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Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables☆

Steven J. Lehotay[a,*], Kyung Ae Son[b], Hyeyoung Kwon[b], Urairat Koesukwiwat[a,c], Wusheng Fu[d], Katerina Mastovska[a,1], Eunha Hoh[a,2], Natchanun Leepipatpiboon[c]

[a] US Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Ln., Wyndmoor, PA 19038, USA
[b] Rural Development Administration, National Academy of Agricultural Science, 249 Seodun-dong, Gweonsun-gu, Suwon 441-707, South Korea
[c] Chromatography and Separation Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
[d] Fujian Center for Disease Control and Prevention, 76 Jin Tai Rd., Fuzhou 350001, China

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ABSTRACT

This article describes the comparison of different versions of an easy, rapid and low-cost sample preparation approach for the determination of pesticide residues in fruits and vegetables by concurrent use of gas and liquid chromatography (GC and LC) coupled to mass spectrometry (MS) for detection. The sample preparation approach is known as QuEChERS, which stands for "quick, easy, cheap, effective, rugged and safe". The three compared versions were based on the original unbuffered method, which was first published in 2003, and two interlaboratory validated versions: AOAC Official Method 2007.01, which uses acetate buffering, and European Committee for Standardization (CEN) Standard Method EN 15662, which calls for citrate buffering. LC–MS/MS and GC–MS analyses using each method were tested from 50 to 1000 ng/g in apple–blueberry sauce, peas and limes spiked with 32 representative pesticides. As expected, the results were excellent (overall average of 98% recoveries with 10% RSD) using all 3 versions, except the unbuffered method gave somewhat lower recoveries for the few pH-dependent pesticides. The different methods worked equally well for all matrices tested with equivalent amounts of matrix co-extractives measured, matrix effects on quantification and chemical noise from matrix in the chromatographic backgrounds. The acetate-buffered version gave higher and more consistent recoveries for pymetrozine than the other versions in all 3 matrices and for thiabendazole in limes. None of the versions consistently worked well for chlorothalonil, folpet or tolylfluanid in peas, but the acetate-buffered method gave better results for screening of those pesticides. Also, due to the recent shortage in acetonitrile (MeCN), ethyl acetate (EtOAc) was evaluated as a substitute solvent in the acetate-buffered QuEChERS version, but it generally led to less clean extracts and lower recoveries of pymetrozine, thiabendazole, acephate, methamidophos, omethoate and dimethoate. In summary, the acetate-buffered version of QuEChERS using MeCN exhibited advantages compared to the other tested methods in the study.

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1. Introduction

In 2003, Anastassiades et al. described the "quick, easy, cheap, effective, rugged and safe" (QuEChERS) method for the multiclass, multiresidue analysis of pesticides in fruits and vegetables [1]. The authors questioned the typical conditions previously used for pesticide residue analysis, and through extensive experimentation and novel use of $MgSO_4$ for salting out extraction/partitioning and dispersive solid-phase extraction (d-SPE) for cleanup, they devised a highly streamlined sample preparation method with excellent results for a wide range of pesticide analytes in many types of foods [1]. Unlike many previous methods developed for traditional chromatographic detection systems (e.g. UV/vis absorbance, fluorescence, element-selective detectors), the QuEChERS approach takes advantage of the wide analytical scope and high degree of selectivity and sensitivity provided by gas and liquid chromatography (GC and LC) coupled to mass spectrometry (MS) for detection. GC–MS and LC–MS(/MS) have become the main analytical tools in most pesticide monitoring laboratories to meet world standards,

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* Corresponding author. Tel.: +1 215 233 6433; fax: +1 215 233 6642.
E-mail address: steven.lehotay@ars.usda.gov (S.J. Lehotay).
[1] Current address: Covance Laboratories, 671 South Meridian Rd., Greenfield, IN 46140, USA.
[2] Current address: Graduate School of Public Health, San Diego State University, San Diego, CA 92182, USA.

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thus the streamlined features, practical benefits and excellent results provided by the QuEChERS sample preparation approach combined with GC–MS and LC–MS/MS have helped lead to the great popularity of QuEChERS concepts. At the time of writing, there are more than 10 companies marketing QuEChERS products and the original paper [1] has been cited in the literature >210 times according to the ISI Web of Knowledge citation index [2].

A limited number of GC-amenable pesticides was evaluated in the original QuEChERS study and although this version has been demonstrated to yield excellent results for hundreds of pesticides in dozens of commodities [1,3–5], subsequent experiments showed some pesticides gave lower stability and/or recoveries depending on pH of the matrix [3,6,7]. The original authors of the QuEChERS approach realized that buffering at pH-5 during extraction gave the optimum balance to achieve acceptably high recoveries (>70%) for certain pH-dependent pesticides (e.g. pymetrozine, imazalil, thiabendazole) independent of the fruit/vegetable matrix [6,7]. Lehotay et al. modified the method to use relatively strong acetate buffering conditions [6] and Anastassiades et al. chose to use weaker citrate buffering conditions [7] in terms of ionic strength. Both versions of these methods went through extensive interlaboratory trials entailing ≈50,000–100,000 data points for dozens of pesticides at fortified and incurred at different levels in different matrices and using different types of GC–MS and LC–MS/MS conditions and instruments. Both methods successfully met statistical criteria for acceptability from independent scientific standards organizations, with the acetate-buffering version becoming AOAC Official Method 2007.01 [8] and the citrate-buffering version being named European Committee for Standardization (CEN) Standard Method EN 15662 [9].

The QuEChERS approach is very flexible and it serves as a template for modification depending on the analyte properties, matrix composition, equipment and analytical technique available in the lab. The template is also very rugged in that high recoveries will be achieved for many pesticides in many matrices even if different ratios and types of sample size, solvent, salts and sorbents are used in modifications. The ruggedness characteristics of the QuEChERS approach have been thoroughly evaluated in the original [1] and subsequent publications by the originators [3–18]. In multiclass, multiresidue pesticide analysis, the sample preparation method inherently necessitates broad analytical scope which makes it impossible to obtain a high degree of cleanup without reducing recoveries for some pesticides. However, greater cleanup can be achieved by using different sorbents in d-SPE if the application has reduced analytical scope.

Reviews of QuEChERS are starting to appear in the literature [15,19] and the original method has evolved into a flexible template for modification in several applications. In addition to pesticide residue analysis in foods [1,3–76], QuEChERS concepts (including d-SPE) have been used for acrylamide [77,78], clinical [79,80], veterinary drug residue [81–90], food quality [91], supplement testing [92], perfluorinated compounds [93,94], polycyclic aromatic hydrocarbons [95], alkaloids [96], environmental [97–100] and mycotoxin [101] analytical applications. Mol et al. developed a universal sample preparation approach for all kinds of chemical contaminants in foods and feeds and QuEChERS concepts contributed to their proposed approach [102].

Due to the great flexibility of the QuEChERS approach, there are so many permutations that vendors of QuEChERS products have difficulties in providing products to meet all the demands (weighing powders in the lab is time-consuming and has higher potential for contamination). The primary application of QuEChERS is for multiclass, multiresidue analysis of pesticides in fruits and vegetables and as part of a training exercise in the USDA lab, we decided to conduct a comparison study to determine if one of the three QuEChERS approaches that have been evaluated among multiple labs [3,8,9] gave more suitable performance for the food commodities in this study. Not only would we compare trueness and precision of results, but also evaluate analyst performance and matrix co-extractives in terms of their amount, effects on quantification and analyte detection interferences. Due to a recent worldwide shortage of acetonitrile [103], we also decided to conduct additional experiments to ascertain if ethyl acetate could be substituted without other changes in the method.

2. Experimental

2.1. Materials

The selected representative matrices consisted of apple–blueberry sauce (a mix of common fruits), peas (a green vegetable) and limes (a citrus fruit), which we purchased from a local organic food store. The 32 representative pesticides for study consisted of acephate, atrazine, azoxystrobin, carbaryl, cis-chlordane, chlorothalonil, chlorpyrifos, chlorpyrifos-methyl, coumaphos, cyprodinil, diazinon, dichlorvos, dimethoate, endosulfan sulfate, ethoprop, folpet, heptachlor, imazalil, imidacloprid, linuron, methamidophos, methomyl, mevinphos, omethoate, permethrin, phosalone, phosmet, procymidone, pymetrozine, tebuconazole, thiabendazole and tolylfluanid. The analytes have diverse properties and several of them are particularly challenging to analyse (e.g. chlorothalonil, folpet, tolylfluanid). The tebuconazole was added during sample comminution in the chopper to evaluate subsampling homogeneity. The internal standards (added prior to extraction) used for QuEChERS sample preparation were atrazine, diazinon and procymidone. Triphenylphosphate (TPP) was added to all final extracts as a quality control measure for the GC–MS and LC–MS/MS analytical steps.

