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Evaluation of a septumless mini-cartridge for automated solid-phase extraction cleanup in gas chromatographic analysis of >250 pesticides and environmental contaminants in fatty and nonfatty foods

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ABSTRACT

The QuEChERSER mega-method has recently been introduced to quantify and identify a wide range of chemical residues (pesticides, veterinary drugs, environmental contaminants, among others) in nearly all types of foods. The approach calls for taking a small amount of the initial extract to cover analytes amenable to liquid chromatography, and the remainder is salted out for analysis by gas chromatography (GC), both with mass spectrometry (MS) based detection. In the case of GC-MS(/MS), the extract undergoes automated robotic mini-cartridge solid-phase extraction (SPE) cleanup in a technique known as μSPE or instrument-top sample preparation (ITSP). In 2022, a septumless mini-cartridge for μSPE was introduced to improve upon the ITSP design. The new design houses a bed of 20 mg anhydrous $MgSO_4$, 12 mg each of C18 and primary secondary amine sorbents, and 1 mg of graphitized carbon black, the latter substituting for CarbonX used in the ITSP product. The septumless μSPE mini-cartridge employs a different gripping mechanism with the syringe needle that allows leak-free operation at higher flow rates (e.g. 10 μL/s), whereas the ITSP design is limited to 2 μL/s. Based on cleanup and analyte elution profiles, the extract load volume and flow rate was increased in μSPE for QuEChERSER from 300 μL at 2 μL/s to 500 μL at 5 μL/s, which improved accuracy of results, sped the cleanup step, and obviated the need for micro-vial inserts in the receiving vials. The new design also reduced both the amount and consistency of dead (void) volumes in the mini-cartridges from 83 ± 14 μL to 52 ± 7 μL for 200-600 μL load volumes. Normalization of peak areas to internal standards led to recoveries between 80 and 120% with typical RSDs <5% in low-pressure GC-MS/MS of 227-242 out of 252 pesticides, polychlorinated biphenyls, polybrominated diphenyl ethers, and polycyclic aromatic hydrocarbons in hemp powder, spinach, whole milk, egg, avocado, and lamb meat.

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1. Introduction

Automated solid-phase extraction (SPE) cleanup in a mini-cartridge format using a robotic autosampler hyphenated with the analytical instrument was first described by Morris and Schriner in 2015 [1]. This concept is commercially known as instrument-top sample preparation (ITSP) or μSPE. In 2016, Lehotay et al. [2] published their work to evaluate and optimize the ITSP product for cleanup of many diverse nonfatty and fatty food types in conjunction with low-pressure gas chromatography – tandem mass spectrometry (LPGC-MS/MS) for analysis of a wide range of pesticides, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and flame retardants, including polybrominated diphenyl ethers (PBDEs).

Initially, mini-cartridge SPE served to replace dispersive (d)-SPE for cleanup in the "quick, easy, cheap, effective, rugged, and safe" (QuEChERS) approach [3] for sample preparation [1,2,4–8]. The ITSP mini-cartridge, containing a 45 mg mixture consisting of 20 mg anhydrous (anh.) $MgSO_4$, 1 mg CarbonX, and 12 mg each of primary secondary amine (PSA) and octyldecylsilane (C18) sorbents, was demonstrated to provide better cleanup than d-SPE without sacrificing analyte recoveries. Furthermore, the automation of just-in-time cleanup in parallel with chromatographic analysis saves time and labor for greater laboratory efficiency and higher sample throughput. These advantages have also been studied and described in other μSPE and ITSP publications involving robotic automation for sample preparation [9–12], pharmaceutical analysis

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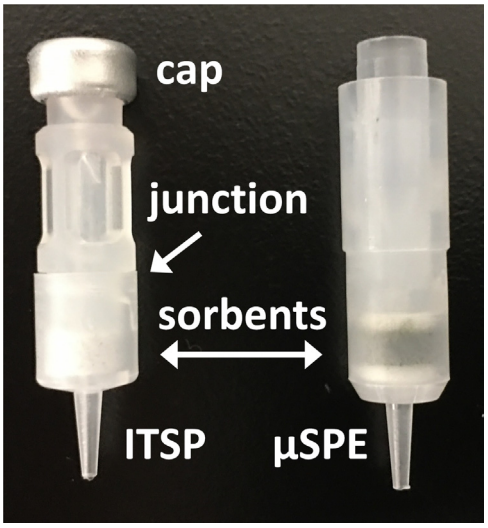

Fig. 1. Picture of mini-cartridge designs for ITSP (unused) and µSPE (used for cleanup of spinach extract).

[13,14], cleanup of PAHs from food oils [15], and application notes [16,17].

Due to its advantages, mini-cartridge SPE has been integrated into the QuEChERSER mega-method (more than QuEChERS is also "efficient and robust"), which is a generic sample preparation approach covering pesticides, veterinary drugs, environmental contaminants, and other analytes in a wide range of sample types [18–24]. To cover such wide scope, the initial QuEChERSER extracts are split into two portions for separate cleanup tailored to LC- and GC-amenable analytes. Although mini-cartridge cleanup has been applied in several LC-based analyses [1,13,14,16,17], SPE cleanup inevitably retains some analytes when dealing with a broad range of physicochemical properties. This was demonstrated in a comparison of 4 different sample preparation methods for analysis of veterinary drug residues in meat and fish [25]. The precipitation-based cleanup used for LC-amenable analytes in QuEChERSER was found to yield higher recoveries for more analytes than SPE methods. Thus, mini-cartridge SPE is only used prior to GC analysis in QuEChERSER to determine several types of GC-amenable pesticides and environmental contaminants.

One of the points of caution with this approach is not to overload the small amounts of sorbent, and since QuEChERS typically yields extracts of ≈1 g equivalent sample per mL, the extracts can overfill the sorbents in the case of highly complex samples. QuEChERSER extracts are 4 times less concentrated (≈0.25 g/mL), which make it less likely for co-extracted matrix to break through the mini-cartridges. However, dried hemp powder and flower were still found to be too complex for the ITSP product in QuEChERSER [21], and further dilution was needed.

Other limitations with ITSP relate to the practical aspects of reliable automation and performance due to particulars of the design. Fig. 1 shows two different designs for mini-cartridge SPE. For the purposes of this report, "ITSP" refers to the mini-cartridge product on the left, and "µSPE" signifies the newly introduced design on the right (although commercially the latter term may also relate to ITSP, depending on the vendor [16,17]). In the ITSP design, the mini-cartridge consists of 3 external components (septum cap, needle guide, and sorbent holder). The mini-cartridge is held by the rubbery septum squeezing the syringe needle upon being pierced. The success of robotic operations in this design depends on the precise alignment of the needle to consistently penetrate with 180° verticality near the exact center of every cartridge on the 96-well plastic holding tray (a 54-position metal tray is also an option). If the robot, tray, and cartridges are not aligned properly, or if the needle is slightly bent, then the needle will not form a good seal with the septum. This can lead to a dropped cartridge or a leak between the needle and septum that affects performance. Excessive tilting of the mini-cartridge is another problem that can lead to failure due to misalignment with the receiving vial openings.

Even if the alignment is perfect, the maximum flow rate in ITSP is 2 µL/s due to leakage of liquid up through the septum at higher flow rates. The seal between the needle guide and sorbent holder in the ITSP mini-cartridge design is another source of potential leakage, which has been observed in practice, albeit infrequently. Sorbent particles can infiltrate the space between the plastic pieces to break the seal, in which case backpressure from the sorbent bed and underlying filter will force the liquid up through the gap rather than through the sorbents.

In 2022, CTC Analytics (Zwingen, Basel-Landschaft; Switzerland) introduced the septumless µSPE mini-cartridge design shown in Fig. 1 (right). This µSPE design consists of two pieces of polypropylene (PP) pressed so tightly together that the possibility of the second type of leak described above is essentially eliminated. The likelihood of the first type of upwards leakage around the needle is also much lower in µSPE because a different mechanism to hold and seal the mini-cartridge obviates the need for the septum cap. In the new version, the robotic liquid handler presses the syringe needle into the tapered opening of the mini-cartridge forcefully enough to create a gripping seal. This connection enables the robotic autosampler to reliably hold, lift, and move the mini-cartridges as needed. Furthermore, this sealing mechanism between the syringe and mini-cartridge permits higher flow rates (*e.g.* >10 µL/s) without leakage. In ITSP, the spent mini-cartridges are released via a downward force applied from a spring-loaded ring encircling the needle beneath the syringe barrel, but in the µSPE design, a motor-activated lever is used. Additional details about the robotics and automation are described elsewhere [10–12,16,17]. Furthermore, the µSPE mini-cartridges potentially accommodate a larger sorbent bed, which is limited to 45 mg in ITSP.

The intent of this study was to assess the recently introduced septumless mini-cartridges and re-optimize the automated µSPE cleanup conditions in QuEChERSER for the LPGC-MS/MS analysis of >250 pesticides, PCBs, PAHs, and PBDEs in different types of food. Nonfatty matrices (hemp pellets and spinach) containing chlorophyll, and diverse types of fatty matrices (egg, whole milk, avocado, and lamb meat) were to be tested. In the µSPE product, 1 mg graphitized carbon black (GCB) was substituted for 1 mg CarbonX used in ITSP, which was another factor in the evaluation and re-optimization. The original sources of the reagents and processes to form the sorbent beds in the mini-cartridges were also different. Performance parameters were to be evaluated in terms of analyte recoveries, precision, degree of cleanup, reliability in automation, level of background interferences, and consistency of volumes.

2. Materials and methods

2.1. Standards and reagents

Analyte and internal standards were obtained from the EPA National Pesticide Repository (Fort Meade, MD; USA), Dr. Ehrenstorfer GmbH (Augsburg; Germany), ChemService (West Chester, PA; USA), Sigma-Aldrich (St. Louis, MO; USA), C/D/N Isotopes (Pointe-Claire, Quebec; Canada), Cambridge Isotope Labs (Andover, MA; USA), and AccuStandard (New Haven, CT; USA). For the salting out step in QuEChERSER, 2 g 4/1 (*w/w*) anh. $MgSO_4$/NaCl in 15 mL PP centrifuge tubes were acquired from Agilent (Little Falls, DE; USA) and UCT (Bristol, PA; USA). Deionized water (18 MΩ-cm) came from a Barnstead (Dubuque, IA; USA) E-Pure Model D4641, and HPLC-

grade acetonitrile (MeCN) was from Fisher Scientific (Pittsburgh, PA; USA). Shikimic acid used as an analyte protectant in LPGC-MS/MS was from Sigma-Aldrich.

The PAL µSPE mini-cartridges originating from CTC Analytics were provided by Archer Scientific (Lake Elmo, MN; USA). The cartridges contained 20 mg anh. $MgSO_4$, 12 mg each of C18 and PSA, and 1 mg GCB (Carbograph-1). ITSP mini-cartridges were obtained from ITSP Solutions (Hartwell, GA; USA), which contained the same amounts of each sorbent in the mix except CarbonX replaced GCB.

