Characterization of the New PAL Micro-SPE Cartridge for Pesticides Extract Clean-up

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PAL SYSTEM
Ingenious sample handling

Overview

Since more than ten years micro-SPE (µSPE) emerged as a micromethod for sample preparation and clean-up in food safety, proteomics, forensic, environmental and analysis. Applications are wide-ranging and cover drugs, environmental contaminants, and, in particular, the QuEChERS extract clean-up in multiresidue pesticide analysis. The automation of the µSPE sample preparation steps led to the desired increase in sample throughput, unification of the used sorbent materials for food commodities, and the potential for the online hyphenation with GC-MS and LC-MS instrumentation.

With the increasing demand and the use of automated µSPE workflows on PAL Systems, the requirement for extended functionality evolved due to mechanical and also analytical limitations of the initially employed ITSP micro-SPE cartridge (ITSP Solutions Inc., Hartwell GA, USA). With the new PAL µSPE cartridge CTC Analytics introduced a novel septumless cartridge design dedicated to an extended application range and a reliable high throughput automation. The evaluation of the new cartridge design is presented with results for pesticides analysis from leading laboratories.

Introduction

Design of the new PAL µSPE cartridge

The novel PAL µSPE cartridge is septumless and consists of two parts only, as shown in **Fig. 1**. The polymer material used is chemically inert and free from leachables. It is compatible with MeCN, EtOAc, MeOH, DCM, hexane and aqueous pH of 1 to 12. The outer dimensions are 35 ± 2 mm in height and 8.5 mm outer diameter.

The outer shell provides a high capacity and flexible volume for sorbent materials separated by filter disks. There is a labelling option for the cartridge type for GLP purposes.

The bottom nozzle is designed to penetrate pre-slit septa of vials and also delivers directly to LC injection ports.

The inner body provides critical functionality with a predefined and reproducible compression of the sorbent/filter layers, and a precise needle guide for safe and always upright positioning. The move of cartridges on the PAL System can be achieved by syringe needle or pipette tip transport. Of key importance here is the resulting high pressure resistance and the leak free seal against a syringe needle of gauge 22 with flat tip for increased extract load speeds and volumes. Instead of sealing through a septum, it is sealed via a constant force in a needle seat (analogous to an LC injector). This patented technology allows flow rates of up to 500 µL/min with the CTC µSPE cartridges.

Analytical Strategy

The cartridge based µSPE QuEChERS extract clean-up is fully automated using a PAL RTC system to perform the complete workflow including the sample extraction. One sample tray on the PAL System carries sample rack, elution rack and the µSPE cartridge reservoir on the conditioning rack as shown in **Fig. 2**. The racks and cartridge reservoir can be of 54 or 96 format, allowing also MTP plate operation.

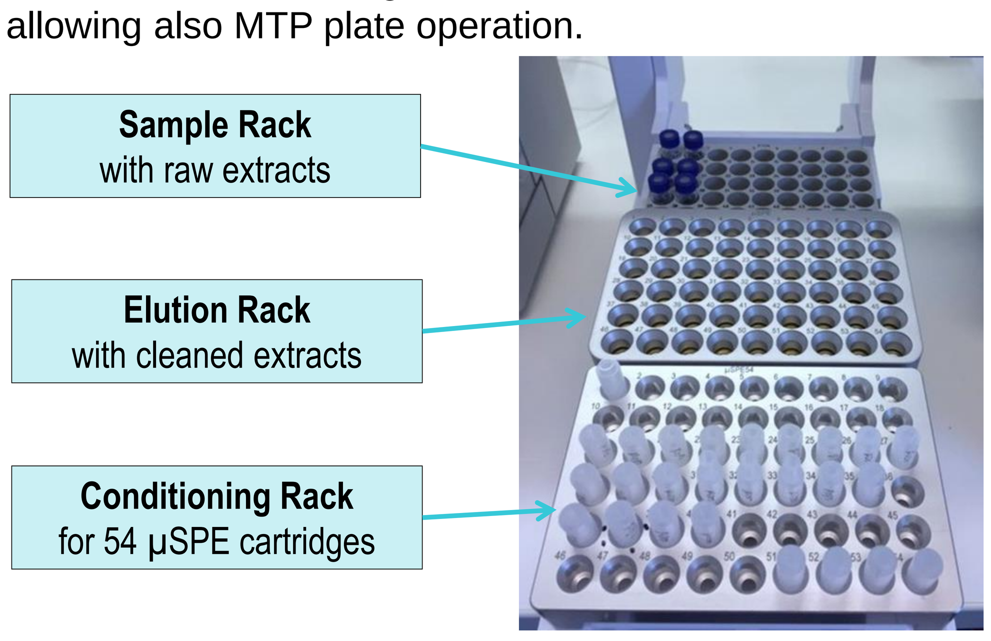

Figure 2. PAL System µSPE trayholder in 54 pos. format.