All pesticide standards were high purity obtained from the Environmental Protection Agency's National Pesticide Repository (Fort Meade, MD, USA), Chemservice (West Chester, PA, USA), or Dr. Ehrenstorfer GmbH (Augsburg, Germany). Stock solutions of 2000 ng/μL were prepared in acetonitrile (MeCN) containing 0.1% acetic acid (HOAc) or toluene. MeCN and ethyl acetate (EtOAc) were obtained from Burdick & Jackson (Muskegon, MI, USA) and toluene from Sigma–Aldrich (St. Louis, MO, USA), were all HPLC-grade or better quality. Ultrapure water from a Barnstead (Dubuque, IA, USA) water purification system was used for preparing the LC mobile phase and other aqueous solutions. Glacial HOAc (ACS reagent grade) and double distilled formic acid (88% purity) were obtained from J.T. Baker (Phillipsburg, NJ, USA) and GFS Chemicals (Columbus, OH, USA), respectively. The purity was not taken into account when making acid solutions, thus the % indicates the volume fractions of acid solutions (e.g. 1% HOAc in MeCN was prepared by mixing 10 mL glacial HOAc with 990 mL MeCN). We prepared 5N sodium hydroxide (NaOH) solution for use with the limes in CEN method EN 15662.

QuEChERS materials were obtained from commercial suppliers. For the initial extraction step, we used Restek (Bellfonte, PA, USA) RESPREP Q150 for the acetate-buffered version, which consisted of 50 mL plastic centrifuge tubes containing 6 g anh. magnesium sulfate ($MgSO_4$) plus 1.5 g anh. sodium acetate (NaOAc). Restek RESPREP Q110 for CEN Standard Method EN 15662 consisted of 50 mL plastic centrifuge tubes containing 4 g anh. $MgSO_4$, 1 g sodium chloride (NaCl), 1 g trisodium citrate dihydrate ($Na_3Cit{\cdot}2H_2O$) and 0.5 g disodium hydrogencitrate sesquihydrate ($Na_2HCit{\cdot}1.5H_2O$). For the original method, we used UCT (Bristol, PA, USA) product ECMSSC which consisted of 50 mL plastic centrifuge tubes containing 4 g anh. $MgSO_4$ and 1 g NaCl.

For cleanup of pea and lime extracts in the citrate-buffered version, we used Restek Resprep Q213, which consisted of 2 mL

mini-centrifuge tubes containing 150 mg anh. $MgSO_4$, 25 mg primary secondary amine (PSA) sorbent and 7.5 mg graphitized carbon black (GCB). For apple–blueberry sauce in all methods and for all matrices with the unbuffered and acetate-buffered versions, we used Restek Resprep Q251 (2 mL mini-centrifuge tubes containing 150 mg anh. $MgSO_4$, 50 mg PSA and 50 mg C_{18}).

For the experiment to compare the use of MeCN with EtOAc (both containing 1% HOAc) in the acetate-buffered method, we repeated the full experiment using both solvents for the 3 matrices with 4 replicates each at 3 spiking levels, but used Waters (Milford, MA, USA) DisQuE product, which consisted of 50 mL tubes containing 6 g anh. $MgSO_4$ plus 1.5 g anh. NaOAc and 2 mL tubes containing 150 mg anh. $MgSO_4$ plus 50 mg PSA sorbent for d-SPE cleanup.

We prepared standard solutions of 2 ng/μL TPP in 0.5% formic acid in MeCN (quality control spike for the analytical step); 200 ng/μL tebuconazole in MeCN (subsample homogeneity spike); and spiking solutions of 5 ng/μL, 25 ng/μL and 100 ng/μL for the 50 ng/g, 250 ng/g and 100 ng/g sample spikes, respectively, of the 28 pesticide analytes in MeCN plus a constant 20 ng/μL each of atrazine, diazinon and procymidone (internal standards) to yield 200 ng/g. Calibration spike solutions were prepared in 0.05% formic acid in MeCN to each contain 2 ng/μL of the internal standards plus 0.25 ng/μL, 0.5 ng/μL, 2.5 ng/μL and 10 ng/μL of the other 29 pesticides (including tebuconazole). These solutions yielded 25 ng/g, 50 ng/g, 250 ng/g and 1000 ng/g equivalent concentrations in the calibration standards in matrix and solvent-only for both GC–MS and LC–MS/MS, with 200 ng/g equivalent concentrations of the internal standards. The formic acid in the MeCN solutions was included to improve stability of base-sensitive pesticides [103]. Furthermore, all solutions were stored in dark glass vials in the freezer when not in use.

2.2. Apparatus and conditions

For GC–MS, a Leco (St. Joseph, MI, USA) Pegasus time-of-flight (TOF) MS instrument coupled with an Agilent (Wilmington, DE, USA) 6890 GC was used. An Atas (Veldhoven, The Netherlands) Optic 3 programmable temperature vaporizer served as the GC injector for 10 μL injection of the final extracts into sintered glass liners. A 5 m integrated guard column was coupled to the 20 m, 0.25 mm i.d., 0.25 μm film thickness Restek Rtx 5-MS analytical column and as the transfer line, we used a 1.5 m, 0.1 mm i.d., 0.1 μm film thickness Restek CLP-Pesticides2 column (the instrumental set-up was capable of 2-dimensional GC, but this feature was not used in this study). The Leco ChromaTOF software (version 3.22) enabled instrument control and data processing, including a feature to calculate the equivalent column dimensions for constant flow control when the columns of different dimensions were coupled. The injector program started at 75 °C for 18 s, which was ramped at 8 °C/s to 280 °C where it was held for 8 min (and then set at 250 °C). A pressure pulse of 3 mL/min with split vent closed for 2 min was used and then column flow was 1.5 mL/min (the split vent was opened at 50:1 split ratio for 2 min and then 20:1) until the end of the run. Ultrahigh purity He was the carrier gas. The GC oven program was 60 °C initial oven temperature for 2 min, 20 °C/min ramp rate–180 °C, 5 °C/min–230 °C, 20 °C/min–280 °C, 40 °C/min–300 °C where it was held for 12 min. The transfer line temperature was 280 °C; 250 °C ion source; −70 eV filament setting; 10 spectra/s data collection; 390 s filament delay. The same GC–MS conditions were employed whether MeCN or EtOAc extracts were injected. A table of GC–MS retention times and the quantitation ions are given in supplementary information.

For LC–MS/MS, an Applied Biosystems (Toronto, ON, Canada) API-3000 triple quadrupole MS/MS with electrospray ionization (ESI) in the positive mode coupled to an Agilent 1100 LC, which includes a binary pump, column temperature control and autosampler, were used in the study. Applied Biosystems Analyst 1.5 software provided instrument control and data collection. The analytical column was a Phenomenex (Torrance, CA, USA) Prodigy ODS-3, 5 μm particles, 150 mm long and 3 mm i.d., which was integrated with a 4 mm long and 3 mm i.d. Security Guard ODS C18 column (kept at 30 °C in the method). Injection volume was 20 μL and flow rate was 0.3 mL/min. Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in MeCN. The gradient program was 70% A from the start ramped to 100% B over the course of 8 min and held until 13.5 min. A Valco (Houston, TX, USA) Model EHMA solenoid-driven divert valve was used before the MS to avoid introduction of the early (<1.4 min) and late-eluting (>13 min) non-analyte components into the detector.

The MS/MS conditions were optimized using direct infusion into the ESI source in positive mode to provide highest signal/noise ratio for the quantification ion of each analyte. A second MS/MS transition was made in case chemical interferences were observed in the quantitation ion chromatogram and for qualitative purposes. The source temperature was 550 °C, ion spray potential was 4500 V and entrance potential was 10 V. All transitions had dwell times of 50 ms in the method. A table of LC–MS/MS retention times and ion transitions is given in supplementary information.

A Robotcoupe (Ridgeland, MS, USA) RSI 2Y1 chopper was used to comminute samples and a Sorvall® Legend RT (Kendro, Osterode, Germany) was used for centrifugation. Other items needed for experiments included analytical and top-loading balances, a freezer, pipettes, solvent dispensers, graduated cylinders, spatulas, funnels, 50 mL polypropylene centrifuge tubes, gloves, beakers, filters and vials.

2.3. Methods

We prepared 750 g each of apple–blueberry sauce, limes and peas separately in the chopper on the first day of experiments with each commodity. The apple–blueberry sauce was mixed at room temperature, the peas were homogenized while frozen (no dry ice added) and the limes were cut into quarters, frozen and then comminuted using dry ice. To measure processing recovery and subsample homogeneity, 0.75 mL of 200 ng/μL tebuconazole was added to the samples during the comminution step. Approximately 250 g were transferred to 3 sealable containers (bags or bottles) for each sample type. One subsample was used for extraction on the same day and the other two were stored in the freezer until they were extracted by the different method versions 1–2 days later.

The experiments were done in the following order: Days 1–3: apple–blueberry sauce by the unbuffered, citrate-buffered and acetated-buffered versions of QuEChERS, respectively; Days 4–6: peas by the acetate-buffered, citrate-buffered and unbuffered QuEChERS methods, respectively; and Days 7–10: limes by the citrate-buffered, acetate-buffered and unbuffered versions, respectively. The GC–MS and LC–MS/MS analyses were conducted concurrently overnight on each day of sample preparation procedures.