2.2. Sample preparation

Food samples were purchased at local grocery stores, and hemp powder was provided by the California Department of Food and Agriculture. For generation of QuEChERSER extracts, 2 g test portions of the food samples were weighed into 15 mL PP centrifuge tubes. Then, 10 mL of 4/1 (*v*/*v*) MeCN/water was dispensed into the tubes, which were capped and vortexed for 10 min using a Glas-Col (Terre-Haute, IN; USA) platform pulsed shaker at maximum intensity with 80% pulsation. This was followed by a 3 min room temperature centrifugation step at 4150 rpm (3711 rcf) with a Kendro (Osterode, Lower Saxony; Germany) Sorvall Legend RT swinging bucket centrifuge. For salt-out partitioning, each extract was decanted into a 15 mL PP centrifuge tube containing 2 g 4/1 (*w*/*w*) anh. $MgSO_4$/NaCl. Next, the extracts were vortexed for 1 min on the platform shaker followed by centrifugation as before. For µSPE (or ITSP), 1.5 mL portions of the upper phase (MeCN extracts) were transferred to amber glass autosampler vials. In the final µSPE conditions, 500 µL extract was loaded at 5 µL/s into the mini-cartridges for collection in the receiving autosampler vials without micro-vial inserts. The inserts were needed for elution volumes <300 µL for direct injection of final extracts (note: use of 400 µL load volume required transfer of a portion of the eluted volume into micro-vial inserts to raise the liquid level high enough for the autosampler needle to consistently reach the solution for injection).

Experiments were conducted to evaluate the effect of different extract load volumes (100, 200, 300, 400, 500, and 600 µL) with flow of 2 µL/s in the case of hemp pellets and 5 µL/s for reagent blank (6% water in MeCN by volumes). To evaluate flow rates of 1, 2, 3, 4, 5, and 6 µL/s, spinach extracts were used with a constant 500 µL load volume. The effect of mini-cartridge storage conditions over 30 days were assessed using the final µSPE conditions, as were cleanup and recoveries of the analytes in avocado, whole milk (bovine), lamb muscle, and chicken egg. Spikes of 252 pesticides and 13 internal standards (ISTDs) were made in quadruplicate at the concentrations listed in Table S1 (supplemental) just prior to the µSPE step. Matrix blanks also underwent cleanup at the same conditions as the spiked extracts, and matrix-matched (MM) calibration standards were prepared by adding the spiking and ISTD solutions to the eluents at the equivalent sample concentrations of the spiked analytes.

For µSPE (or ITSP) cleanup, a CTC Analytics PAL3-RTC robotic liquid handler was used with a hand-held controller. The robotic device was set up on a bench for independent stand-alone operation in this study, but it can be readily coupled with an analytical instrument for usage as a robotic autosampler for just-in-time cleanup conducted in parallel with chromatographic analysis. The vials and mini-cartridges were all placed on the same 3-slot tray holder, and the liquid handler contained two tray holders to permit up to 108 samples in a batch. Each slot consisted of 54-position racks containing: 1) autosampler vials of the salt-partitioned extracts; 2) receiving vials for the final extracts covered by a metal guide to help align the mini-cartridges over the vials; and 3) a metal holder for the mini-cartridges.

A gas-tight 1 mL "PAL Smart" syringe (22 gauge, flat-tipped needle) was installed in the µSPE tool, which was always pre-wetted and washed to waste once with 1 mL MeCN before moving to a sample vial in slot 1. To both avoid bubbles in the syringe and mix the sample before cleanup, the syringe first pumped 100 µL of sample up and down 3 times, then pulled up the desired sample load volume at 30 µL/s. Then, the syringe needle grabbed via downward pressure on the associated mini-cartridge held in slot 3. The mini-cartridge was lifted into place over the matching receiving autosampler vial in slot 2 (Note: Pre-slit "+" septa were installed in the vial caps to allow access to the mini-cartridge tips; leakage may occur if a seal forms between the septum and tip because displaced air by the liquid needs to escape, thus the use of appropriate septa is important). The loaded sample was passed through the mini-cartridge into the receiving vial at the set flow rate, and the mini-cartridge was discarded into a waste container. To thoroughly mix the sample prior to analysis, the syringe pumped 200 µL of final extract up and down 3 times. Lastly, the syringe was cleaned with 700 µL each of: 1) an equal parts mixture of MeCN/MeOH/water/acetone; and 2) MeCN. The total cycle time was 5.33 min in the final method, which would have extended to ≈7.5 min when used on the instrument-top due to the switching back and forth to a tool containing the 10 µL syringe for injection plus its washing steps.

2.3. Analysis

Extracts were analyzed using an Agilent 7890A/7010 GC-MS/MS system coupled with a Gerstel (Linthicum, MD; USA) MPS3 as the autosampler. For LPGC, a 5 m, 0.18 mm i.d. uncoated guard/restrictor capillary connected to a 15 m, 0.53 mm i.d., 1 μm thickness film Rtx-5MS analytical column plus an extra 1 m uncoated 0.53 mm i.d. integrated transfer line capillary served as the column set (part #11800; Restek; Bellefonte, PA; USA) [26]. A Restek Topaz low-pressure drop splitless precision liner with glass wool was installed in the Agilent split/splitless injector. Injection volume was 3 μL final extract + 1 μL of 1 µg/µL shikimic acid solution separated by a 1 μL air gap. Inlet temperature was 280°C, and a pressure pulse of 40 psi was applied for 0.75 min. Initial oven temperature was 80°C for 1 min, ramped to 320°C at 45°C/min and held until 10 min. The high purity helium carrier gas started at 2.25 mL/min for 3 min, followed by 1.5 mL/min until 10 min. The ion source was set at 320°C and the transfer line was 280°C. Electron ionization (EI) was applied at -70 eV with 100 μA filament current. Ion transitions listed in Table S1 were applied using dynamic multiple reaction monitoring. For assessment of cleanup, everything was the same except injection volume was 2 µL without added shikimic acid and data acquisition was full-scan MS (*m/z* 75-800). MassHunter 10.0 software was used for instrument control and data processing.

Cleanup efficiency was also evaluated using a Tecan (Männedorf, Zürich; Switzerland) Safire^2 multidetection 96-well plate reader that measured the absorbance of 200 µL solutions at 37°C with scan range of 300-700 nm in 1 nm increments.

3. Results and discussion

3.1. Operational performance

In terms of automated robotic operations, none of the ≈120 µSPE cleanup procedures underwent a stoppage in this study. In experiments, the weights of each loading and receiving vial were measured using an analytical balance before and after performing automated µSPE. As reported in Section 3.2, densities of the extract solutions were determined by weighing fixed volumes from

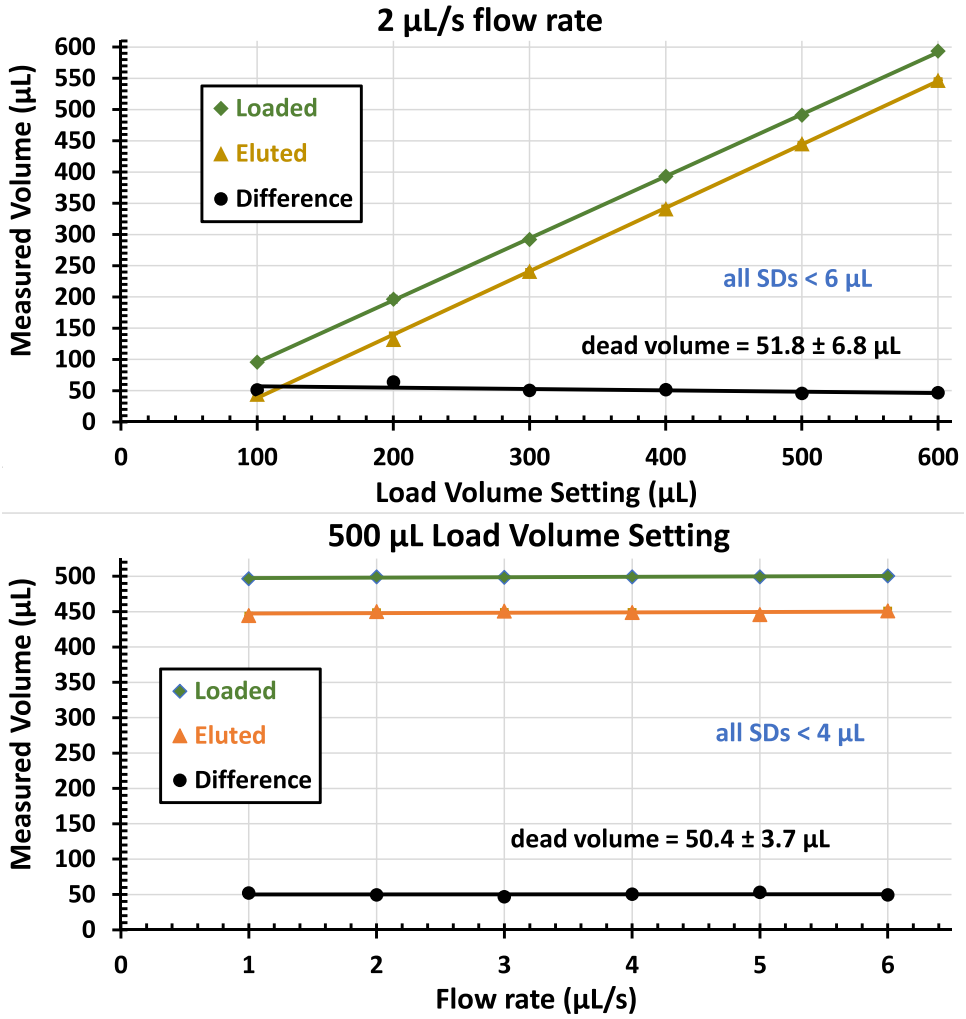

Fig. 2. Measurement of load, elution, and dead volumes in µSPE experiments for hemp pellets (top) and spinach (bottom) using different volume and flow conditions. One vial out of 60 had 85 µL less elution volume than average, and two were 20–25 µL different, which were thought to result from transcription errors in recording weights.

calibrated piston displacement pipettors. The actual loading, elution, and dead (void) volumes during the cleanup step were then calculated from the densities and weight differences of the vials.

Fig. 2 shows the results for the volume measurements in the experiments in which load volumes and flow rates were varied. The error bars, representing standard deviation (SD) with n=5 for each data point, are included in the plots, but they are typically smaller than the size of the symbols. An outlier of 85 µL less elution volume than average for one of the 60 replicates in Fig. 2 (300 µL load volume) was confirmed by visual inspection. Also, a result with 25 µL difference from average occurred, but no difference in volume in the micro-vial insert was observed even though that difference would have been noticeable. This outlier was believed to be due to a transcription error, which was also very likely to be the case for another replicate in the flow rate experiment. These suspicious results were not included in the plots in Fig. 2.

In the volume calculations, none of the SDs exceeded ±6 µL. The initial assessment of ITSP indicated similar degree of variability in the dead volumes for a given load volume, but in that design, dead volumes ranged from 75-90 µL with a trend of 3.6 µL increasing dead volume per 100 µL load volume [2]. As shown in Fig. 2 (top), the dead volume using the septumless µSPE mini-cartridges remained a consistent 52 ± 7 µL independent of load volume. The ≈30 µL lower and more consistent dead volumes in the µSPE design indicated that the packing of the sorbents was tighter than in the case of ITSP. If the sorbent bed is packed too tightly in ITSP, the high backpressure increases the chance of upward leakage, but the design of the µSPE mini-cartridges tolerates the higher backpressure without leakage. In retrospect, the previous finding in ITSP that the void volume of the mini-cartridges increased by 3.6% in direct proportion with the load volume was most likely a result of slight leakages, not an artefact of the measurement process [2]. Furthermore in µSPE, Fig. 2 (bottom) shows that flow rate also did not make a difference in the calculated dead volumes. The fixed load volume of 500 µL (499 µL measured) for spinach extracts led to elution volumes of 449 ± 4 µL. This improvement in the reliability, consistency, and elution volume efficiency of µSPE over ITSP laid the fundamental groundwork for improved analytical performance, as will be discussed in the following sections.

