Automated µSPE Clean-up of Pesticide Ethylacetate Extracts

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PAL SYSTEM Ingenious sample handling

Overview

Purpose: The goal of this development was to establish a generic clean-up procedure for the extracts of the ethylacetate extraction method which is useful for all incoming food types without requirement for different clean-up procedures dedicated to different food types. An automated extract clean-up was envisioned for an improved sample throughput on GC-MSMS systems providing less manual variability and more reproducible pesticides recoveries for a wide variety of food commodities.

Methods: A mixture of PSA, C18, Carboxen™ and $MgSO_4$ in micro-SPE cartridges (60101-45GC Thermo Scientific GC µSPE Cartridge) is used for an automated load and elute workflow on an x,y,z-robotic system. The clean-up procedure is executed in-time for online GC-MS analysis.

Results: The comparison of the automated µSPE workflow with an earlier manual dSPE method showed very good compliance within the normal and accepted error range in pesticide analysis for a wide range of food commodities with additional advantages for data quality and sample throughput.

Introduction

Generic extraction methods for the multi-compound pesticides analysis for food have found their solid place in private, industrial, and governmental laboratories. Ethylacetate 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 laborious clean-up of the raw extracts. The direct injection to GC is impaired by the high matrix content, resulting in large matrix effects and requiring frequent maintenance. A suitable and also easy to handle clean-up procedure was missing to complement the very capable extraction using acetonitrile (*aka* QuEChERS method [1]) or ethylacetate (*aka* SweEt method [2,3]) as solvents.

Using dispersive SPE (dSPE), it turned out in practice that different food commodities required different adequately modified sorbent mixes to optimally handle the many diverse matrix components like chlorophyl, carbohydrates or lipids without sacrificing the recovery of the large set of target pesticides. It is reported that ethylacetate achieves high recoveries for a wide range of 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) was applied for ethylacetate extracts as a clean-up method, in particular for fat containing samples[4].

Methods

Sample Extraction with Ethylacetate

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 $MgSO_4$ and 1.5 g sodium acetate. 10 mL of ethylacetate are added. The tube is shaken for 5 minutes. After centrifugation 1 mL of the supernatant is transferred to a regular 2 mL autosampler vial.

Clean-up of the Ethylacetate Extract

An automated procedure using µSPE cartridges on an x,z,y-robotic system was established for the extract clean-up[5,6]. The cartridges in use for the purification of the GC-MS samples contain 45 mg of a mixture of PSA, C18EC, CarbonX and $MgSO_4$ sorbent materials, as specified in **Table 1**.

***Table 1**: µSPE cartridge sorbent material composition*

Sorbent	Bedmass	Units	%
PSA	12	mg	27
C18EC	12	mg	27
CarbonX	1	mg	2
$MgSO_4$	20	mg	44
Total	**45**	**mg**	**100**

Robotic System Configuration

As x,y,z-robot a Thermo Fisher Scientific TriPlus RSH system was used. The configuration with the dedicated µSPE trayholder is shown in **Figures 1 and 3**.

The vials with the ethylacetate raw extract are placed into slot 1 of the µSPE tray holder of the TriPlus RSH robot. Slot 3 in the front holds the µSPE cartridges. The eluted and cleaned extracts are collected in empty 2 mL vials located in slot 2 in the centre of the tray holder under the aluminium cover.

Prep-Ahead Clean-up Workflow

The clean-up processing of the raw EtOAc sample extracts is illustrated in **Figure 2**. The workflow steps are executed in the so-called *prep-ahead* mode including the online injection of the cleaned extract to GC-MSMS. 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. The extract purification is prepared in-time and avoids compound degradation by different and increasingly long wait times in larger sample series.

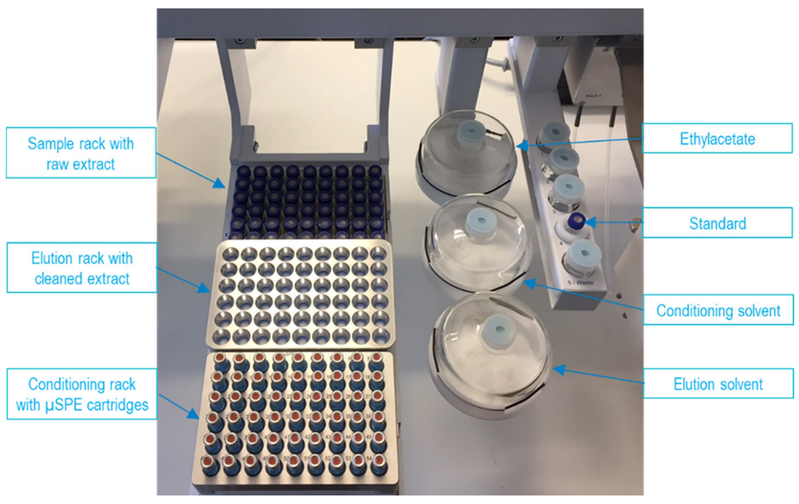


***Figure 1.** µSPE tray holder configuration (left) with dedicated standard and solvent positions (right) on the TriPlus RSH robotic system.*

