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Quick, Easy, Cheap, Effective, Rugged, and Safe Approach for Determining Pesticide Residues

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Summary

This chapter describes a simple, fast, and inexpensive method for the determination of pesticides in foods and potentially other matrices. The method, known as the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method for pesticide residues involves the extraction of the sample with acetonitrile (MeCN) containing 1% acetic acid (HAc) and simultaneous liquid–liquid partitioning formed by adding anhydrous magnesium sulfate ($MgSO_4$) plus sodium acetate (NaAc), followed by a simple cleanup step known as dispersive solid-phase extraction (dispersive-SPE). The QuEChERS method is carried out by shaking a fluoroethylenepropylene (FEP) centrifuge tube that contains 1 mL 1% HAc in MeCN plus 0.4 g anhydrous $MgSO_4$ and 0.1 g anhydrous NaAc per gram wet sample. The tube is then centrifuged, and a portion of the extract is transferred to a tube containing 50 mg primary secondary amine (PSA) and 50 mg C18 sorbents plus 150 mg anhydrous $MgSO_4$ per milliliter extract (the dispersive-SPE cleanup step). Then, the extract is centrifuged and transferred to autosampler vials for concurrent analysis by gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–tandem mass spectrometry (LC–MS/MS). Different options in the protocol are possible depending on alternate analytical instrumentation available, desired limit of quantitation (LOQ), scope of targeted pesticides, and matrices tested.

Key Words: Food; fruits; gas chromatography; liquid chromatography; mass spectrometry; pesticide residue analysis; sample preparation; vegetables.

1. Introduction

Multiresidue analysis of pesticides in fruits, vegetables, and other foods is a primary function of many regulatory, industrial, and contract laboratories throughout the world. It is estimated that more than 200,000 food samples are analyzed worldwide each year for pesticide residues to meet a variety of purposes. Once analytical quality requirements (trueness, precision, sensitivity, selectivity, and analytical scope) have been met to suit the need for any particular analysis, all purposes for analysis favor

practical benefits (high sample throughput, ruggedness, ease of use, low cost and labor, minimal solvent usage and waste generation, occupational and environmental friendliness, small space requirements, and few material and glassware needs).

A number of analytical methods designed to determine multiple pesticide residues have been developed in the time since this type of analysis became important *(1–10)*. However, few if any of these methods can simultaneously achieve high-quality results for a wide range of pesticides *and* the practical benefits desired by all laboratories. In 2003, the QuEChERS (quick, easy, cheap, effective, rugged, and safe) method for pesticide residue analysis was introduced *(11)*; it provides high-quality results in a fast, easy, inexpensive approach. Follow-up studies have further validated the method for more than 200 pesticides *(12)*, improved results for the remaining few problematic analytes *(13)*, and tested it in fat-containing matrices *(14)*.

The QuEChERS method has several advantages over most traditional methods of analysis in the following ways: (1) high recoveries (>85%) are achieved for a wide polarity and volatility range of pesticides, including notoriously difficult analytes; (2) very accurate (true and precise) results are achieved because an internal standard (ISTD) is used to correct for commodity-to-commodity water content differences and volume fluctuations; (3) high sample throughput of about 10–20 preweighed samples in about 30–40 min is possible; (4) solvent usage and waste are very small, and no chlorinated solvents are used; (5) a single person can perform the method without much training or technical skill; (6) very little labware is used; (7) the method is quite rugged because extract cleanup is done to remove organic acids; (8) very little bench space is needed, thus the method can be done in a small mobile laboratory if needed; (9) the acetonitrile (MeCN) is added by dispenser to an unbreakable vessel that is immediately sealed, thus minimizing solvent exposure to the worker; (10) the reagent costs in the method are very inexpensive; and (11) few devices are needed to carry out sample preparation.

This chapter provides the protocol for the QuEChERS method that is currently undergoing an extensive interlaboratory trial for evaluation and validation by pesticide-monitoring programs in several countries. In brief, the method uses a single-step buffered MeCN extraction while salting out water from the sample using anhydrous magnesium sulfate ($MgSO_4$) to induce liquid–liquid partitioning. For cleanup, a simple, inexpensive, and rapid technique called dispersive solid-phase extraction (dispersive-SPE) is conducted using a combination of primary secondary amine (PSA) and C18 sorbents to remove fatty acids among other components and anhydrous $MgSO_4$ to reduce the remaining water in the extract. Then, the extracts are concurrently analyzed by liquid chromatography (LC) and gas chromatography (GC) combined with mass spectrometry (MS) to determine a wide range of pesticide residues.

The final extract concentration of the method in MeCN is 1 g/mL. To achieve <10-ng/g limit of quantitation (LOQ) in modern GC–MS, a large volume injection (LVI) of 8 µL is typically needed, or the final extract can be concentrated and solvent exchanged to toluene (4 g/mL), in which case 2-µL splitless injection provides the anticipated degree of sensitivity. If MS instruments are not available in the laboratory, other options are also possible to analyze the samples using LC and GC coupled to element-selective detectors. These aspects are discussed in more detail in **Subheadings 1.2., 1.3.**, and **3.**

Table 1 lists the many pesticides that have been successfully evaluated with the QuEChERS method. Many other untested pesticides in the same classes can be analyzed by the method, and the final choice of analytes for this protocol depends on the analyst's particular needs. The only pesticides that have failed to be validated successfully in studies thus far include asulam, daminozide, dicofol, captan, folpet, pyridate, and thiram. The method does not work for carboxylic acids, such as daminozide, because of their strong retention on PSA during the cleanup step: dicofol degrades rapidly to dichlorobenzophenone in samples, which is why it was not found in the extracts; asulam, pyridate, and thiram are exceptionally difficult and are not currently analyzed in multiclass, multiresidue methods; and the problems with captan and folpet are not likely to be because of the QuEChERS sample preparation method, but related to their GC–MS analysis, which is especially difficult because of their degradation on active sites in the GC system. (Unfortunately, these pesticides cannot be readily analyzed by LC–tandem mass spectrometry [MS/MS] either.)

1.1. Calibration in Pesticide Methods

In any quantitative method, the accuracy of the result cannot be better than the accuracy of the calibration. Pesticide residue analysis using chromatographic methods nearly always utilizes external calibration, in which analyte solutions of known concentrations are injected contemporaneously (in the same sequence) as the sample extracts, and the intensity of the analyte peaks in the standards is compared with those from the samples to determine the pesticide concentrations. The number of calibration standards needed in the determination and their concentrations depend on the quality assurance (QA) requirements for the analysis or laboratory, but generally four calibration standards (plus the matrix blank, or zero standard) dispersed from the LOQ to the highest expected analyte concentration are accepted practice *(15,16)*. Reported results should not list concentrations outside the concentration range covered by the calibration standards.

Furthermore, QA guidelines generally dictate that analytical methods be evaluated to determine the effects of matrix components in the extracts on the quantitative results *(15,16)*. If it is demonstrated that no differences are observed between analyte peak intensities in matrix extracts vs those in solvent only over the entire concentration range, then calibration standards may be prepared in solvent-only solutions. Each pesticide–matrix pair must be evaluated in this case, which can be a great deal of work. Otherwise, the matrix effects must be overcome empirically because the determined results may not be altered with a "fudge factor" in most pesticide analysis applications.