3.2. Hydration of $MgSO_4$ during storage

The removal of water in the extracts has been shown to be one of the important factors to yield better cleanup for GC-based analysis in QuEChERS or QuEChERSER. Anh. $MgSO_4$ was chosen for use in both the salt-out partitioning and SPE steps in either approach because it most effectively reduces water content in the MeCN extracts compared to common alternatives [3,27]. Not only is cleanup by PSA, C18, and GCB improved in drier extracts [2], water should

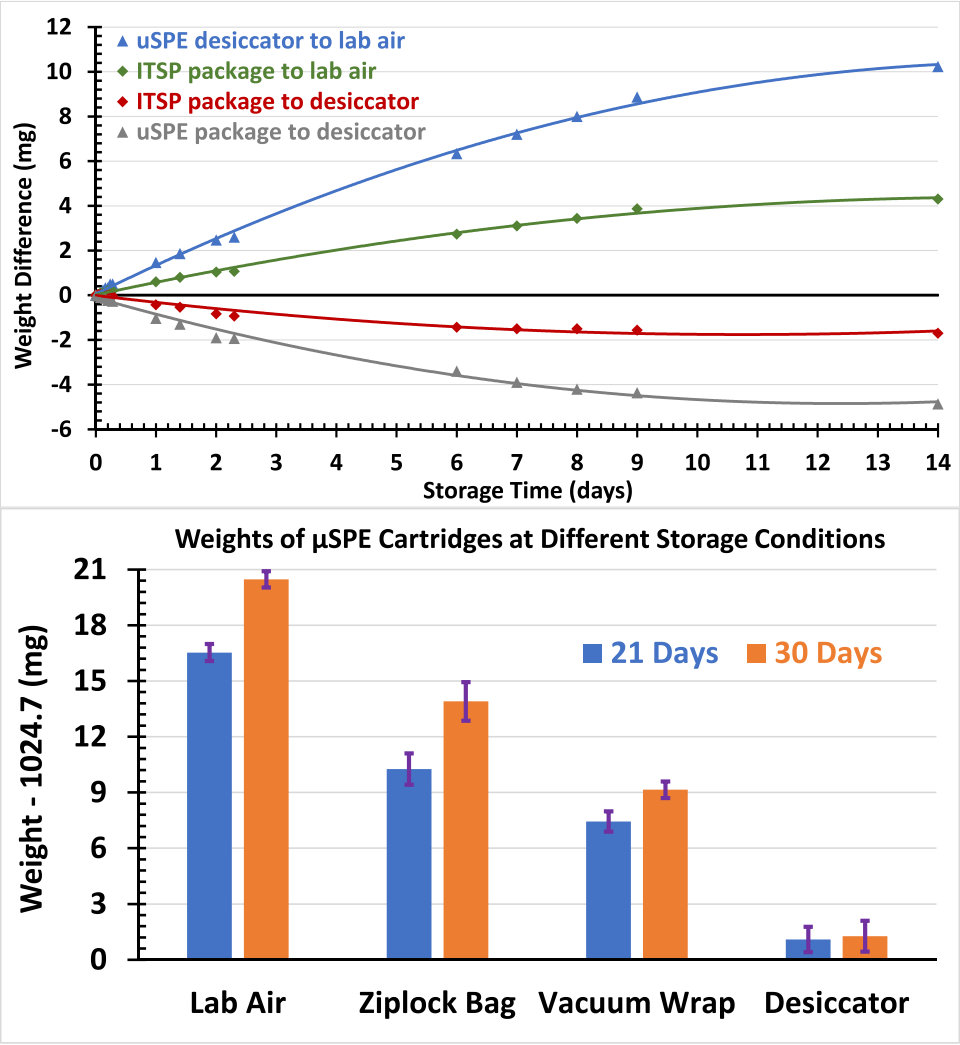

Fig. 3. Comparison in the increase or decrease of weight in the µSPE and ITSP mini-cartridges (n=3 each) over time and different storage conditions due to (de)hydration of the 20 mg $MgSO_4$ salt (capable of retaining 20.9 mg water). Ideally, the mini-cartridges should be stored in a desiccator for several days before usage. It takes about a month for the $MgSO_4$ in the µSPE design to fully hydrate in an air-conditioned lab (51% humidity at 20°C). Note: the approximate weight of the cartridges (1024.7 mg) was subtracted from the measured weights in the bottom chart.

be avoided in GC-MS to improve performance and to extend the operational lives of the EI filament and column stationary phase.

With respect to water removal efficiency, the 20 mg anh. $MgSO_4$ (0.166 mmol) in each µSPE or ITSP mini-cartridge has the capacity to hydrate with up to 1.16 mmol of water, or 20.9 µL (mg) at ambient conditions (each molecule of $MgSO_4$ can retain 7 molecules of water). However, this depends on the initial state of hydration of the $MgSO_4$ salt. As shown in Fig. 1, the septum-less µSPE mini-cartridges are open to the atmosphere at both ends whereas the ITSP design includes a cap at the top. A comparison was made between the storage conditions for the mini-cartridges over time to assess the state of (de)hydration of $MgSO_4$ via weight measurements.

Fig. 3 charts mini-cartridge weight differences over time at different storage conditions. The upper plot indicates the degree of initial $MgSO_4$ hydration in the mini-cartridges freshly removed from the packaging. Initially, the mini-cartridges were 24% hydrated in the case of µSPE and 9.5% for ITSP, but in light of these results, better desiccation will likely be provided in future packaging in both cases. Fig. 3 also shows how long it took for the $MgSO_4$ in the mini-cartridges to partially hydrate when exposed to the laboratory environment (20°C and 51% humidity), as well as the rate of dehydration when placed in a desiccator. Clearly, the cap on the ITSP design limited diffusion, leading to slower rates of (de)hydration than with the µSPE design. It took about 2 weeks in a desiccator for both types of mini-cartridges to fully dehydrate. In the case of µSPE, it took about a month for the $MgSO_4$ to fully hydrate (20.9 mg additional weight) when stored in the open lab air. To keep hydration to <10%, the desiccated mini-cartridges should be used within about 40 h for µSPE or 4 days for ITSP on the robotic autosampler tray. This corresponds to 185 to 440 samples, respectively, in the case of QuEChERSER using LPGC-MS/MS with 13 min cycle times. Unfortunately, the storage experiments were conducted last in this study, and the µSPE results presented in the following sections involved the use of mini-cartridges fresh from the packaging that already contained ≈5 mg water.

3.3. Water content of extracts

As stated in Section 3.1, the densities of the salted-out QuEChERSER extracts and reagent blanks before and after µSPE were measured in the experiments. The density of the aqueous MeCN solutions could be used to reasonably determine the %water (*v*/*v*) in the solutions. Recently, comparison of results using this approach *vs.* nuclear magnetic resonance (NMR) spectroscopy to measure water content in lamb meat extracts showed similar accu-

Table 1
Calculated %water (*v/v*) in MeCN for the extracts before and after µSPE in the experiments based on the density calibration plot presented on p.3 of supplemental figures. $MgSO_4$ hydration indicates the amount of water removed from the extract at 5 µL/s flow rate relative to the full capacity of the 20 mg anh. $MgSO_4$ in the mini-cartridges (add 24% to each listed value to account for the initial degree of hydration).

Sample	Before µSPE	After µSPE					Avg (hydration)
		200 µL	300 µL	400 µL	500 µL	600 µL	
Reagent blank	6.0	4.0	4.3	4.3	4.5	3.8	4.2 ± 0.2
($MgSO_4$ hydration):		(14%)	(20%)	(28%)	(32%)	(58%)	
Whole milk	6.8	**500 µL load volume and 5 µL/s flow rate:**					4.3 (54%)
Egg	6.4						5.0 (30%)
Avocado	5.9						4.1 (39%)
Lamb meat	6.1						4.3 (39%)

racy and trustworthiness between the methods [24]. Supplemental figures (p. 3) includes the calibration line of measured density vs. %water (*v/v*) in MeCN solutions prepared contemporaneously with the reagent blank experiment. This was also used to estimate the moisture contents in another experiment, as compiled in Table 1.

Previously, the QuEChERSER salting out and ITSP steps were found to reduce water content of the extracts to 6% and 2-4%, respectively [2,24]. As shown in Table 1, the pre-µSPE extracts for fatty matrices averaged 6%, which matched the previous result for lamb [24], leading to use of a 6% water solution as the reagent blank. Table 1 shows that the final extracts were calculated to be 4-5% water independent of matrix or load volume at 5 µL/s flow rate.

If hydration is 100% complete (ignoring the 24% initial hydration level), 349 µL of a solution containing 6% water would fill the drying capacity of the 20 mg anh. $MgSO_4$, corresponding to ≈400 µL load volume when taking the ≈50 µL void volume into account. However, Table 1 indicates that hydration efficiency was less than 100%, and that the amount of water retained increased as the load volume increased. As shown for the fatty matrices, effective hydration efficiencies were 39-54% at the final conditions of 500 µL load volume and 5 µL/s flow rate (corresponding to 63-78% actual hydration of the $MgSO_4$ accounting for the initial 24%). Other volume and flow parameters could have been used to slightly improve cleanup efficiencies, as discussed in Section 3.4.2, but analytical performance (and speed) was preferred at these conditions. Perhaps a greater amount of anh. $MgSO_4$ should be used in the future to yield drier final extracts, and the possibility for a larger sorbent bed in the µSPE design could accommodate that option.

3.4. Optimization of final conditions

To choose the optimal load volume and flow rate settings, multiple measurement techniques were employed to assess the degree of µSPE cleanup of QuEChERSER extracts for different matrices. Recoveries and RSDs (n=4) were also determined in each case to assess the robustness of the conditions. Supplemental information (pp. 2-18) contains many figures demonstrating the background levels for reagent blanks and the degree of cleanup of hemp pellets, spinach, milk, egg, lamb meat, and avocado at preliminary or final conditions, including UV/Vis absorbance spectra (minus MeCN background) from 300-700 nm and full-scan (*m/z* 75-800) total ion current (TIC) chromatograms. Elution profiles in µSPE of many noteworthy analytes with or without normalization to ISTDs are also presented in the cases of hemp pellets using 100-600 µL load volumes at 2 µL/s flow rate and spinach using 500 µL load volume at 1-6 µL/s flow rates.

3.4.1. Rinse and storage options

Fig. 4 and supplemental figures (pp. 2-6) display the TIC chromatograms for reagent blanks. One of the options in mini-cartridge SPE is to first wash sorbent bed with solvent prior to loading the sample extracts for cleanup [8]. This approach has only one advantage as shown in Fig. 4 (top): chemicals that may be present in the plastic, filters, and sorbents are washed to waste. Perhaps prewetting of the sorbents with MeCN affects their performance, but this facet has not yet been demonstrated in this application.

