Automated micro-SPE Clean-up of QuEChERS Extracts for Multi-Residue Pesticide Analysis

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Introduction

The extraction and clean-up of pesticides from food still raises particular challenges due to the high number and difference in chemical nature of the compounds embedded in food matrices of high complexity and highly varying compositions. Due to their polarity, molecular weight, stability, or ionization characteristics compounds are detected by GC and LC separation typically using MS/MS or accurate mass instrumentation (Raina 2011; Scherbaum 2008)

In recent years the QuEChERS (Quick Easy Cheap Effective Rugged Safe) methodology (Anastassiades 2003) has been introduced in many food safety laboratories for pesticides. While the extraction step has been well standardized (AOAC 2007.1; CEN 15662) the clean-up of the resulting extracts still differs widely for different matrices and laboratories with the common goal to extend the GC, LC and MS maintenance cycles for increased productivity (Jiao 2016), and reducing matrix effects. The potential for high sample throughput using the QuEChERS extraction is impeded by laborious maintenance work (Hildmann 2014; Stahnke 2012).

Analytical limitations of the dispersive SPE (dSPE) step used in the QuEChERS methodology with high matrix load resulting in poor clean-up of lipid-rich foods in particular. In consequence several matrix dependent clean-up strategies are found in literature to overcome matrix effects (Anastassiades 2013; Chamkasem 2013; Jiao 2016). “As for clean-up stage by dSPE, type and amount of sorbent and MgSO4 and their selectivity were the main problematic issues” (Rejczak 2015). With the versatility of the QuEChERS extraction step an equally matrix independent and robust clean-up solution for extracts is highly desirable.

Methods

Unified clean-up using micro solid phase extraction

It has been shown that the application of a mini-column solid phase extraction (cSPE) instead of using dSPE maintains the high potential of the QuEChERS extraction for high sample throughput and recovery for a large number of pesticides in different even those high in lipids (Morris 2014, 2015). The miniaturization of the clean-up step to a microliter scale solid phase extraction (µSPE) reduces solvent use and waste, and offers a unique potential for full automation of the clean-up on an x,y,z robotic autosampler, as a standalone solution or integrated into GC-MS or LC-MS analysis, typically using selected ion monitoring (SIM), the triple quadrupole MS/MS multi reaction monitoring (MRM), or high resolution accurate mass (HRAM) detection.

Recent publications showed the important role of zirconia-based sorbent materials for the clean-up step of fat containing matrices which were not employed in the original QuEChERS method development (Anastassiades 2003). Using µSPE cartridges comprising zirconia-based sorbent material extends the clean-up of QuEChERS extracts to higher fat containing matrices (Lozano 2013; Morris 2015; Stenerson 2013).

µSPE Workflow

Using miniaturized SPE cartridges

The application of µSPE employs miniaturized SPE cartridges in approx. dimensions of 33 mm height x 8 mm OD, less than typical autosampler vials (pat. ITSP, Hartwell, GA, USA). These SPE cartridges are unique in that they behave with packed sorbents exactly like LC columns allowing separations at optimum van Deemter flow rates. Unlike classical SPE using a vacuum manifold the flow rates can be precisely controlled with the syringe plunger drive of the PAL RTC autosampler (see Table 1) (Hayward 2016).

Classical SPE	µSPE
Limited selectivity	High selectivity
Limited flow control	Precise flow control
Sample volumes > 10 mL	Sample volumes < 1 mL
Elution volume > 1 mL	Elution volume < 100 µL
Requires evaporation step	Ready to use, no sample dilution
Typically manual operation	Fully automated
Offline	Offline or online to LC or GC
QC difficult	Fully traceable

Table 1: Comparison of classical SPE and µSPE

The sorbent types to be used depend on the type of application using commercially available sorbent materials, even filter materials, in the volume of typically 10 to 50 mg.

The cartridge is sealed by a septum above the sorbent bed, allowing the syringe to push solvents or extracts through the sorbent bed. The syringe replaces the vacuum system of the classical SPE, working as an LC pump with defined flow rates of only low µL/s for sharp analyte/matrix separation (Fig.1).

