

# GC/MS Application Note

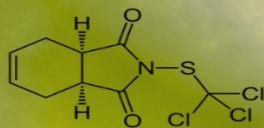
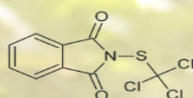


ENVIRONMENTAL



FOOD SAFETY

**Routine Pesticide Analysis using  
Micro-SPE**





# Routine Pesticide Analysis using Micro-SPE

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## Keywords

food, pesticides, multi-compound method, ethyl acetate extraction, automated clean-up, micro-SPE, high lipid content, avocado, liver, spices, cereals, captan, folpet, PTV, GC-MSMS

## Introduction

The department of pesticides analysis of the Official Food Control Authority of the Canton of Zürich in Switzerland examines goods from production and trade and analyses food for pesticide residues. Starting from Steve Lehotay's publication<sup>1</sup> and presentation at the 5<sup>th</sup> Latin American Pesticide Residue Workshop (LAPRW), the department of pesticide analysis introduced the automated clean-up of extracts in their routine lab as early as 2020. The methodology of generic extraction with ethyl acetate is well established for many years already, but clean-up procedures turned out to be a major obstacle for the steadily increasing sample throughput due to the additional manual workload addressing separately the different kind of food commodities of a governmental laboratory. Two years of experience led to a thorough understanding of the new automated micro-SPE ( $\mu$ SPE) clean-up workflow and its successful implementation into the laboratory routine procedure for pesticides analysis. This report presents for the first time the application of  $\mu$ SPE for the clean-up of the raw extracts using ethyl acetate as extraction solvent for pesticides from different also more complex food commodities.

## Project Goal

Generic extraction methods for food have found their solid place for the multi-compound pesticides analysis in private, industrial, and governmental laboratories. Ethyl acetate and acetonitrile extraction methods have been developed as fast and easy to handle standard multi-compound methods. An up to recently unsolved bottleneck became the clean-up of the raw extracts as the direct injection to GC is impaired by the high matrix content. Also, the LC analyses are affected by matrix effects and frequent maintenance requirements. A suitable and also easy to handle clean-up procedure was missing to complement the very capable extraction using acetonitrile (aka QuEChERS method) or ethyl acetate (aka SweEt method<sup>2,3</sup>) as solvents. Using the suggested dispersive SPE (dSPE), it turned out in practice that different food commodities required different adequate sorbent mixes to handle the many diverse matrix components (like chlorophyll, carbohydrates or lipids) of varying food commodities optimally without losses of the target pesticides. It is reported that ethyl acetate achieves high recoveries also for polar pesticides but also extracts a large amount of non-polar co-extractives, such as lipids and wax materials, which must be removed before the chromatographic determination. Typically, an additional gel permeation chromatography (GPC) is applied for ethyl acetate extracts as a clean-up method, in particular for fat containing samples<sup>4</sup>.

<sup>1</sup> Anastassiades, M., Lehotay, S.J., et al. "Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and 'Dispersive Solid-Phase Extraction' for the Determination of Pesticide Residues in Produce." *Journal of AOAC International*, 86(2) 2003, 412–31.

<sup>2</sup> Ekroth, S. „Simplified Analysis of Pesticide Residues in Food Using the Swedish Ethyl Acetate Method (SweEt)“. National Food Administration (NFA) Sweden, 2011. [http://www.laprw2011.fq.edu.uy/pdf/Lunes/Susanne Ekroth.pdf](http://www.laprw2011.fq.edu.uy/pdf/Lunes/Susanne%20Ekroth.pdf).

<sup>3</sup> Ekroth, S. „The SweEt Method: An Efficient Alternative to Analyze Pesticide Residues in Food“. National Food Administration (NFA) Sweden, presented at the LAPRW Conference, San Jose, Costa Rica, 2017.

<sup>4</sup> Barceló, D. "Food Contaminants and Residue Analysis." N.A., edited by Y. Picó, In: *Comprehensive Analytical Chemistry*, Vol. 51, Elsevier B.V., 2008.