In general, GC methods for organochlorine insecticides are not affected by matrix, and LC using non-MS detection techniques dos not encounter matrix effects (unless there are chemical interferences in the signals). However, LC–MS techniques, particularly using electrospray ionization (ESI), are susceptible to ion suppression effects from coeluting components in the chromatogram, even though direct interference in the MS spectrum is seldom observed *(17)*. This indirect matrix effect in LC–MS tends to yield falsely low results in the samples when compared to standards that do not contain matrix components. In the case of GC, matrix components tend to fill active sites in the system (mainly in the injector liner and capillary column). This reduces the

Table 1
Possible Pesticide Analytes That Have Been Shown to Yield >90% Recoveries Using the QuEChERS Method

acephate,[a] acetamiprid, Acrinathrin, aldicarb, aldicarb sulfone, aldicarb sulfoxide, Aldrin, azaconazole, azamethiphos, azinphos-methyl, azoxystrobin, Bifenthrin, bitertanol, Bromopropylate, bromuconazole, Bupirimate, buprofezin, butocarboxim, butocarboxim sulfone, butocarboxim sulfoxide, Cadusafos, carbaryl, carbendazim, carbofuran, 3-hydroxy-carbofuran, chlorbromuron, (α-, γ-)Chlordane, (α-, β-)Chlorfenvinphos, Chlorpropham, Chlorpyrifos, Chlorpyrifos-methyl, Chlorthaldimethyl, Chlorothalonil,[a] Chlozolinate, clofentezine, Coumaphos, cycloxydim,[a] (λ-)Cyhalothrin, cymoxanil, Cypermethrin, cyproconazole, cyprodinil, (2,4′-, 4,4′-)DDE, (2,4′-, 4,4′-)DDT, Deltamethrin, demeton, demeton-*O*-sulfoxide, demeton-*S*-methyl, demeton-*S*-methyl sulfone, desmedipham, Diazinon, dichlofluanid,[a] Dichlorobenzophenone, dichlorvos, diclobutrazole, Dicloran, dicrotophos, Dieldrin, Diethofencarb, difenoconazole, Diflufenican, dimethoate, dimethomorph, diniconazole, Diphenyl, Diphenylamine, disulfoton, disulfoton sulfone, diuron, dmsa, dmst, dodemorph, (α-, β-)Endosulfan, Endosulfan sulfate, EPN, epoxiconazole, Esfenvalerate, etaconazole, ethiofencarb sulfone, ethiofencarb sulfoxide, Ethion, ethirimol, Ethoprophos, etofenprox, Etridiazole, Famoxadone, fenamiphos, fenamiphos sulfone, Fenarimol, Fenazaquin, fenbuconazole, fenhexamid,[a] Fenithrothion, fenoxycarb, Fenpiclonil, Fenpropathrin, Fenpropidine, fenpropimorph, fenpyroximate, Fenthion, fenthion sulfoxide, Fenvalerate, florasulam,[a] Flucythrinate I and II, Fludioxonil, flufenacet, Flufenconazole, flusilazole, Flutolanil, Fluvalinate, Fonophos, fosthiazate, Furalaxyl, furathiocarb, furmecyclox, Heptachlor, Heptachlor epoxide, Heptenophos, Hexachlorobenzene, hexaconazole, hexythiazox, imazalil, imidacloprid, Iprodione, iprovalicarb, isoprothiolane, isoxathion, kresoxim-methyl, Lindane, linuron, Malathion, malathion oxon, Mecarbam, mephosfolan, Mepronil, Metalaxyl, metconazole, methamidophos,[a] Methidathion, methiocarb, methiocarb sulfone,[a] methiocarb sulfoxide, methomyl, methomyl-oxime, metobromuron, metoxuron, Mepanipyrim, Mevinphos, monocrotophos, monolinuron, myclobutanil, nuarimol, Ofurace, omethoate, oxadixyl, oxamyl, oxamyl-oxime, oxydemeton-methyl, paclobutrazole, Parathion, Parathion-methyl, penconazole, pencycuron, (*cis*-, *trans*-)Permethrin, phenmedipham, *o*-Phenylphenol, Phorate, phorate sulfone, Phosalone, Phosmet, Phosmet-oxon, phosphamidon, Phthalimide, picoxystrobin, Piperonyl butoxide, pirimicarb, pirimicarb-desmethyl, Pirimiphos-methyl, prochloraz, Procymidone, profenofos, Prometryn, Propargite, Propham, propiconazole, propoxur, Propyzamide, Prothiofos, pymetrozine,[a] Pyrazophos, pyridaben, pyridaphenthion, pyrifenox, pyrimethanil, Pyriproxyfen, Quinalphos, Quinoxyfen, Quintozene, sethoxydim,[a] spinosad, spiroxamine, tebuconazole, tebufenozide, Tebufenpyrad, tetraconazole, Tetradifon, Tetrahydrophthalimide, Terbufos, Terbufos sulfone, thiabendazole, thiacloprid, thiamethoxam, thiodicarb, thiofanox, thiofanox sulfone, thiofanox sulfoxide, thiometon, thiometon sulfone, thiometon sulfoxide, thiophanate-methyl, Tolclofos-methyl, tolylfluanid,[a] triadimefon, triadimenol, Triazophos, trichlorfon, tricyclazole, tridemorph, trifloxystrobin, trifluminazole, Trifluralin, Triphenylphosphate, vamidothion, vamidothion sulfone, vamidothion sulfoxide, Vinclozolin

GC-amenable pesticides are capitalized; those preferentially analyzed by LC–MS/MS are not capitalized; those that can be analyzed by either technique are underlined.

[a]Or >70%.

number of active sites exposed to those analytes that also tend to adsorb on the sites. Therefore, the common effect of matrix in GC is to cause greater response of the susceptible analytes in the sample extracts than in solvent only because more of the analytes are lost to active sites in calibration standards in solvent-only solutions *(18–25)*. Those pesticides most strongly affected in GC tend to contain hydroxy, amino, phosphate, and other relatively polar groups *(21)*.

Several approaches have been devised in an attempt to overcome matrix effects in LC–MS *(26–28)* and GC *(11,19–25)*, but in both instrumental methods, the most common approach is the use of matrix-matched calibration standards *(20–23)*. Matrix matching has been shown to work better than most other approaches, but it is not ideal because it requires many blank matrices (which may be hard to find), entails extra extractions, and reduces ruggedness by introducing more matrix to the analytical instrument in a sequence than would be injected otherwise.

In the future, enough evidence may accumulate for two promising approaches to replace matrix-matching calibration: (1) the echo technique in LC–MS and (2) analyte protectants in GC. The echo technique involves injection of a calibration standard in solvent only just prior to (or immediately after) the sample extract when the mobile phase gradient has just started. This leads to two peaks adjacent to each other per analyte; one is the standard, and the other is from the sample. If ion suppression effects are the same for both peaks, then this will lead to accurate results *(26–28)*. In GC, the use of analyte protectants takes advantage of the increased response provided by the matrix-induced enhancement effect to equalize the signals of susceptible analytes in sample extracts and calibration standards alike *(11,19,24,25)*. This is done by adding a high concentration of components (analyte protectants) with multiple hydroxy groups to sample extracts and calibration standards in solvent. The analyte protectants have been shown to work well in providing accurate results, better peak shapes, and lower LOQ, and they surprisingly increase ruggedness of the analysis by continuing to work even in a very dirty GC system *(11,24,25)*.