In the study, 4 replicate spikes each at 50 ng/g, 250 ng/g, and 1000 ng/g were made (each trainee chemist was responsible for one spike at each level) and 1 mL extracts were taken for d-SPE in all cases. Calibration stds in solvent-only solutions and in blank matrix extracts were prepared at 25 ng/g, 50 ng/g, 250 ng/g and 1000 ng/g equivalents for analysis. The sequence of samples for each instrument and day was as follows: (1) 25 ng/g std in solvent, (2) 25 ng/g std in matrix, (3–6) 50 ng/g spikes A–D, (7) 50 ng/g std in matrix, (8) 50 ng/g std in solvent, (9) 250 ng/g std in solvent, (10) 250 ng/g std in matrix, (11–14) 250 ng/g spikes D–A, (15) matrix blank, (16–19) 1000 ng/g spikes A–D, (20) 1000 ng/g std in matrix, (21) 1000 ng/g std in solvent and (22) reagent blank. The first injection in each sequence also served as a system suitability check and

instrument maintenance (changing of the liner and cutting 50 cm from the guard column in GC–MS and rinsing the shield plate in LC–MS/MS) was conducted before the first sequence of each new matrix. The last injection was used to check for analyte carry-over from the high standard injection.

2.3.1. QuEChERS methods protocols

(1) Appropriately label all tubes and vials needed for the analysis: matrix and reagent blanks, 50 ng/g, 250 ng/g and 1000 ng/g spikes (4 replicates each) and 4 extra d-SPE tubes and autosampler vials for calibration standards in matrix and MeCN-only (25 ng/g, 50 ng/g, 250 ng/g and 1000 ng/g equivalents for both GC–MS and LC–MS/MS analyses).
(2) Place an empty, uncapped 50 mL polypropylene centrifuge tube in a styrofoam rack on a top-loading balance.
(3) For citrate-buffered and unbuffered versions, add 10.0 ± 0.1 g of thoroughly comminuted sample into the labeled centrifuge tube. For limes with the citrate-buffered method, add 0.6 mL of 5 N NaOH aqueous solution. For the acetate-buffered version, add 15.0 ± 0.1 g. For reagent blanks, use 10 mL or 15 mL ultrapure water as the sample.
(4) Add 100 μL (citrate-buffered and unbuffered) or 150 μL (acetate-buffered) of the appropriate spiking solutions to the samples. Add 100 μL or 150 μL MeCN to the blanks. Cap the tubes well and vortex for 1 min. Allow 15 min to let the pesticides better integrate into the samples.
(5) For the citrate-buffered and unbuffered versions, add 10 mL MeCN to each sample in the tubes. For the acetate-buffered version, add 15 mL of 1% HOAc in MeCN. Cap the tubes well and shake vigorously by hand for 30 s.
(6) Pour the mixed extract to the appropriate tube containing 4 g anh. $MgSO_4$ + 1 g NaCl (unbuffered version); 6 g anh. $MgSO_4$ + 1.5 g NaOAc (acetate-buffered version); or 4 g anh. $MgSO_4$ + 1 g NaCl + 1 g $Na_3Cit{\cdot}2H_2O$ + 0.5 g $Na_2HCit{\cdot}1.5H_2O$ (citrate-buffered version).
(7) Seal all the tubes well and shake the tubes vigorously by hand for 1 min ensuring that the solvent interacts well with the entire sample and that crystalline agglomerates are broken up sufficiently during shaking.
(8) Centrifuge the tubes at 3450 rcf for 2 min at room temperature. For the lime samples in the citrate-buffered method, place the tubes in the −20 °C freezer for 1 h, then remove the top layer of "oil/wax" with a Pasteur pipet. Let the extract reach room temperature.
(9) Transfer 1 mL of the extracts (upper layer) to the appropriate d-SPE tubes. For all apple–blueberry extracts and for peas and limes in the unbuffered and acetate-buffered versions, use 50 mg PSA + 50 mg C_{18} + 150 mg anh. $MgSO_4$. For the citrate-buffered version for peas and limes, use 25 mg PSA + 7.5 mg GCB + 150 mg anh. $MgSO_4$. For matrix blanks, transfer five 1 mL aliquots to 5 different d-SPE tubes (matrix blank plus 4 matrix-matched calibration standards).
(10) Seal the tubes well and shake vigorously or vortex for 30 s.
(11) Centrifuge the d-SPE tubes at 3450 rcf for 2 min at room temperature.
(12) Transfer 0.5 mL of extracts to the autosampler vials, except for the matrix blanks, which are first combined into a small beaker and then 0.5 mL aliquots are transferred to vials.
(13) Transfer 0.5 mL of MeCN to 4 vials for the solvent-only calibration standards.
(14) Add 50 μL of 2 ng/μL TPP in 0.5% formic acid in MeCN solution to all vials.
(15) Add 50 μL of calibration standard spiking solutions to the appropriate vials for matrix-matched and solvent-only standards.
(16) Add 50 μL of MeCN to the spiked sample extracts and matrix and reagent blanks.
(17) Cap all vials and shake thoroughly to mix.
(18) Transfer 200 μL of each solution to a 2nd set of appropriately-labeled vials with low-volume inserts for GC–MS analysis.
(19) Add 1 mL of 0.1% formic acid solution in water to the 0.4 mL extract remaining in the 1st set of vials for LC–MS/MS analysis.

Note: for the limes in all cases and peas in the citrate-buffered method, a precipitate appeared in the LC extracts after the 0.1% formic acid solution was added. The lime extracts were filtered through 0.45 μm PVDF filters (Mini-UniPrep vials, Whatman, Florham Park, NJ, USA), and centrifugation was able to remove the precipitate in the case of peas.

For the EtOAc comparison experiments, we first repeated the MeCN (containing 1% HOAc) extractions for all 3 matrices and replicates as before, but used Waters DisQuE products. Then, we repeated the full experiment again except substituted MeCN with EtOAc (including 1% HOAc) in the acetate-buffered method. For LC–MS/MS, the 0.4 mL EtOAc extract in the vial was evaporated with nitrogen to just dryness and then 1.4 mL of mobile phase A solution was added.

A detailed Excel spreadsheet template was prepared in which the integrated peak areas were cut and pasted into the appropriate cells from the instrument data files. The recoveries with and without use of an internal standard (diazinon) were calculated by direct proportional comparison with the matrix-matched calibration standard at the given spiking level (50 ng/g, 250 ng/g and 1000 ng/g). Least linear squared calibration plots were also calculated in matrix and solvent-only to determine matrix effects. All results were compiled through linkage to a second Excel spreadsheet file, in which the recoveries were split into different categories depending on method, matrix, spiking level and analyst. No results were removed from the data compilation even if statistical outliers may have occurred (certain pesticides in limes could not be analysed by GC–MS).

3. Results and discussion

Analytical chemists have a common saying that, "Analytical methods are like toothbrushes, everybody uses their own." As evidenced in the literature [1–101], there are many different permutations of the QuEChERS approach, some of which serve a useful purpose to improve results or practical efficiency for the given analyte(s)/matrix(es) applications, but some others have differences only due to personal preferences. The 3 versions of QuEChERS we compare in this study particularly stand out because they have been extensively evaluated in many labs for a wide range of pesticides in many fruits and vegetables [1,3–9]. Commercial products from at least 10 vendors are available for these 3 different versions, to further simplify use of the approach in routine practice.

In this study, the 3 versions of QuEChERS were compared as part of a training course to demonstrate the differences in the methods and to answer the simple question: "Which version is better?" It adds to the interest of the participants (and instructors) and value of the training program to perform useful experiments at the same time as provide training. In a previous training course, the host laboratory conducted QuEChERS experiments for participant-requested pesticide/matrix combinations in which different ways to address LC–MS/MS matrix effects were also investigated [18]. To ensure validity of the comparison in the current study, the experiments were planned and conducted carefully, systematically and consistently. To help ensure high quality results, the 4 analyst trainees competed for a reward to the person who achieved the highest accuracy. Peak integrations in GC–MS and LC–MS/MS were done

Table 1
Compilation of average pesticide recovery results in LC–MS/MS and GC–MS for the 3 versions of QuEChERS spiked at 50 ng/g, 250 ng/g and 1000 ng/g (4 replicates, one per analyst, at each level) in apple–blueberry sauce, peas, and limes.

