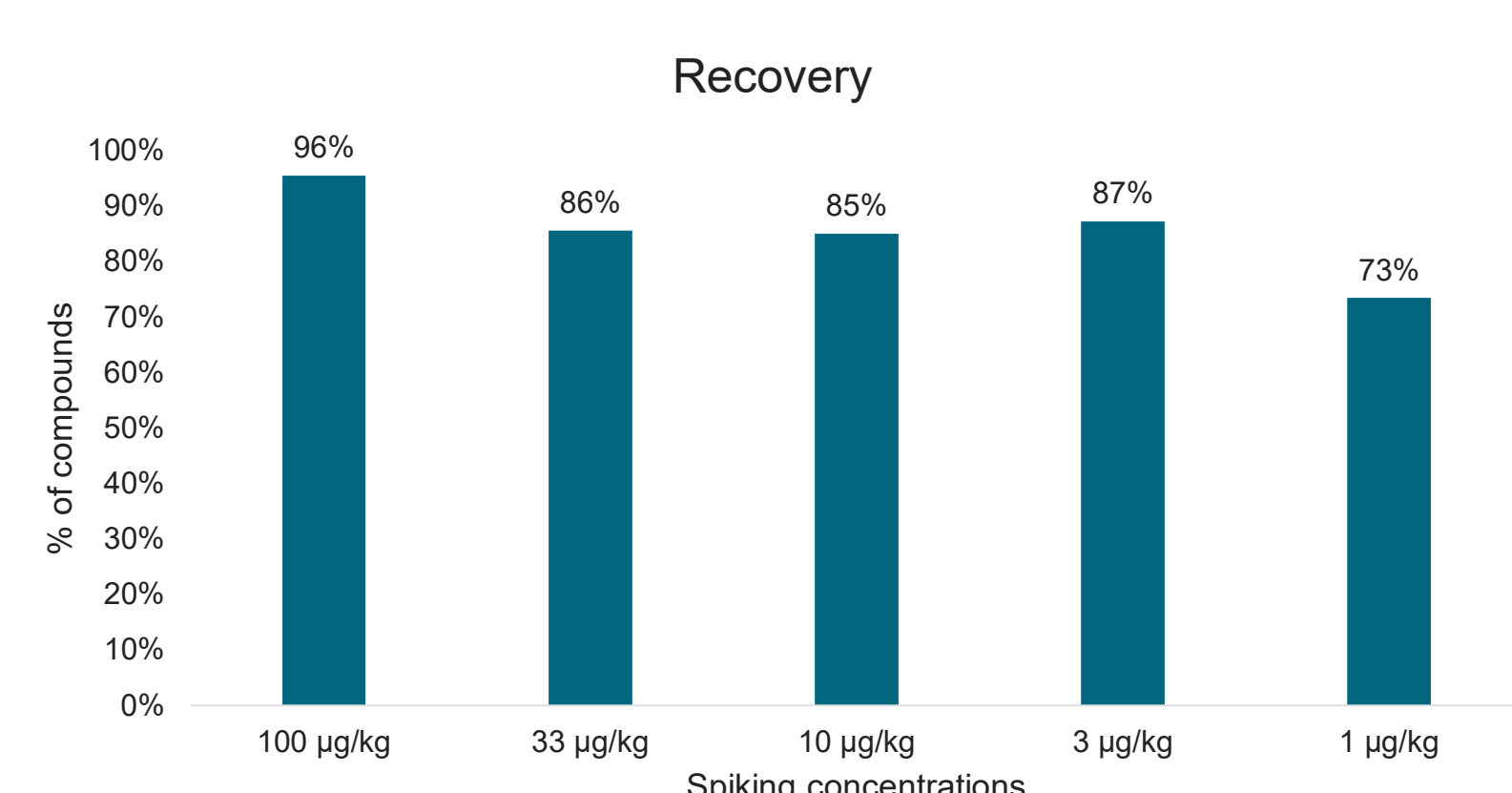


Recovery efficiency

In order to stay compliant with SANTE guidelines, recovery needs to stay within the range 70-120%, although recovery values outside this range can be accepted if the results are consistent and a correction factor can be applied.

Figure 5 shows the percentage of compounds recovered after clean-up at five different spiked concentration levels.

Figure 5. Percentage of compounds with recovery in the range 70-120% after clean-up for rye matrix sample spiked at five concentration levels



At the 10 μ g/Kg level, 85% of the pesticides showed good recovery. The slightly lower recovery rate at 1 μ g/Kg were possibly due to adsorption onto the μ -SPE sorbent during the clean-up, or to loss into the injector considering that some pesticides in cleaner extracts are possibly more prone to degradation as they are less protected in the presence of lower concentrations of matrix.

To ensure accurate quantitation for compounds showing low recovery, a semi-procedural standard calibration by spiking the blank matrix before the clean-up step, is recommended, compensating for recovery losses, especially at low concentration levels.

In this validation study², a semi-procedural calibration with a blank rye matrix was used to validate 170, 159, 142, 130, and 127 compounds in rye, wheat, barley, oat, and rice, respectively, with recovery in the range 70-120% and RSD < 20%.