Although the two alternate approaches may become the standard methods in the near future, it is too early to make this assertion now. Also, the careful choice of analytes quantified by LC–MS/MS and GC–MS may bypass matrix effects altogether. In the meantime, instructions in this protocol (*see* **Subheading 3.3.**) are given for the use of four matrix-matched calibration standards (plus the zero standard) to cover the concentration range of the pesticides that need to be detected in the samples.

1.2. Analysis of GC-Amenable Pesticide Residues

Traditionally, selective detectors in GC have been used to detect individual classes of GC-amenable pesticides, such as organochlorines, organophosphates, and organonitrogens *(1–6)*. Either multiple injections were necessary or split flows would be made to multiple detectors. GC–MS has become the primary approach to analyze all classes of GC-amenable pesticides in the same chromatogram *(7–10)*. Traditionally, GC–MS was mainly used for confirmation of analytes previously detected by selective detectors, but modern GC–MS instruments are sensitive, easy to use, reli-

able, and affordable for most laboratories. GC–MS has become a standard laboratory instrument and can provide qualitative and quantitative information for essentially any GC-amenable analyte in a single injection. Especially when fitted with LVI, GC–MS can provide comparable sensitivity of selective detectors even in complicated extracts.

Several MS techniques are available, the most common of which use a quadrupole design that is very rugged and practical. Ion trap MS instruments provide the advantages of lower LOQ in full-scan operation and the option for conducting MS^n of targeted analytes. Time-of-flight (TOF) instruments are more expensive, but may provide greater speed or higher mass resolution in the analysis. Magnetic sector is a fourth MS instrument option, but they are very large and expensive and generally reserved for special applications. Any of these MS techniques may be coupled with GC for pesticide residue analysis and should produce equal high-quality results *(26,27)*. Any difference in analytical accuracy between these types of MS systems is most likely a function of the injection process and not related to detection *(10)*.

Each MS approach also has multiple modes of operation. The most common ionization approach for GC–MS analysis of pesticides is electron impact (EI) ionization, which often yields many mass fragments to aid analyte identification. EI at 70 eV is the standard used for generating spectra with commercial instruments, and mass spectral libraries are available that contain full-scan spectra for as many as 300,000 compounds at these conditions. Another facet in MS analysis involves whether selected ion monitoring (SIM) or MS^n should be employed to provide lower LOQ and greater selectivity in the analysis of *targeted* pesticides *(8,9)*, or whether full-scan MS should be conducted to potentially identify *any* GC-amenable chemical in the chromatogram *(7)*. The targeted approach limits the number of analytes to about 60 that can be detected in a typical 30- to 40-min GC chromatogram, but full-scan operation permits a nearly unlimited number of analytes in a single injection. The analyst should refer to the literature if needed for further discussion *(29–31)*.

1.3. Analysis of LC-Type Pesticide Residues

Since the development of robust atmospheric pressure ionization (API) ion source designs, which consist of ESI and atmospheric pressure chemical ionization (APCI), very powerful and reliable LC–MS instruments have been introduced commercially. Depending on the source design, APCI works equally well or better as ESI for many pesticides, but APCI heats the analytes more than ESI, which potentially leads to problems for thermolabile pesticides. Thus, ESI has greater analytical scope and has become the primary ionization technique in LC–MS, but if all of the analytes in a method are compatible with APCI, then APCI may provide benefits of fewer ion suppression effects and a higher flow rate.

Because of the soft ionization nature of API, high background of LC mobile phases, and relatively low separation efficiency of LC, tandem MS (or high resolution) is often required to determine pesticide residues in complex extracts. Just as quadrupole, ion trap, TOF, and magnetic sector instruments may be coupled to GC, they may also be used in LC with the same advantages and disadvantages *(30)*. Moreover, just as trueness and precision in the analytical result are generally influenced by injection in

GC–MS more than detection, the performance of the ion source is typically the limiting factor in LC–MS techniques.

LC–MS/MS is rapidly becoming an indispensible analytical tool in analytical chemistry, and most pesticide-monitoring laboratories in developed countries have access to LC–MS/MS instruments. Many modern pesticides are not GC amenable, and if they do not fluoresce or contain a strong chromophore for ultraviolet/visible absorption, then LC–MS/MS is the only way to detect the chemical in its underivatized form. Derivatization of these types of analytes followed by GC analysis was often done in the past, but such methods are usually problematic to develop and implement in practice, and they do not lend themselves to multiclass, multiresidue applications *(1,7)*. Despite the great capital expense of the instruments, the powerful attributes of LC–MS/MS provide exceptional analytical performance, save time in method development, and can be used robustly in a variety of routine or special projects *(12,17,26–28,32)*.

The quality of LC–MS/MS analyses and instruments has reached the point that LC–MS/MS provides superior results than GC–MS even for many GC-amenable pesticides. This is indicated in **Table 1**; 90% of the underlined pesticides are not capitalized, which means that LC–MS/MS provided better sensitivity, greater trueness, or more precision than GC–MS for that pesticide *(12)*. The broad peaks in LC separations allow plenty of time in the MS/MS data collection process to monitor many other coeluting peaks without affecting quality of the results. Thus, hundreds of pesticide analytes can be monitored by LC–MS/MS in a single chromatogram *(12,26–28)*, which is not possible in GC–MS using SIM or MS^n techniques. Alternate methods for LC analysis using selective detectors rely on the LC separation to resolve the difference analytes from each other and matrix interferences. This is acceptable in a few multiresidue applications, such as *N*-methyl carbamate insecticides *(1,6–8)*, but traditional LC methods cannot meet multiclass, multiresidue analytical needs.

Indeed, the concurrent use of LC–MS/MS and (LVI) GC–MS for nearly any pesticide constitutes the state-of-the-art approach to multiclass, multiresidue analysis of pesticides in a variety of matrices. The QuEChERS method is an effective sample preparation procedure that very efficiently produces sample extracts suitable for both of these powerful analytical tools. This approach can be improved further in the near future by integrating other advanced techniques, such as direct sample introduction *(33–35)* and fast-GC–MS separations *(30,36–38)*, which may someday become the ultimate approach to pesticide residue analysis. The following protocol is an important step to meeting that challenge.

2. Materials
2.1. Sample Comminution
1. Food chopper (e.g., Stephan or Robotcoupe vertical cutters).
2. Probe blender (e.g., Ultraturrax) or Polytron homogenizers.
3. Container jars.
4. Blank sample verified to contain no detectable analytes.
5. Samples to be analyzed.
6. Freezer.