Pesticide		Ave. %recovery (n = 36)			%RSD (n = 36)			Analyst %RSD (n = 9)			
		Original	CEN	AOAC	Original	CEN	AOAC	A	B	C	D
Acephate	LC	87	90	94	8	7	7	10	8	6	7
Acephate[a]	GC	86	92	92	14	8	18	17	12	11	14
Atrazine[b]	LC	94	90	96	10	7	13	n/a	n/a	n/a	n/a
Atrazine[b]	GC	89	89	88	13	7	5	n/a	n/a	n/a	n/a
Azoxystrobin	LC	104	103	112	8	7	11	10	10	11	7
Azoxystrobin[a]	GC	105	104	106	10	14	16	13	19	10	10
Carbaryl	LC	102	100	105	6	5	9	9	8	6	6
Carbaryl	GC	109	101	102	14	12	18	19	13	15	12
Chlorothalonil	**GC**	**73**	**61**	**91**	**56**	**64**	**57**	**72**	**59**	**56**	**50**
Chlorpyrifos	LC	98	100	100	9	16	16	15	14	15	10
Chlorpyrifos	GC	101	99	98	9	8	6	8	9	8	6
Chlorpyrifos-Methyl	LC	95	102	107	10	9	15	13	13	12	11
Chlorpyrifos-methyl	GC	103	100	101	9	6	4	7	7	7	5
Cis-chlordane	GC	97	97	93	9	8	7	8	10	8	7
Coumaphos	GC	110	104	104	15	11	11	10	15	12	15
Cyprodinil	LC	98	93	100	6	9	8	7	6	10	10
Cyprodinil	GC	102	96	95	12	9	9	10	11	10	10
Diazinon[b]	LC	92	97	87	6	6	9	n/a	n/a	n/a	n/a
Diazinon[b]	GC	103	111	102	15	19	16	n/a	n/a	n/a	n/a
Dichlorvos	LC	100	99	104	8	5	7	9	8	6	5
Dichlorvos	GC	98	96	106	16	19	12	17	19	12	15
Dimethoate	LC	101	99	104	7	5	8	8	8	7	4
Dimethoate	GC	104	103	100	12	8	12	12	9	12	9
Endosulfan sulfate	GC	106	102	103	14	12	13	13	14	11	14
Ethoprop	LC	99	101	104	7	7	9	9	9	8	5
Ethoprop	GC	102	100	100	6	6	4	7	6	5	4
Folpet	**GC**	**66**	**63**	**69**	**72**	**82**	**56**	**75**	**79**	**61**	**68**
Heptachlor	GC	95	96	94	9	7	5	8	8	8	5
Imazalil	LC	95	92	92	9	12	9	11	11	9	10
Imazalil[a]	GC	91	96	86	17	12	12	12	16	16	13
Imidacloprid	LC	98	99	103	6	6	7	8	8	7	5
Linuron	LC	100	101	104	6	6	8	8	8	7	5
Methamidophos	LC	80	85	88	8	10	8	9	14	9	8
Methamidophos[a]	GC	80	83	85	10	10	11	14	9	8	11
Methomyl	LC	100	100	101	6	6	8	8	8	7	5
Mevinphos	GC	111	99	100	21	13	18	16	22	13	20
Omethoate	LC	91	90	92	10	6	7	10	8	7	6
Omethoate[a]	GC	90	100	94	12	13	25	24	18	15	15
Permethrin	GC	102	101	95	14	13	9	11	15	11	11
Phosalone	GC	109	105	103	13	13	12	12	15	12	11
Phosmet	LC	103	100	107	5	5	7	7	6	7	5
Phosmet	GC	110	106	103	13	12	16	12	14	13	15
Procymidone[b]	GC	99	98	98	7	10	5	n/a	n/a	n/a	n/a
Pymetrozine	LC	**31**	**34**	82	**100**	**51**	7	**65**	**63**	**65**	**62**
Tebuconazole[c]	LC		102			8		n/a	n/a	n/a	n/a
Tebuconazole[c]	GC		88			13		n/a	n/a	n/a	n/a
Thiabendazole	LC	84	78	85	24	19	16	20	19	24	18
Thiabendazole[a]	GC	89	91	94	13	15	16	12	16	16	15
Tolylfluanid	**LC**	**63**	**60**	**76**	**69**	**71**	**50**	**67**	**65**	**63**	**58**
Tolylfluanid	**GC**	**71**	**63**	**66**	**70**	**70**	**57**	**68**	**68**	**61**	**68**
TPP[d]	LC		n/a			8		n/a	n/a	n/a	n/a
TPP[d]	GC		n/a			12		n/a	n/a	n/a	n/a
Overall average		97.9	97.4	98.3	10.6	9.7	10.9	11.5	11.7	10.3	9.5
Average LC		95.8	95.8	99.2	8.4	8.1	9.6	10.1	9.7	9.2	7.4
Average GC		99.6	98.8	97.6	12.4	11.0	11.6	12.6	13.2	11.1	11.3

n/a = not applicable.
Diazinon was used as the internal standard in both LC–MS/MS and GC–MS. The combined %RSD results are also given for each analyst, which were averaged excluding values in bold text (recoveries that varied depending on matrix).

[a] GC results for limes were excluded (n = 24).
[b] Internal standard recovery at 200 ng/g not corrected for diazinon.
[c] Combined result for tebuconazole, which was added at 200 ng/g during the chopping step (n = 108).
[d] TPP was added at 200 ng/g to all standards and extracts prior to the analytical step (n = 153).

alternately by the trainees paired in groups and Excel spreadsheets of calculations and compiled results were shared with input and review by all involved.

The different versions were compared through different empirical means. The main test of accuracy in the residue analysis entailed measurement of trueness through recovery experiments and precision from replication (intraday and interday). Other aspects included analytical scope and concentration range, which were assessed by the range of analytes and matrices included in spikes at different concentration levels. Practical issues of sample throughput, ease of performance, costs, safety and waste generated were evaluated by the analysts. Ruggedness was determined by measuring matrix effects and instrument performance over time. Analyst performance was done by verifying that the

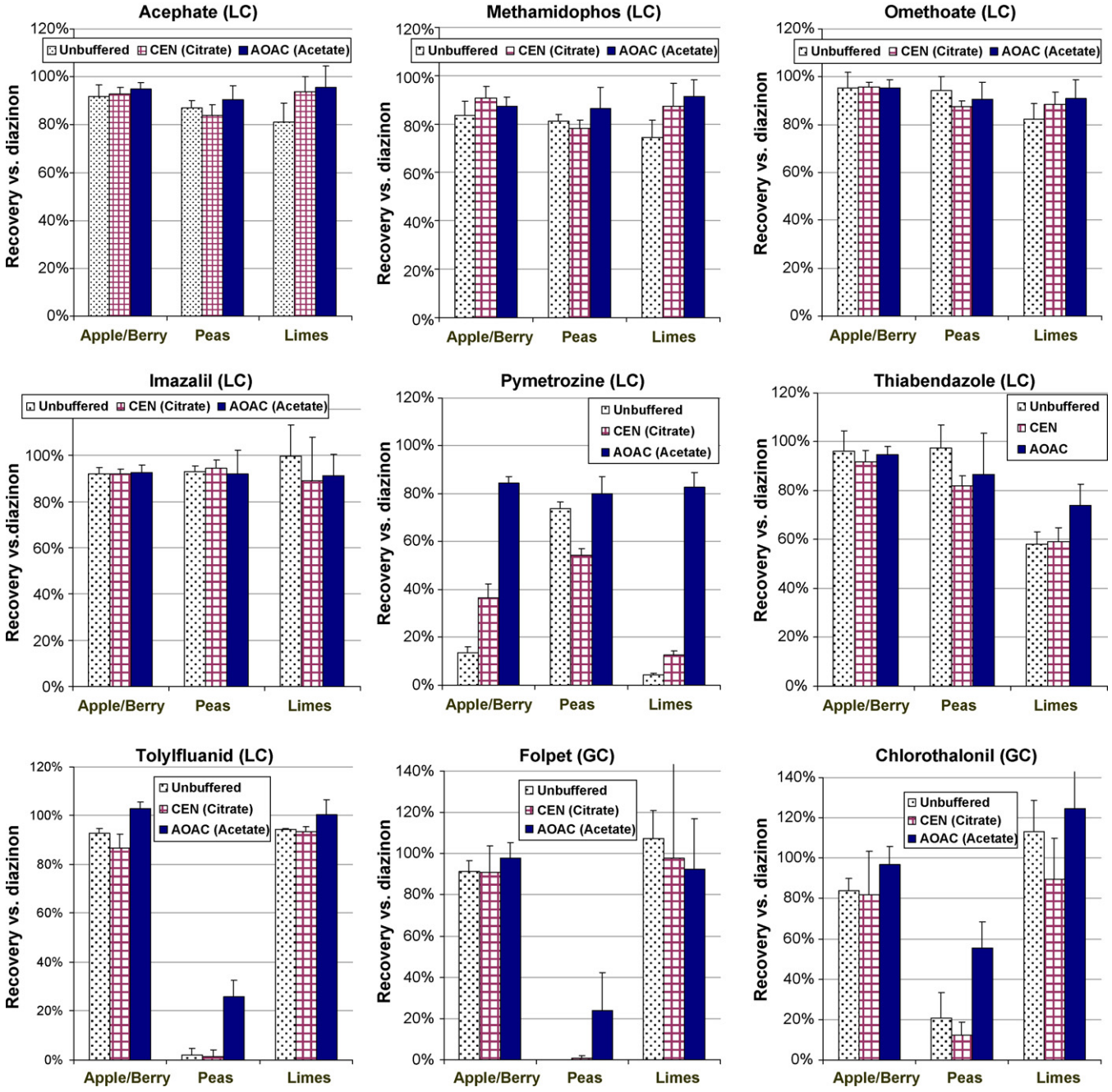

Fig. 1. Average recoveries (and standard deviations) of pesticides that showed differences among the different matrices using the 3 different versions of QuEChERS (4 replicates each at 50 ng/g, 250 ng/g and 1000 ng/g spiking levels, $n = 12$).

different chemists could all achieve acceptable results with the methods.

