

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 μ L.

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 (<30 μ L), 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 μ SPE cartridge: features

- Small dead volume (< 20 μ L): 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

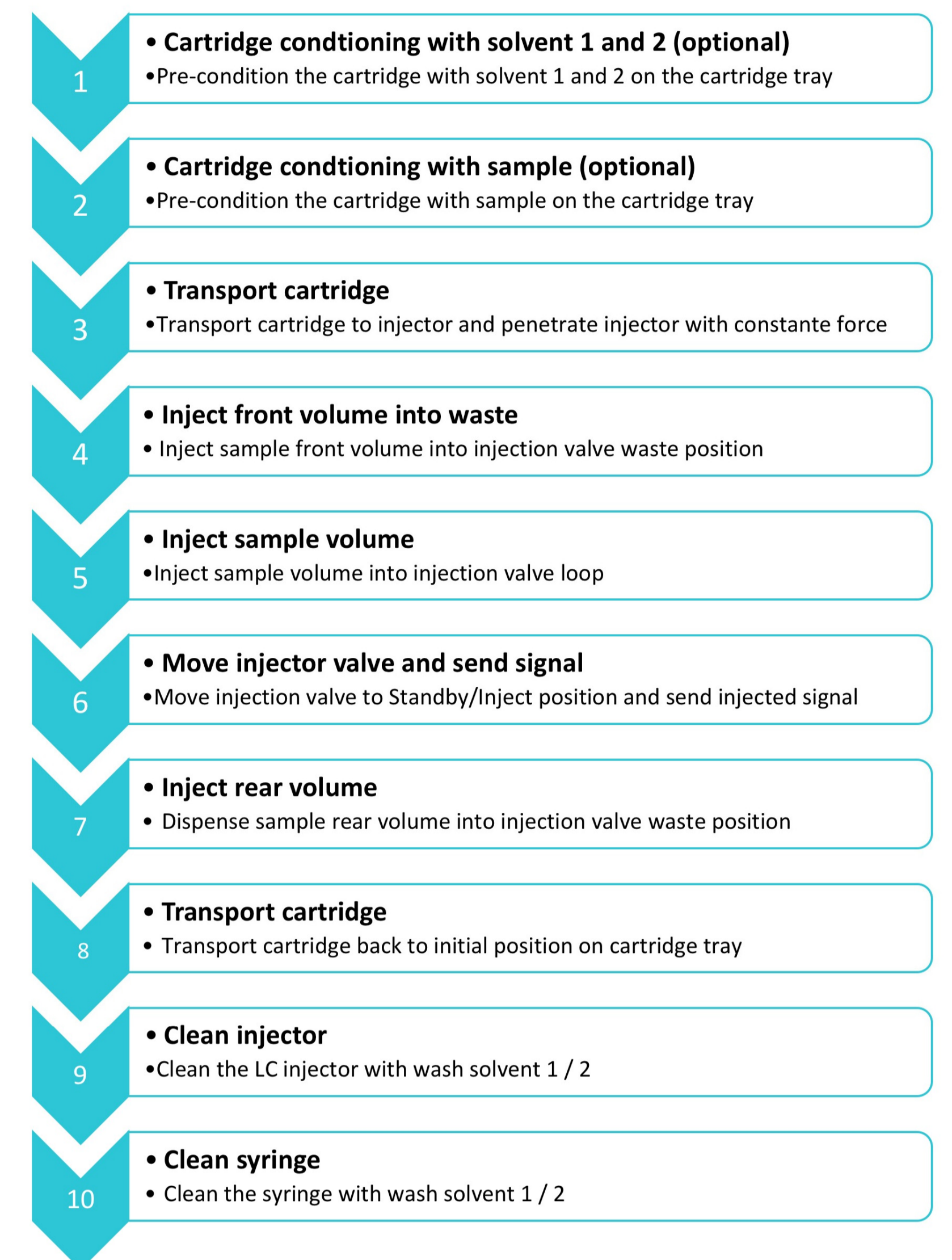


All measurements were performed on a Shimadzu LC-2030 Plus LC-UV instrument. The column used was a HALO C18, 2.7 μ M, 2.1 x 50 mm, run in isocratic conditions (50% acetonitrile + 0.1% FA and 50% water + 5% CH₃CN + 0.1 % FA) at 0.50 mL/min. The UV detector was operated at 30°C and 254 nm. LC runtime was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