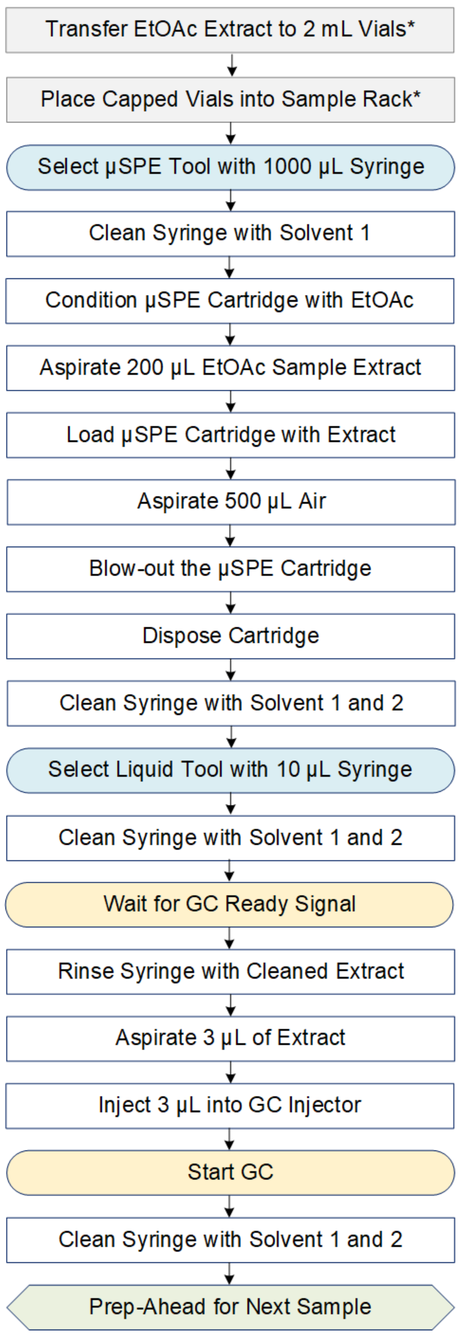


***Figure 2.** Automated workflow steps for clean-up of ethylacetate extract on the TriPlus RSH robotic system, and µSPE cartridge used.*

µSPE Clean-up Workflow

The automated clean-up workflow as shown in **Figure 2** starts with the conditioning of the cartridges held ready in slot 1 with 300 µL ethylacetate from the reservoir. After the cartridge conditioning the syringe loads 200 µL of the raw extract in ethylacetate from a sample vial in slot 1 and first moves to the cartridge tray to pick the conditioned cartridge by inserting the needle. The cartridge is then moved by the syringe to the elution tray and inserted into an empty vial (held ready below the cover) at slot 2. Here the raw extract is pushed through the sorbent bed of the cartridge with a constant speed of 2 µL/s by the syringe. The sample matrix is retained on the cartridge and the cleaned extract elutes the pesticides into the vial below. Additionally, a blow-out step using the syringe can be added.

After an automated change of the preparation syringe tool to a regular 10 µL GC injection syringe, 3 µL of the cleaned extract are injected to the GC-MSMS system. The syringes are cleaned with polar and less polar solvents.

GC-MSMS Analysis

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 for online µSPE extract clean-up and injection.

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 the 3 µL 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.

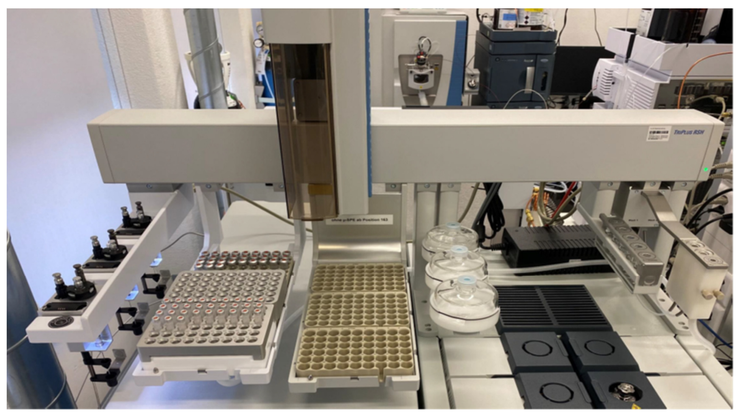
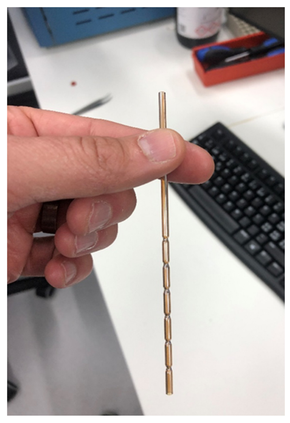


***Figure 3.** TriPlus RSH System for automated online µSPE extract clean-up.*

A DB-5ms Ultra Inert GC column (Agilent Technologies Inc.) is used with only 15 m length, 0.25 mm ID, and 0.25 µm film thickness. A standard baffled inlet liner without glass wool is used (Restek Corporation, Bellefonte, PA, USA, **Figure 4**).

Results

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



As a result of the applied online µSPE 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 can be seen in **Figure 4**.

***Figure 4.** PTV inlet liner after more than 100 food extract analyses during the weekly liner change.*

The low matrix burden after the online µSPE 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.

Conclusion

- The comparison of the automated µSPE workflow with the manual dSPE clean-up with optimized sorbent mixes for a particular food commodity showed very good compliance within the normal and accepted error range in pesticide analysis.
- Only one type of cartridge with a standard sorbent mix is used for a wide variety of food commodities. No further optimization of the mix for different food types was required.
- All samples are treated on the identical timeline which avoids uncontrolled decomposition during waiting times in long sample series thus improving reproducibility of the recovery of the target analytes.
- Folpet and captan, two typical but difficult GC analytes were successfully analysed with the described EtOAc extraction and automated µSPE clean-up workflow.
- The PAL Method Composer, used for creating the shown clean-up procedure, is a versatile tool for the adaption of the µSPE clean-up. The described µSPE workflow could be developed in less than 3 days without the need of any programming knowledge.
- The described µSPE workflow has been in routine operation for two years now and showed high reliability and is also applied for unattended overnight runs, releasing time from earlier manual workload to be used for other duties such as data evaluation and quantitation[7].

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