However, pre-wetting the sorbents has the following disadvantages: (1) the eluted extracts are diluted by the extra solvent, which varies to the same extent as the void volume; (2) the extra time needed causes the cleanup method to extend longer than the analysis cycle time in LPGC and other rapid methods, which reduces sample throughput; (3) extra reagents are needed and solvent waste is generated for disposal; and (4) adding rinsing steps in the method require more accessories, programming, and movements of the robotic liquid handler, which increases cost, complications, wear-and-tear on the parts, and opportunities for failures. In particular, the need to penetrate the septum cap twice for each sample in ITSP leads to greater chance of upward leakage, needle slippage, and/or dropping of a mini-cartridge. The septumless design and mechanism in µSPE may reduce those chances, but this has not been studied.

Easier solutions can be followed rather than rinsing the cartridges. For one, cleaner and/or pre-rinsed cartridges can be provided by the manufacturers in the first place. For another, increased selectivity in analysis can avoid possible interferences from the leachates, rendering the issue moot. Indeed, LPGC-MS/MS of the 252 targeted ion transitions for quantification based on summation function peak integration [28] used in this study showed that only two analytes yielded peak heights >10,000 counts (naphthalene and acenaphthalene). As shown in supplemental figures (p. 5), the identification criteria were met for those relatively volatile 2-ring PAHs, but background concentrations were <0.1 ng/mL in the reagent blanks. Post-receipt storage of the µSPE mini-cartridges under vacuum for 21 days in a plastic bag reduced the background levels of these PAHs, but otherwise, no notable differences between the different storage conditions were observed in the ≈450 µL elutions of 94/6 (*v/v*) MeCN/water at 5 µL/min (see Fig. 4 and supplemental p. 6). In theory, storage of the mini-cartridges in a vacuum desiccator would be ideal for consistency, but simply using them within a few days out of the packaging as they are provided also works fine, as demonstrated in this study.

3.4.2. Cleanup of matrices

The chemical noise in the final extracts coming from food commodities is typically higher than the background level originating from the reagent blanks. This is another reason that rinsing of the mini-cartridges was not felt to be worthwhile in the final method. However, comparison of the full-scan LPGC-MS TIC chromatograms of post-µSPE matrix *vs.* reagent blanks indicated less difference than expected (see supplemental pp. 2-18). As also shown previously for ITSP [2,4–8,15–24], the automated µSPE cleanup procedure is highly effective for fatty and nonfatty commodities alike. Supplemental information (pp. 10-18) includes many figures show-

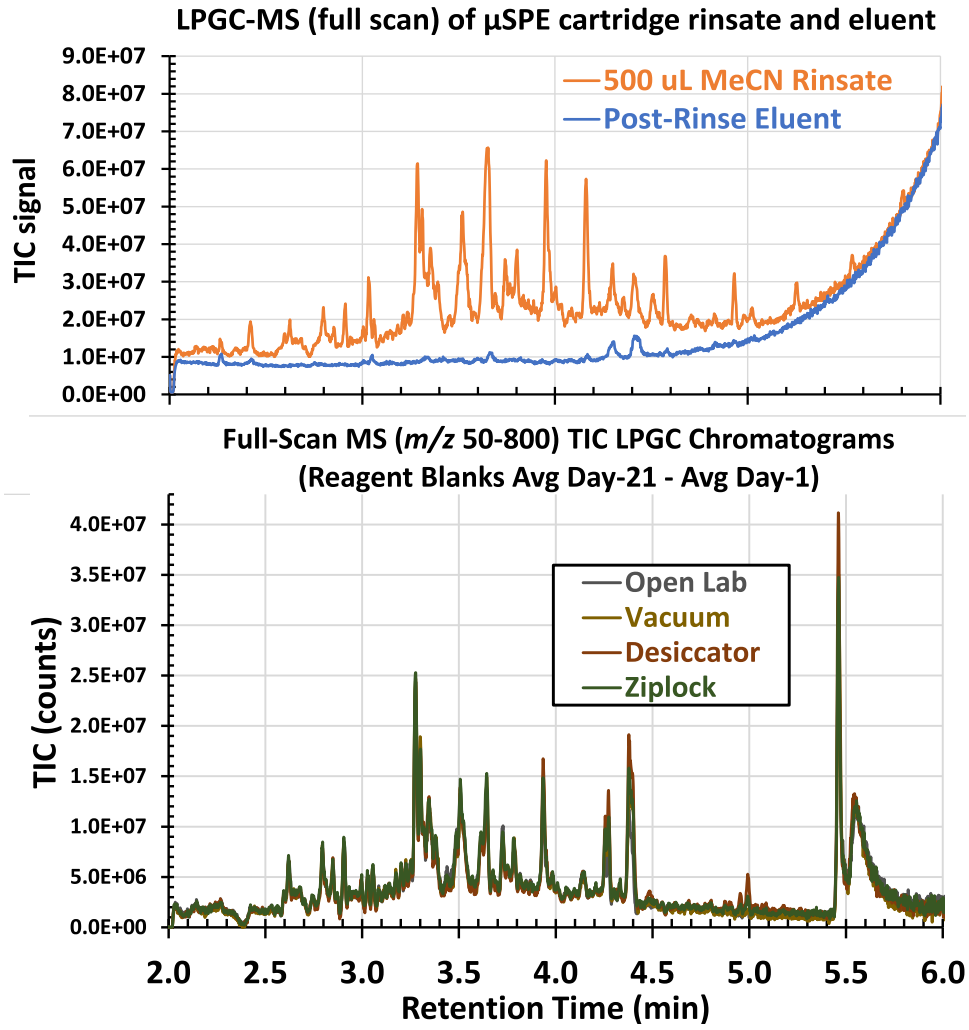

Fig. 4. Full-scan (*m/z* 50-800) total ion current (TIC) LPGC-MS chromatograms: (top) 500 µL acetonitrile (MeCN) passed through a fresh µSPE cartridge followed by 500 µL of 94/6 (*v/v*) MeCN/water both at 5 µL/s; (bottom) apparent reagent background increase after storage of the mini-cartridges for 21 days in different environments. No significant background interferences occurred for the analyte ion transitions in LPGC-MS/MS without the rinsing step (see supplemental figures p. 7).

ing how µSPE in this study removed up to ≈90% of integrated chemical matrix backgrounds in both UV/Vis and LPGC-MS. In the case of lamb muscle, Ninga et al. further demonstrated that the scaled-up sorbent combination used in ITSP removed >99% of the original sample test portion [24].

With respect to the effect of load volumes and flow rates, Fig. 5 demonstrates that better cleanup is achieved at lower volume and slower flow. However, rather small differences in cleanup efficiencies resulted between 100 and 600 µL load volumes at 1-6 µL/s, at least in the cases of hemp and spinach. Taking analyte recoveries and speed of µSPE into account (as discussed in Section 3.4.3), the final conditions were chosen to be 500 µL load volume (leading to ≈450 µL elution volume) and 5 µL/s flow rate.

3.4.3. Recoveries and RSDs vs. load volume and flow rate

Supplemental figures (beginning on p. 19) show the effects of load volume, flow rate, and normalization to an ISTD in the µSPE recoveries of up to 265 compounds spiked into QuEChERSER extracts of the different nonfatty and fatty matrices. Error bars designate the SDs in the measurements with n=4, which appear even when they are narrower than the symbols used in the plots. RSDs of analyte recoveries in the experiments were typically 0-5% (see Table 2), which is exceptional for GC-based analyses. This shows how the consistency of elution volumes pays off in similar consistency in analytical signals, even without normalization to ISTDs. In fact, normalization often increased the RSDs, demonstrating that random sources of noise were predominant in those instances.

The µSPE results can be compared with similar plots during development of the optimized ITSP method presented previously in an open access publication, including its supplemental information [2]. In that case, only load volume was evaluated because the time needed to complete ITSP conducted in parallel with LPGC-MS/MS required use of the maximum flow rate of 2 µL/s. In fact, the 13 min LPGC analytical cycle time also dictated that the load volume had to be 300 µL in ITSP. Just as importantly, this was the maximum volume that would not overfill the 300 µL micro-vial inserts used in ITSP, including the addition(s) of analyte protectant and/or calibration standard solutions.

The capability for greater flow rates and load volumes in the septumless µSPE design than in the ITSP product led to several benefits: (1) faster cleanup potentially leading to higher sample throughput and turn-around time; (2) higher and more consistent recoveries for more analytes; (3) elimination of the need for micro-vial inserts in the receiving autosampler vials; (4) more final extract volume for storage and re-analysis, if needed; (5) increased robustness in results, even if leakage occurs to reduce elution volume; and (6) increased flexibility in the final µSPE methods depending on the application. If desired, elution volumes as large as

Table 2
Average %recoveries (%RSDs; n=4) obtained at the final µSPE conditions for the spiked QuEChERSER extracts of different commodities at the concentrations listed in Table S1 (supplemental). The recoveries for the numbered ISTDs are not normalized; they are used for the analytes as given by number. Recoveries <80% or >120% and RSDs >10% appear in bold.