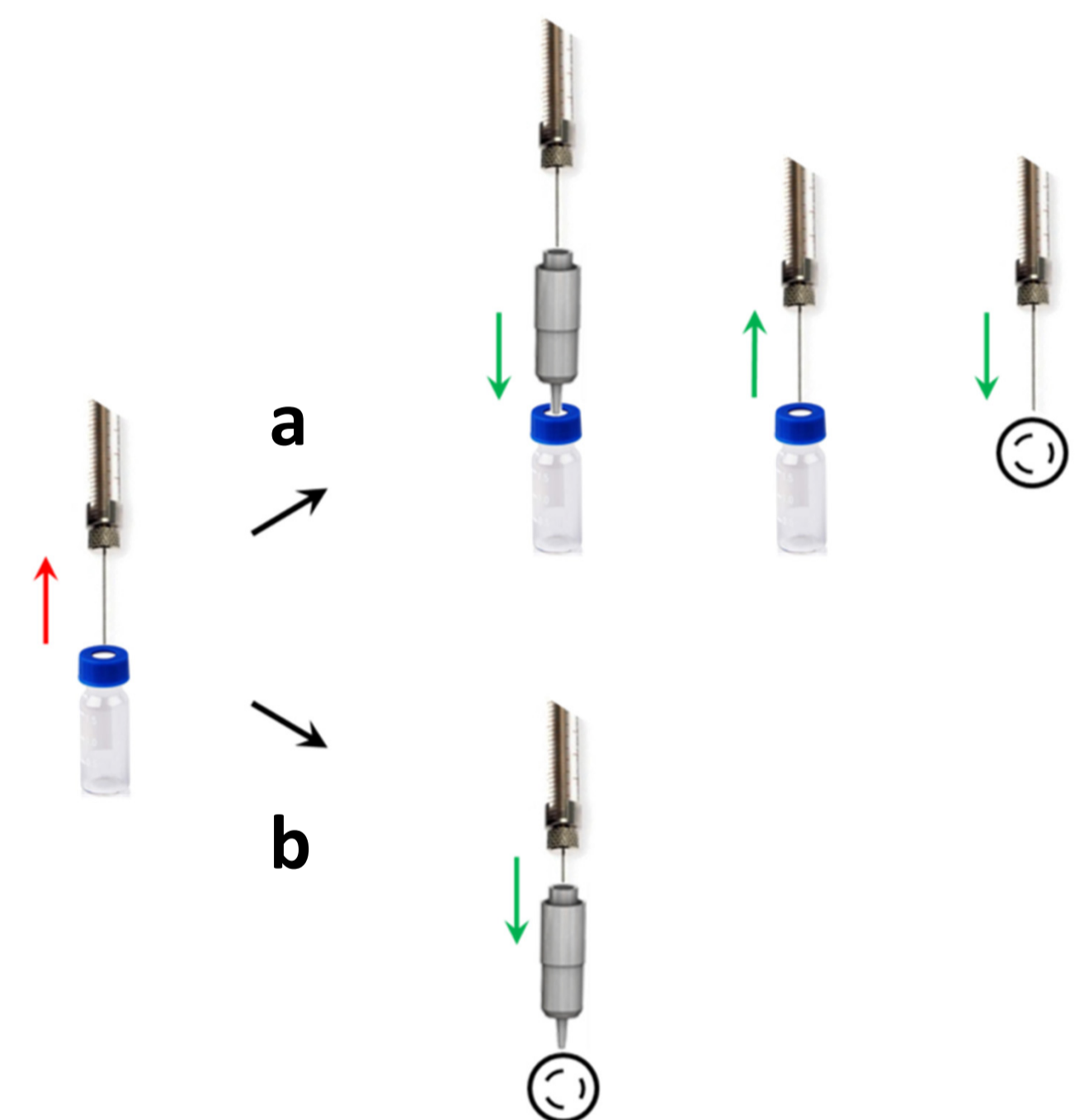
All injections were performed using a PAL System dual head autosampler, operated as a single head instrument. A 250 μ L LCMS tool was used to inject raw samples into μ SPE cartridges.

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

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



Reduced labor time with the automated direct μ SPE injection



Contrary to a conventional μ SPE workflow (Fig. 1a), where a sample filtrate is first collected into an elution vial, and subsequently aspirated to be injected into LC, a direct μ SPE-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 μ SPE cartridge into an LC injection valve.