Optional items are:

1. Dry ice or liquid nitrogen.
2. Cryogenic chopper.

2.2. QuEChERS Sample Preparation

1. Analytical-grade MeCN.
2. High-performance liquid chromatographic (HPLC)-grade glacial acetic acid (HAc).
3. 1% HAc in MeCN (v/v) (e.g., 10 mL glacial HAc in 1 L MeCN solution).
4. Reagent-grade anhydrous sodium acetate (NaAc) (*see* **Note 1**).
5. Powder form anhydrous $MgSO_4$ > 98% pure (*see* **Note 2**).
6. PSA sorbent with 40-μm particle size (e.g., Varian, Harbor City, CA) (*see* **Note 3**).
7. Analytical-grade toluene.
8. Pesticide reference standards, typically above 99% purity (e.g., Chemservice, Accustandard, Dr. Ehrenstorfer).
9. Pesticide stock solutions (10 mg/mL): add 5 mL toluene to each 50 mg pesticide reference standard in 8-mL dark glass vials with Teflon-lined caps and store at 20°C or below (*see* **Note 4**).
10. ISTD stock solution (2 mg/mL): add 5 mL toluene to 10 mg d_{10}-parathion (e.g., C/D/N Isotopes or Cambridge Isotope Laboratories) in 8-mL dark glass vial with Teflon-lined cap and store at 20°C or below (*see* **Note 5**).
11. Triphenylphosphate (TPP) stock solution (2 mg/mL): add 5 mL toluene to 10 mg TPP in 8-mL dark glass vial with Teflon-lined cap and store at 20°C or below.
12. Working standard pesticides solution (40 ng/μL): add 400 μL of each pesticide stock solution at room temperature (RT) to a 100-mL volumetric flask containing 10 mL 1% HAc in MeCN and dilute with MeCN to the mark. Transfer four roughly equal portions of the solution to 40-mL dark glass vials with Teflon-lined caps and store at 20°C or below (*see* **Note 6**).
13. ISTD working solution (20 ng/μL): add 250 μL of the ISTD stock solution at RT to a 25-mL volumetric flask and dilute with MeCN to the mark. Transfer the solution to a 40-mL dark glass vial with Teflon-lined cap and store at 20°C or below.
14. TPP working solution (2 ng/μL): add 25 μL of the TPP stock solution at RT to a 25-mL volumetric flask and dilute with 1% HAc in MeCN to the mark. Transfer the solution to a 40-mL dark glass vial with Teflon-lined cap and store at 20°C or below (*see* **Note 7**).
15. Calibration standard spiking solutions *w*, *x*, *y*, and *z* (for *w*, *x*, *y*, and *z* standards): add 50 μL of ISTD stock solution, 2.5 mL of 1% HAc in MeCN solution, and 12.5•(*w*, *x*, *y*, and *z*) μL of the 40-ng/μL working standard pesticides solution at RT per (*w*, *x*, *y*, and *z*) ng/g desired equivalent calibration standard concentration into a 25-mL volumetric flask and fill to the mark with MeCN. For example, if the *w* standard is to be 10 ng/g, then add 125 μL of the 40-ng/μL working standard pesticides solution to the flask. Transfer the solutions to four 8-mL dark glass vials with Teflon-lined caps and store at 20°C or below.
16. 50-mL Fluoroethylenepropylene (FEP) centrifuge tubes (e.g., Nalgene 3114-0050 or equivalent) (or 250-mL FEP centrifuge bottles for 16- to 75-g samples).
17. Top-loading balance.
18. Solvent dispenser (15 mL for 15-g sample) and 1- to 4-L bottle.
19. Centrifuges.
20. Vials containing anhydrous NaAc plus anhydrous $MgSO_4$: add 1.5 g anhydrous NaAc plus 6 g anhydrous $MgSO_4$ to each vial for use with 15-g sample size (*see* **Note 8**).

21. Sealable centrifuge tubes (2–15 mL) containing powders for dispersive SPE: add 50 mg PSA sorbent plus 150 mg anhydrous $MgSO_4$ per 1 mL of extract to undergo cleanup (*see* **Note 8**).

Optional items:

1. Mechanical shaker, probe blender, or sonication device.
2. C18 sorbent with 40-μm particle size (*see* **Note 9**).
3. Graphitized carbon black (GCB; e.g., Supelco or Restek) (*see* **Note 10**).
4. Vortex mixer.
5. Minicentrifuge.
6. Evaporator (e.g., Turbovap or N-Evap).
7. Graduated centrifuge tubes (10–15 mL) for use in evaporator.
8. Calibration standard spiking solutions w, x, y, and z in toluene (for w, x, y, and z standards): add 50 μL of ISTD stock solution and 12.5•(w, x, y, and z) μL of the 40-ng/μL working standard pesticides solution at RT per (w, x, y, and z) ng/g desired equivalent calibration standard concentration into a 25-mL volumetric flask and fill to the mark with toluene. For example, if the w standard is to be 10 ng/g, then add 125 μL of the 40-ng/μL working standard pesticides solution to the flask. Transfer the solutions to four 8-mL dark glass vials with Teflon-lined caps and store at 20°C or below.

2.3. Analysis of GC-Amenable Pesticides

1. GC–MS system.
2. Programmable temperature vaporizer for LVI.
3. Autosampler.
4. A 30-m analytical capillary column with 0.25 mm id, 0.25 μm of (5% phenyl)-methylpolysiloxane low-bleed stationary phase (e.g., DB-5ms or equivalent).
5. Retention gap such as a 1- to 5-m, 0.25 mm id deactivated capillary column.
6. Helium at 99.999% purity.

Alternatives:

1. GC system(s) coupled with selective detector(s) such as pulsed flame photometric detector, flame photometric detector, halogen-specific detector, electron capture detector, electrolytic conductivity detector, atomic emission detector, nitrogen–phosphorus detector.
2. Split/splitless injector.

2.4. Analysis of LC-Type Pesticides

1. LC–MS/MS system.
2. ESI ion source.
3. Automated divert valve placed between analytical column and ion source.
4. Syringe pump for direct infusion of solutions into ion source.
5. Autosampler.
6. HPLC-grade methanol (MeOH).
7. HPLC-grade water.
8. Double-distilled, 88% formic acid.
9. 5 m*M* Formic acid in MeOH: add 214 μL formic acid to MeOH in 1 L solution.
10. 5 m*M* Formic acid in water: add 214 μL formic acid to water in 1 L solution.
11. 6.7 m*M* Formic acid in water: add 72 μL formic acid to water in 250 mL solution.
12. 15 cm long, 3.0 mm id, 3-μm particle size C18 analytical column.
13. 4 cm long, 3.0 mm id C18 guard column.

Step	Procedure
0.	Comminute >1 kg sample with vertical cutter; Homogenize ≈200 g subsample with probe blender
1,2.	Transfer 15 g subsample to 50 mL FEP tube
3-5.	Add 15 mL 1%Hac in MeCN + 1.5 g anh. NaAc + 6 g anh. $MgSO_4$ + 150 µL I.S. solution
6,7.	Shake vigorously for 1 min; Centrifuge >1500 rcf for 1 min
8,9.	Transfer 1-8 mL to tube with 150 mg anh. $MgSO_4$ + 50 mg PSA per mL extract and shake briefly
10.	Centrifuge >1500 rcf for 1 min
11-16a.	Transfer 0.5-1 mL extract to GC vial and add TPP; Transfer 0.15-0.3 mL to LC vial and add 0.45-0.9 mL 6.7 mM formic acid
11-14b.	Transfer 0.25 mL from step 10 to LC vial; Add TPP and 0.86 mL 6.7 mM formic acid
15b.	Transfer 4 mL from step 10 to grad. cent. tube; Add TPP and 1 mL toluene
16-18b.	Evaporate at 50°C with N_2 to 0.3-0.5 mL; Add toluene to make 1 mL
19-21b.	Add 0.2 mL anh. $MgSO_4$ and swirl >6 mL mark; Centrifuge >1500 rcf for 1 min; Transfer ≈0.6 mL to GC vial
17a/22b.	Analyze by (LVI/)GC/MS and LC/MS-MS

Fig. 1. Outline of the protocol in the QuEChERS method.