The goal of the project was to establish in the pesticides laboratory of the Official Food Control Authority of the Canton of Zürich a generic clean-up procedure for the extracts of the applied ethyl acetate extraction method which is useful for all incoming food types without requirement for a food type dedicated clean-up procedure. An automated extract clean-up was envisioned for an improved sample throughput on the three already installed GC-MSMS systems providing less manual variability and more reproducible pesticides recoveries. Based on the early publications by Bruce Morris and Richard Schriener from Hill Laboratories, Hamilton, New Zealand<sup>5</sup>, as well as by Steve Lehotay, US Department of Agriculture, Wyndmoor, PA, USA<sup>6</sup>, the application of the reported  $\mu$ SPE clean-up promised to be available as a viable solution.

## Instrumentation

Three triple quadrupole GC-MSMS systems are in operation (TSQ 8000 Evo and TSQ 9000, Thermo Fisher Scientific, Austin, TX, USA) equipped with TriPlus RSH robotic systems (Thermo Fisher Scientific, Austin, TX, USA) for online  $\mu$ SPE extract clean-up and injection (**Figure 1**). One unit carries in addition a headspace tool and agitator module for the determination of dithiocarbamate pesticides after acid cleavage and  $\text{CS}_2$  detection. Thanks to the automated tool change (ATC) both methods can be executed without user interaction, the TriPlus RSH robot automatically selects the appropriate tool for online  $\mu$ SPE extract clean-up or the acid cleavage for  $\text{CS}_2$  detection. The TraceFinder software (Thermo Fisher Scientific) is used for the execution of the automated sample preparation workflow and the GC-MS instrument control, data acquisition and reporting.



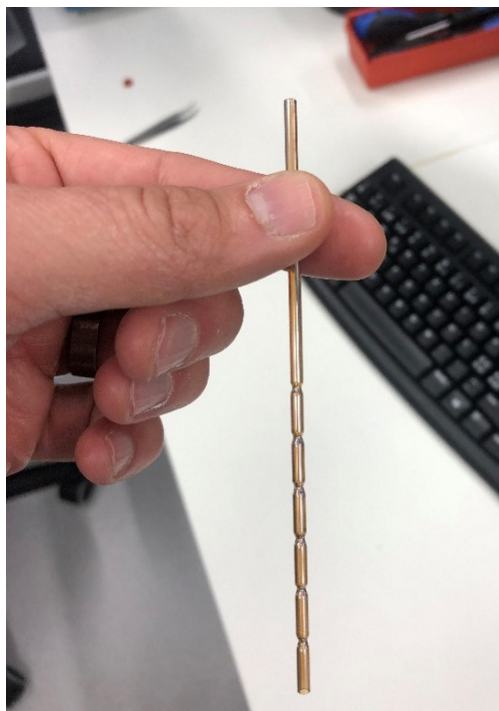
**Figure 1.** TriPlus RSH System for automated online  $\mu$ SPE extract clean-up

All GCs are equipped with a temperature programmable injector (PTV) allowing the injection at a low temperature of 55 °C for performance improvements of the lower volatile components. Excess solvent vapor of a 3  $\mu$ L injection is vented by applying 3 s of split flow, followed by a splitless completion of the vaporization and transfer to the column to minimize loss of the higher volatile compounds. A DB-5ms Ultra Inert GC column (Agilent Technologies Inc.) is used with only 15 m length, 0.25 mm ID, and 0.25  $\mu$ m film thickness. A standard baffled inlet liner without glass wool is used as displayed in **Figure 2** (Restek Corporation, Bellefonte, PA, USA).

Each GC-MS system runs about 100 sample injections per week, in addition to the calibration and system suitability checks.

<sup>5</sup>Morris, B.D., and R.B. Schriener. "Development of an Automated Column Solid-Phase Extraction Cleanup of QuEChERS Extracts, Using a Zirconia-Based Sorbent, for Pesticide Residue Analyses by LC-MS/MS." *J. Agric. Food Chem.*, 63 (2015) 5107–5119. doi:10.1021/jf505539e.

