

Automated Clean-up and Analysis of Pesticides in Spices by Mini-SPE

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PAL SYSTEM
Ingenious sample handling

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Overview

Objective: Develop an automated clean-up method with high recovery and sufficient clean-up for GC-MS.

Methods: The automated sample preparation workflow presented involves an x,y,z-robotic autosampler. Mini-SPE cartridges comprising the clean-up sorbent for all spice matrices are used. Spice samples were extracted by using QuEChERS. The raw extract is placed into the autosampler vial for automated mini-SPE clean-up in parallel to online GC-MS/MS analyses. For an efficient clean-up, three different sorbent compositions were compared along with various automated workflows.

Results: Commonly used pesticides found in spices were used for method development. For the relatively simple matrixes (e.g., coriander, cumin, and cardamom) the LOQ achieved was 10 ng/g. In the more complex matrixes of e.g., turmeric, chili powder, and black pepper an LOQ of 10 ng/g for around 77% of the compounds was achieved. The remainder of the compounds had satisfactory recoveries at 20 ng/g and higher levels.

Highlights: The study describes the effectiveness of the automated mini-SPE clean-up in multiresidue analysis of pesticides in a range of spice matrixes. The clean-up cartridge uses one standardized sorbent mix for all spices. The mini-SPE clean-up can be automated and executed in few minutes only in parallel to an ongoing GC-MS/MS analysis for improved productivity. The method facilitates high-throughput residue analysis in compliance with the regulatory requirements of sensitivity and method performance.

Introduction

Pesticide residues are routinely tested in spices for trade compliance. The high sample load for food testing laboratories demands for automation in sample preparation. The complex matrix of spices makes clean-up of QuEChERS extracts challenging and time consuming. No study on efficient and automated clean-up is available to date on spices. Spices of economic importance, e.g., black pepper, cardamom, chili powder, coriander, cumin, turmeric, and control samples for each of the spices, were selected for this study.

Methods

QuEChERS Sample Preparation

2 g ground powder of each spice was soaked in 10 mL water for 30 min in a 50 mL centrifuge tube. 10 mL acetonitrile was added and vortexed for 1 min. 4 g activated MgSO₄ and 1 g NaCl were added and vortexed for 2 min, then centrifuged at 2800g for 5 min [1]. The supernatant raw extract was transferred to 2 mL vials for the automated mini-SPE clean-up.

GC-MS Analysis

A Thermo Scientific TRACE™ 1300 GC coupled with a TSQ™ 8000 Evo triple quadrupole MS and TriPlus™ RSH liquid auto sampler were employed. The analysis was performed by 2 µL injection of the cleaned extract in splitless mode into a single tapered liner with glass wool. A TraceGOLD™ TG-5 MS column (30m, 0.25mm, 0.25µm) was used for separation with the oven temperature program 70°C (1 min), 25°C/min to 150°C, 5°C/min to 200°C, and 10°C/min to 285°C (5 min hold). The total run time was 27.7 min. The MS ion source was set to 250°C, the transfer line to 285°C.

The compound detection was performed in the timed-SRM mode with transitions directly imported from the TraceFinder compound database. Three matrix-selective SRM transitions were selected for each analyte.