Ideally, limits of quantification (LOQs) would also be determined empirically to find the lowest spiking level that can be recovered with consistent signal/noise >10. The LOQs of the different QuEChERS versions have been assessed previously to be <10 ng/g, depending on the analytical instrumentation and conditions used. Results at the LOQ tend to be variable independent of the sample preparation method, thus we chose to avoid this complication by spiking well above the LOQs with 50 ng/g being the lowest spiking level. The final sample amount to extract volume ratios were all the same in each method (1 g/mL), and matrix-matched calibration slopes and S/N ratios for the pesticide analytes were much the same among the 3 versions for any given concentration, which indicated that the LOQs were the same in the different methods.

3.1. Results from spiking recovery experiments

The descriptions of the experiments are given in detail in Experimental. Table 1 lists the recoveries and variabilities for the different QuEChERS versions averaged from the 36 spikes (4 replicates by 4 analysts at 3 levels in 3 matrices over 9 days). The diazinon-normalized recoveries were exceptional (98% on average with 10% RSD) and they were only slightly lower and more variable when an internal standard was not used (the uncorrected recoveries are given for diazinon, atrazine and procymidone). As observable from the typically very low %RSD values, there were few differences among recoveries in the different matrices and levels. The %RSDs of results per pesticide for each analyst are also given in the table and their performances were essentially the same (10–12% RSD on average).

The recovery of the quality control spike (tebuconazole) for the sample homogenization step was between 88 and 102% in the method with 8–13% RSD (LC–MS/MS results were somewhat more precise than GC–MS for tebuconazole). No real differences in homogeneity results were noted among the different matrices, but it was clear that apple–blueberry sauce and peas were easy to homogenize and dry ice was needed to properly comminute the limes. The variability of tebuconazole when using a 10 g or 15 g subsample for extraction were also similar, which was limited by the analysis, not the sample processing procedure.

The variability of the quality control spike for the analytical step (TPP) was 8% RSD in LC–MS/MS and 12% RSD in GC–MS. This is in keeping with the average variabilities in the results of most of the pesticides in the study. The relative ease or difficulty in the analysis for each analyte and analytical method (LC or GC) can be assessed by comparing the individual results to the overall averages.

3.2. pH-dependent pesticides

In agreement with earlier results, nearly all pesticides gave exceptionally good results overall, except for those appearing in bold text in Table 1, which include chlorothalonil, folpet and tolylfluanid. These pesticides are known to be base-sensitive and they are unstable and problematic in pesticide residue analysis by any current multiresidue method [104]. Pymetrozine also showed differences, but only for reasons of pH during the extraction step, not degradation [6]. Fig. 1 shows the recoveries of the highlighted pesticides and other analytes of interest that showed smaller differences (acephate, methamidophos, omethoate, imazalil and thiabendazole) with respect to matrix and method.

As Fig. 1 results demonstrate, the QuEChERS version using the strong acetate buffering at pH-4.8 [6] more often gave higher and more consistent recoveries for the problematic, pH-dependent pesticides than the unbuffered method (as expected) and the citrate-buffered version, which uses citrate buffering of weaker strength and slightly higher pH of 5–5.5 [7]. Tolylfluanid, folpet and chlorothalonil mostly degraded in the peas prior to extraction, but the use of the strong acetate buffering led to somewhat higher recoveries in the green vegetable. This can be helpful at least to screen for these fungicides, which are registered for application in a wide range of commodities [105].

The most striking example of recovery differences between the matrices and methods is pymetrozine, which is also registered for use in a wide range of commodities including citrus fruits [105]. Thus, it is important to achieve high recoveries for pymetrozine (and the other common pesticides) independent of matrix. In particular, the situation with low recoveries of pymetrozine in citrus and highly variable recoveries for pH-dependent pesticides in general, was the impetus that Lehotay et al. modified the original QuEChERS method to use buffering in the first place [3,6,8]. As shown in Table 1 and Fig. 1, pymetrozine gave 82% average recovery with 7% RSD using the acetate-buffered QuEChERS version among all 3 commodities, whereas the citrate-buffered version gave overall recovery of ≈30% (same as unbuffered version) with only somewhat better consistency (51% RSD) than the unbuffered version (100% RSD). However, the lower recovery of this pesticide using the citrate-buffered method is sufficient for screening purpose [7,106].

Shia [107] conducted a similar comparison study of recoveries using the 3 different QuEChERS methods for a diverse range of pesticides (including chlorothalonil, tolylfluanid and pymetrozine) in a variety of commodities (grapes, oats, oranges and avocadoes). In all cases, the AOAC (acetate buffering) version gave higher recoveries for the pH-dependent pesticides than the CEN (citrate-buffering) version, confirming the results obtained in this study. As we also found, Shia's study did not show notable differences between the different method results for all other pesticides spiked into the different matrices.

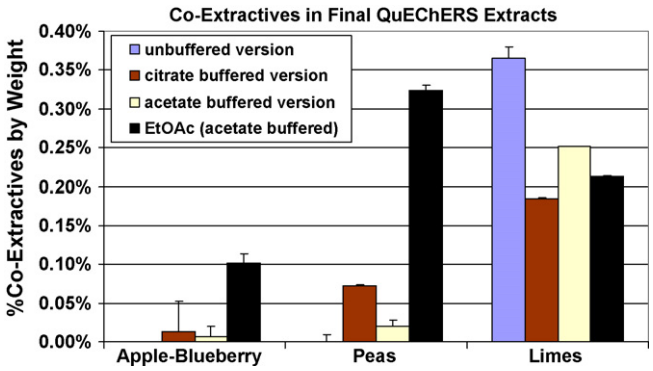

Fig. 2. Amount of co-extractives as determined by weight differences of QuEChERS final extracts taken to dryness (2 replicates each of 5 g sample equivalents). The unbuffered version gave 0% measured co-extractives in the case of apple–blueberry and pea.

A clear conclusion can be made that the acetate-buffered version achieves higher and more consistent recoveries for the pH-dependent pesticides in fruit and vegetable matrices. Both buffering versions, as well as the original method and other less-extensively-studied modified versions, achieve equally high quality results for many common pesticides applied to agricultural commodities.

3.3. Co-extracted matrix components

Anastassiades et al. chose to use citrate buffering rather than acetate buffering for reasons described in ref. [7]. For one, they asserted that the use of the more concentrated acetate buffer system resulted in "visibly worse cleanup results compared to the original QuEChERS method." In our comparison, visual observations of the initial and final extracts indeed showed slight differences in color and color intensity in the different extracts (see Supplementary data, Fig. I), but it was not possible to determine that one extract was actually "cleaner" than another just by looking at them. Therefore, we also assessed the degree of cleanup in the extracts in the same empirical manner as done before [1,6,7,10,11,13,18] through gravimetric measurements, chromatography and determination of matrix effects on quantitation . Anastassiades et al. found that the CEN QuEChERS version gave 0.1% co-extractives in red currant whereas the AOAC version gave 0.25% co-extractives [7].

In the gravimetric measurements, we combined the excess initial sample extracts for each method/matrix pair (only 1 mL of the 10–15 mL extract was taken for d-SPE cleanup). We transferred duplicate 5 mL portions (5 g equivalent) to pre-weighed glass test tubes (the moisture had been removed by heating the tubes for 1 h at 110 °C prior to weighing). Similarly, we scaled up the d-SPE cleanup steps 10-fold by adding 10 mL extract and the contents of 10 d-SPE tubes to 15 mL centrifuge tubes (in duplicate for each matrix/method). Then, 5 mL (5 g equivalent) of each extract after cleanup was transferred to additional pre-weighed test tubes. All extracts in the tubes were taken to dryness using nitrogen stream evaporation and the tubes were again heated at 110 °C for 1 h to remove moisture from the glass prior to weighing on a calibrated analytical balance. The weight difference was recorded to estimate the amount of co-extracted matrix in the initial and final extracts. Fig. 2 give the results from the experiment for the final extracts.

In the case of apple–blueberry sauce, the initial QuEChERS extracts contained ≈0.2% of matrix components (unbuffered = 0.23%, citrate-buffered = 0.17% and acetate-

buffered = 0.13%). The use of 50 mg PSA + 50 mg C_{18} + 150 mg anh. $MgSO_4$ per mL extract for d-SPE in all 3 versions essentially removed the apple–blueberry co-extractives to the extent that they could no longer be measured by the gravimetric approach (as shown in Fig. 2). The inclusion of C_{18} in this step is a deviation from all 3 original published versions, but it has been shown that the C_{18} has no effect on recoveries and can only help, not harm, the d-SPE performance [1,7,10–15,107]. The measured weight of the apple-blueberry co-extracts in the final extracts strictly using acetate-buffered method was 0.05% rather than 0.01% when the C_{18} was also used. It is regrettable that Lehotay did not include the C_{18} in d-SPE as part of the protocol in AOAC Official Method 2007.01 [8,15]. In order to isolate only the effect of citrate vs. acetate buffering in this matrix comparison, we also used twice as much PSA for the apple–blueberry as the original and CEN QuEChERS versions called for in the published protocols [1,3–5,7,14]. The QuEChERS approach is very flexible and such minor adjustments in these sorbent amounts have little impact on pesticide recoveries (unlike pH and solvent which can have major impact on pH-dependent pesticides).