Alternatives:

1. LC system(s) coupled with selective detector(s) (e.g., fluorescence, diode array detector, ultraviolet/visible absorbance).
2. Postcolumn derivatization system and reagents.

3. Methods

Figure 1 shows a flowchart of the overall protocol of the approach, including the QuEChERS sample preparation method and its two main options that essentially depend on the desired LOQ in GC–MS. **Option A** relies on LVI to achieve the low LOQ if needed, and **Option B** entails solvent evaporation and exchange to toluene to increase the amount of equivalent sample injected in splitless mode. Once all the mate-

rials are ready and the 15-g homogenized subsamples have been weighed into the 50-mL tubes, a single analyst can prepare 10–20 extracts with the QuEChERS method in approx 30–40 min in **Option A**. The solvent exchange and evaporation step in **Option B** approximately doubles the time needed for the analyst to complete the method.

3.1. Sample Comminution

For food samples, an appropriate chopper (e.g., vertical cutter) must be used to comminute large, representative sample portions up to 9 kg *(1)*. Blend the sample until it gives a consistent texture. Transfer approx 200 g to a sealable container for freezer storage after further comminution with a probe blender. Blend the subsample with the mixer until it is homogeneous. A second subsample (e.g., 15 g) is taken for extraction immediately, and the container is then sealed and stored in the freezer in case reanalysis is necessary (*see* **Notes 11** and **12**).

3.2. QuEChERS Sample Preparation

The QuEChERS method may be scaled appropriately to any subsample size shown to be adequately representative of the original sample. If LVI is not used for GC–MS, then 12 g or more must be extracted to typically detect <10 ng/g of the pesticides in food. The method is designed for samples with >75% moisture. If needed, add water to hydrate drier samples so that moisture becomes approx 80% and pores in the sample are more accessible to the extraction solvent. The following instructions are scaled for 15-g samples (after hydration, if needed) extracted in 50-mL FEP centrifuge tubes. *Safety note*: Work with pesticides and solvents in a hood and wear appropriate laboratory safety glasses, coat, and gloves; ensure that the centrifuge is balanced and do not exceed the safety limits of the tubes or rotors used.

3.2.1. Extraction and Cleanup

1. Weigh 15 g sample into each tube (use 13 mL water for a reagent blank).
2. Weigh 15 g blank(s) to attain enough extract for five matrix-matched calibration standards as described in **Subheadings 3.2.2.** and **3.2.3.** Add 75 μL working standard pesticides solution to an additional matrix blank (this will yield 200 ng/g) as a quality control (QC) spike for evaluating recoveries.
3. Add 15 mL 1% HAc in MeCN into each tube using the solvent dispenser.
4. Add 150 μL of ISTD solution (this will yield 200 ng/g) to samples, reagent blank, and QC spike, but not to blank(s) used for matrix-matched calibration standards (*see* **Note 13**).
5. Add 6 g anhydrous $MgSO_4$ plus 1.5 g anhydrous NaAc (or 2.5 g NaAc•$3H_2O$) to all tubes (the extract will reach 40–45°C) and seal the tubes well (ensure that powder does not get into the screw threads or rim of the tube).
6. Shake the tubes vigorously by hand for 1 min (using a motion from the arms more than the wrist) with 3–5 tubes at once in each hand, ensuring that the solvent interacts well with the entire sample and that crystalline agglomerates are broken up sufficiently during shaking. (*see* **Note 14**).
7. Centrifuge the tubes at more than 3000*g*. The greater the force, the better for forming a solid sample plug and providing potentially cleaner extracts.
8. Transfer needed amount (1–8 mL) of the MeCN extract (upper layer) at RT to the dispersive-SPE tubes containing 50 mg PSA (and C18 for fatty samples) plus 150 mg anhy-

drous MgSO$_4$ per milliliter extract. For matrix blanks to be used for the five matrix-matched calibration standards, first combine the blank extracts (if multiple blanks were extracted), then either transfer the needed amounts (1–8 mL) into separate dispersive SPE tubes as with the sample extracts or proportionally scale up the dispersive SPE step to obtain the extract volume needed for the standards after cleanup (*see* **Subheadings 3.2.2.** and **3.2.3.** for further explanation).

9. Seal the tubes well and mix by hand (or use a vortex mixer) for approx 30 s.
10. Centrifuge the dispersive SPE tubes at more than 3000*g*.

3.2.2. Options for Handling Extracts for Analysis

Depending on the LOQ needed, the chosen pesticide analytes, and analytical instruments and techniques used, 1–8 mL of the extract will be taken for dispersive-SPE cleanup. This cleanup technique loses half of the extract volume to the powder reagents, and the extraction method yields 1-g/mL equivalent sample concentrations. For GC–MS, approx 8 mg should be injected to generally achieve an LOQ below 10 ng/g, assuming that matrix interferences are not the limiting source of noise. If this degree of sensitivity is needed, then either LVI (e.g., 8-μL injection) must be used or the extracts must be concentrated. LVI is the simpler option, but if such a device is not available on the GC instrument (or it does not provide acceptable results for certain pesticide analytes), then splitless injection of the concentrated extract is the remaining option. When performing the MeCN evaporation step in this option, it is convenient to exchange solvent to toluene, which acts as a good keeper for the pesticides and has benefits in traditional GC analysis (e.g., smaller vaporization expansion volume). Further details, including a comparison of GC injection solvents, are provided elsewhere for this application *(13,39)*.

In **Option A**, if the desired LOQ can be achieved in GC with injection of the MeCN extract (using LVI or not), then a 1-mL aliquot is taken to minimize reagent costs (or a larger volume is taken, and the procedure is scaled up appropriately at slightly greater materials cost). In **Option B**, if direct injection of the MeCN extract in GC cannot achieve the necessary LOQ using the available instrumentation, then 8 mL is taken for dispersive SPE cleanup, and an extract concentration and solvent exchange step is performed prior to GC analysis (LC injection volume can be more easily increased, thus extract concentration is less of an issue in that case). Each of these options is described as follows:

Option A. Use 1 mL extract in **step 8**, and then after **step 10**:

11a. Transfer 500 μL of the final extracts from the dispersive SPE tubes (or five 500-μL aliquots of the combined matrix blank extract after dispersive SPE) to autosampler vials for (LVI) GC–MS.
12a. Add 50 μL of the 2-ng/μL TPP working solution at RT to all extracts (to make 200-ng/g equivalent concentration and 0.09% HAc, which improves stability of certain pesticides).
13a. Add 25 μL of MeCN to all sample extracts, the QC spike, the reagent blank, and the zero standard (to compensate for the volume to be added to the calibration standards in the next step).
14a. Follow procedures described in **Subheading 3.2.3.**, **Option A**, for the four matrix blank extracts to be used for matrix-matched calibration standards (*w*, *x*, *y*, and *z* standards).

15a. Cap and shake the vials to mix solutions, then uncap them.