<sup>6</sup>Lehotay, S.J., Han, L., et al. "Automated Mini-Column Solid-Phase Extraction Cleanup for High Throughput Analysis of Chemical Contaminants in Foods by Low-Pressure Gas Chromatography-Tandem Mass Spectrometry." *Chromatographia*, 79 (2016) 1113–30. doi:10.1007/s10337-016-3116-y.



**Figure 2.** PTV inlet liner after more than 100 analyses during the weekly liner change.

As a result of the applied online  $\mu$ SPE raw extract clean-up, a liner exchange is performed only once a week, reducing system downtime significantly. Even at the time of change after about 100 sample runs, the liner still appears to be clean without visible residues, as shown in **Figure 2**.

The low matrix burden after the online  $\mu$ SPE raw extract clean-up also shows up with the extended lifetime of the GC column in use. The column gets clipped about half a meter only after six months of use and more than 2600 sample analyses run on the system. An MS ion source maintenance is performed, when deemed necessary, approximately once a month. Thanks to the Thermo Fisher 'Never Vent'<sup>®</sup> technology the ion source exchange is a maintenance procedure that only takes about two hours until the system is ready again to process the next samples.

## Workflow

### Ethyl Acetate Extraction

The processing of food samples starts manually from a bulk sample by cryomilling to achieve a representative test portion. From the homogenized test portion about 10 g are weighed into a regular 50 mL centrifuge extraction tube-containing 6 g  $\text{MgSO}_4$  and 1.5 g sodium acetate. 10 mL of ethyl acetate are added, and the tube is shaken mechanically for 5 minutes. After centrifugation 1 mL of the supernatant is transferred to 2 mL autosampler vials.

**Table 1.**  $\mu$ SPE cartridge sorbent material composition

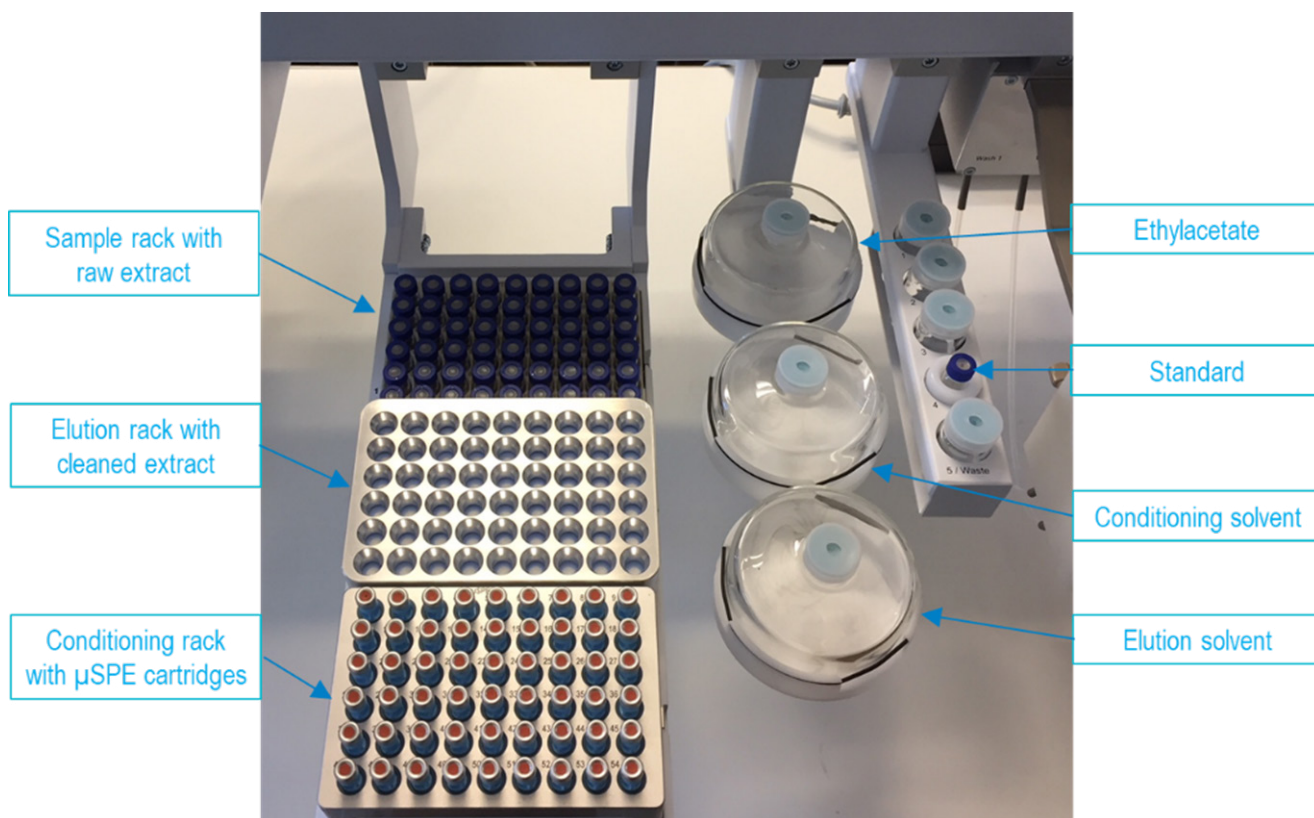
Sorbent	Bedmass	Units	%
PSA	12	mg	27
C18EC	12	mg	27
CarbonX	1	mg	2
$\text{MgSO}_4$	20	mg	44
<b>Total</b>	<b>45</b>	<b>mg</b>	<b>100</b>