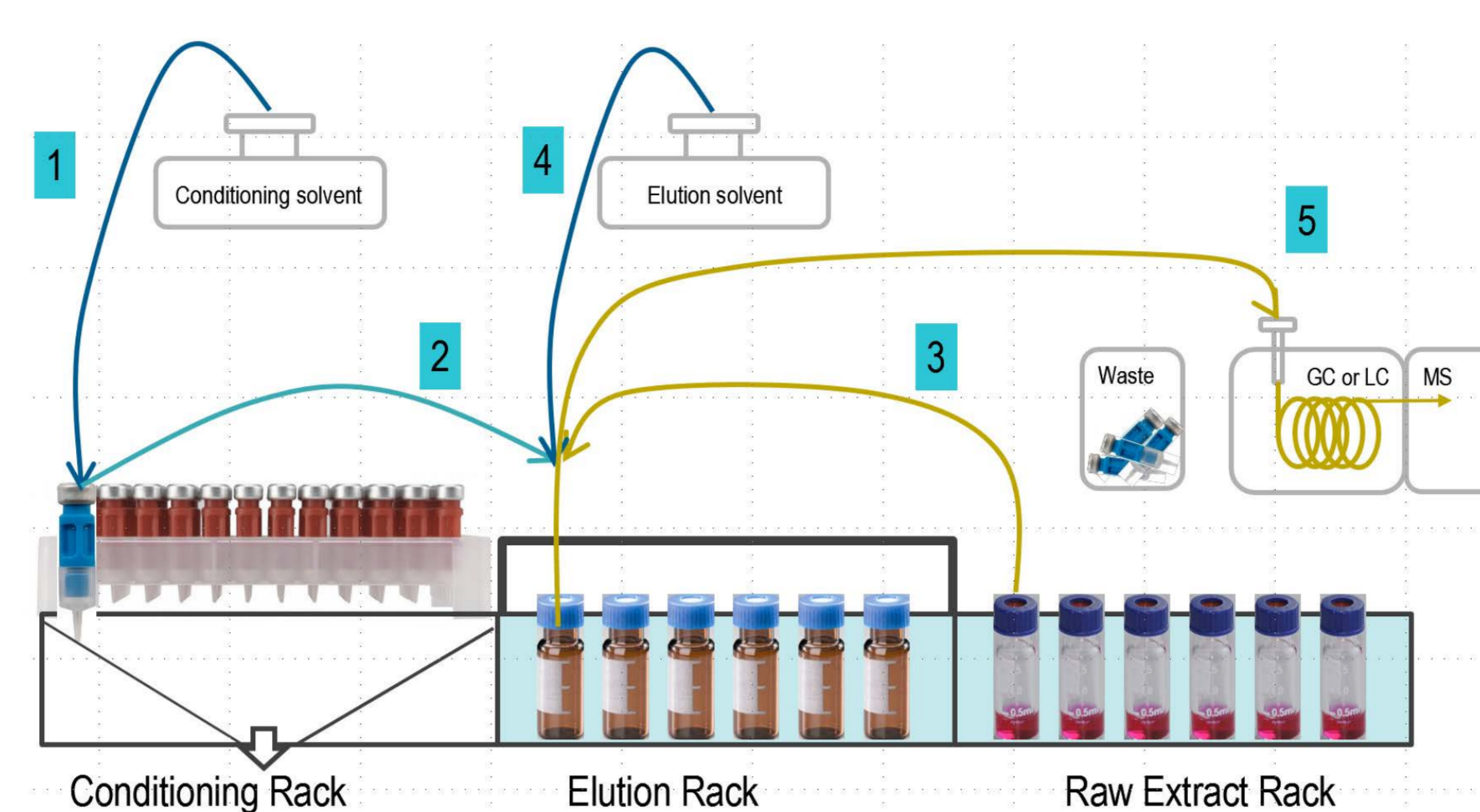
A linear calibration curve was established in a range of 0.5–50 ng/mL in coriander matrix.

Mini-SPE Clean-up

A CTC Analytics PAL RTC System equipped for mini-SPE standalone operation was used for the automated raw extract clean-up. The performance of the automated mini-SPE clean-up was optimized using different sorbent material mixes.

Figure 1 shows the clean-up stepwise and the required trayholder configuration. The raw extracts in 2 mL vials are placed into the raw extract rack. A conditioning rack provides the cartridges with the SPE sorbent material mix for clean-up. The elution rack between them is used to collect the cleaned extract in 2 mL empty vials with split septa. From here the cleaned extract is taken for online or offline analysis.