16a. Transfer 150 µL of the extracts from each vial to a counterpart LC autosampler vial into which 0.45 mL of 6.7 m*M* formic acid solution has been added (this is done to match the organic solvent and formic acid contents in the initial LC mobile phase of 5 m*M* formic acid in 25% MeOH).

17a. Cap all vials and conduct (LVI) GC–MS and LC–MS/MS analytical sequences according to **Subheadings 3.3.** and **3.4.**

Option B. Use 8 mL extract in **step 8**, and then after **step 10**:

11b. Transfer 250 µL of the MeCN extracts from the dispersive SPE tubes (or five 250-µL aliquots of the combined matrix blank extract after dispersive SPE) to autosampler vials for LC–MS/MS.

12b. Add 25 µL of the 2-ng/µL TPP working solution at RT to all vials and 12.5 µL of MeCN to all sample extracts, the QC spike, the reagent blank, and the zero standard.

13b. Follow procedures described in **Subheading 3.2.3.**, **Option B**, for the four matrix blank extracts to be used for the *w*, *x*, *y*, and *z* standards.

14b. Add 860 µL of 6.7 m*M* formic acid solution to achieve the acid concentration and organic solvent content at the initial LC mobile phase and cap all vials.

For evaporation and solvent exchange to toluene for GC–MS (without LVI):

15b. Transfer 4 mL of each extract (or five 4-mL aliquots of the combined matrix blank extract after dispersive SPE) to 10- to 15-mL graduated centrifuge tubes containing 1 mL of toluene and 400 µL of the 2-ng/µL TPP working solution added at RT.

16b. Evaporate the extracts at 50°C and sufficient N$_2$ gas flow until volume is 0.3–0.5 mL.

17b. Follow procedures described in **Subheading 3.2.3.**, **Option B**, for the four matrix blank extracts to be used for the *w*, *x*, *y*, and *z* standards.

18b. Add toluene to take each extract up to the 1-mL mark

19b. Add anhydrous MgSO$_4$ to reach the 0.2-mL mark on the tube and swirl to rinse above the 6-mL mark.

20b. Centrifuge the tubes at more than 600*g*.

21b. Transfer ≈0.6 mL of the final extract to the GC autosampler vials, and cap all vials.

22b. Conduct (LVI/)GC/MS and LC/MS-MS analytical sequences according to **Subheadings 3.3.** and **3.4.**

3.2.3. Preparation of Matrix-Matched Calibration Standards

The concentration range of the matrix-matched calibration standards is to be decided by the analyst, and these concentrations are listed as *w*, *x*, *y*, and *z* (given as nanograms-per-gram equivalent concentrations with respect to the original sample). As an example, if the LOQ of the method is 10 ng/g, then the four suggested concentrations of the standards are 10, 50, 250, and 1250 ng/g. In continuation of the procedures above, the instructions for the preparation of the matrix-matched calibration standards are as follows:

Option A. If 1- to 2-mL aliquots of the extracts are taken for dispersive SPE in **step 8**, then only a single 15-g matrix blank is typically needed to provide enough extract for the zero, *w*, *x*, *y*, and *z* standards. For the 0.5-g equivalent extracts described in **step 14a**, add 25 µL of the respective calibration standard spiking solution (*w*, *x*, *y*, and *z*) at RT to the appropriate four matrix blank extracts (*w*, *x*, *y*, and *z* standards). Similarly, if

2-mL aliquots are taken in **step 8,** then 1-mL extracts are to be transferred in **step 11a**, in which case add 50 μL of the respective calibration standard spiking solution (*w*, *x*, *y*, and *z*) to the appropriate four matrix blank extracts (*w*, *x*, *y*, and *z* standards) in **step 14a**.

Option B. At least 22 mL of matrix blank extract is needed after dispersive SPE cleanup (or ≥44 mL of initial extract) to prepare the zero, *w*, *x*, *y*, and *z* standards. Depending on the matrix and water content, a 15-g sample will typically yield 11 mL MeCN extract after centrifugation, thus four (but maybe five) 15-g blank samples need to be extracted. For the *w*, *x*, *y*, and *z* standards in LC–MS/MS described in **step 13b**, add 12.5 μL of the respective calibration standard spiking solutions *w*, *x*, *y*, and *z* at RT. For the *w*, *x*, *y*, and *z* standards in toluene for GC–MS as described in **step 17b**, add 200 μL of the respective calibration standard spiking solution (*w*, *x*, *y*, and *z*) at RT. The calibration standard spiking solutions for GC in this case should preferably be in toluene. If the spiking solution is in MeCN, then 200 μL MeCN should also be added to the other extracts in **step 18b**. Be aware that the presence of 20% MeCN may lead to poor chromatography, and MeCN should not be added if an N-sensitive GC detector (e.g., nitrogen–phosphorus detector) is used without a detector bypass vent.

3.3. Analysis of GC-Amenable Pesticides

Generic conditions are given next and in **Table 2** for the GC–MS analysis of selected pesticides from the list in **Table 1**. The analyst may use many different sets of conditions that offer equally valid results in the separation and detection of pesticides of their particular interest. In fact, the analyst should optimize the given conditions to yield the lowest LOQ for their chosen analytes in the shortest amount of time. The selected ions for quantitation and identification should be made to maximize S/N ratios of the analytes while avoiding matrix interferences. Information about the expected retention times (t_R) and intense ions in the mass spectra for hundreds of pesticides are listed elsewhere *(1,7,8)*. Commercial mass spectral libraries (e.g., National Institute of Standards and Technology [NIST] and Wiley) also contain the EI spectra of hundreds of pesticides, which can help determine their t_R and choose quantitation masses when optimizing the GC conditions.

Otherwise, the way to determine the t_R and mass spectrum for a pesticide is to inject > 1 ng and look for the peak(s). The presence of the molecular ion (M^+) in the spectrum helps ensure that the pesticide does not degrade during injection, and if no library spectrum is available, it should be verified that the spectrum makes sense relative to the structure of the pesticide. In general, the analyst should choose the ion(s) for quantitation with the highest intensity at higher mass, but all selections should be verified to meet LOQ requirements in the matrix(es) of interest. Proper choice of quantitation ions can often substantially reduce LOQ, especially in complex backgrounds.

For extracts in MeCN, inject only as much as needed to achieve the LOQ desired in the analysis. Split mode (e.g., 10:1 split ratio for a 1-μL injection) may be all that is needed for applications designed to detect pesticides >1 μg/g in the samples, but LVI is required for maximal sensitivity. In most applications, 8 mg equivalent sample injected onto the column should be sufficient to achieve <10 ng/g LOQ for most pesticides. This would necessitate 8-μL injection in LVI of the 1-g/mL MeCN extracts from **Subheading 3.2.2.**, **Option A**. In this case, it is suggested to program the pro-

Table 2
Conditions for the GC–MS Analysis of Selected Analytes, TPP, and the ISTD Using the Generic GC Method Described in the Text

Analyte	t_R (min)	Quant. ion(s) (m/z)	Analyte	t_R (min)	Quant. ion(s) (m/z)
Methamidophos	5.95	94,95, 141	Dichlorobenzophenone	13.85	139, 250
Dichlorvos	6.02	109,185	Cyprodinil	14.39	224
Propoxur	8.96	110,152	Penconazole	14.57	213,248
Ethoprophos	9.22	158,200	Tolylfluanid	14.68	137,181,238
Hexachlorobenzene	10.15	282,284,286	Heptachlor epoxide	14.80	237,353
Lindane	10.79	181,183,219	cis-Chlordane	15.80	237,272,375
Diazinon	10.89	179,276,304	p,p'-DDE	16.47	246,316,318
Chlorothalonil	11.26	264, 266,268	Dieldrin	16.63	263,277
Chlorpyrifos-methyl	12.17	199,286,288	Endosulfan sulfate	18.27	237,272,387
Carbaryl	12.49	115,116,144	TPP (QC)	18.50	326
Dichlofluanid	13.20	123, 224	cis-Permethrin	20.54	183
Chlorpyrifos	13.44	199,314,316	trans-Permethrin	20.66	183
d_{10}-Parathion (ISTD)	13.61	301	Coumaphos	20.73	226,334,362

Quant., quantitation.