The µSPE extract clean-up workflow

The classical SPE knows two different operation principles, so is µSPE. Often, the analytes are enriched on the sorbent and the matrix washed off before elution. In the scavenging mode the matrix is retarded, and analytes elute.

Enrichment mode:

- µSPE cartridge conditioned in the Conditioning Rack
- Sample loaded in the Conditioning Rack
- Matrix washed off in the Conditioning Rack
- Cartridge transfer to the Elution Rack
- Analyte elution in the Elution Rack

Scavenging mode:

- µSPE cartridge conditioned in the Conditioning Rack
- Cartridge transfer to the Elution Rack
- Sample loaded in the Elution Rack
- Analyte elution in the Elution Rack
- Matrix stays in the sorbent, disposed

The clean-up of QuEChERS pesticides extracts uses the scavenging mode. Liquid syringes or pipette tools on a PAL RTC System are employed for the applied sample and solvent volumes of several 100 µL only (**Fig. 3**).

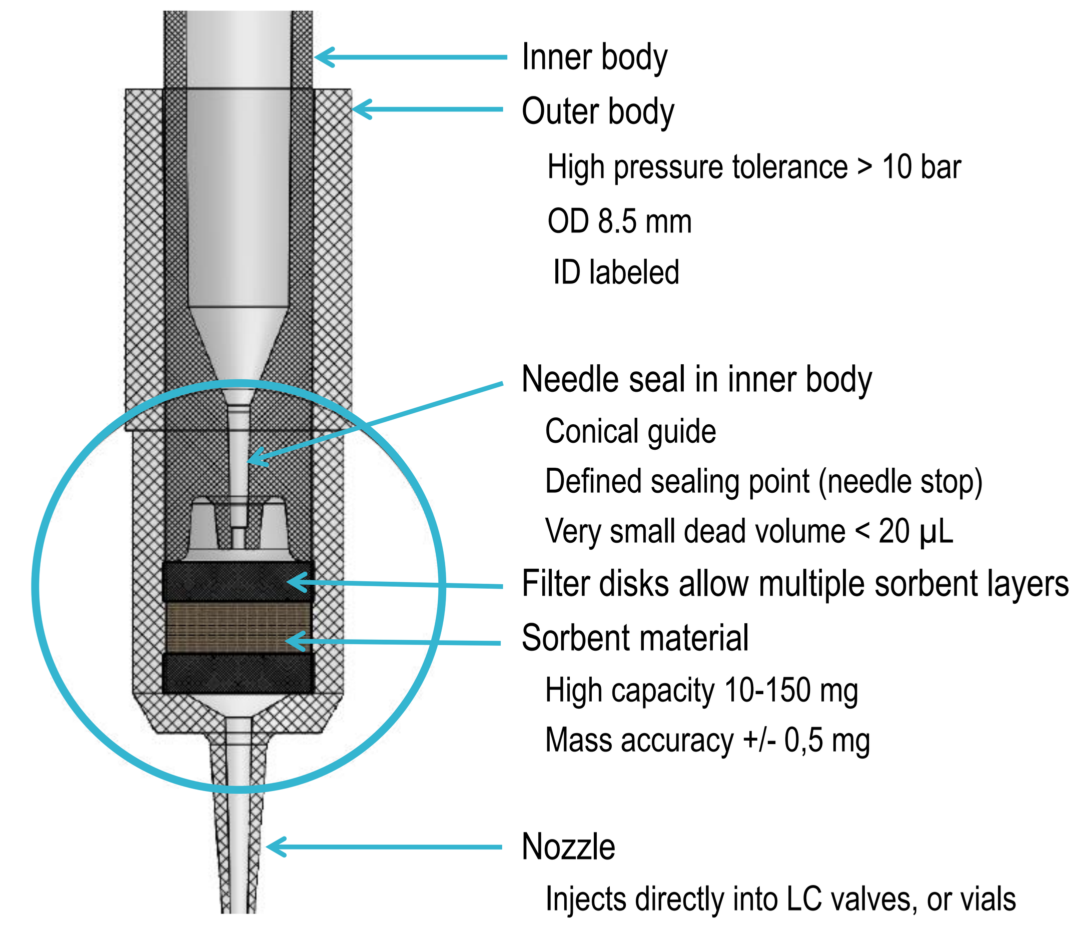

Figure 1. The novel PAL µSPE cartridge (cross section)