Analyte	ISTD	Hemp*	Spinach	Avocado	Milk	Egg	Lamb
internal standards (ISTDs)							
malathion-d10	#1	100 (1)	106 (1)	88 (5)	94 (1)	92 (0)	91 (2)
atrazine-d5	#2	99 (1)	104 (1)	89 (4)	93 (1)	92 (1)	90 (2)
pyridaben-d13	#3	98 (1)	101 (0)	**79** (8)	91 (0)	87 (1)	86 (2)
$^{13}C_{12}$-DDE, p,p'	#4	99 (1)	101 (1)	87 (4)	90 (2)	89 (1)	89 (3)
$^{13}C_{12}$-PCB 153	#5	96 (1)	97 (1)	84 (3)	90 (2)	85 (3)	86 (2)
phenanthrene-d10	#6	94 (1)	100 (0)	**77** (4)	**74** (1)	**74** (1)	**76** (1)
FBDE 126	#7	**79** (4)	89 (3)	**52** (10)	**49** (3)	**51** (4)	**53** (0)
fluoranthene-d10	#8	**76** (2)	85 (1)	**49** (7)	**38** (2)	**41** (6)	**45** (0)
pyrene-d10	#9	**71** (1)	82 (2)	**46** (8)	**34** (3)	**37** (7)	**42** (1)
benzo(a)pyrene-d12	#10	**5** (**21**)	**8** (5)	**3** (**42**)	**3** (**15**)	**4** (**29**)	**4** (**11**)
benzo(ghi)perylene-d12	#11	**2** (5)	**2** (8)	**1** (**38**)	**3** (**20**)	**3** (**24**)	**3** (**20**)
acenaphthylene-d8	#12	100 (1)	103 (1)	89 (3)	93 (1)	89 (0)	88 (1)
naphthalene-d8	#13	102 (0)	97 (3)	85 (3)	95 (1)	91 (3)	91 (4)
triphenylphosphate-d15	#14	98 (1)	102 (1)	92 (3)	91 (1)	90 (1)	88 (2)
organophosphorus pesticides							
acephate	2	90 (1)	93 (1)	90 (7)	99 (1)	98 (0)	97 (1)
azinphos-ethyl	3	101 (4)	97 (0)	93 (5)	98 (1)	101 (2)	105 (1)
azinphos-methyl	3	99 (0)	104 (3)	82 (10)	93 (1)	94 (4)	93 (0)
bromophos	1	96 (2)	92 (1)	100 (2)	98 (3)	101 (1)	100 (2)
cadusafos	2	97 (1)	95 (1)	104 (1)	106 (1)	104 (1)	105 (1)
carbophenothion	1	98 (2)	93 (2)	99 (2)	100 (2)	103 (3)	101 (2)
chlorfenvinphos	1	95 (1)	93 (1)	101 (3)	102 (1)	106 (1)	102 (1)
chlorpyrifos	1	98 (4)	99 (4)	93 (4)	95 (3)	95 (2)	95 (3)
chlorpyrifos-methyl	1	97 (1)	94 (1)	106 (1)	103 (1)	101 (1)	102 (0)
coumaphos	1	114 (9)	104 (5)	**79** (3)	**74** (4)	84 (11)	83 (6)
diazinon	2	99 (0)	96 (0)	105 (3)	105 (2)	104 (2)	105 (2)
dichlorvos	2	98 (1)	97 (1)	106 (2)	108 (1)	105 (0)	108 (2)
dimethoate	2	99 (1)	98 (1)	102 (3)	105 (1)	101 (0)	106 (0)
di(mono)crotophos [1]	2	95 (2)	96 (1)	97 (7)	105 (1)	104 (1)	102 (1)
disulfoton	2	97 (0)	95 (1)	101 (1)	106 (1)	105 (1)	107 (1)
ethion	1	96 (1)	93 (2)	101 (3)	104 (1)	105 (2)	102 (1)
ethoprophos	2	99 (1)	96 (1)	104 (2)	105 (1)	104 (1)	106 (1)
fenamiphos	1	94 (4)	88 (1)	93 (9)	101 (2)	103 (3)	100 (3)
fenitrothion	1	96 (1)	94 (3)	103 (2)	103 (3)	105 (3)	103 (2)
fensulfothion	1	91 (4)	95 (2)	83 (**13**)	99 (3)	102 (4)	98 (3)
fenthion	1	98 (1)	94 (1)	105 (1)	105 (1)	103 (1)	103 (2)
fenthion sulfone	1	94 (4)	97 (2)	95 (8)	100 (1)	102 (3)	102 (3)
fonophos	2	98 (0)	95 (1)	108 (2)	105 (1)	106 (1)	106 (1)
fosthiazate	1	107 (6)	88 (5)	103 (6)	117 (3)	102 (8)	110 (7)
heptenophos	2	98 (1)	97 (0)	104 (1)	106 (0)	105 (1)	107 (1)
isocarbofos	1	96 (2)	96 (4)	111 (4)	105 (4)	104 (6)	105 (5)
isofenphos	1	97 (2)	91 (3)	109 (1)	105 (2)	102 (2)	102 (1)
methamidophos	2	96 (1)	98 (1)	97 (5)	101 (1)	101 (2)	102 (0)
methidathion	1	99 (1)	97 (2)	99 (4)	103 (0)	106 (1)	105 (2)
omethoate	2	96 (2)	97 (1)	97 (8)	105 (1)	104 (1)	103 (2)
parathion	1	98 (2)	95 (2)	99 (4)	106 (1)	105 (1)	101 (1)
parathion-methyl	1	96 (1)	97 (0)	107 (2)	106 (1)	107 (1)	103 (1)
phenthoate	1	97 (1)	96 (1)	105 (3)	108 (2)	106 (2)	104 (1)
phorate	2	99 (1)	96 (2)	103 (2)	103 (1)	103 (3)	105 (2)
phosalone	3	98 (1)	97 (1)	104 (1)	100 (1)	101 (1)	103 (1)
phosmet	3	100 (1)	100 (1)	95 (7)	103 (1)	101 (3)	102 (1)
pirimiphos	1	97 (3)	96 (1)	107 (3)	102 (2)	102 (4)	105 (2)
pirimiphos-methyl	1	96 (1)	95 (2)	105 (2)	104 (1)	105 (1)	105 (0)
profenofos	1	97 (3)	94 (3)	102 (3)	103 (2)	101 (2)	99 (2)
propetamphos	2	98 (1)	97 (2)	105 (1)	106 (1)	106 (1)	108 (2)
sulprofos	1	97 (2)	91 (2)	103 (1)	101 (2)	103 (0)	101 (2)
temephos	3	105 (5)	84 (**23**)	**125** (**15**)	107 (**27**)	104 (7)	110 (**13**)
terbufos	2	97 (1)	96 (2)	104 (1)	105 (2)	106 (2)	106 (1)
tetrachlorvinphos	1	98 (1)	95 (2)	99 (2)	105 (2)	103 (1)	102 (1)
triazophos	1	95 (2)	95 (1)	95 (4)	102 (1)	101 (1)	101 (1)
tribufos	1	94 (2)	94 (3)	98 (3)	100 (2)	102 (3)	100 (1)
organochlorine pesticides							
aldrin	1	93 (4)	95 (3)	97 (4)	101 (4)	97 (2)	96 (3)
chlordane, cis-	4	96 (8)	95 (3)	107 (4)	105 (7)	101 (9)	103 (4)
chlordane, trans-	4	100 (4)	98 (3)	105 (5)	115 (6)	98 (4)	98 (6)
chlordecone (kepone)	6 [a]	82 (5)	86 (2)	**75** (7)	115 (1)	106 (1)	100 (2)
DDD, o,p'-	4	101 (2)	96 (5)	113 (9)	106 (4)	**122** (8)	105 (**15**)
DDD, p,p' + DDT, o,p'	4	98 (1)	97 (2)	101 (1)	104 (3)	102 (2)	104 (2)
DDE, o,p'-	4	98 (0)	97 (2)	102 (2)	103 (3)	100 (1)	100 (3)
DDE, p,p'-	4	97 (3)	96 (3)	101 (3)	101 (0)	101 (2)	97 (2)
DDT, p,p'-	4	101 (3)	90 (2)	88 (3)	95 (2)	98 (2)	94 (3)
dichlorobenzophenone	1	94 (3)	95 (2)	100 (3)	95 (3)	97 (2)	98 (3)

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Table 2 (*continued*)

Analyte	ISTD	Hemp[a]	Spinach	Avocado	Milk	Egg	Lamb
dieldrin	1	109 (7)	99 (8)	106 (4)	102 (4)	99 (**12**)	109 (5)
endosulfan I	1	90 (1)	89 (4)	108 (5)	102 (1)	100 (3)	101 (6)
endosulfan II	1	98 (5)	96 (2)	106 (2)	112 (5)	98 (6)	111 (9)
endosulfan sulfate	1	92 (7)	110 (**13**)	108 (**16**)	98 (2)	104 (5)	108 (4)
endrin	1	97 (5)	104 (10)	102 (7)	95 (10)	101 (6)	102 (6)
endrin ketone	3	101 (5)	103 (3)	**121** (7)	99 (7)	107 (5)	99 (2)
HCH, alpha	2	101 (3)	94 (2)	98 (2)	105 (2)	102 (2)	102 (1)
HCH, beta	2	94 (3)	100 (2)	98 (2)	101 (1)	102 (2)	103 (2)
HCH, delta	2	97 (2)	98 (2)	100 (2)	101 (2)	99 (2)	100 (3)
HCH, gamma (lindane)	2	98 (0)	96 (1)	106 (1)	107 (1)	107 (2)	108 (2)
heptachlor	1	93 (2)	94 (2)	98 (2)	100 (3)	96 (3)	96 (1)
heptachlor epoxide	1	95 (6)	95 (3)	98 (7)	103 (3)	93 (5)	102 (5)
hexachlorobenzene	6	87 (1)	87 (1)	81 (2)	**78** (2)	**77** (2)	80 (3)
methoxychlor	3	103 (1)	98 (3)	105 (1)	101 (1)	105 (2)	106 (1)
mirex	4	93 (1)	94 (0)	98 (2)	98 (2)	97 (1)	98 (3)
oxychlordane	1	96 (**12**)	82 (5)	96 (3)	93 (3)	107 (2)	107 (4)
quintozene	3	96 (1)	95 (1)	114 (6)	95 (1)	101 (2)	102 (2)
pyrethroid pesticides							
allethrin	1	95 (2)	93 (1)	98 (3)	104 (1)	107 (1)	102 (4)
bifenthrin	3	97 (0)	96 (0)	114 (4)	103 (2)	106 (1)	106 (0)
cyfluthrin	3	92 (3)	93 (7)	105 (6)	100 (7)	103 (4)	103 (5)
cyhalothrin, lambda	3	95 (2)	97 (0)	109 (1)	102 (3)	106 (2)	105 (2)
cypermethrin	3	93 (2)	95 (1)	104 (3)	104 (2)	102 (2)	105 (1)
cyphenothrin	3	100 (3)	103 (2)	109 (2)	101 (3)	107 (3)	109 (6)
deltamethrin	3	**67** (6)	88 (4)	83 (7)	99 (6)	103 (7)	96 (7)
es+fenvalerate	3	95 (5)	97 (1)	99 (3)	104 (0)	104 (1)	103 (1)
etofenprox	3	97 (1)	96 (0)	101 (1)	98 (1)	100 (2)	101 (1)
fenpropathrin	3	98 (1)	97 (2)	110 (4)	106 (3)	108 (1)	111 (2)
fluvalinate, tau	3	92 (7)	96 (2)	93 (7)	103 (1)	103 (2)	102 (1)
permethrins	3	95 (3)	100 (4)	107 (4)	103 (1)	105 (2)	106 (1)
phenothrin	3	97 (2)	96 (1)	109 (4)	102 (1)	105 (2)	103 (1)
resmethrin	1	80 (3)	**78** (8)	**71** (2)	101 (5)	103 (4)	100 (3)
tetramethrin	3	96 (1)	97 (1)	109 (2)	103 (2)	106 (2)	105 (2)
carbamate pesticides							
carbaryl	1	97 (1)	100 (1)	100 (4)	100 (3)	101 (2)	103 (2)
carbofuran	2	100 (1)	96 (1)	100 (4)	103 (1)	104 (1)	104 (1)
chinomethionate	9	**38** (6)	**74** (3)	89 (5)	105 (6)	104 (5)	91 (2)
chlorpropham	2	99 (0)	98 (0)	107 (1)	106 (1)	105 (1)	107 (1)
fenobucarb	2	98 (1)	97 (1)	106 (1)	107 (0)	106 (1)	107 (1)
fenoxycarb	3	99 (0)	100 (1)	94 (7)	103 (1)	105 (3)	107 (1)
iprovalicarb	1	96 (1)	95 (2)	96 (6)	104 (1)	103 (0)	100 (2)
methiocarb	1	97 (2)	96 (2)	99 (5)	104 (2)	104 (1)	104 (1)
pirimicarb	1	94 (1)	95 (1)	105 (1)	103 (1)	102 (1)	105 (1)
promecarb	2	99 (2)	95 (1)	106 (1)	104 (1)	104 (1)	106 (1)
propham	2	99 (1)	97 (1)	107 (0)	106 (1)	106 (1)	107 (1)
propoxur	2	99 (1)	98 (1)	104 (2)	107 (0)	107 (1)	108 (1)
triazole pesticides							
bitertanol	3	97 (1)	98 (0)	99 (4)	103 (2)	105 (1)	106 (1)
cyproconazole	1	95 (1)	93 (1)	98 (3)	103 (1)	102 (0)	103 (1)
difenoconazole	3	94 (6)	96 (3)	83 (**17**)	98 (3)	99 (1)	97 (4)
fenbuconazole	3	96 (3)	98 (1)	93 (9)	98 (2)	101 (1)	98 (2)
flusilazole	1	97 (1)	94 (1)	102 (2)	101 (1)	101 (1)	105 (1)
flutriafol	1	97 (1)	95 (1)	102 (4)	102 (1)	103 (1)	102 (1)
hexaconazole	1	97 (3)	96 (4)	99 (3)	102 (2)	103 (2)	102 (1)
myclobutanil	1	98 (1)	94 (1)	102 (3)	103 (1)	102 (1)	103 (1)
paclobutrazol	1	97 (1)	94 (2)	101 (4)	103 (2)	102 (0)	101 (2)
penconazole	1	96 (0)	95 (1)	102 (1)	101 (1)	104 (0)	103 (1)
propiconazole	1	95 (2)	92 (3)	104 (3)	101 (2)	101 (1)	103 (2)
tebuconazole	1	94 (2)	94 (1)	97 (5)	101 (2)	99 (1)	98 (4)
tetraconazole	1	98 (1)	92 (1)	104 (1)	103 (2)	103 (1)	105 (1)
triadimenol	1	97 (1)	95 (1)	102 (1)	102 (1)	104 (2)	105 (2)
triadimephon	1	97 (4)	94 (2)	109 (3)	102 (2)	106 (4)	104 (2)
other pesticides							
acequinocyl	9	**62** (5)	**75** (6)	101 (**13**)	**146** (**21**)	**141** (**28**)	112 (10)
atrazine	2	98 (2)	97 (1)	108 (1)	106 (2)	105 (2)	105 (1)
azoxystrobin	3	89 (10)	96 (3)	84 (**21**)	102 (3)	102 (1)	99 (4)
benfluralin	2	95 (1)	95 (1)	105 (2)	106 (2)	105 (1)	106 (2)
benoxacor	1	98 (1)	96 (2)	108 (1)	106 (2)	107 (2)	102 (2)
bifenazate	3	100 (3)	95 (3)	98 (7)	95 (4)	107 (7)	109 (3)
boscalid	3	96 (2)	98 (1)	86 (10)	99 (1)	101 (3)	96 (2)
bromopropylate	3	102 (1)	98 (2)	114 (4)	104 (1)	110 (1)	107 (1)
bromoxynil	6[b]	**34** (10)	**77** (2)	**77** (**12**)	97 (5)	87 (5)	**79** (2)
bupirimate	1	95 (2)	94 (3)	105 (3)	103 (2)	102 (2)	104 (1)
buprofezin	1	101 (3)	96 (3)	106 (2)	105 (2)	107 (5)	101 (2)
captan	1	95 (6)	93 (4)	97 (**15**)	89 (5)	107 (5)	99 (4)