<sup>7</sup>2 g for dry spices, 5 g for grain, dry samples wetted with water (10 mL) prior to extraction

## Extract Clean-up

The automated procedure using  $\mu$ SPE cartridges (60101-45 GC Thermo Scientific GC SPE Cartridge) was established as extract clean-up. The cartridges in use for the purification of the GC-MS samples contain 45 mg of a mixture of PSA, C18EC, CarbonX and MgSO<sub>4</sub> sorbent materials<sup>5</sup>, as specified in **Table 1**.

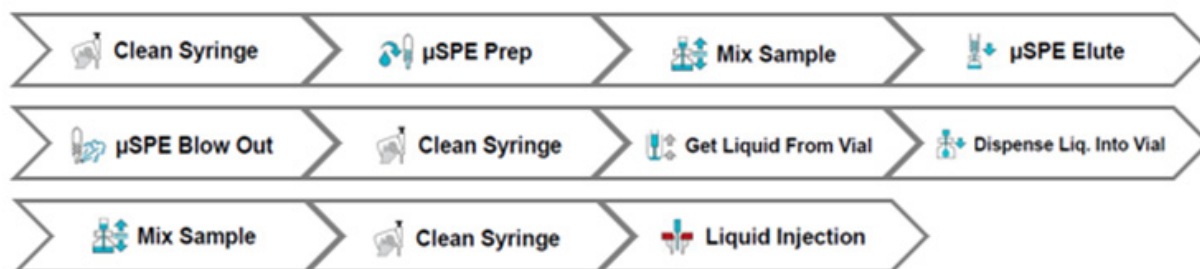
The configuration of the TriPlus RSH robotic system with the dedicated  $\mu$ SPE trayholder is shown in **Figure 3**. The vials with the ethyl acetate raw extract are placed into slot 1 of the  $\mu$ SPE tray holder of the TriPlus RSH robot. Slot 3 in the front holds the  $\mu$ SPE cartridges. The eluted and cleaned extracts are collected in empty 2 mL vials in slot 2 in the center of the tray holder. The processing of the sample is executed serially including the online injection of the cleaned extract to GC-MS.