Extended automation to dilution and calibration standards

Extending the automation capability to additional steps of the entire workflow enhances the labor-saving capability and the sample throughput, with further benefits of removing error-prone manual operations.

At the DTU-food laboratory, a TriPlus RSH bench station has been used to develop a workflow combining automated preparation of matrix-matched calibration standards, and μ -SPE clean-up of sample extracts.

Figure 6 reports the sequence of steps for the automated dilution/calibration. The dilution steps allows for the preparation of up to 8 levels of concentration, with dilution from 1:5 up to 1:10,000. The dilution is done in matrix, and different matrices can be used if required.

Optionally, up to three blanks can be prepared before the calibration dilution steps, and two IS can be added to the calibration dilution steps.

A pre-dilution step is optional in case required before the direct calibration dilution. A 10x and 100x dilution stock are prepared.

For executing the automated dilution/calibration workflow, a dedicated vial tray is added to the TriPlus configuration along with a large solvent station, as shown in Figure 7.

Figure 6. Steps for the automated dilution/calibration workflow executed by the TriPlus RSH autosampler

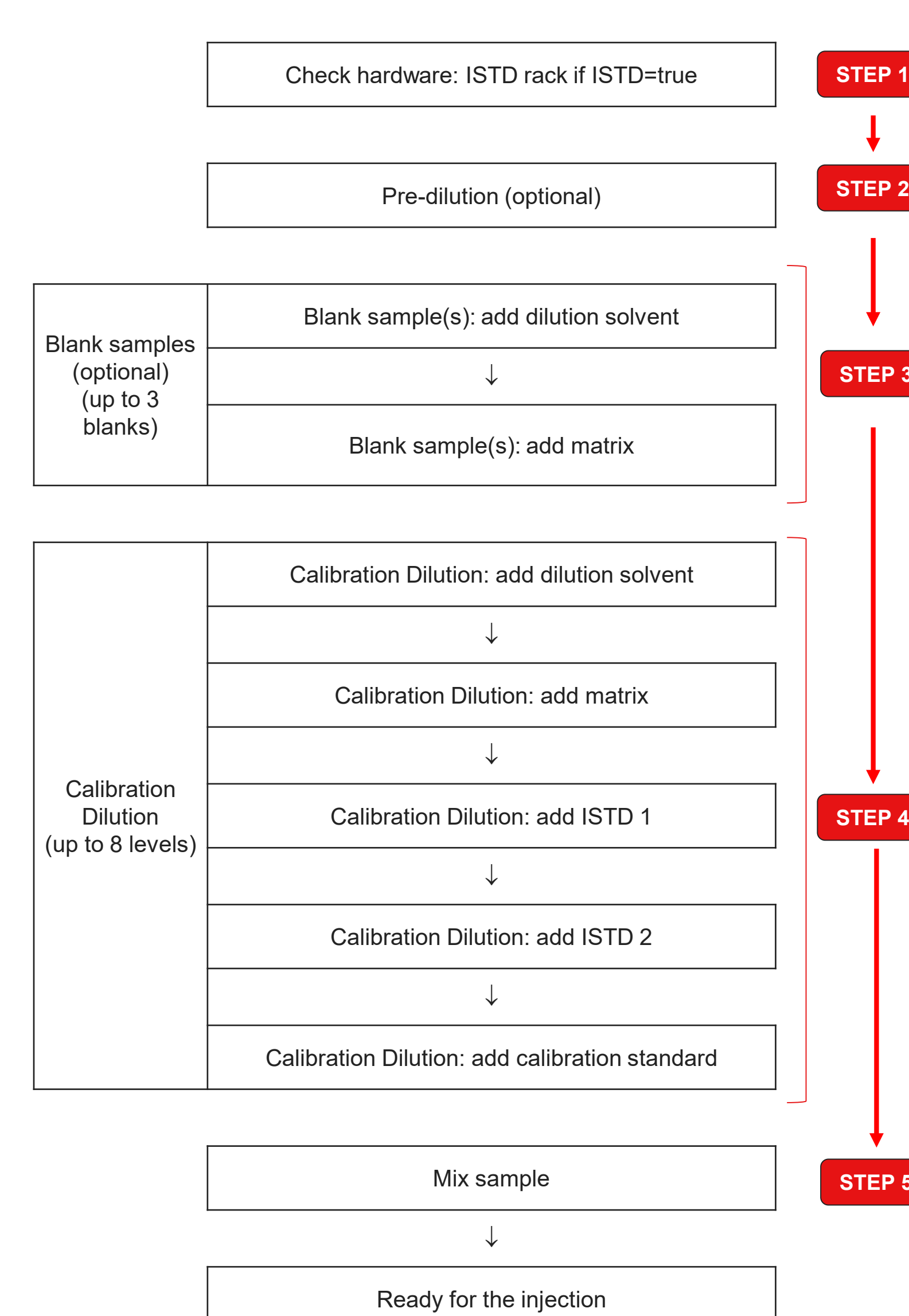
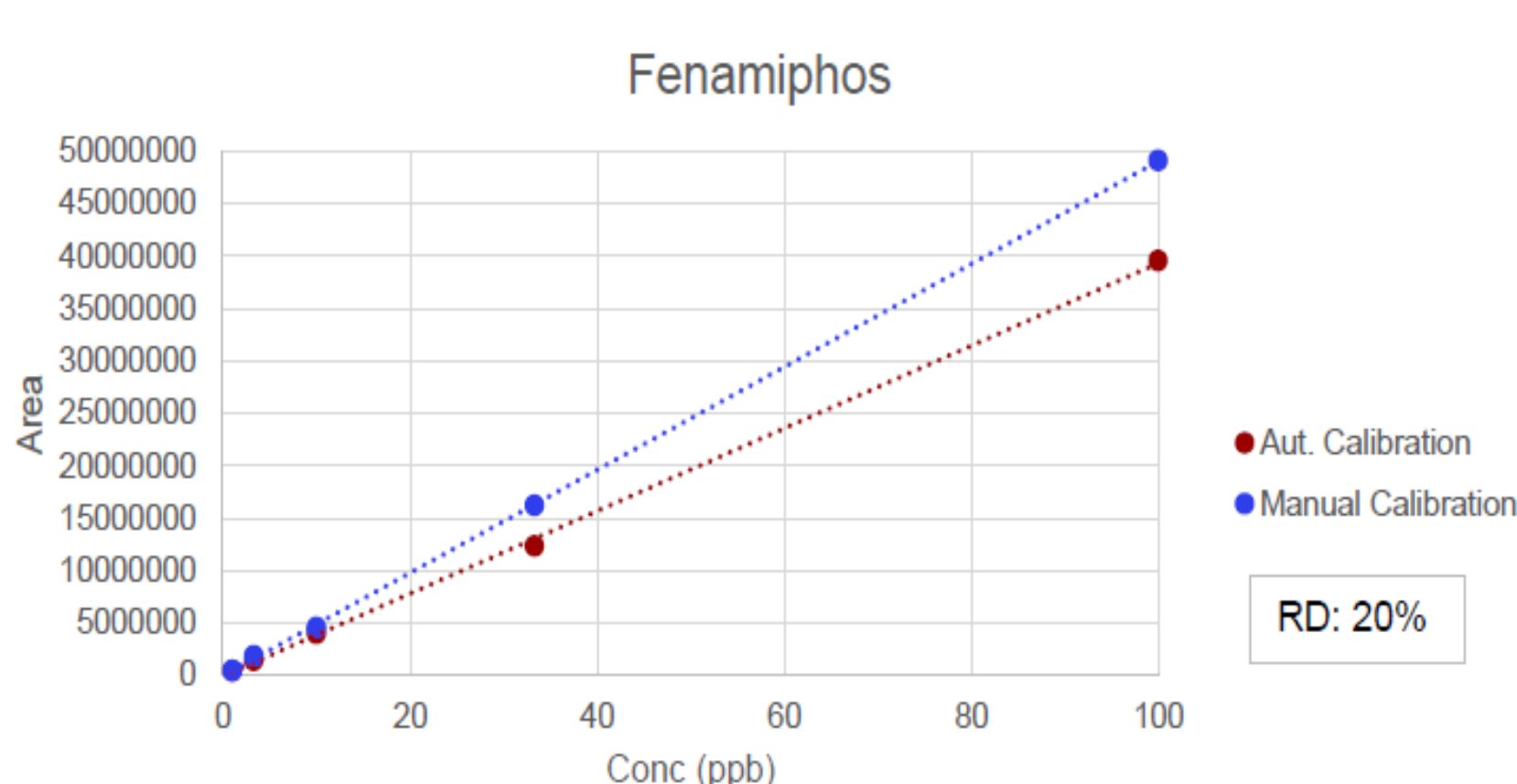


Figure 7. TriPlus RSH bench station equipped for automated dilution/calibration and clean-up



Figure 8. Example of automated calibration vs manual calibration for Fenamiphos



Conclusions

This validation study performed which is currently on-going at DTU-Food laboratory highlights several benefits of an automated approach for sample preparation in pesticides analysis:

- Automation increases sample throughput and saves labor time through unattended operations, removing manual operations with possible related human errors and delivering precise and reliable results.
- The μ -SPE clean-up has been demonstrated to be very efficient, with good recovery for most of the pesticides under study, in five different cereals matrix. The automated workflow has been demonstrated to be compliant with SANTE guidelines in term of recovery, precision and sensitivity for hundreds of pesticides.
- The μ -SPE cartridge is available with an optimized sorbent mix dedicated for GC or LC analysis, which can be used for many different type of food matrices. Part of the future work in DTU will be to extend the validation scope to more difficult matrices.

References

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- Elena Hakme, Mette Erecius Poulsen, *Journal of Chromatography A*, 1652, (2021), [462384]

Acknowledgements

We would like to thank Thomi Preiswerk from CTC Analytics (Switzerland) for providing the scripts for the TriPlus RSH autosampler, and for the continuing valuable support.

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