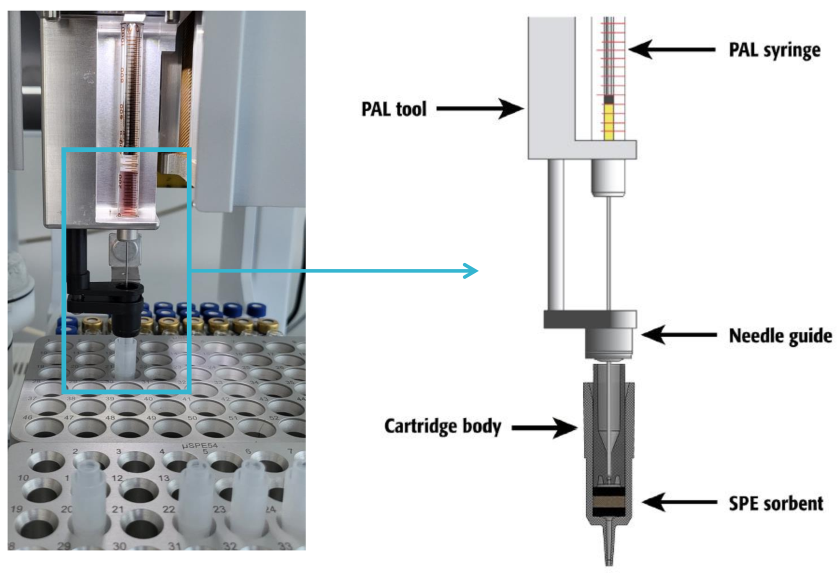

Figure 3. Principle of the PAL µSPE operation

Evaluation

The novel PAL µSPE cartridges were evaluated in routine laboratories for pesticides analysis. A typical sorbent mixture often used for clean-up of QuEChERS extracts consists of 20 mg anh. $MgSO_4$, 12 mg each of C18 and PSA, and 1 mg GCB (Carbograph-1).

Load volume and flow rate for GC-MS analysis

In the recent publication Nicolas Michlig and Steve Lehotay of the US DoA in Wyndmore, PA, USA [1] reported about the optimization and limits of the QuEChERS extract load volume and load speed with respect to the clean-up performance. Different food commodities including fatty and high chlorophyl containing foods were tested (hemp, spinach, avocado, milk, egg, lamb) using the novel PAL µSPE cartridges with the above-mentioned sorbent mixture for LPGC-MS analysis.

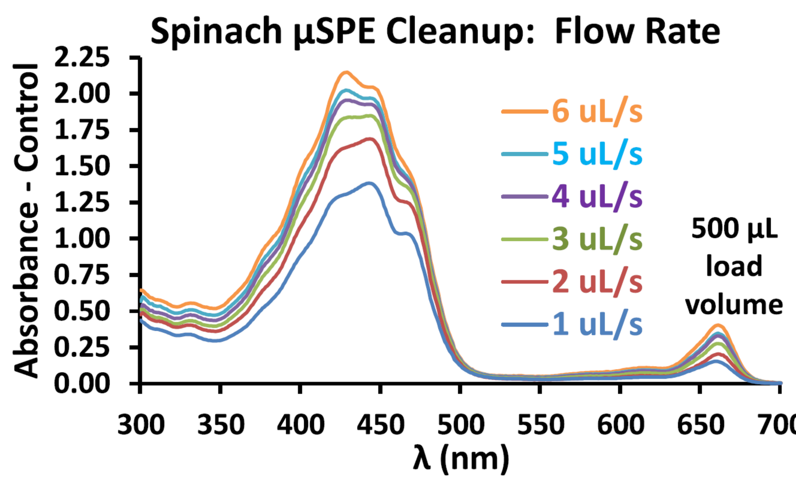

Figure 4. PAL µSPE clean-up performance at different flow rates monitoring chlorophyl removal [1].