(*continued on next page*)

Table 2 (*continued*)

Analyte	ISTD	Hemp*	Spinach	Avocado	Milk	Egg	Lamb
carfentrazone	1	97 (1)	95 (1)	101 (3)	103 (2)	102 (1)	106 (3)
chlorfenapyr	1	96 (3)	98 (5)	98 (5)	107 (5)	104 (7)	108 (4)
chloroneb	2	97 (1)	95 (2)	106 (3)	102 (5)	102 (6)	102 (2)
clofentazine	3	94 (4)	105 (4)	91 (10)	113 (7)	103 (4)	109 (1)
clopyralid	9 [c]	89 (9)	90 (7)	**24** (7)	**41** (8)	**32** (**11**)	**20** (6)
cyprodinil	3	96 (1)	96 (3)	103 (6)	91 (2)	92 (2)	93 (2)
dichlormid	2	99 (1)	96 (2)	103 (1)	107 (1)	106 (1)	107 (2)
diclofop-methyl	1	96 (0)	96 (1)	99 (2)	100 (1)	101 (2)	101 (1)
dicloran	2	97 (1)	98 (0)	103 (3)	103 (1)	101 (1)	101 (1)
dimethomorph	3	87 (12)	96 (6)	86 (**18**)	102 (1)	109 (5)	98 (4)
diphenylamine	2	99 (0)	98 (1)	107 (2)	107 (1)	105 (1)	107 (1)
diuron	2	112 (8)	92 (2)	96 (9)	99 (3)	94 (5)	100 (9)
dodemorph	6	**76** (4)	**76** (3)	80 (1)	86 (2)	87 (1)	86 (2)
ethalfluralin	2	97 (2)	96 (2)	106 (4)	104 (2)	107 (4)	101 (3)
ethofumesate	1	99 (2)	92 (1)	110 (3)	107 (2)	105 (1)	109 (2)
ethoxyquin	2	101 (2)	**70** (3)	**76** (1)	**58** (0)	107 (0)	**2** (7)
etoxazole	3	101 (1)	95 (1)	110 (3)	105 (1)	109 (1)	108 (1)
etridiazole	2	98 (1)	95 (2)	102 (1)	105 (1)	103 (1)	105 (1)
famoxadone	3	90 (8)	97 (2)	**68** (**28**)	95 (3)	102 (3)	100 (4)
fenamidone	3	98 (1)	99 (2)	114 (4)	107 (1)	109 (2)	107 (2)
fenarimol	3	102 (2)	98 (1)	109 (2)	105 (1)	107 (1)	110 (1)
fenazaquin	3	92 (1)	92 (1)	93 (0)	83 (1)	84 (1)	85 (1)
fenhexamide	7	90 (5)	96 (1)	**74** (**12**)	105 (4)	97 (5)	102 (2)
fenoxaprop-ethyl	3	102 (3)	97 (2)	90 (4)	87 (1)	87 (4)	97 (2)
fipronil	1	93 (1)	98 (4)	101 (10)	106 (6)	107 (5)	106 (3)
fipronil sulfide	1	99 (4)	94 (2)	107 (1)	110 (2)	107 (5)	103 (7)
fipronyl desulfinyl	1	98 (1)	95 (3)	110 (4)	107 (3)	104 (3)	107 (3)
flonicamid	2	98 (3)	98 (4)	103 (3)	108 (3)	105 (3)	103 (2)
fludioxonil	1	95 (0)	96 (2)	90 (9)	97 (1)	99 (3)	99 (3)
flufenacet	1	98 (1)	95 (2)	102 (3)	102 (2)	107 (1)	105 (3)
flufenoxuron	2	99 (6)	104 (5)	106 (2)	105 (5)	96 (3)	100 (5)
fluopyram	1	99 (3)	94 (1)	101 (2)	102 (2)	104 (1)	103 (2)
fluridone	3	90 (**13**)	94 (4)	81 (**19**)	82 (**14**)	95 (**18**)	**74** (6)
fluroxypyr-meptyl	1	91 (10)	97 (4)	100 (10)	94 (3)	95 (5)	95 (3)
flutolanil	1	97 (0)	96 (0)	101 (2)	104 (0)	103 (1)	104 (1)
folpet	1	**79** (2)	80 (2)	80 (5)	86 (1)	85 (4)	91 (3)
hexazinone	1	96 (1)	94 (1)	95 (6)	101 (0)	101 (2)	100 (2)
imazalil	1	93 (5)	92 (3)	90 (9)	96 (3)	106 (2)	92 (7)
indoxacarb	3	103 (1)	98 (1)	101 (7)	107 (2)	108 (1)	109 (1)
iprodione	1	96 (7)	94 (**13**)	106 (7)	106 (8)	97 (**11**)	102 (7)
isoproturon	1	97 (6)	99 (**11**)	99 (6)	95 (**14**)	103 (8)	112 (5)
kresoxim-methyl	1	97 (1)	96 (1)	106 (1)	105 (0)	105 (1)	104 (1)
lactofen	3	96 (1)	100 (1)	102 (7)	108 (5)	111 (2)	107 (2)
linuron	1	98 (3)	94 (2)	98 (3)	102 (2)	103 (2)	102 (3)
metalaxyl	1	96 (2)	97 (3)	111 (2)	101 (3)	107 (3)	109 (2)
methoprene	1	94 (2)	88 (1)	101 (2)	99 (4)	101 (2)	99 (5)
metribuzin	1	95 (0)	96 (1)	106 (1)	106 (2)	107 (1)	105 (1)
napropamide	1	96 (1)	93 (2)	99 (3)	101 (1)	100 (2)	104 (2)
nitenpyram	2	88 (6)	89 (8)	117 (3)	109 (4)	106 (10)	106 (3)
norflurazon	1	94 (2)	91 (0)	88 (10)	101 (4)	103 (3)	101 (2)
oxadiazon	1	98 (2)	91 (2)	115 (4)	108 (2)	105 (1)	105 (3)
oxadixyl	1	96 (0)	95 (1)	103 (2)	104 (2)	103 (1)	101 (1)
oxyfluorfen	1	95 (3)	91 (5)	100 (3)	103 (2)	104 (1)	101 (4)
pendimethalin	1	94 (3)	89 (1)	96 (3)	103 (2)	101 (1)	100 (2)
penthiopyrad	1	97 (3)	96 (2)	98 (5)	107 (2)	103 (1)	104 (2)
o-phenylphenol	1	98 (1)	108 (9)	99 (6)	101 (4)	104 (2)	103 (3)
picoxystrobin	1	96 (3)	98 (2)	102 (2)	101 (3)	102 (3)	108 (3)
piperonyl butoxide	1	94 (2)	94 (1)	97 (3)	98 (1)	99 (1)	98 (2)
prochloraz	3	94 (2)	96 (2)	90 (4)	95 (4)	96 (10)	96 (3)
procymidone	1	99 (2)	93 (1)	107 (1)	102 (1)	101 (1)	105 (2)
propanil	1	96 (1)	96 (0)	96 (6)	101 (1)	100 (1)	100 (1)
propargite	1	103 (4)	94 (4)	108 (4)	99 (1)	103 (5)	108 (5)
propazine	2	96 (2)	95 (2)	109 (1)	106 (1)	107 (1)	105 (0)
propyzamide	2	98 (2)	98 (2)	105 (2)	108 (1)	107 (1)	107 (0)
pyraclostrobin	5	92 (4)	101 (2)	**55** (**19**)	**78** (5)	**71** (7)	**75** (1)
pyrazophos	3	99 (6)	94 (4)	91 (7)	**78** (4)	**77** (8)	83 (4)
pyridaben	3	97 (1)	97 (0)	104 (1)	105 (1)	105 (1)	105 (1)
pyrimethanil	2	96 (1)	95 (1)	98 (1)	97 (1)	95 (2)	95 (1)
pyriproxyfen	3	99 (1)	98 (1)	109 (0)	104 (1)	105 (1)	107 (1)
quizalofop-ethyl	3	93 (3)	95 (1)	**74** (**11**)	**78** (1)	78 (8)	81 (2)
spirodiclofen	3	92 (**13**)	87 (3)	115 (**13**)	115 (5)	107 (8)	96 (5)
spiromesifen	1	88 (1)	98 (2)	99 (1)	99 (4)	100 (4)	102 (2)
tebufenpyrad	3	100 (1)	96 (2)	111 (3)	102 (1)	107 (1)	107 (1)
tebuthiuron	2	97 (1)	96 (1)	102 (3)	105 (1)	105 (0)	106 (0)
terbuthylazine	2	99 (1)	98 (1)	106 (1)	107 (2)	106 (1)	105 (1)