Caffeine, acetonephthone 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 μ SPE.

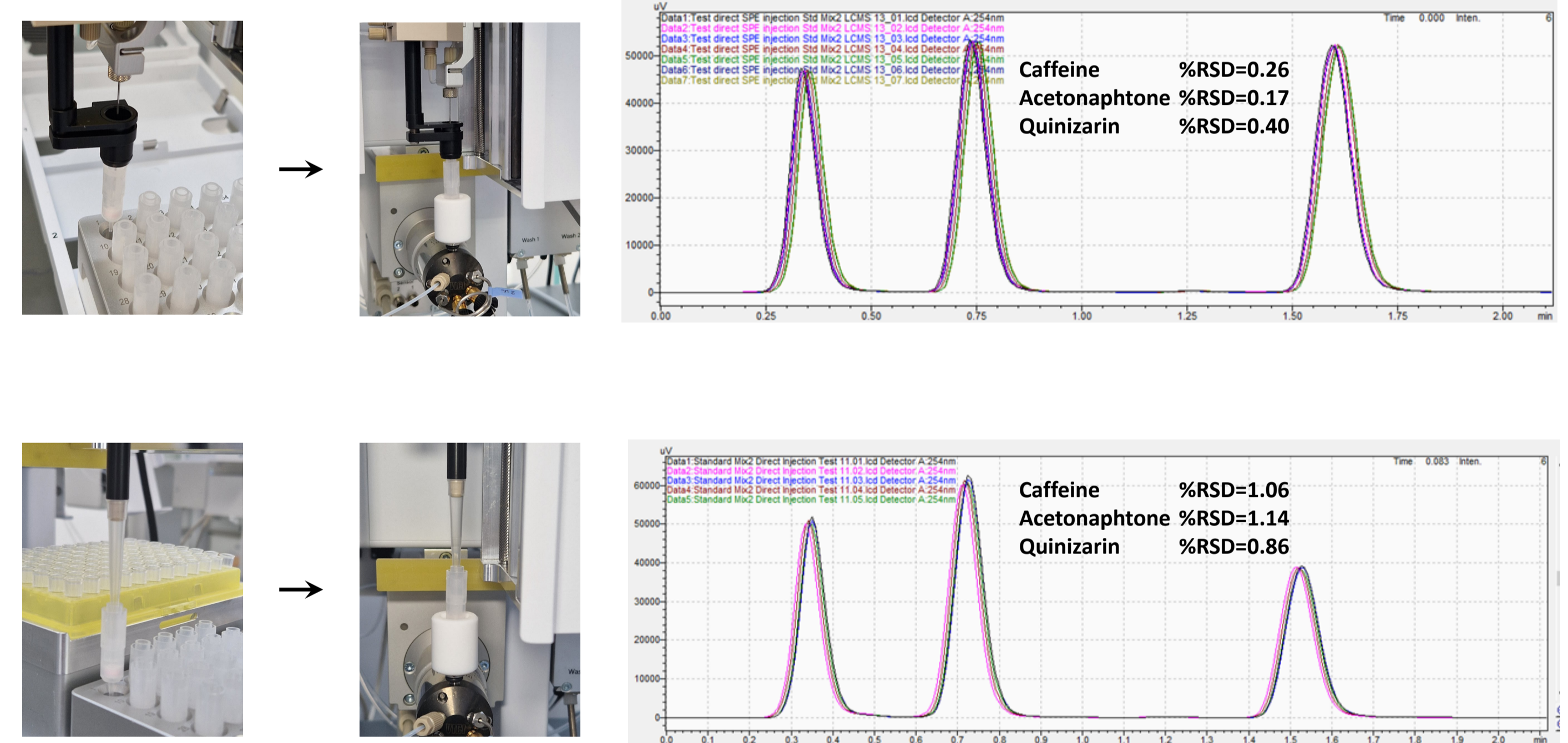


Fig.1: comparison between conventional (a) and direct (b) injection μ SPE workflows.