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grammable temperature vaporizer to start at 75°C for 3 min followed by a 200°C/min temperature ramp to 275°C. A solid plug of Carbofrit or inert sorbent is typically needed to contain the liquid solvent during the vaporization process in LVI *(12,40,41)*. For the 4-g/mL extracts in toluene, 2-μL splitless injection at 250°C should be satisfactory to achieve <10 ng/g LOQ. It is recommended to use a 1– 5-m phenylmethyl-deactivated guard column as a retention gap to minimize solvent condensation effects and better protect the analytical column. This also serves to reduce the effect of GC maintenance on the t_R of the analytes because the retention gap is shortened, not the analytical column.

For GC, set helium head pressure on the column at 10 psi or flow to 1 mL/min with systems capable of electronic pressure/flow control. After an appropriate time for solvent delay (e.g., approx 1.5 min in splitless and approx 4 min in LVI), a generic oven temperature program for MeCN extracts is 75°C initial temperature ramped to 175°C at 25°C/min, then to 225°C at 5°C/min, followed by a 25°C/min ramp to 290°C, at which it is held for 10 min. In the case of toluene injections, the initial oven temperature should be increased to 100°C and everything else kept the same. Of course, other temperature programs may be used, but in any case, peak shapes should be Gaussian, and peak widths at half-heights should be less than 5 s.

For the MS, the analyst should follow the instructions provided by the instrument manufacturer to optimize the system for detection of the pesticide analytes. Prior to injection of the sequence, a system suitability test should be made, such as autotuning of the MS, to help ensure that analytical quality is acceptable *(15,16,42)*. It is suggested that the calibration standards be dispersed throughout the sequence to demonstrate adequate instrument performance over the entire time-frame that the samples are injected.

3.4. Analysis of LC-Type Pesticides

In the case of LC–MS/MS, the analyst should follow the instrument manual guidelines to set the ion source temperature, gas flows, voltage potentials, and other general parameters for the particular instrument and analytical needs. Based on the LC conditions described below, the MS/MS detection parameters for each analyte should be optimized by using a syringe pump to infuse approx 1 ng/μL of the pesticide in 5 m*M* formic acid in 1/1 MeOH/water solution at 0.3 mL/min into the source. Most of the pesticides will ionize well in ESI-positive (+) mode, thus the M^{+1} mass spectral peak should first be optimized, and then conditions for collision-induced dissociation to maximize the S/N ratio of the first MS/MS transition should be determined. Most instruments have automated programs to optimize the parameters with little analyst intervention. It is not difficult also to test the signals in negative mode for comparison purposes, but nearly all of the LC-type analytes listed in **Table 1** ionize sufficiently well in ESI$^+$. In some cases, Na$^+$ adducts with the ionized pesticides form in the ion source. This is not necessarily problematic if all of the analyte generates the adduct and if the result is quantitatively reproducible.

Each instrument will give somewhat different optimized settings, even for the same model, but sensitivity will not typically be a problem. With the latest instruments,

Table 3
Conditions for the LC–MS/MS Analysis of Selected Pesticides in a Triple Quadrupole Instrument Using ESI⁺ Mode at the LC Conditions Given in the Text

Analyte	t_R (min)	Precursor ion (m/z)	Product ion (m/z)	Collision energy (V)
Methamidophos	9.6	141.8	112.0	17
Pymetrozine	10.0	217.9	105.0	27
Acephate	11.1	138.8	143.0	19
Carbendazim	11.9	191.8	160.0	25
Thiabendazole	13.2	201.8	174.9	37
Imidacloprid	15.9	255.9	209.0	21
Imazalil	16.3	296.8	159.0	31
Thiophanate-methyl	18.8	342.8	151.0	29
Dichlorvos	19.0	220.7	127.0	23
Carbaryl	19.3	202.2	145.0	13
Dichlofluanid	20.9	332.7	223.8	17
Ethoprophos	21.2	242.8	173.0	21
Cyprodinil	21.2	225.9	108.0	35
Tolylfluanid	21.3	346.7	237.9	15
Penconazole	21.5	283.8	159.0	39

LOQ < 10 ng/g can typically be achieved with a 10-µL injection volume for the extracts of 0.25-g/mL equivalent sample concentrations. The water and acid contents of the extract closely match the initial LC mobile phase; thus, a larger volume may be injected without seriously affecting peak shapes. Therefore, a lower LOQ can usually be achieved by injecting a larger volume of the extract if needed.

As an example, generic LC conditions for the analysis of multiple pesticide residues are as follows: 0.3-mL/min flow rate; reservoirs to contain 5 m*M* formic acid in (1) water and (2) MeOH; gradient program of 25% solution B ramped to 100% linearly over 15 min, then held for an additional 15 min. After 30 min, the flow can be increased to 0.5 mL/min and the mobile phase returned to 75% solution A over the course of 2 min and allowed to equilibrate for 6 min. A divert valve should be placed between the column outlet and ion source to eliminate the introduction of salts and early eluting matrix components into the MS instrument before the t_R of the first analyte and any coextracted matrix components that may elute after the last pesticide of interest. Some pesticides may give broad or dual peaks if the mobile phase pH is not acidic enough. In this case, the formic acid content may be increased in an attempt to provide better chromatographic peak shapes. **Table 3** lists sample conditions for 15 selected pesticides in LC–MS/MS.

Just as with GC–MS, QA protocols should be followed and system suitability tests conducted prior to analyzing a sequence of samples. Regular preventive maintenance must be done to ensure adequate operation of the instruments. Inject the matrix blank to determine if a significant interferant is present at the t_R of the analytes. No evidence of carryover should be present in the reagent blank, which should be injected after the most highly concentrated standard in the sequence.