(*continued on next page*)

Table 2 (*continued*)

Analyte	ISTD	Hemp*	Spinach	Avocado	Milk	Egg	Lamb
tetradifon	3	103 (1)	103 (2)	110 (4)	106 (2)	110 (4)	109 (4)
tetrahydrophthalimide	2	101 (1)	100 (3)	**121** (2)	115 (1)	118 (7)	112 (2)
thiamethoxam	1	100 (6)	87 (3)	109 (**15**)	**74** (**20**)	**125** (**19**)	107 (**18**)
thiobencarb	1	95 (1)	95 (0)	104 (1)	104 (0)	105 (1)	105 (1)
tolclofos-methyl	1	98 (1)	95 (1)	108 (3)	104 (0)	105 (1)	105 (2)
tralkoxydim	6	**56** (3)	**73** (4)	112 (**15**)	98 (2)	96 (4)	84 (4)
triallate	2	96 (1)	95 (2)	107 (2)	104 (2)	103 (1)	104 (1)
tridiphane	1	97 (2)	93 (3)	103 (2)	102 (2)	106 (3)	100 (2)
trifloxystrobin	1	98 (1)	96 (1)	100 (2)	102 (1)	103 (2)	104 (1)
triflumizole	1	97 (2)	93 (2)	100 (1)	103 (1)	104 (2)	102 (2)
trifluralin	2	96 (1)	96 (1)	106 (1)	106 (1)	104 (1)	107 (1)
vinclozolin	1	98 (0)	96 (3)	104 (3)	109 (2)	104 (3)	106 (2)
PCBs							
PCB 77	6	93 (6)	84 (7)	**73** (**11**)	**62** (5)	**60** (10)	**72** (8)
PCB 81	7	96 (3)	98 (4)	107 (10)	86 (6)	89 (2)	95 (3)
PCB 105	5	99 (3)	99 (4)	100 (3)	93 (4)	102 (4)	94 (6)
PCB 123+118	5	81 (3)	95 (1)	98 (3)	93 (3)	101 (6)	99 (3)
PCB 126	9	92 (3)	92 (4)	95 (6)	86 (3)	90 (**11**)	91 (3)
PCB 156+157	5	99 (3)	94 (1)	92 (1)	93 (1)	96 (4)	93 (2)
PCB 167	5	**77** (**11**)	96 (7)	98 (6)	97 (7)	94 (**11**)	93 (4)
PCB 169	7	**56** (7)	**69** (1)	**50** (2)	**35** (4)	**45** (8)	**41** (1)
PCB 170	5	92 (9)	100 (3)	99 (4)	99 (8)	105 (6)	96 (2)
PCB 180	5	81 (2)	94 (3)	100 (4)	107 (5)	92 (6)	97 (5)
PCB 189	5	98 (7)	94 (3)	92 (6)	83 (1)	86 (6)	92 (3)
PBDEs							
PBDE 28	5	100 (2)	100 (0)	102 (6)	102 (5)	98 (6)	105 (4)
PBDE 47	5	95 (6)	97 (3)	93 (8)	96 (6)	102 (10)	95 (2)
PBDE 99	3	94 (7)	88 (4)	108 (8)	92 (7)	102 (3)	82 (**11**)
PBDE 100	5	112 (7)	94 (4)	92 (5)	88 (6)	100 (3)	90 (5)
PBDE 153	5	93 (4)	97 (10)	100 (**19**)	**75** (9)	84 (**20**)	92 (**13**)
PBDE 154	5	81 (9)	83 (10)	86 (3)	**77** (10)	95 (**19**)	83 (7)
PBDE 183	7	115 (8)	100 (**16**)	**129** (**26**)	**177** (**38**)	**125** (**18**)	**140** (**26**)
PAHs							
acenaphthene	12	96 (2)	97 (1)	99 (1)	101 (1)	100 (1)	101 (1)
acenaphthylene	12	99 (1)	98 (1)	99 (1)	101 (1)	99 (1)	102 (0)
benz(a)anthrac(hrys)ene [2]	9	**35** (8)	**50** (1)	**39** (10)	**24** (7)	**37** (**16**)	**38** (7)
benzo(a)pyrene	10	99 (**11**)	97 (2)	**174** (**36**)	**152** (10)	**150** (**22**)	**122** (**21**)
benzo(bjk)fluoranthene	10	**137** (1)	**180** (1)	**191** (**21**)	**129** (8)	**138** (9)	**140** (4)
benzo(c)fluorene	9	82 (1)	89 (1)	80 (3)	**75** (3)	**77** (5)	84 (2)
benzo(ghi)perylene	11 [d]	107 (8)	115 (**13**)	85 (**75**)	**144** (**33**)	115 (**24**)	98 (**17**)
cyclopenta(cd)pyrene	9	**35** (**11**)	**50** (1)	**37** (**11**)	**26** (8)	**35** (**16**)	**40** (7)
dibenz(ah)anthracene	11 [d]	113 (**13**)	**123** (4)	**69** (**20**)	**159** (6)	103 (**20**)	106 (7)
dibenzo(ae)pyrene	10 [e]	**49** (**15**)	**45** (4)	**19** (2)	**78** (**12**)	**52** (**11**)	**24** (2)
dibenzo(ah)pyrene	10 [e]	**75** (**18**)	114 (**21**)	**21** (**13**)	**40** (**20**)	**28** (6)	**25** (10)
dibenzo(ai)pyrene	10 [e]	**66** (**18**)	88 (3)	**38** (**16**)	**57** (**15**)	**43** (**12**)	**48** (**18**)
dibenzo(al)pyrene	10	**78** (**13**)	**71** (1)	92 (**26**)	**170** (**16**)	**139** (**18**)	**137** (**13**)
fluoranthene	8	100 (1)	99 (0)	104 (2)	101 (1)	101 (1)	99 (1)
fluorene	2	94 (1)	96 (1)	99 (1)	98 (0)	96 (1)	99 (2)
indeno(1,2,3-cd)pyrene	11 [d]	111 (6)	109 (2)	**76** (**11**)	**161** (**12**)	94 (**20**)	92 (8)
naphthalene	13	97 (1)	99 (2)	106 (1)	100 (1)	104 (0)	103 (1)
phen(anthra)cene [3]	6	96 (1)	99 (3)	101 (1)	105 (1)	107 (2)	114 (5)
pyrene	9	109 (6)	111 (5)	**125** (6)	114 (8)	113 (4)	108 (4)

* 2 µL/s
[a] 7 in hemp
[b] 9 in spinach
[c] 10 in spinach
[d] 10 in avocado, milk, egg, and lamb
[e] 9 in avocado, milk, egg, and lamb;
[1] dicrotophos+monocrotophos
[2] benz(a)anthracene+chrysene
[3] phenanthrene+anthracene

the receiving vial (*e.g.* 1.8 mL) with flow rate >10 µL/s could be used in the scavenging µSPE mode (retain matrix), and larger load volumes could be used in the enrichment mode (retain analytes followed by elution with a different solvent).

Fig. 6 plots the number among 252 analytes and 13 ISTDs with average recoveries between 80 and 120% when normalized or not to an appropriate ISTD. The upper plot for hemp pellets compares 100-600 µL load volumes at 2 µL/s and the lower plot for spinach shows the effect of flow rate from 1-6 µL/s for 500 µL load volume. In the latter case of flow rate, the investigated range barely registered a difference in the overall results, and 5 µL/s was chosen for the final method because it reasonably balanced speed of elution with cleanup efficiency. With respect to load volume, 500 µL was chosen in the final method because no additional ISTD-normalized analytes fell within the 80-120% range using 600 µL load volume. Perhaps volumes up to 1.5 mL would have increased recoveries for some (but not all) of the remaining 16 analytes, but this would have resulted in significantly worse cleanup.

Another option involves subsequent addition of MeCN or another solvent to elute more analytes from the sorbents [1,8], but

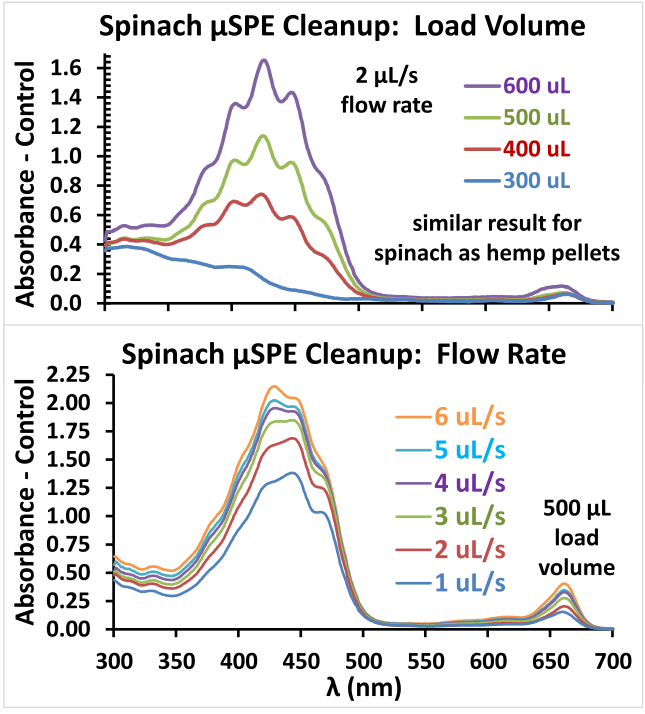

Fig. 5. Effect of load volume at 2 µL/s (top) and flow rate with 500 µL load volume (bottom) in the UV/Vis absorbance spectra of QuEChERSER extracts of spinach after µSPE cleanup. The absorbance background of MeCN served as the control for subtraction. See supplemental figures pp. 10–17 for spectra before and after cleanup and matrix removal efficiencies.

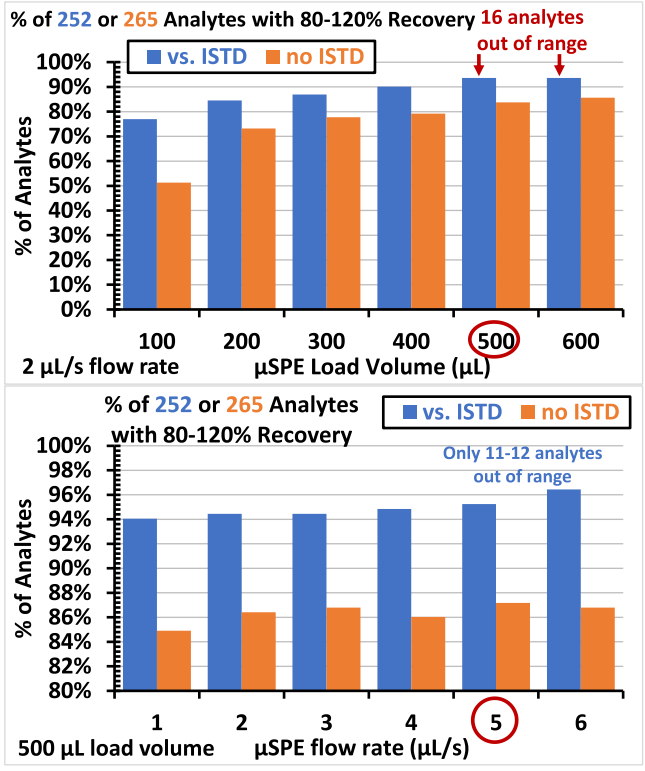

Fig. 6. Range of analyte recoveries in spiked QuEChERSER extracts of hemp pellets (top) and spinach (bottom) in µSPE with and without normalization to internal standards (ISTDs) depending on load volumes and flow rates, respectively. The individual analyte results using the final method conditions of 500 µL at 5 µL/s appear in Table 2.

this also worsens cleanup, complicates programming, dilutes final extracts, increases solvent consumption, and potentially leads to higher detection limits and slower sample throughput. Another option is to pass an air bubble from the syringe through the mini-cartridge to increase elution volume, but greater variability in that volume would likely result; it would be better to simply add 50 µL more load volume to more consistently yield the desired elution volume.