**Figure 3.**  $\mu$ SPE tray holder configuration with standards and solvents on the TriPlus RSH robotic system.

Raw extracts get processed on a self-controlled time axis of the TriPlus RSH robot so that the extract is ready for injection when the GC Ready signal is expected. Extract purification is prepared in-time and avoids compound degradation by different and increasingly long wait times.

The automated clean-up workflow starts with the conditioning of the cartridges held ready in slot 1 with 300  $\mu$ L ethyl acetate. After the cartridge conditioning with elution solvent from the reservoir the syringe loads 200  $\mu$ L of the raw extract in ethyl acetate from a sample vial in slot 1 and moves to the cartridge tray to pick a conditioned cartridge by inserting the needle. The cartridge is moved by the syringe to the elution tray and inserted into an empty vial (held ready below the cover) at slot 2.



**Figure 4.** Automated  $\mu$ SPE clean-up workflow, here shown for online GC-MS injection.

The raw extract is then pushed through the sorbent bed of the cartridge with a constant speed of 2  $\mu\text{L/s}$  by the syringe. The extracted matrix is retained on the cartridge, the cleaned extract elutes and gets collected in the vial below. Additionally, a blow-out step using the syringe can be added. The automated  $\mu\text{SPE}$  clean-up workflow is graphically illustrated in **Figure 4**. After the clean-up procedure the TriPlus RSH robot cleans the preparation syringe with polar and less polar solvents and finally changes to the injection tool with a regular 10  $\mu\text{L}$  GC injection syringe. After dilution and a mixing step, 3  $\mu\text{L}$  of the cleaned extract are injected. The PTV injector is kept at 55  $^{\circ}\text{C}$  during injection with a 3 s split open time, before starting the injector and GC oven heating ramp, see **Table 2**. The described automated clean-up procedure takes 15 min of the total GC-MSMS analysis time of 45 min, see the analysis parameters used for the MS in **Table 3**.

Temperature program:

#	Rate [ $^{\circ}\text{C}/\text{min}$ ]	Temperatur [ $^{\circ}\text{C}$ ]	Hold Time [min]
Initial		55.0	2.00
1	20.0	165.0	0.00
2	3.0	205.0	0.00
3	10.0	290.0	0.00
4	10.0	310.0	3.00

Carrier gas: Helium  
 Carrier gas mode: Constant pressure  
 Carrier gas pressure: 70 kPa (depending on column length)  
 GC Column: DB5-ms UI / 15 m x 0.25 mm x 0.25  $\mu\text{m}$   
 Injection mode: PTV  
 Injection volume: 3  $\mu\text{L}$

**Table 2.** GC parameters

PTV injector temperature program:

Rate [ $^{\circ}\text{C}/\text{s}$ ]	Temp [ $^{\circ}\text{C}$ ]	Time [min]	Split [mL/min]
0	55	0.1	30
2.5	330	12	0

**Table 3.** Parameters of the MSMS system

Ionisation mode: EI pos  
 MS transfer line temp.: 290  $^{\circ}\text{C}$   
 Ion source temp.: 220  $^{\circ}\text{C}$   
 Scan mode: Timed SRM  
 Total scan time: 0.3 s  
 Total compounds: 209 (418 MRM)

It is important to note at this point that the extracts get injected online right after the clean-up step. Waiting times, in particular different waiting times after contact with the sorbent material, are avoided so that all samples are treated on the identical timeline to avoid uncontrolled decomposition thus improving reproducibility of the recovery of the target analytes.

