Direct injection of µ-SPE extracts into LCMS: automated clean-up meets high-throughput analysis

Thomas Preiswerk, Mario F. Mirabelli, Reto Bolliger;
CTC Analytics AG, Zwingen, Switzerland

ABSTRACT

Micro solid phase extraction (µ-SPE) is emerging in recent years as a convenient way to process raw sample extracts, e.g., from a QuEChERS workflow, to perform LC and/or GC mass spectrometric analysis on a clean sample. The advantages of µ-SPE over conventional SPE or dispersive SPE is the lower required sample volume, typically between 100 and 500 µL.
A novel µ-SPE cartridge was developed and used for different analytical workflows, fully automated on PAL System autosamplers. The cartridge was designed to operate at pressure up to 20 bar, to minimize dead volume (<30uL), and to being able to accommodate a broad range of sorbent mass (typically between 5 and 150mg). The cartridge can be used to process raw samples in different types of workflows such as filtration, QuEChERS cleanup and analyte enrichment. This work focused on the direct injection of extracts into LC valves.

Sample preparation and instrumental analysis

PAL System µSPE cartridge: features

- Small dead volume (< 20µL): small sample loss
- 20 bar pressure tolerance: small particle sorbents can be used
- Wide range of sorbent mass: 5-150mg
- Chemically stable (PP/HDPE) and robust
- Multiple injections on one cartridge, safe transport
- Fully automated production, labelling and QC

All measurements were performed on a Shimadzu LC-2030 Plus LC-UV instrument. The column used was a HALO C18, 2.7 µM, 2.1 x 50 mm, run in isocratic conditions (50% acetonitrile + 0.1% FA and 50% water + 5% CH3CN + 0.1 % FA) at 0.50 mL/min. The UV detector was operated at 30°C and 254 nm. LC runtinme was 2 min.

All injections were performed using a PAL System dual head automsampler, operated as a single head instrument. A 250 µL LCMS tool was used to inject raw samples into µSPE cartridges.

The µSPE cartridges used were packed with 15mg of C18 sorbent (end-capped), with average particle size of 30 µm. The sorbent was packed in between two porous polyethylene frits.

The steps of the direct µSPE injection workflow used are reported in the flow chart on the right.

1. **Cartridge condtioning with solvent 1 and 2 (optional)**
   •Pre-condition the cartridge with solvent 1 and 2 on the cartridge tray
2. **Cartridge condtioning with sample (optional)**
   •Pre-condition the cartridge with sample on the cartridge tray
3. **Transport cartridge**
   •Transport cartridge to injector and penetrate injector with constante force
4. **Inject front volume into waste**
   • Inject sample front volume into injection valve waste position
5. **Inject sample volume**
   •Inject sample volume into injection valve loop
6. **Move injector valve and send signal**
   •Move injection valve to Standby/Inject position and send injected signal
7. **Inject rear volume**
   • Dispense sample rear volume into injection valve waste position
8. **Transport cartridge**
   • Transport cartridge back to initial position on cartridge tray
9. **Clean injector**
   •Clean the LC injector with wash solvent 1 / 2
10. **Clean syringe**
    • Clean the syringe with wash solvent 1 / 2

Reduced labor time with the automated direct µSPE injection

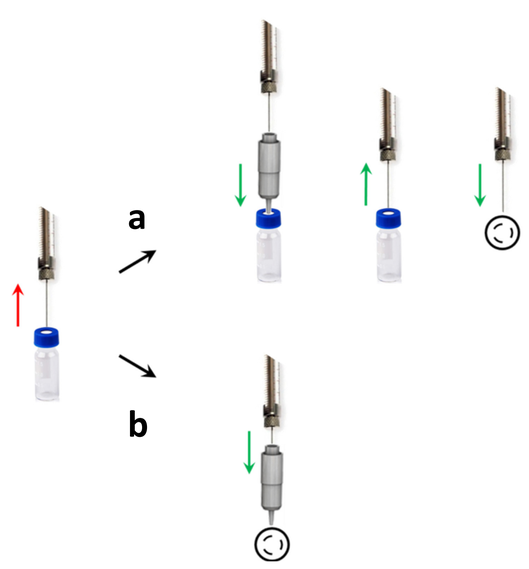

Fig.1: comparison between conventional (a) and direct (b) injection µSPE workflows.

Contrary to a conventional µSPE workflow (Fig. 1a), where a sample filtrate is first collected into an elution vial, and subsequently aspirated to be injected into LC, a direct µSPE-LC injection (Fig. 1b) allowed to save a significant amount of consumables per sample, and to maximize analysis throughput. A straightforward and fast µ-SPE workflow was realized with by directly injecting a raw sample through the µSPE cartridge into an LC injection valve.

Caffeine, acetonaphthone and quinizarin (4mg/mL, 4mg/mL and 20mg/mL in acetonitrile, respectively) were used as model compounds for LC-UV method optimization. Results show that a QuEChERS cleanup workflow can be performed in as little as 2min per sample, as opposed to >5 minutes required for traditional µSPE.