Figure 1: The five automated mini-SPE clean-up steps



- 1 Cartridge conditioning
- 2 Move cartridge to the elution rack
- 3 Load the raw QuEChERS extract onto the cartridge
- 4 Elute and collect pesticides
- 5 Inject the cleaned extract into GC-MS or LC-MS

Table 1: Published Mini-SPE Workflows

	Morris/Shriner (2015)	Lehotay/Han/Sapozhnikova (2016)
Applied to	LC-MS/GC-MS	GC-MS ²⁾
Syringe wash	Elution solvent ²⁾	2x 0.5 mL MeCN
Conditioning ³⁾	150/200 µL	—
Aspirate extract	150/200 µL	300 µL
Load extract	150/200 µL	300 µL
Flow	2 µL/s	2 µL/s
Syringe wash	Elution solvent ²⁾	2x 0.5 mL MeCN
Elute	150/150 µL	—
Collected volume ³⁾	300/250 µL	220 µL
Discard cartridge	yes	yes
Syringe wash	Elution solvent ²⁾	2x 0.5 mL MeCN/MeOH/water
Syringe wash	—	4x 0.5 mL MeCN
Dilution	1200 µL/—	add 25 µL MeCN
Analyte protect.	—	add 25 µL AP mix ⁴⁾
Syringe wash	Elution solvent ²⁾	2x 0.5 mL MeCN/MeOH/water
Syringe wash	—	4x 0.5 mL MeCN
Injection	10.0/1.0 µL	1.0 µL

¹⁾ LC-MS analyses are done without extract clean-up

²⁾ 100 mM formate buffer in MeCN/MeOH (1:1)

³⁾ Cartridge dead volume ca. 80 µL

⁴⁾ Analyte protectants (APs) 25 mg/mL ethylglycerol, 2.5 mg/mL gulonic lactone, d-sorbitol, and 1.25 mg/mL shikimic acid in MeCN/water (3/2, v/v)

Clean-up Workflow

The automated clean-up workflow was published in 2015 by Morris and Schriner [2], applied for LC-MS analysis [3], and further optimized in 2016 by Lehotay for GC-MS applications [4] by skipping the cartridge conditioning and elution step but using a higher extract load, as shown in Table 1. For the spices investigated the Morris/Shriner workflow was chosen based on the achieved recoveries of 70 to 120%, as shown in Figure 2.

Three different sorbent material mixes were evaluated for the spice extract clean-up:

- (1) 30 mg C18/Z-Sep/CarbonX (20.7/8.3/1)
- (2) 30 mg PSA/C18/GCB (5.6/22.2/2.2)
- (3) 45 mg MgSO₄/PSA/C18/CarbonX (20/12/12/1)

The sorbent material mix (3) showed the best recovery for pesticides from spice extracts, shown in Figure 3.

Results

A total number of 69 commonly used pesticides found in spices were used for method development. For the relatively simple matrixes of e.g., coriander, cumin, and cardamom the LOQ for the target pesticides was 10 ng/g. The method provided an LOQ of 10 ng/g for around 77% of the compounds in the relatively complex matrixes of e.g., turmeric, chili powder, and black pepper. The remainder of the compounds had satisfactory recoveries at 20 ng/g and higher levels.

Figure 2. Pesticide recoveries with and without cartridge conditioning and elution