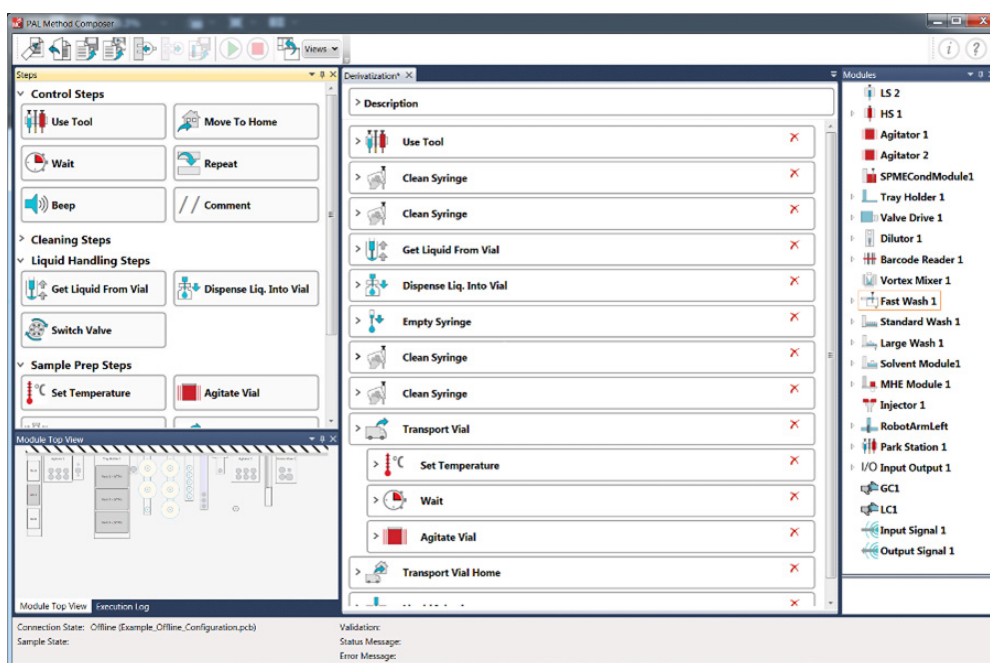
## Workflow Preparation

The TriPlus RSH  $\mu$ SPE clean-up workflow is created using the TriPlus Method Composer software (Thermo Fisher Scientific, Austin, TX, USA) and optimized for the parallel execution ('prep-ahead' mode) with the ongoing GC separation of a previous analysis. The graphical user interface is shown in **Figure 5**. The tools and modules of the used TriPlus RSH configuration are shown on the right side. The available workflow activities for this configuration are offered in the box on the left. The required tasks are pulled by 'drag & drop' into the centre of the screen to build the workflow sequence and customized by adaptation of the default parameters. The saved workflow is selected within the TraceFinder sequence table of the GC-MS systems for execution within the planned sample sequence.

With the typical 'prep-ahead' mode of the TriPlus RSH system the maximum sample throughput for each of the employed GC-MS systems is achieved.

There is no wait time for the GC-MS system. A next analysis run starts right away after the Ready signal of the GC.

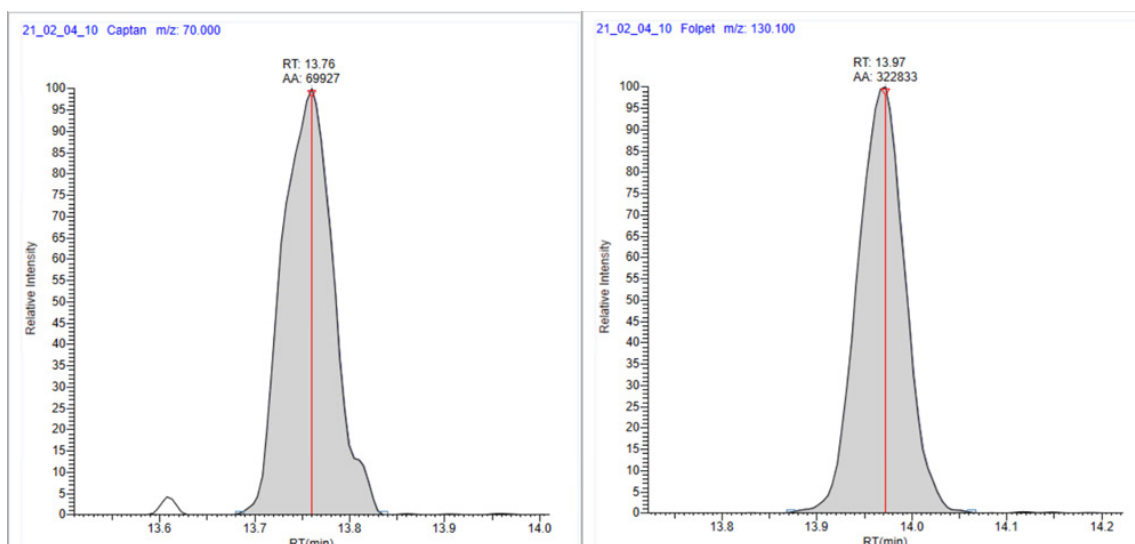
The clean-up method is optimized to match the GC-runtime. The sample preparation of the next sample will start in order to align the readiness of the consecutive sample with the Ready signal for injection to the GC. In case of a faster GC cycle time the TriPlus RSH Method Composer can also be used to further optimize the method to match a faster sample to sample cycle.