The amount of GCB in d-SPE can have a significant effect on the amount of chlorophyll removed and on recoveries of structurally planar pesticides [1,7,11,14,15]. In the case of peas, we used the same d-SPE sorbent combination as in apple–blueberry sauce for the unbuffered and acetate-buffered versions, which had only a slight effect in reducing the green color of the pea extracts (see Supplementary data, Fig. I). However, we used 25 mg PSA + 7.5 mg GCB + 150 mg anh. $MgSO_4$ per mL extract, which was the exact d-SPE sorbent amounts called for in CEN Standard Method EN 15662 for dark green vegetables (technically, we should have used 2.5 mg GCB for the lighter green peas, but we preferred to test the method that provided greater cleanup).

Despite the slightly darker green color of the unbuffered and acetate-buffered pea extracts, Fig. 2 shows that those modified QuEChERS versions gave somewhat cleaner extracts by weight than the citrate-buffered version. The initial pea extracts gave 0.14–0.17% co-extractive amounts in all 3 methods, but the citrate-buffered d-SPE version left 0.07% of co-extractives whereas the greater amount of PSA and use of C_{18} in the other d-SPE step removed the matrix components to the same extent in peas as in apple–blueberry sauce (≈90% of measurable amount removed). Without the inclusion of C_{18} in the acetate-buffered d-SPE step, final co-extracted amount of peas was 0.06% rather than 0.02%, thus C_{18} appeared to have a greater impact than PSA in the cleanup differences between the citrate-buffered and acetate-buffered versions.

Citrus fruits are notoriously difficult matrices for pesticide residue analysis. The acidity is more intense than in other fruits and the peel is full of pectin and complex aromatic compounds that cause precipitation in final extracts, higher matrix effects, more chromatographic interferences and greater need for instrument maintenance. In our experiment to weigh matrix co-extractives from the different methods, limes gave 1.4% initial co-extracted amount in the unbuffered method and 0.6% in the buffered versions, which are 3–7 times greater than in the other 2 matrices. This is in agreement with previous results for orange juice, red currants and lemons [6,7]. All extracts had been stored for multiple days in the freezer prior to the experiment and the very top layer of the extracts were not used in case of lime oil had separated out. Using the same d-SPE sorbents in each QuEChERS version as done with peas, the co-extracted amounts in the final extracts became 0.37%, 0.18% and 0.25% for the unbuffered, citrate-buffered and acetate-buffered versions, respectively, as shown in Fig. 2. Without the C_{18} in the acetate-buffered version, the final amount of co-extracts was 0.32%. In strict accordance with the EN 15662 protocol, we should not have included 7.5 mg GCB in d-SPE for limes, but doing so led to less green color in the extracts (see Supplementary data, Fig. I) and a smaller amount of matrix co-extractives even when less PSA and no C_{18} were used.

The freeze-out step for limes may have reduced the amount of co-extractives in the initial lime extracts [7], but ultimately, the extra PSA and C_{18} in the subsequent d-SPE cleanup step do the same job faster and easier [13]. According to the gravimetric experiment and recovery results, the use of 50 mg PSA + 50 mg C_{18} + 7.5 mg GCB + 150 mg $MgSO_4$ per mL extract would provide greater extent of d-SPE cleanup for all matrices than the exact published protocols without unacceptably affecting recoveries, even for structurally planar pesticides. For example, the planar pesticide, cyprodinil, gave high recoveries in peas and limes with the CEN method using 7.5 mg GCB per mL in d-SPE. Other planar pesticides, chlorothalonil and thiabendazole gave only slightly lower recoveries in those cases, but their results are more complicated to interpret because they are also affected by pH. The use of this sorbent combination will be studied further in the future.

3.4. Matrix effects

Matrix effects are known to be problematic in pesticide residue analysis using LC–MS/MS and GC–MS [108]. In the former, ion suppression can occur in the ion source to cause a reduced signal when matrix co-elutes with the analyte peaks and in GC, the matrix-induced chromatographic response enhancement effect can occur when co-extractives fill active sites in the chromatographic system, which causes higher analyte transfer efficiency, thus greater signal in the presence of matrix [109]. Because alternatives are either less practical or effective, the most common way to avoid matrix effects in both LC–MS/MS and GC–MS is to use matrix-matched calibration standards [18]. In this study involving spiked samples, we had the luxury to use final extracts of the exact same matrix to perfectly match the sample extracts and achieve highly accurate results. In routine analysis, it is unlikely to find perfectly matching blank matrices and compensation for matrix effects will not necessarily be as accurate.

Ideally, the injected extracts will be sufficiently clean so that no matrix effects will occur in the first place. The extent of matrix effects can be measured in each analytical sequence by comparing calibration standards of the same concentrations in solvent-only vs. those in matrix extracts. This was done in every sequence in our experiments and the differences in the best-fit calibration slopes for LC–MS/MS are presented in Fig. 3. We did not use an internal standard in this approach because it can also undergo matrix effects and could give misleading conclusions.

As Fig. 3 shows, none of the QuEChERS versions gave significantly different matrix effects from each other with the instrument and conditions we used. In fact, the patterns observed tracked very well together with respect to matrix, method and pesticide. In the case of apple–blueberry, none of the analytes gave more than 16% differences, which may have been unrelated to matrix effects at all. We do not know why azoxystrobin in all 3 methods or pymetrozine with the citrate-buffered version gave a sensitivity enhancement in the matrix, but the effects were small in any case. Peas gave even less intense (<12%) and more consistent sensitivity differences in matrix than apple–blueberry sauce except for thiabendazole and pymetrozine (the first two analytes to elute from the LC column). In the case of limes, clear matrix suppression effects occurred for all of the pesticides in the LC–MS/MS analyses from 12% (dichlorvos) to 80% (imazalil). Observed differences for each pesticide are too small to tell if one method gave more or less matrix effects than another in limes, or any of the matrices.

In the case of GC–MS, the integrated peak data were treated in the same way as in LC–MS/MS, but signal enhancements were much too variable to make valid comparisons. Without use of the

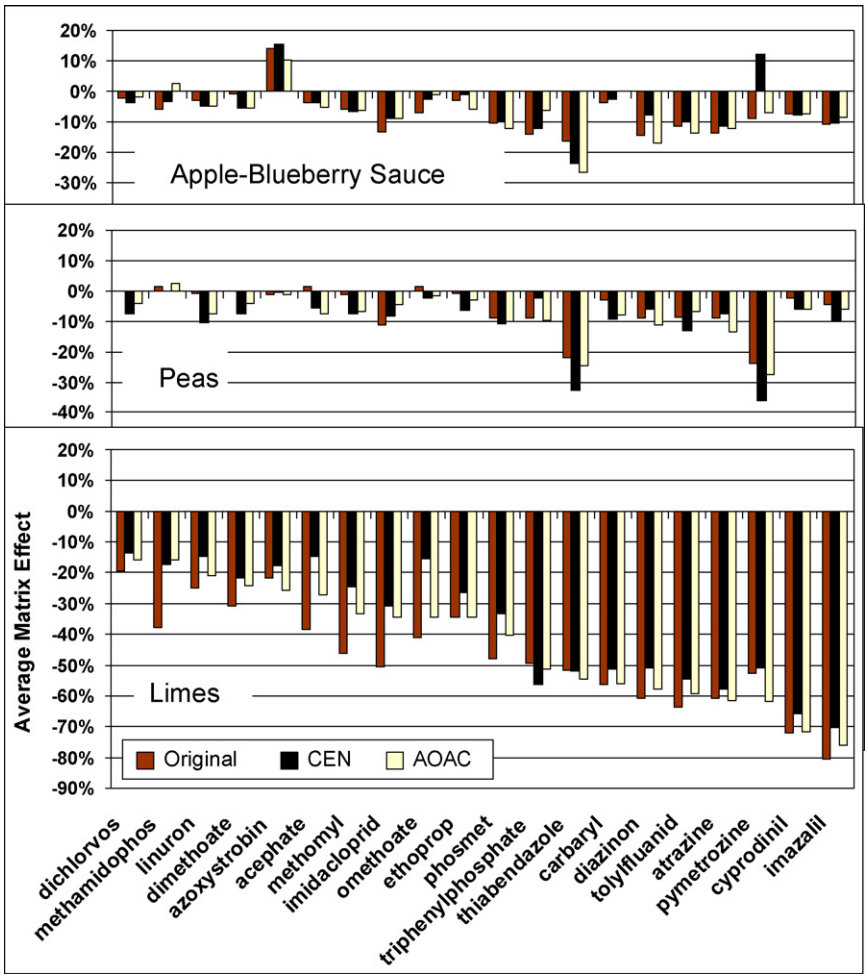

Fig. 3. Comparison of LC–MS/MS matrix effects in the different methods for the different commodities as measured by the %differences in slopes of the calibration curves (without use of the internal standard) from matrix-matching vs. those from standards in solvent-only.