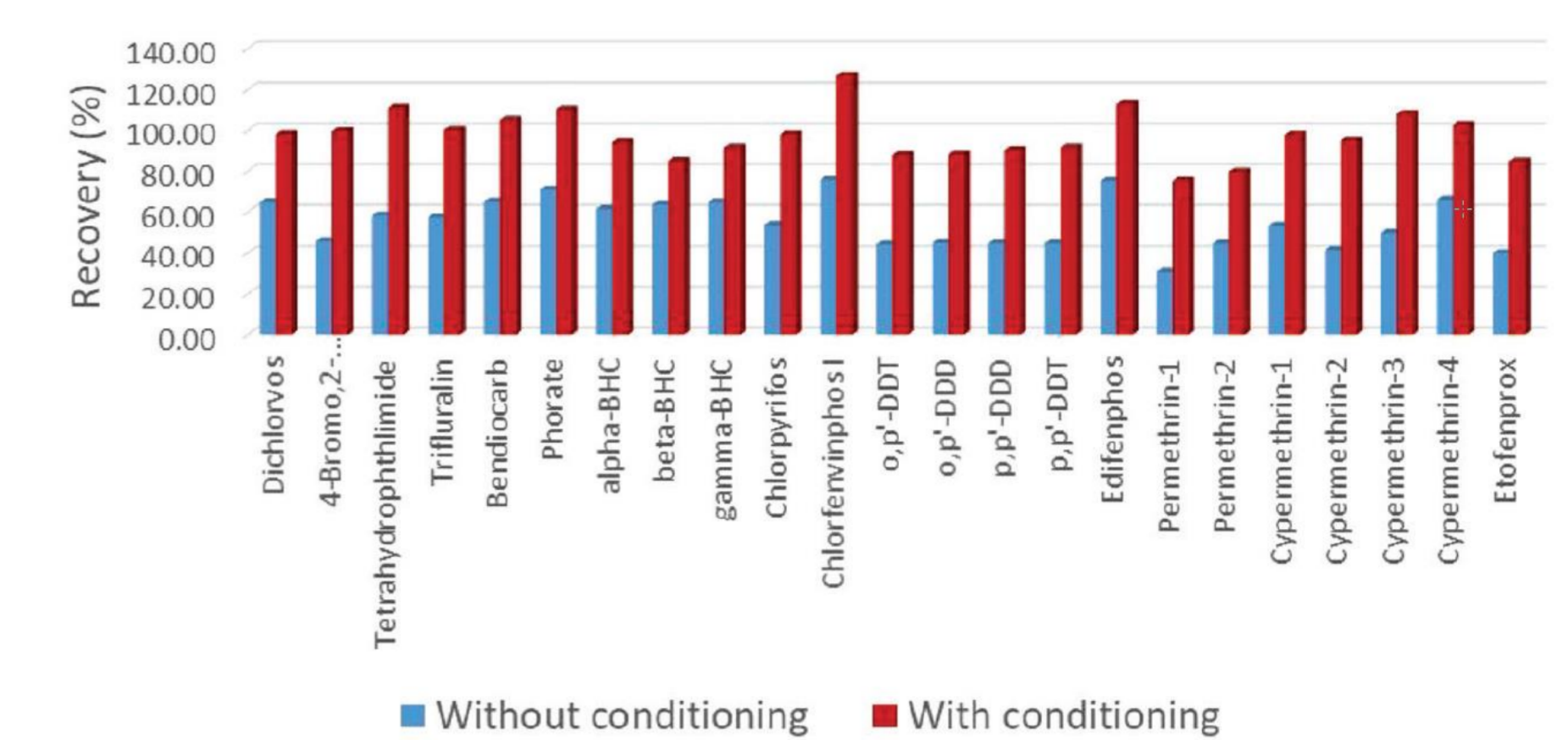