In the chosen workflow setup, the initial cartridge conditioning was skipped and the QuEChERS extract loaded directly onto the cartridge. The optimization experiments led to the choice of 500 µL load volume and 5 µL/s flow rate in 1.7 min exploiting the high pressure resistance of the novel cartridge design (**Fig. 4**). The high flow rate resulted in shorter total clean-up times of 5.33 min per sample well inline with the fast low pressure GC separation (LPGC) allowing the parallel prep-ahead processing during an ongoing GC-MS run.

Recoveries were obtained in the range of 80-120% for 90-96% of 252 pesticides and environmental contaminants, depending on matrix, with typical RSDs of < 5%.

Flexibility in the use of sorbent materials

Commercially available sorbent materials were evaluated with the new PAL µSPE cartridges by Ederina Ninga et al. of the EURL for Cereals and Feeding Stuff in Lyngby, Denmark, for several difficult food matrices [2]. **Table 1** shows the achieved clean-up efficiencies in scavenging mode (% matrix removal) and the 70 to 120% recovery rates.

Sorbent	Amount	Rapeseed Cake			Fish Feed		
	[mg]	Clean-up	GC-MS	LC-MS	Clean-up	GC-MS	LC-MS
# of compounds			132	145		135	145
C18/Z-Sep/GCB	21/8/1	25%	65.2%	82.1%	80%	83.7%	71.2%
C18/PSA/GCB	12/12/1	54%	66.7%	74.5%	83%	86.7%	80.1%
C18	15	6%	25.8%	46.9%	39%	7.4%	73.3%
Chitin	15	2%	44.7%	55.9%	n.a.	59.3%	85.6%
Chitin	30	19%	31.1%	79.3%	n.a.	20.7%	85.6%
Z-Sep	20	50%	80.3%	89.7%	62%	67.6%	82.3%
EMR	15	11%	75.0%	82.8%	25%	91.1%	84.9%
EMR	30	32%	78.0%	76.6%	45%	80.0%	82.2%

Table 1. Clean-up efficiency with different sorbent materials used in customized PAL µSPE cartridges in scavenging mode with 250 µL $MeCN/H_2O$ 1:1 extract, 2 µL/s load speed [2].

The custom prepared cartridges with Z-Sep and EMR sorbents gave the best results with regard to recoveries. Chitin and C18 showed the poorest clean up. For insects' analysis the composition 12 mg C18/ 12 mg PSA/ 1 mg GCB showed the most effective clean up efficiency.

In addition to the µSPE clean-up the automated preparation of calibration dilutions and the addition of QC/IS standards before injection have been integrated into the automated workflow achieving excellent quantitative precision .