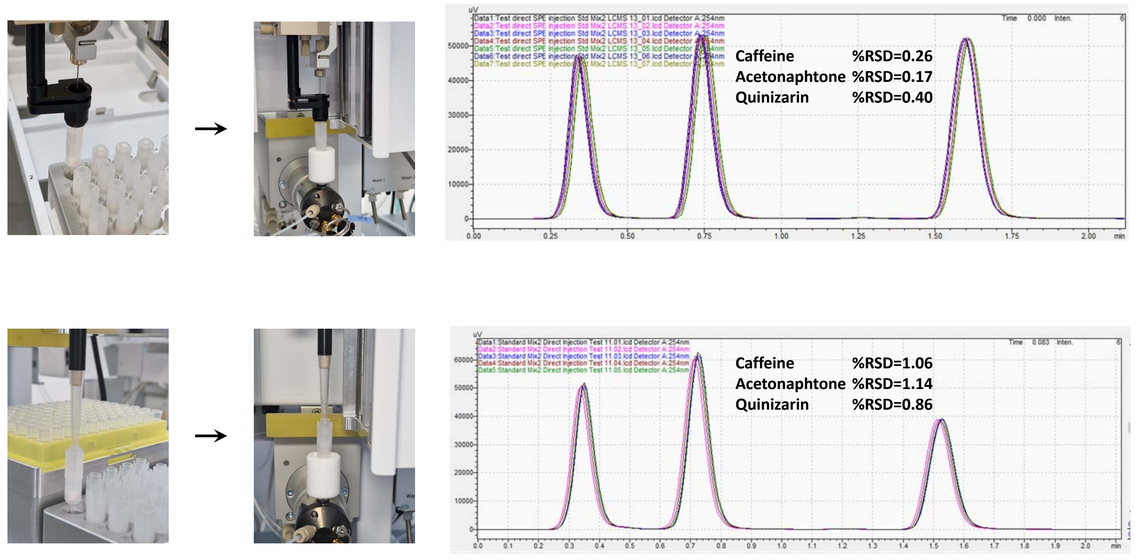

Fig.2: direct injection of µSPE into an LC valve with syringe (top) and pipette (bottom) pick-up/injection. An active wash cup is used to minimize carryover.

Results

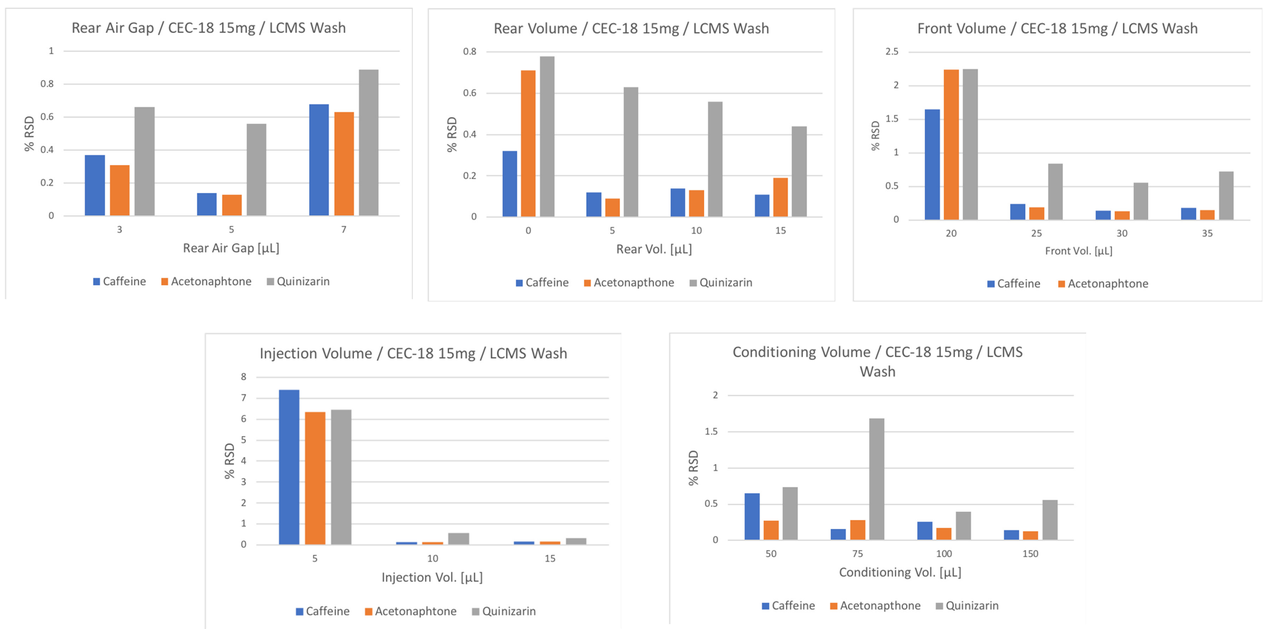

Fig.3: optimization of most relevant µSPE and LC sample preparation parameters: rear air gap, rear volume, front volume, injection volume and conditioning volume.

The optimized parameters for the direct µSPE syringe injections were found to be as follows:

Rear air gap:	5µL
Rear Volume:	5µL
Front volume:	30µL
Conditioning Volume:	150µL
Injection Volume :	10µL

The precision of the direct injections were <1% RSD, and carryover was minimized by equipping the injection valve with an active wash cup (Fig.2) and by performing a reverse rinsing (a dedicated pump module is connected to the injection valve for its rinsing after injection). Direct µSPE pipette injections also allowed to reach <1.2% RSD.
Cycle times of the direct injection approaches based on the optimized parameters (Fig.3) are summarized in the table below:

	RSD%	Cycle time
Conventional uSPE workflow (syringe)	<1	5 min
Direct uSPE injection (Syringe)	1-2%	2 min
Direct uSPE injection (Pipette)	2%	2 min

CONCLUSION

The µSPE direct injection workflow has been optimized and used for the high-throughput cleanup of liquid samples. Excellent RSD% values were obtained, comparable with the conventional µSPE workflows, but with a much-simplified approach requiring less time and less consumables (i.e., no sample collection vials required). Several µ-SPE cartridges are currently being tested by LCMS for the cleanup of complex sample matrices containing various pesticides and drugs, in order to further validate the performance of the direct µ-SPE-LCMS workflow in different use cases.