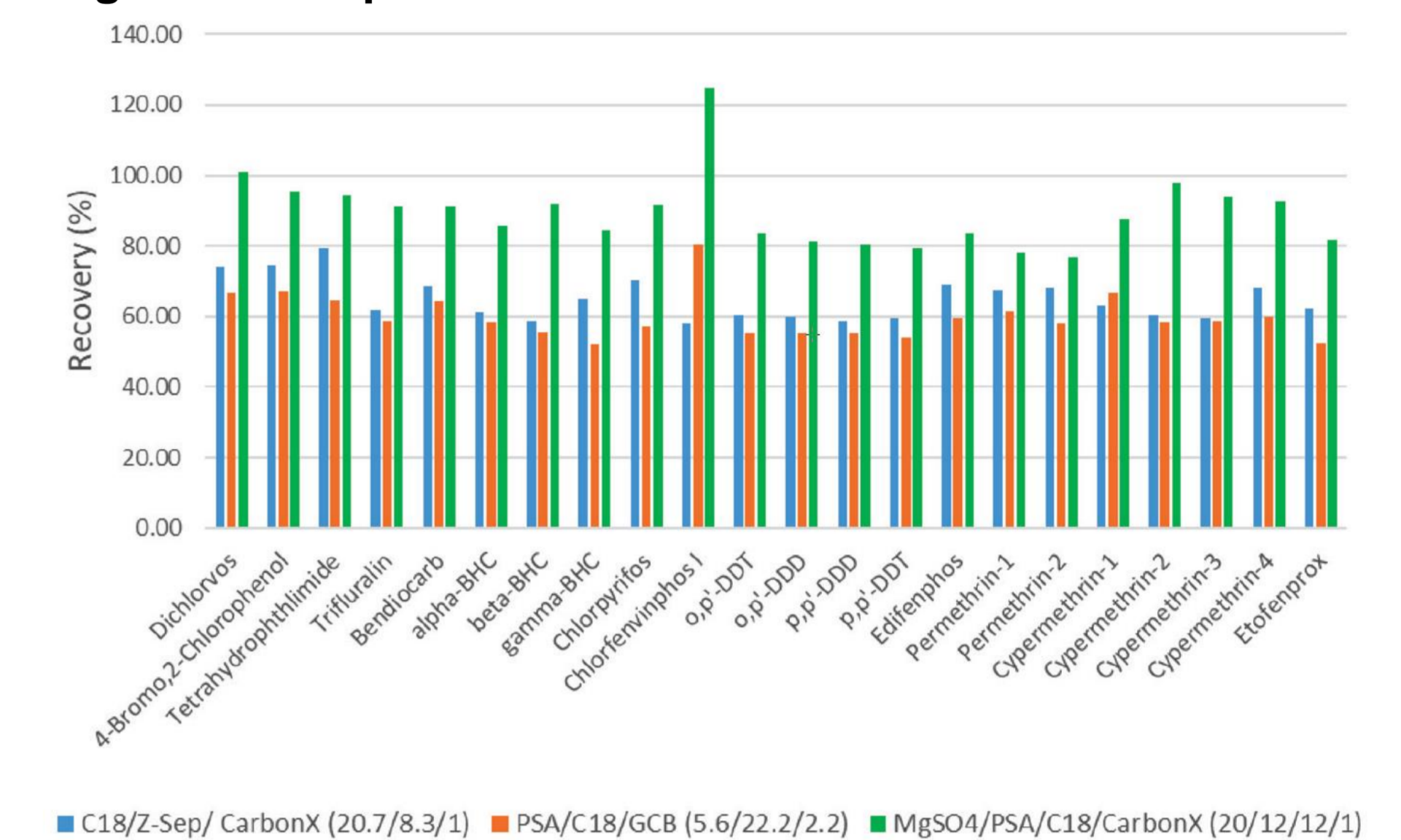