3.4.4. Choice of ISTDs

As shown in Fig. 6 (bottom), all but a dozen of the 252 analytes at the final conditions for spinach did not fall within the 80-120% range of average recoveries when normalized to an appropriate ISTD. Table 2 lists the ISTD used for each analyte and all recoveries and RSDs for the 6 matrices at the final µSPE conditions (except 2 µL/s for hemp pellets). The actual recoveries and RSDs of the ISTDs appear at the top of the table, and non-normalized recoveries of each analyte can be calculated by multiplying the listed result by the average recovery of the particular ISTD used.

As discussed previously for ITSP [2], the choice of ISTD for each analyte can be very important when elution is not complete from the mini-cartridge, which is also shown for µSPE in many supplemental figures (pp. 19-34). However, increasing elution volume from ≈220 µL in ITSP to ≈450 µL in µSPE led to complete elution of many more analytes (and ISTDs). This rendered the choice of ISTD unimportant for the vast majority of analytes, in which case malathion-d10, atrazine-d5, $^{13}C_{12}$-p,p'-DDE, pyridaben-d13, naphthalene-d8, or phenanthrene-d10 could have been used with little effect on the results. Nonetheless, the choice of ISTD still remained critical for certain analyte/matrix combinations as shown in Table 2. For example, $^{13}C_{12}$-PCB 153, FBDE 126, fluoranthene-d10, and pyrene-d10 averaged between 80 and 95% recovery at the final conditions, and depending on the commodity, their choice sometimes affected which analytes yielded acceptable normalized recoveries. These instances are footnoted in Table 2.

In the most extreme cases, the 5- and 6-ring PAHs, benzo(a)pyrene-d12 and benzo(ghi)perylene-d12, respectively, were strongly retained (>90%) by the 1 mg GCB in the mini-cartridges. These were used to compensate for the few highly-retained analytes, but the concern in those cases was that recoveries sometimes exceeded 120% due to the differences in the actual recoveries between the analyte and ISTD.

Alternatively, it could be better to compensate for actual validated recoveries for all analytes rather than normalize to ISTDs at all. This approach is common practice in some applications, such as an example of lipophilic analytes in QuEChERS of fatty matrices [29]. Although some regulatory chemists oppose this practice involving enforcement actions in food applications, compensation for known and consistent thoroughly validated recoveries due to physicochemical reasons (*e.g.* MeCN/water salt-out partitioning constants) is not only analytically justifiable, it is preferable. Even when normalization is made to an ISTD, it should ideally cover the full method of sample preparation and analysis, and validation should include extraction efficiencies for incurred samples in each commodity analyzed. Note that the recoveries in Table 2, Fig. 6, and all supplemental figures were isolated only to the µSPE step, whereas full method validation fell outside the scope of this study. QuEChERSER has previously been validated extensively using ITSP [18–21,23,24], and future studies will continue along this path involving diverse analytes and matrices using µSPE.

3.4.5. Partially retained analytes in µSPE

Although the automated cleanup step worked well for the vast majority of the 252 tested pesticides and environmental contaminants, some analytes were still partially retained by the sorbents at

the final conditions. The chemical structures of those and other interesting analytes often appear within the graphs in supplemental figures (pp. 19-34), and arrows and/or colors designate the functional groups that tend to more strongly interact with the PSA, C18, and/or GCB sorbents. As a weak anion exchanger, PSA tends to interact with hydroxyls and/or non-tertiary amines, often delaying elution of chemicals containing those functional groups, especially carboxylic acids such as clopyralid. Other examples of this retention mechanism appearing in supplemental figures include acephate (p. 23), ethoxyquin (p. 24), fenhexamide (p. 25), cyprodinil (p. 26), and tralkoxydim (p. 33). C18 tends to retain chemicals with long alkyl chains, including acequinocyl among the supplemental figures (p. 32).

The most sensitive of the retention mechanisms among the sorbents entails π-π bond interactions between GCB and structurally (co-)planar functional groups, typically involving analytes with cyclic aromatic rings. The µSPE mini-cartridges used in this study merely contain 1 mg of Carbograph-1 GCB, but certain pesticides, PCBs, and PAHs were highly retained depending on the number of co-planar rings in the molecules. For example, naphthalene consisting of 2-rings was essentially unretained, but nearly all of each 6-ringed benzoperylene was retained. For PCBs, those with symmetrical structures were co-planar, leading to greater interactions with the GCB than the unsymmetrical biphenyls in which the chlorine groups caused the rings to twist. PBDEs also have this same trait as PCBs, except the two rings are separated by an oxygen atom. The choice of the ISTD mostly compensated for those different degrees of retention, but sometimes the ideal ISTD for the particular analyte was not available.

In ITSP, the mini-cartridges contained 1 mg CarbonX [2], which gave somewhat different degrees of retention and cleanup than the GCB used in the µSPE mini-cartridges. Different sources and types of GCBs behave somewhat differently than others, and even different lots of the same brand have been known to affect the results for certain analyte/matrix combinations. Furthermore, the mixing of the sorbents prior to manufacturing of the mini-cartridges in each case do not produce perfectly homogeneous sorbent beds from one mini-cartridge to another. The GCB and CarbonX particles consist of a different size and shape profiles than the more uniformly distributed PSA and C18 particles, which reduce the homogeneity of the mixtures. This is also the case for anh. $MgSO_4$, but the effect of 19.5 *vs.* 20.5 mg (for example) of the salt powder makes little if any observable difference in results, as indicated by different degrees of $MgSO_4$ hydration among the mini-cartridges in this study. Similarly, $\pm$1 mg PSA and/or C18 relative to 12 mg each would not have much practical effect in the outcome. However, 0.9 *vs.* 1.1 mg GCB can lead to significantly different recoveries for the most sensitive analytes, and slightly different amounts of chlorophyll would also be removed from the extracts. Indeed, greater variability occurred in the cases of the PAHs, PBDEs, and PCBs than pesticides, as shown with respect to RSDs in bold text in Table 2.

To avoid this issue, GCB would not need to be added to the mini-cartridge product except for cleanup of green vegetable extracts. In terms of cleanup, the GCB is primarily effective for the removal of chlorophyll. GCB would not be included in the standard product for non-chlorophyll containing commodities, and a separate type of mini-cartridge containing 1 mg GCB would be used for cleanup of green vegetables. Analysis of the environmental contaminants is not important in those matrices anyway.

Despite the issues with GCB, the results using the full complement of sorbents for a wide range of analytes in all matrices was still mostly acceptable, as shown in Table 2. It is easier to use the same product for everything than to introduce multiple products that can confuse users. Although an excessive proliferation of products has been one of the drawbacks with QuEChERS over the years, this has also served as an advantage with respect to flexibility.

3.5. Final method results

The use of µSPE mini-cartridges at the final conditions for pre-GC cleanup in QuEChERSER for fatty and non-fatty foods yielded excellent results for nearly all of the 252 pesticides and environmental contaminants evaluated in this study. From the overall 1590 recovery results for each pair of analyte/matrix shown in Table 2, the normalized recoveries fell within the acceptable range (80-120%) in 91% of the cases. The results for the 214 pesticide analytes were exceptionally good except for the few instances noted by bold text in Table 2.

Hexachlorobenzene (HCB) served as a method suitability test analyte when using the ITSP mini-cartridge design, and at least 300 µL load volume was needed to yield 80% normalized recovery [2]. Using the final method with the µSPE design, HCB recovery was ≈80% even without normalization to an ISTD (see supplemental p. 31). For milk and egg matrices, HCB recoveries were slightly lower (≈78%) likely due to presence of co-extracted lipids.

The effect of GCB on the recovery of structurally planar analytes can more clearly be observed in the results of PAHs in Table 2. These type of molecules have rigid co-planar polyaromatic structures that strongly interact with GCB to yield low recoveries, particularly for analytes with ≥5 aromatic rings (*e.g.* benzopyrenes, benzoperylenes, dibenzopyrenes). However, smaller PAHs with ≤4 aromatic rings, such as anthracene, phenanthrene, naphthalene, and pyrene, yielded high normalized recoveries in all matrix types.

Independent of recoveries, the variabilities observed using the µSPE cartridges was usually very low throughout the study, often with RSDs ≤1% for many analyte/matrix combinations. Among the 1590 results in Table 2, 93.8% had RSDs <10%, and only 1.4% of them gave RSD >20%. Compared to ITSP, the higher degree of consistency observed for µSPE in this study was attributed to the larger and more uniform elution volumes associated with the septumless mini-cartridges.

4. Conclusions

The new µSPE mini-cartridge design evaluated in this study improved upon analytical performance while allowing faster cleanup with reduced chance of leaks or other common failures associated with automation. No stoppages in automation were observed in this or subsequent studies using the µSPE product for >250 samples in our lab thus far. Re-optimization experiments led to the choice of 500 µL load volume and 5 µL/s flow rate in µSPE, which exceeded the practical limits of 300 µL at 2 µL/s when using the ITSP design in the QuEChERSER application. The total time for cleanup at the final method conditions was 5.33 min per sample, which could be sped further if desired. This increased speed would be needed when the automated cleanup step is conducted in parallel with even faster LPGC-MS analysis [26,30].

The reduced dead (void) volume of the µSPE mini-cartridges in comparison to the ITSP design not only allowed greater load volumes but also provided excellent elution consistency. The sorbent mix combining anh. $MgSO_4$, PSA, C18 and GCB continued to provide exceptional cleanup for extracts from fatty and nonfatty matrices alike. Although GCB retained some co-planar analytes, such as >4-ring PAHs, the higher load volume and flow rate with the µSPE design, plus normalization to an appropriate ISTD, helped to overcome potential losses. Acceptable 80-120% recoveries were obtained for 90-96% of the 252 pesticides and environmental contaminants, depending on matrix, and RSDs were typically <5%, with <10% RSDs achieved for 94% of the 1590 analyte/matrix pairs in

the study. The cleanup provided using the septumless µSPE mini-cartridges proved to be an efficient and effective alternative in automated SPE cleanup in the QuEChERSER sample preparation approach, meeting the needs of routine high-throughput food analysis. Independent of detection, the final µSPE extracts have 0.25 or 1 g/mL sample equivalent in QuEChERSER or QuEChERS, respectively, which led to detection limits <5 ng/g in this study, as well as others [2,4–6,18–24] using LPGC-MS/MS for nearly all analytes and matrices.

Credit author statement

Both authors contributed to the conception, execution, and reporting of this work.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2022.463596.

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