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

Results

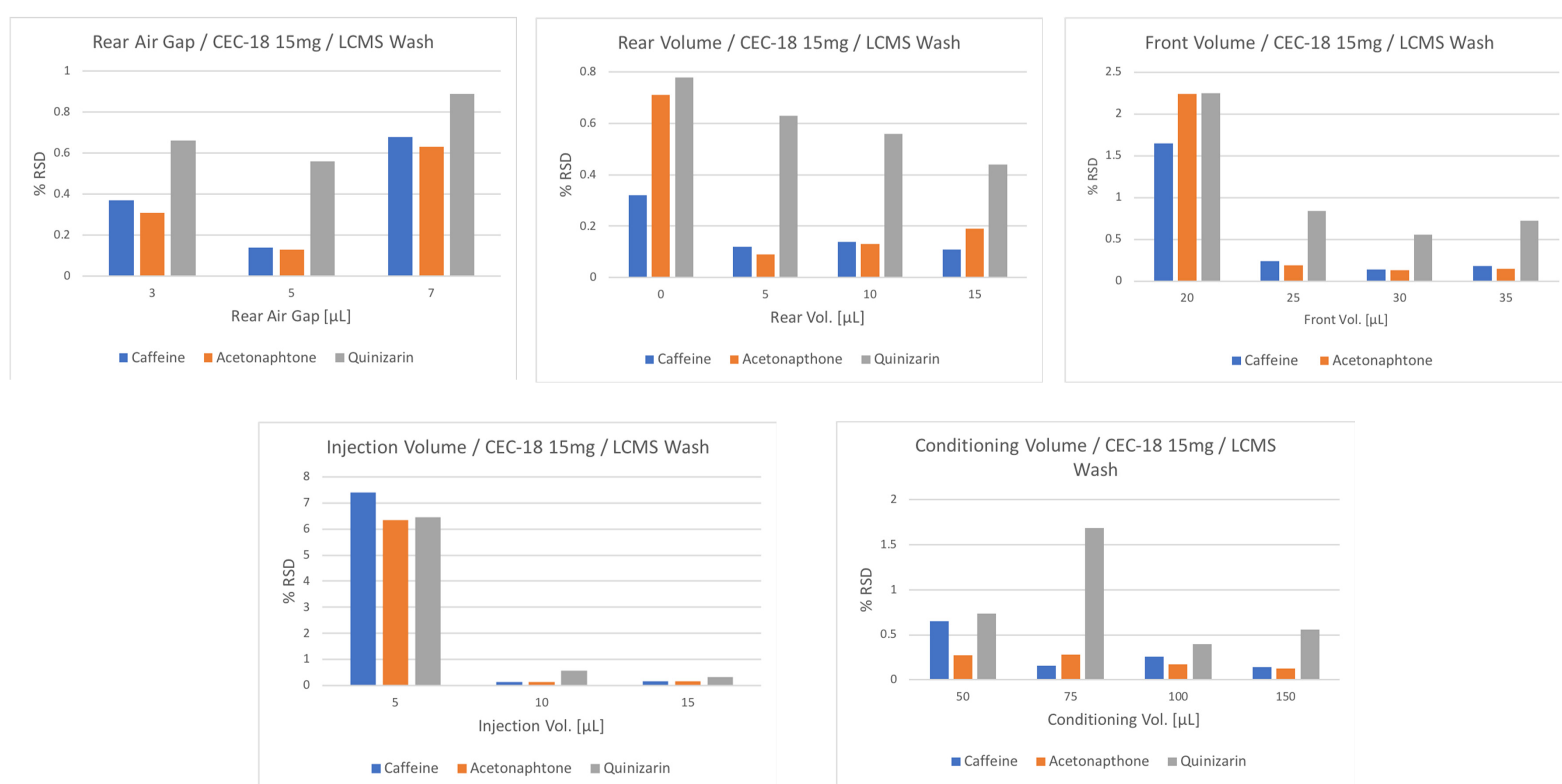


Fig.3: optimization of most relevant μ SPE and LC sample preparation parameters: rear air gap, rear volume, front volume, injection volume and conditioning volume.

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

- Rear air gap: 5 μ L
- Rear Volume: 5 μ L
- Front volume: 30 μ L
- Conditioning Volume: 150 μ L
- Injection Volume: 10 μ L

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 μ SPE 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 μ SPE workflow (syringe)	<1	5 min
Direct μ SPE injection (Syringe)	1-2%	2 min
Direct μ SPE injection (Pipette)	2%	2 min

CONCLUSION

The μ SPE 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 μ SPE 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.