3.5. Data Analysis

Quantitation is based on least-linear-squared calibration of analyte peak areas divided by the ISTD peak areas plotted vs analyte concentration. The ratio of analyte peak area to ISTD peak area becomes the signal S. The analyte concentrations in the matrix-matched standards on a per-sample basis (nanogram per gram) can be determined by multiplying the volume (microliters) added to the extract by the analyte concentrations in the added solutions (nanograms per microliters) and dividing by the equivalent amount (grams) of sample in the extract. The concentrations (in nanograms per gram) C of the pesticide analytes in the samples and QC matrix spike are determined from the equation

$$C = (S - y \text{ intercept})/\text{Slope}$$

If there are no interferences, the y-intercept should be nearly zero, and the correlation coefficient of the slope should be >0.99. In some circumstances, a nonlinear relationship occurs in the calibration plot and a quadratic best-fit curve may provide better correlation and results. The TPP is a QC measure to isolate the variability of the analytical step from the sample preparation method. The volumes of the final extracts and each preceding step are carefully controlled in the sample preparation protocol, and ideally the ISTD would not need to be used to achieve equally accurate results. Pipets, syringes, and balances should be periodically calibrated to ensure accuracy. However, random and systematic errors in volumetric transfers are inherent in analytical methods, and the ISTD should improve the accuracy of the results. The recoveries of the ISTD can be assessed by comparing the peak areas of the ISTD in the samples with those from the calibration standards. The TPP/ISTD peak area ratio should remain consistent (<10% relative standard deviation) in the method, and if any extract gives a substantially different ratio from the others, then the results from that extract should be questioned. Furthermore, if the QC spike yields recoveries <70% or >120%, then the results from all the samples should be questioned. If all pesticide recoveries outside the acceptable range are the same, then a systematic bias is indicated. If variable recoveries are obtained, then a systematic bias is not likely to be the source of the problem. Many pesticides can be analyzed by both LC–MS/MS and GC–MS, and the comparison of their results from both distinct methods can be invaluable in isolating any problems that may occur with one of the instruments or techniques.

4. Notes

1. NaAc•3H_2O may be substituted for anhydrous NaAc, but 1.7 g per gram sample must be used rather than 1 g anhydrous NaAc per gram sample.
2. Heat bulk quantities of anhydrous $MgSO_4$ to 500°C for longer than 5 h to remove phthalates and any residual water prior to its use in the laboratory, but this is not critical.
3. Aminopropyl SPE sorbent can be substituted for PSA, but 75 mg per milliliter of extract should be used.
4. Toluene is the most suitable solvent for long-term storage (>10 yr) of pesticide stock solutions in general because of its slower evaporation rate, miscibility with MeCN, and the higher solubility and stability of pesticides in toluene compared to other solvents. However, not all pesticides can be dissolved at 10 mg/mL in toluene. In these cases,

MeCN, acetone, MeOH, or ethyl acetate should be used, but long-term stability may become an issue, and old solutions should be replaced more often.

5. The choice of ISTD is very important because it must not already be present in the sample, but be completely recovered in the method for detection in both GC–MS and LC–MS/MS (otherwise, a pair of ISTDs may be used). A relatively inexpensive deuterated pesticide (d_{10}-parathion) was chosen as the ISTD in this protocol. Deuterated chlorpyrifos or chlorpyrifos-methyl are more suitable for a greater range of selective GC detectors, but they are more expensive. It is also possible to use an uncommon compound as the ISTD, but its suitability would have to be determined.

6. In this protocol, 250 is the maximum number of pesticides that can be added to make this solution, which will consist of 100% toluene if the stock standards are all in toluene. This is not ideal for spiking of the sample or preparation of calibration standard spiking solutions. The stock standards can be prepared in MeCN, but some pesticides will have reduced stability, which is significantly improved in 0.1% HAc solution, but degradation is not eliminated *(39)*. An alternative approach is to prepare mixtures of pesticides in stock solutions by dissolving multiple pesticide reference standards in the same vial.

7. The TPP working standard is prepared in MeCN with 1% HAc because when it is added to the MeCN extracts in **steps 12a** or **12b** and **15b**, the extract will contain 0.09% HAc for improved stability of certain pesticides (e.g., chlorothalonil, captan, folpet, tolylfluanid, dichlofluanid, carbaryl).

8. To speed the process greatly, the density of the powders can be determined and scoops made of the appropriate volume, but weighing should still be done to check consistency (reagent weights ± 5% deviation from the stated amount are acceptable). The containers should be sealed during storage and can be refilled and reused without cleaning between uses. A commercial product (#CUMPSC2CT) containing 50 mg of PSA + 150 mg anh. $MgSO_4$ is available from United Chemical Technologies.

9. If samples contain ≥ 1% fat, add the same amount of C18 sorbent as PSA (in addition to PSA) in centrifuge tubes for dispersive SPE. As the fat content increases, a third phase (lipids) will form in the extraction tube, and the recoveries of nonpolar pesticides will decrease as they partially partition into the lipid layer. The most nonpolar pesticides (e.g., hexachlorobenzene, DDE) will give < 70% recovery at approx 5% fat content, but relatively polar GC-amenable and LC-type pesticides are completely recovered at >15% fat *(14)*. A commercial product (#CUMPSC18CT) containing 50 mg each of PSA and C18 + 150 mg anh. $MgSO_4$ is available from United Chemical Technologies.

10. If none of the analytes have planar structures, then GCB can be used to provide additional cleanup, especially for removal of chlorophyll, sterols, and planar matrix coextractives. In this case, add the same amount of GCB as PSA and C18 (50 mg each per 1 mL of extract) in centrifuge tubes for dispersive-SPE. Planar pesticides include terbufos, thiabendazole, hexachlorobenzene, and quintozene, among many others *(11)*.

11. The advantages of this approach include (1) the extracted portion is highly representative of the initial sample; (2) the sample is well comminuted to improve extraction by shaking rather than blending; (3) less time is spent on the overall homogenization process than trying to provide equivalent homogenization of the large initial sample using the chopper alone; and (4) a frozen subsample is available for reanalysis if needed. The sample homogenization step is a critical component in the overall sample preparation process; unfortunately, many analysts do not pay adequate attention to this important step. If the sample is not homogenized properly, then the analytical results will not be as accurate as they could be, independent of the performance of the sample preparation and analytical steps.

12. To provide the most homogeneous comminuted samples, frozen conditions, sufficient chopping time, and appropriate sample size to chopper volume should be used. Use of frozen samples also minimizes degradative and volatilization losses of certain pesticides (e.g., dichlorvos, chlorothalonil, dichlofluanid). If best results of susceptible pesticides are needed, then cut the food sample into 2- to 5-cm^3 portions with a knife and store the sample in the freezer prior to processing. Cryogenic blending devices, liquid nitrogen, or dry ice may also be used (but make sure all dry ice has sublimed before weighing samples and ensure that water condensation is minimal, especially in a humid environment). For further information about sample processing in pesticide residue analysis of foods, the analyst should refer to several publications on the topic *(43–48)*.

13. An uncommon or deuterated pesticide standard may be spiked into the sample during homogenization to determine the effectiveness of the procedure through the measurement of recovery and reproducibility using the technique and specific devices. For typical applications, the recovery should be > 70%, with relative standard deviation < 20% for a 100- to 500-ng/g fortification level.

14. Alternately, do not seal the tubes and use a probe blender for extraction, taking care not to overheat the extract. Another option is to extract using sonication. These stronger measures may be needed to ensure that any bound residues are extracted. Fruits, vegetables, and other high-moisture samples do not typically interact strongly with the residues, and shaking alone is usually acceptable for extraction of nearly all pesticides. However, dry or porous/sorptive sample types, such as grains and soils, require blending, higher temperature, more acidic or basic conditions, or more time to completely extract those residues prone to strong matrix interactions.

Acknowledgment

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