Using the PAL RTC autosampler

The small dimensions allow the scale-down of the clean-up workflow to low microliter level using a x,y,z-robotic autosampler. In addition the cartridge septum allows the transport to any location on the autosampler. The new PAL RTC platform has been employed to develop a seamless µSPE-clean-up process for the extracts delivered by the QuEChERS extraction step with integrated injection to GC-MS and LC-MS analysis (Table 2).

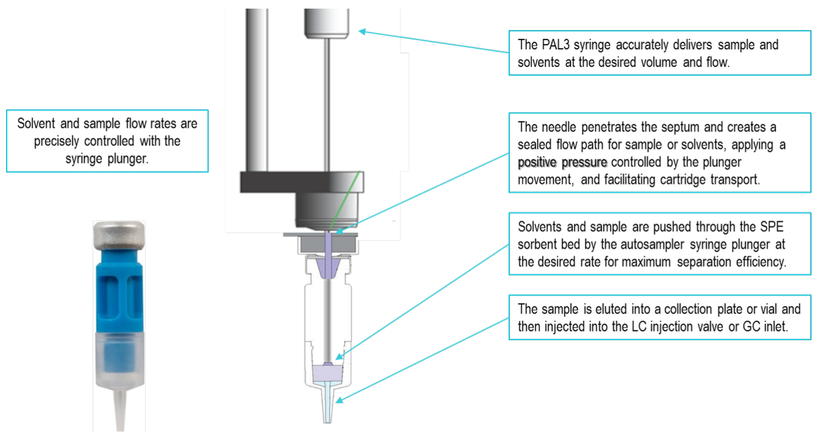


Figure 1: µSPE controlled elution from low particle size sorbent bed (Courtesy ITSP, Hartwell GA, USA)

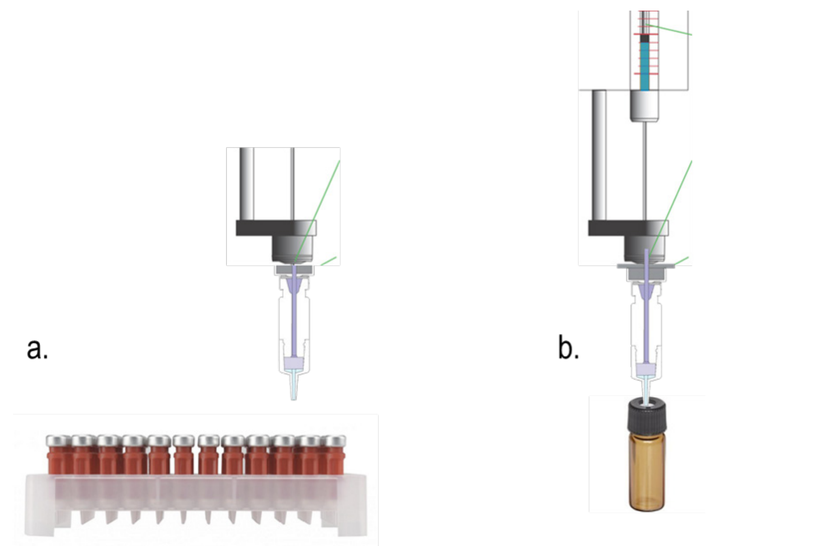


Figure 2: Cartridge needle transport (a) and sample load/elute (b)

1. Clean	syringe with elution solvent
2. Condition	µSPE cartridge with elution solvent
3. Move	µSPE cartridge to elution tray
4. Load	extract onto cartridge
5. Clean	syringe with elution solvent
6. Elute	extract from µSPE cartridge with elution solvent
7. Discard	used cartridge
8. Change	tool for injection syringe
9. Inject	into GC-MS or LC-MS
10. Clean	injection syringe

Table 2: Workflow for QuEChERS extract clean-up

Challenges

Challenges arising from an automated workflow

The starting point of the automated clean-up workflow is the centrifuged extract of the standardized QuEChERS extraction. As with manual dSPE applications a sequence of optimized working steps are necessary until final extract collection, covering the three major steps