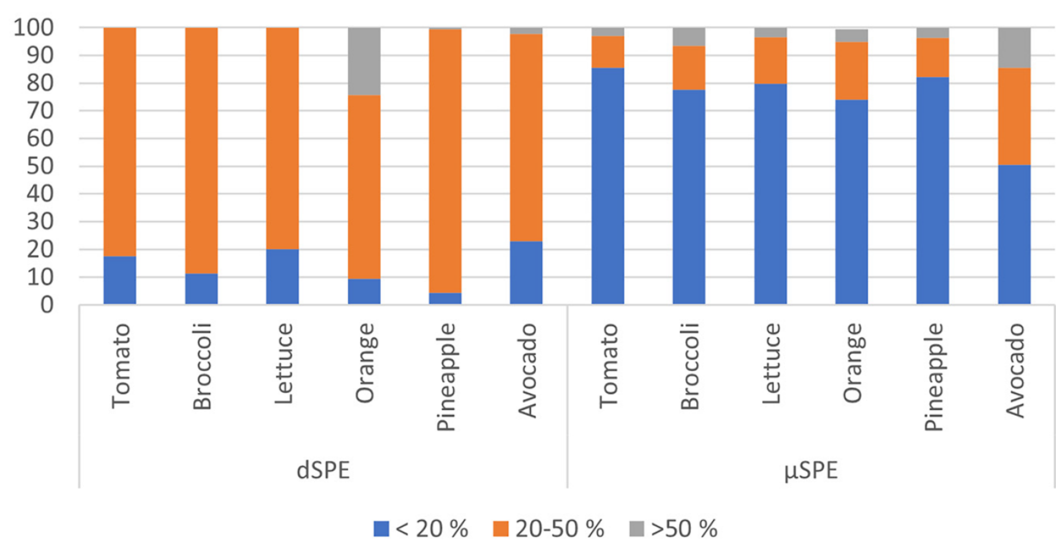

Figure 5. LC-MS matrix effects observed with dSPE and µSPE clean-up [3]

Matrix effect reduction for LC-MS analysis

The reduction of matrix effects in LC-MS using dSPE and µSPE and the clean-up performance for polar compounds in QuEChERS extracts from tomato, orange, rice, avocado and black tea matrices were compared by Lorena Manzano Sánchez and Florencia Jesús et al. at the EURL for Pesticide Residues in Fruit & Vegetable in Almeria, Spain [3].

The PAL µSPE cartridges comprised 45 mg $MgSO_4$/PSA/C18/ CarbonX (20/12/12/1, w/w). 100 µL of MeCN conditioned the cartridge before the QuEChERS extract load of 200 µL. Elution took place with 100 µL MeCN with 5% formic acid.

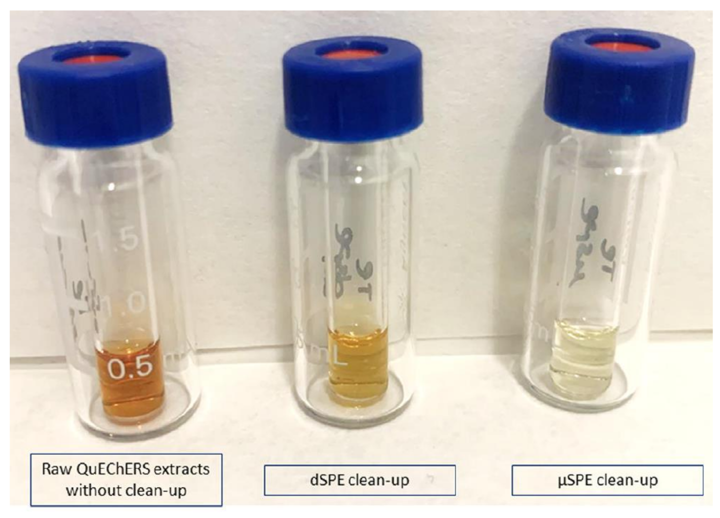

Figure 6. Visualization of the dSPE and µSPE clean-up for black tea [3].

The observed LC-MS matrix effect using automated µSPE was considered low with < 20% for more than 50 percent of the 243 studied compounds (**Fig. 5**). In contrast with dSPE >60% of the compounds showed a moderate matrix effect. The visual effect of the µSPE clean-up for black tea is demonstrated in **Fig. 6**.

Polar compounds are reported to be positively affected by the µSPE clean-up, such as the acidic compounds fluazifop, haloxyfop and quizalofop with recoveries of 70% to 120%, while manual dSPE obtained recoveries < 50%. Also, sulfonylureas such as flazasulfuron, orthosulfamuron, oxasulfuron gave better recoveries when using the PAL automated µSPE clean-up with certain commodities like rice.

Conclusion

The novel septumless PAL µSPE cartridge design showed high reliability in automated and fast high throughput workflows with excellent clean-up results and improved recoveries for GC-MS and LC-MS. The cartridge design successfully showed flexibility for different types and volumes of sorbent materials.

- The automated performance and clean-up efficiency for pesticide analysis is excellent.
- The use of the automated µSPE clean-up reduces manual laboratory work and allows increased sample throughput in routine analysis.
- A single sorbent material mix is equally employed for the clean-up all food commodities.
- PAL µSPE cartridges can be provided with customized sorbent materials.
- High quality calibration curves are generated as part of the workflow avoiding typical quantitation errors.
- Automated µSPE avoids the qualitative and quantitative errors that are oberved when dSPE is applied.

References

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