Figure 3. Comparison of SPE sorbent material mixes



Comparison to dSPE Clean-up

For all compounds investigated the mini-SPE clean-up provided acceptable recoveries from spice extracts, also for the late-eluting compounds like cypermethrin, deltamethrin, etofenprox, fenvalerate (Figure 4).

Figure 4. Comparison of mini-SPE and dSPE clean-up

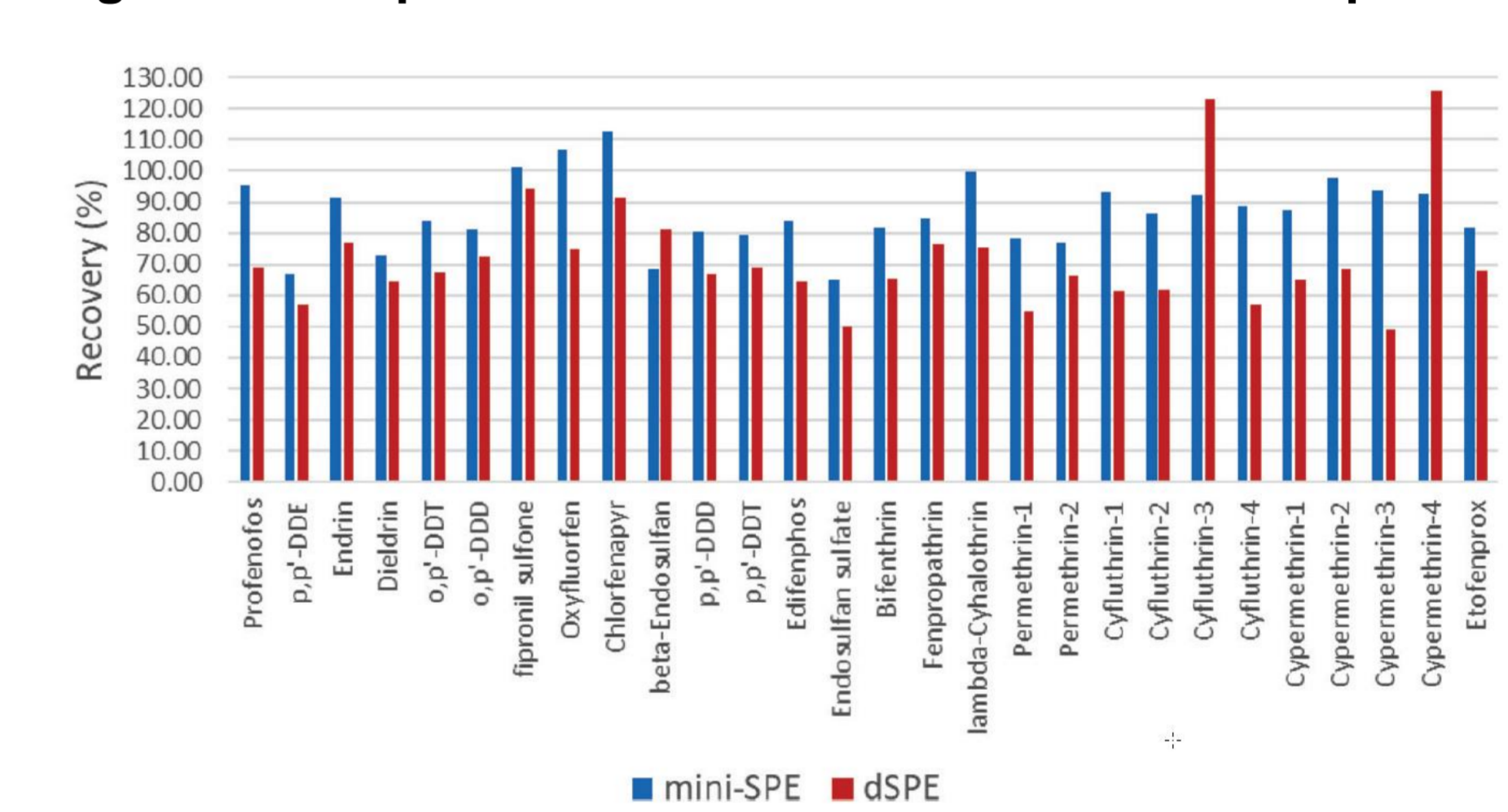


Figure 5. Turmeric, chilli, and red wine before and after automated mini-SPE clean-up.



Conclusion

The automated mini-SPE clean-up workflow improved the efficiency of analysis and significantly increased the sample throughput. The method performance ensured precise and accurate residue estimations. It was possible to identify all the compounds at the 10 ng/g level in coriander, cumin, and cardamom matrixes. For more complex matrixes, e.g. turmeric, chili powder, and black pepper, the LOQs were either 10 or 20 ng/g [5]. The results demonstrate that the used cartridge composition can work efficiently for a wide range of spices with no specific requirements of clean-up optimization for individual matrixes.

References

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