- Cartridge conditioning
- Extract loading
- Pesticides elution

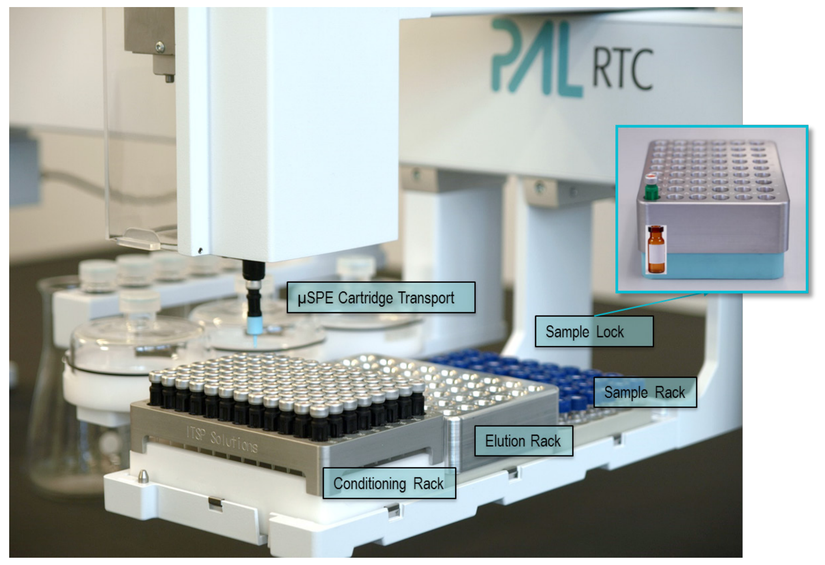
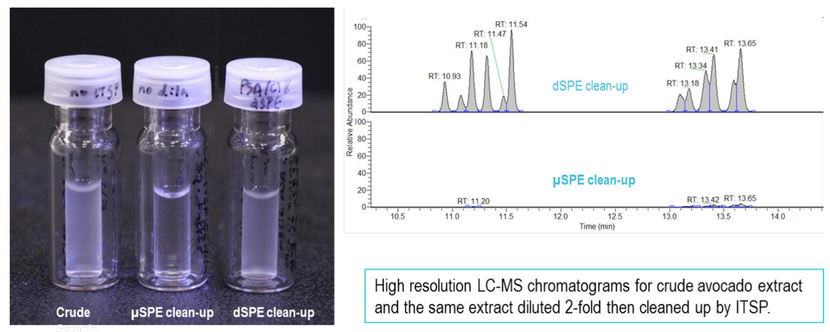


Figure 3: PAL RTC 120 (G7370A) µSPE clean-up workflow setup

Results

Procedure step	LCMS	GCMS
Clean syringe with elution solvent		
Condition µSPE cartridges in the conditioning rack	150 µL	200 µL
Transfer cartridge to the elution rack		
Load QuEChERS extract from the sample vial onto the cartridge	150 µL	100 µL
Clean syringe with elution solvent		
Elute the cartridge with elution solvent	150 µL	150 µL
Collect eluates in 2 mL vial, total volume	300 µL	250 µL
Discard cartridge to waste		
LCMS: Dilute combined extract and mix with syringe (opt.)	1200 µL	
GCMS: Add analyte protectant solution (opt.)		30 µL
Dilute combined extract with EtOAc and mix with syringe (opt.)		250 µL
Inject to GCMS or LCMS	10 µL	3 µL

Table 3: PAL RTC 120 automated clean-up workflow volumina (Morris/Schriner 2015)



High resolution LC-MS chromatograms for crude avocado extract and the same extract diluted 2-fold then cleaned up by ITSP.

Figure 4: Avocado µSPE clean-up (Courtesy Hill Labs, Hamilton, NZ)

The clean-up cycle is completed in less than 10 min. Average pesticides recoveries are in the range of 70 to 120 % (Morris 2015). In particular, when applied to extracts of high-lipid foods like Avocado, the advantage of a more robust separation from matrix is evident (Fig.4), leading to increased productivity with higher uptimes and less instrument maintenance of GC injectors and MS ion sources. The described workflow is in use for more than 1000 samples/week. More than 900 matrices for 270 pesticides have been analysed using standardized µSPE cartridges for GC-MS and LC-MS.

Conclusions

- Clean extracts from many matrices (apple, kiwi, carrot, kale, orange, black olive, wheat grain, dried basil, pork, salmon) lead to increased uptime of the LC-MS (1) or GC-MS systems (2).
- Micro-SPE yielded accurate results in rugged, high-throughput operations with minimal labor and data review. The described workflow is used for > 1000 samples/week, in 900 matrices for 270 pesticides

References

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