**Figure 5.** PAL Method Composer Software including the  $\mu$ SPE clean-up workflow steps shown in the center.

## Experience with selected pesticides and critical matrices

It could be shown that the recovery of the pesticides folpet and captan benefit from the GC injection volume of 3  $\mu$ L as demonstrated in **Figure 6**. Smaller injection volumes of less diluted extracts are contra-productive for these two difficult to analyse fungicides. Nevertheless, it is also crucial for the analysis of captan and folpet that the liner and the analytical column are in good condition as it can be achieved in routine with the  $\mu$ SPE clean-up.



**Figure 6.** Pak-Choi spiked with 100  $\mu$ g/kg captan and folpet, measured on a TSQ 8000 EVO

The current experience and setup allow the clean-up of samples with a lipid content of up to 15 % which for instance is the approximate fat content of avocados. Also, liver samples can be run without the need of a separate freeze-out of fats. Critical matrices like spices with a high content of essential oils (e.g. chilly, paprika, etc.) are cleaned-up online as well. 2 g of spices are treated with 10 mL of water before extraction with ethyl acetate. The raw extract is then cleaned as described above.

A pre-treatment is also required for grains and cereals. 5 g of sample material is soaked in 10 mL of water before extraction, then the raw extract is automatically cleaned-up as described.

New matrices are treated using the described workflow without any additional dilution.

## Quantitation

A screening procedure is used to identify potential non-compliant samples. The non-compliant residues in the selected samples are quantified by using the standard addition method with the identified pesticide taking into account any potential matrix effect. Three data points are prepared in an automated workflow with online GC-MS injections. A processing (procedural) standard is added at the beginning to correct for possible losses of pesticides during extraction and clean-up by  $\mu$ SPE.

## Conclusion

The comparison of the earlier manual method using the optimized dSPE clean-up sorbent mix for a particular food commodity with the automated  $\mu$ SPE workflow showed very good compliance within the normal and accepted error range in pesticide analysis.

Folpet and captan, two typical but difficult GC-analytes, were successfully analysed with the automated  $\mu$ SPE workflow.

The PAL Method Composer is a versatile tool for the adaption of the  $\mu$ SPE workflow to the specific needs as published by Steve Lehotay. After a short learning phase of less than a day, the described  $\mu$ SPE workflow could subsequently be developed in less than 3 days without the need of any programming knowledge.

The described  $\mu$ SPE workflow has been in routine operation for two years now and showed high reliability also applied for unattended overnight runs, releasing time from earlier manual workload to be used for other duties such as data evaluation and quantitation.

## Future work

As the utilized cartridges showed excellent potential for lipid removal, the scope of samples using the described workflow will be extended to cheese and liver samples to avoid the time-consuming manual freeze-out process of the contained fat, after extraction. Also under investigation is the applicability of the clean-up procedure for the analysis of PCBs and PAH contaminations of the food samples.

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