internal standard, the results were not consistent enough for different pesticides even within the same sequence and day-to-day matrix effect differences were too large even if the same extracts were re-injected. This is because the GC–MS matrix effects were more dependent on the condition of the instrument (inlet liner and retention gap) moreso than on the method or matrix. Also, the volatilization injection process in GC is less precise than liquid injection in LC and the use of the triple quadrupole MS/MS approach in LC provided more selectivity and easier peak integration in the complex extracts than full spectrum TOF data acquisition. Furthermore, a combination of matrix enhancement for pesticides susceptible to degradation on active sites occurs in GC at the same time as matrix diminishment effects due to build-up of non-volatile materials in the inlet. These are reasons why analyte protectants, which are substance added to all injected samples in a sequence to maximize matrix enhancement and reduce diminishment effects, can provide valuable advantages in GC–MS [1,110,111], particularly in selected ion monitoring and MS/MS modes. Unfortunately, the analyte protectants complicate full spectrum MS analysis, and we did not use them in this study, in part because we wished to assess matrix effect differences without them.

3.5. Chromatographic interferences

Another way to compare and assess matrix co-extractives in the different methods is to check for chemical interferences in the chromatograms. In LC–MS/MS, there were no chromatographic peaks that could be mistaken for the analytes even in the lime extracts. The pesticide peaks were easy to integrate and data processing was fast and simple using LC–MS/MS. However, the situation was not so simple in the GC–MS analysis and more care was needed to conduct analyte peak integrations, even when using mass spectral deconvolution and reconstructed selected ion chromatograms for each pesticide. However, direct chemical interferences >25 ng/g equivalent concentrations did not occur in the analyses except for certain pesticides in lime extracts for all 3 methods.

Fig. 4 shows the GC–MS total ion chromatograms of the matrix blank extracts for each commodity from the different methods; the chromatograms were very similar within each sample type for all 3 methods. All of the same chromatographic peaks occurred for each matrix with only small intensity differences. Two prominent peaks of amides that appeared in the unbuffered method in all 3 matrices were traced to the reagent blanks, as indicated by asterisks in Fig. 4. Otherwise, the peaks appearing in the reagent blanks were much fewer and less intense than those in the sample extracts. As shown in Fig. 4, some prominent peaks from fatty acids appeared in the apple–blueberry extracts and some sterols appeared in pea extracts, but these did pose problems in the pesticide analyses. However, some pesticides were very difficult to integrate due to the complexity of the matrix background in the case of limes and the GC–MS results for those analytes in limes were excluded from the recovery compilation, as noted in Table 1.

The only observable differences among the unbuffered and buffered QuEChERS versions occurred in the chromatograms

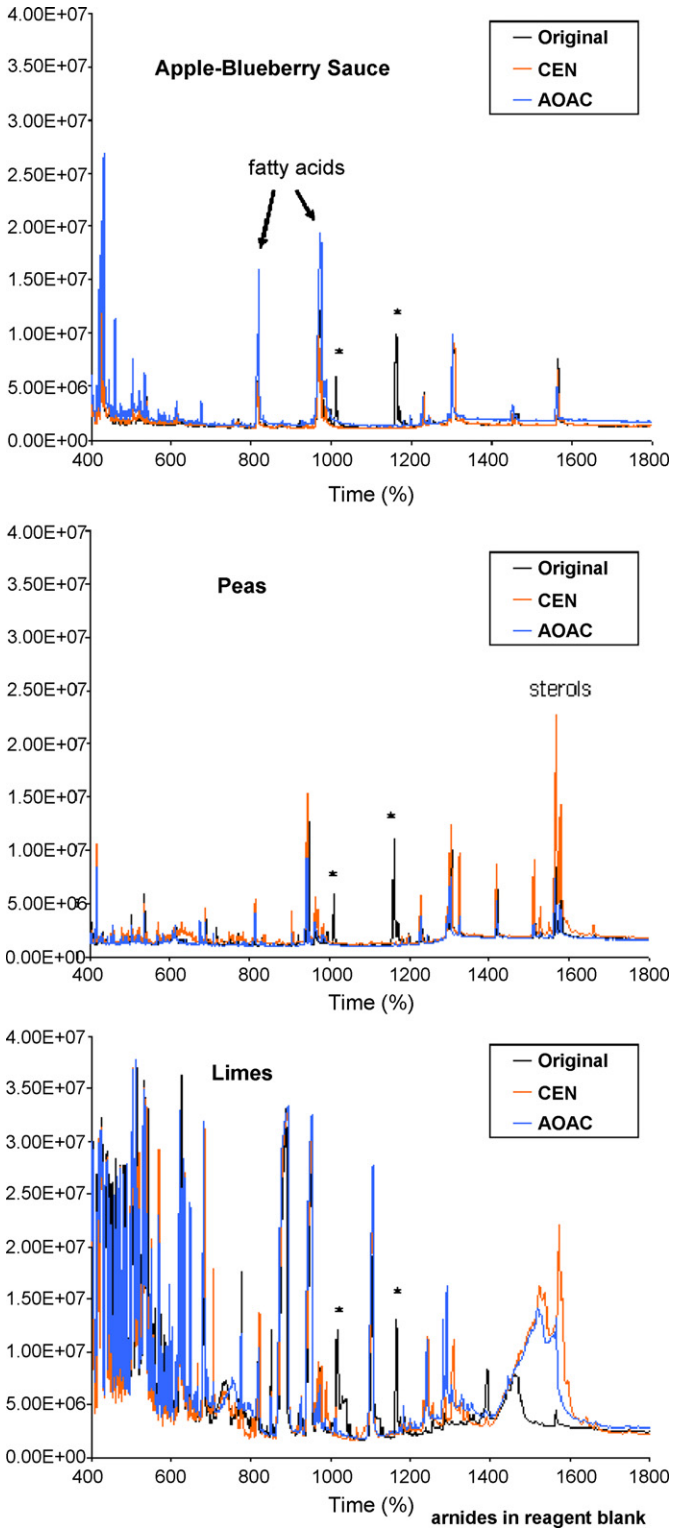

Fig. 4. Total ion chromatograms in GC–MS (TOF) of the different blank matrix extracts using the different QuEChERS method versions.

of lime extracts from 1200 to 1700 s. The buffering in either the citrate- or acetate-buffered methods led to more intense late-eluting (less volatile) matrix components in the total ion chromatograms than with the unbuffered version. As shown in a previous study [12], the AOAC acetate-buffered version had more co-extractives (mainly fatty acids) in GC–MS than the unbuffered version in cereal grains. The unbuffered method using an additional amount of PSA to reduce fatty acid co-extractives should be used with corn, oats and similar grains [12]. However, in our study, the co-extracted matrix differences for limes were not so dramatic and the much improved recoveries of pymetrozine (and thiabendazole, tolylfluanid, chlorothalonil, and folpet to a lesser extent) in citrus fruit makes the acetate-buffered version more advantageous.

Indeed, the comparative assessment of the different QUEChERS methods for the representative pesticide residues in the representative fruits and vegetable tested in this study do not show any notable differences in matrix effects or co-extractives in GC–MS or LC–MS/MS. In the different co-author labs in the US, South Korea, and Thailand, we have made countless hundreds of injections of many different food matrices using buffered QuEChERS in GC–MS and LC–MS/MS instruments without maintenance concerns.

The only observed advantage of CEN Standard Method EN 15562 over AOAC Official Method^{SM} 2007.01 was that the use of 7.5 mg/mL GCB in d-SPE removed some of the green color in extracts while still obtaining adequate recoveries of structurally planar pesticides. The use of this amount of GCB can be adopted easily into the other versions, too. In terms of practical aspects, the original and acetate-buffered versions were nearly exactly the same in terms of ease, sample throughput, labor, cost, waste generated, labware needs, glassware washing and safety issues. The citrate-buffered method was also very similar to perform, but it entailed more complications (base addition and freeze-out steps in limes and 3 d-SPE options depending on sample type, plus additions of 4 salts to the tubes, which was done by a vendor in our case). Also, a precipitate occurred in the pea extracts for LC–MS/MS using the citrate-buffered method, but not in the others (precipitates occurred in all 3 versions when the 0.1% formic acid aqueous solution was added to the lime extracts for LC–MS/MS). Overall, the acetate-buffered version gave the most advantages in the comparison study, with no observed disadvantages vs. the other versions.

3.6. Substitution of MeCN with EtOAc in AOAC Official Method^{SM} 2007.01

In the fall of 2008 until the spring of 2009, a worldwide shortage of MeCN occurred because of an economic downturn in acrylonitrile production and temporary shut downs of manufacturing plants due to a hurricane in Texas and the Olympics in Beijing [103]. The cost of MeCN increased greatly and some labs had difficulties to obtain the solvent. The QuEChERS method uses only 10–15 mL MeCN per sample, but labs needed an alternative solvent in case MeCN was not available.

In the original QuEChERS study, MeCN was shown to be the most advantageous solvent for extraction of pesticide residues from food and EtOAc was found to be second best overall [1]. Indeed, EtOAc has been used in pesticide residue analysis applications since the 1960s, and it has been used in methods employing QuEChERS concepts before [16,29–34]. The physicochemical and practical advantages and disadvantages of MeCN vs. EtOAc in QuEChERS have been described previously [1,15]. However, EtOAc had not been evaluated using the acetate-buffering version before and we hypothesized that the strong buffering may improve the results of pH-dependent pesticides that typically are not fully extracted using EtOAc without control of pH. We also hoped to improve results for folpet and chlorothalonil in GC–MS by employing EtOAc.

In this study using the same matrices and experimental design as already described, we followed the AOAC Official Method 2007.01 protocol exactly using MeCN + 1% HOAc and EtOAC + 1% HOAc side-by-side as the extraction solvents. Fig. 2 shows the results of the gravimetric experiment in comparison with the MeCN-based QuEChERS versions. The reason that the EtOAc gave more co-extractives in the apple–blueberry final extracts was not because it extracted more matrix components, but because it is

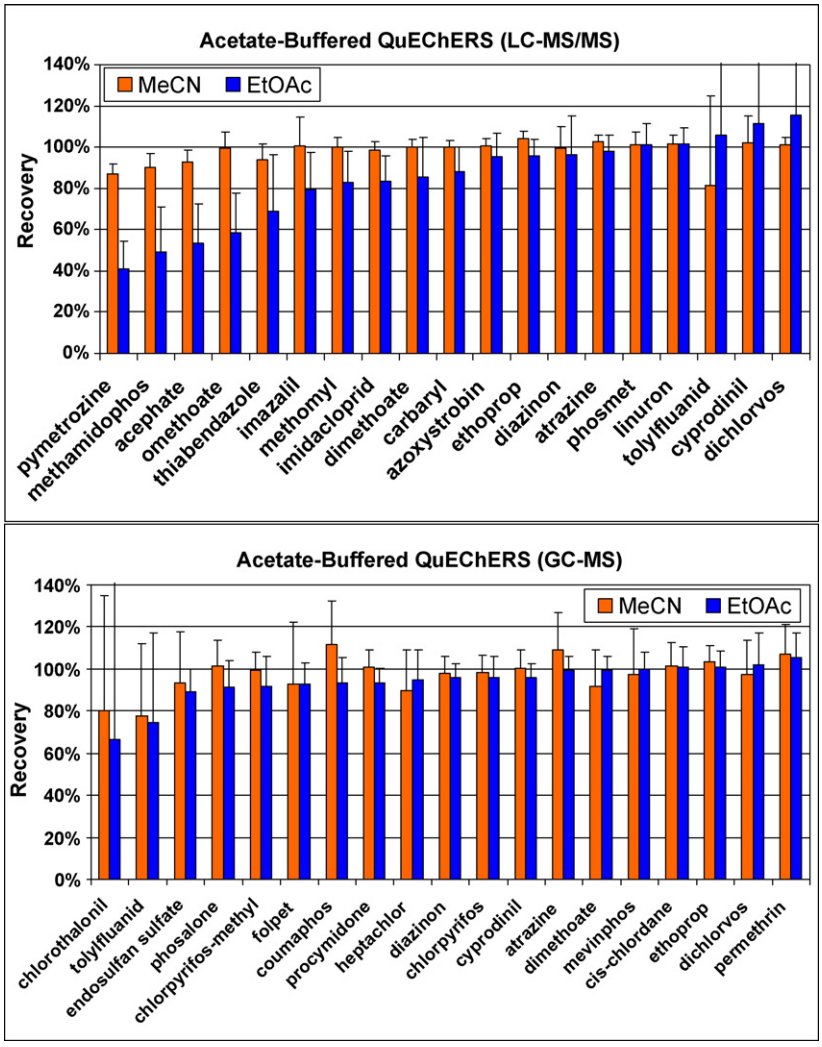

Fig. 5. Pesticide recovery comparison between AOAC Official Method[SM] 2007.01 (acetate buffering) using MeCN or EtOAc as the extraction solvent for LC- and GC-amenable pesticides spiked at 50 ng/g, 250 ng/g and 1000 ng/g in apple–blueberry sauce, peas and limes ($n = 36$).

less effective for cleanup in d-SPE than MeCN. The initial EtOAc extracts actually gave slightly less (0.11% vs. 0.14%) co-extractives in apple–blueberry sauce, but the final extracts were still 0.10% co-extractives (vs. 0.05% using MeCN without C_{18}). In peas, the initial EtOAc-based extracts contained 0.35% matrix co-extractives vs. 0.16% with MeCN. Again, d-SPE with EtOAc offered very little cleanup and final extracts remained at 0.32% co-extractives by weight whereas MeCN gave 0.06% final extractives using the same d-SPE sorbents (50 mg PSA + 150 mg anh. $MgSO_4$). In the case of limes, EtOAc was found to give cleaner initial and final extracts than using the same protocol with MeCN (0.46% vs. 0.60% and 0.21% vs. 0.32%, respectively).

In terms of pesticide recoveries, Fig. 5 summarizes the comparison of EtOAc and MeCN in the experiment for all spiking levels and matrices (normalized to atrazine internal standard in both GC and LC). Interestingly, the acetate buffering indeed improved the recoveries of pH-dependent pesticides in all matrices vs. EtOAc results without buffering (from previous experience). However, acetate-buffered MeCN still gave more accurate (true and precise) results for all analytes in LC–MS/MS than EtOAc did. The results using acetate-buffered EtOAc are even more consistent from matrix to matrix than the citrate-buffered QuEChERS version using MeCN. It may be possible to correct for recoveries if results remain very consistent using EtOAc if MeCN is not available.

As Fig. 5 shows, EtOAc gave high variability in the LC–MS/MS analysis of dichlorvos, cyprodinil and tolylfluanid (but not in GC–MS for the same analytes). EtOAc is not a good injection solvent in reversed-phase LC and it had to be evaporated in the final LC extracts, which led to probable losses of dichlorvos due to volatility and poor peak shapes for omethoate and imazalil.

Conversely, EtOAc is a better solvent for GC than MeCN, as demonstrated by the slightly more consistent recoveries and reproducibilities overall in GC–MS using EtOAc than MeCN. Folpet in particular gave better results with EtOAc than MeCN, but not dramatically so and our hopes were misplaced that EtOAc would yield better results for chlorothalonil and tolylfluanid, too. In conclusion, EtOAc gave essentially equivalent results as MeCN for GC-amenable pesticides and worse results for pH-dependent and certain other LC-amenable pesticides. It gave a similar degree of matrix co-extractives in limes, but final extracts for apple–blueberry and peas were dirtier when using EtOAc.

4. Conclusions

Multiclass, multiresidue analysis of pesticide residues in foods does not lend itself easily to fine tuning. The differences of even the same commodity types from one source to another, as well as reagent properties from batch-to-batch and instruments from lab-

to-lab make sensitive optimizations to fine tune matrix effects vs. pesticide recoveries a continual, complicated pursuit. Just as buildings and bridges require extra strength to withstand anomalies of high winds and other stresses, it is better to devise sample preparation conditions to provide high recoveries of as many pesticides as feasible in as many matrices as possible.

The QuEChERS approach is so flexible and rugged that most pesticides give excellent results when different amounts and types of solvents and salts are used for extraction and different sorbents and amounts are used in d-SPE. There could be dozens of protocols with slight modifications that achieve high recoveries, but with no real advantages over each other depending on the matrices. The use of a single protocol with a single set of reagents is much easier and more efficient than using different methods for the same application. Our study showed that protocol subtleties with respect to base additions, different amounts of d-SPE sorbents and use of a freeze-out step provided no observed benefit. In fact, such steps only add to the time, complication and cost of the method, which undermines the QuEChERS concept.

Only a few pesticides are problematic with the approach, depending on matrix and buffering is essential to improve the results for pH-dependent pesticides. It is only for those pesticides for which buffering is needed, therefore, and the results for those pesticides must not be compromised or sacrificed by trying to "fine tune" the protocol. The use of weak citrate buffering as in CEN Standard Method EN 15662 does not meet the need to achieve acceptably high recoveries of pymetrozine in different matrices vs. the previously-developed acetate-buffered QuEChERS method [6,8]. In d-SPE, the use of 7.5 mg GCB with 50 mg (or possibly more) each of PSA and C_{18} and 150 mg $MgSO_4$ per mL extract provides slightly better cleanup of food extracts without severely affecting pesticide recoveries. A final QuEChERS protocol may be harmonized in this way after further assessments and interlaboratory trials.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.01.044.

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