RESIDUES AND TRACE ELEMENTS

Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce

MICHELANGELO ANASTASSIADES[1] and STEVEN J. LEHOTAY[2]
U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 E. Mermaid Ln, Wyndmoor, PA 19038
DARINKA ŠTAJNBAHER
Public Health Institute, Environmental Protection Institute, Prvomajska 1, 2000 Maribor, Slovenia
FRANK J. SCHENCK
U.S. Food and Drug Administration, Office of Regulatory Affairs, Southeastern Regional Laboratory, 60 Eighth St, Atlanta, GA 30309

A simple, fast, and inexpensive method for the determination of pesticide residues in fruits and vegetables is introduced. The procedure involves initial single-phase extraction of 10 g sample with 10 mL acetonitrile, followed by liquid–liquid partitioning formed by addition of 4 g anhydrous $MgSO_4$ plus 1 g NaCl. Removal of residual water and cleanup are performed simultaneously by using a rapid procedure called dispersive solid-phase extraction (dispersive-SPE), in which 150 mg anhydrous $MgSO_4$ and 25 mg primary secondary amine (PSA) sorbent are simply mixed with 1 mL acetonitrile extract. The dispersive-SPE with PSA effectively removes many polar matrix components, such as organic acids, certain polar pigments, and sugars, to some extent from the food extracts. Gas chromatography/mass spectrometry (GC/MS) is then used for quantitative and confirmatory analysis of GC-amenable pesticides. Recoveries between 85 and 101% (mostly >95%) and repeatabilities typically <5% have been achieved for a wide range of fortified pesticides, including very polar and basic compounds such as methamidophos, acephate, omethoate, imazalil, and thiabendazole. Using this method, a single chemist can prepare a batch of 6 previously chopped samples in <30 min with approximately $1 (U.S.) of materials per sample.

Received September 4, 2002. Accepted by JS October 25, 2002.

[1] Current address: Chemisches und Veterinäruntersuchungsamt Stuttgart, Schaflandstrasse 3/2, 70736 Fellbach, Germany.

[2] Author to whom correspondence should be addressed; e-mail: slehotay@arserrc.gov.

Mention of brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture above others of a similar nature not mentioned.

Pesticide residue analysis of food and environmental samples has been performed in numerous government and private laboratories throughout the world for approximately 40 years. However, the methods used for analysis of common pesticides are far from ideal. Some residue monitoring laboratories still use methods developed 30 years ago when analytical needs were less demanding, solvent usage was less of an issue, extended analysis time and manual labor were the norm, and technology was less capable than today. Modern residue monitoring programs, however, are expected to be responsive to the latest developments in agriculture and new legislation. The introduction of new, more rapid, and effective analytical approaches, therefore, is essential for laboratories to improve overall analytical quality and laboratory efficiency.

Without question, the most efficient approach to pesticide analysis involves the use of multiclass, multiresidue methods (MRMs). The first notable MRM was the Mills method developed in the 1960s by U.S. Food and Drug Administration (FDA) chemist P.A. Mills (1). At that time, nonpolar organochlorine insecticides (OCs) were the main focus for analysis. With the Mills method, OCs and other nonpolar pesticides were extracted from nonfatty foods with acetonitrile (MeCN), which was then diluted with water, and the pesticides were partitioned into a nonpolar solvent (petroleum ether). As a consequence, relatively polar pesticides, such as certain organophosphorus insecticides (OPs), were partially lost during this step. The need to analyze more polar OPs and other pesticides in agriculture initiated the development of alternative procedures to determine compounds not extracted by the Mills method. These methods often simply modified the Mills procedure by using the initial MeCN extract but with different partitioning, cleanup, and determinative steps (2–4).

In the 1970s, new methods were developed to extend the analytical polarity range to cover OCs, OPs, and organonitrogen pesticides (ONs) in a single procedure (5, 6). These multiclass MRMs differed from the Mills approach in that acetone, rather than MeCN, was used for the initial extraction. However, the new methods still used nonpolar sol-

vents (dichloromethane or dichloromethane–petroleum ether) to remove the water in a liquid–liquid partitioning step. Furthermore, NaCl was added to the water phase in both methods during the partitioning step, the amount of which had a direct effect on the polarity range covered by the methods. Becker (5), who developed the first MRM of this kind, added an NaCl solution to the initial extract, which only partially saturated the water phase with salt. Luke et al. (6) and Specht and Tilkes (7), however, added solid NaCl to saturate the water phase, which forced more acetone into the organic layer, thus increasing its polarity and leading to higher recoveries of the polar analytes.

Soon after their introductions, the Becker method became official in Germany as the DFG-S8 procedure, and the Specht method became the official DFG-S19 procedure (8). The Luke method, which replaced the Mills method in the FDA, became the official PAM 302 E1 procedure (9), and several years later became AOAC Official Method **985.22** (10, 11). These multiclass MRMs and their many variations are still widely used by pesticide residue monitoring laboratories worldwide.

Since the 1980s, environmental and health concerns related to the use of chlorinated solvents have led to the development of many new methods in which such solvents were avoided. Specht et al. (12) and Anastassiades and Scherbaum (13) used a mixture of cyclohexane–ethyl acetate (1 + 1) instead of dichloromethane (or dichloromethane–petroleum ether, 1 + 1) to induce partitioning. Casanova (14) and Nordenmeyer and Thier (15) used solid-phase extraction (SPE) to extract pesticides from diluted acetone extracts, thus completely avoiding liquid–liquid partitioning. Luke et al. (16) added a combination of fructose, $MgSO_4$, and NaCl to separate water from acetone in the initial extract without using nonpolar solvents. Schenck et al. (17) also investigated the use of salts to form a water–acetone partition in extracts. Parfitt (18) studied exposure of the initial Luke extracts to low temperatures to remove the water phase by freezing it out of solution. All these separation approaches have one or more disadvantages in practice because acetone is simply too miscible with water to be easily separated without using nonpolar solvents.

MeCN is more easily and effectively separated from water than acetone (17, 19) upon addition of salts. In the case of MeCN, salt alone can be used to form a satisfactory separation from water, and Lee et al. (20) used NaCl for this purpose rather than the nonpolar co-solvent of the Mills method. Several other multiclass MRMs have been published since then, following this salting-out principle with MeCN (21–27).

A third extraction solvent commonly used in multiresidue MRMs is ethyl acetate (EtAc; 28–32). EtAc has the advantage of partial immiscibility with water, which makes the addition of other nonpolar solvents to separate water from the extract unnecessary. However, a problem with EtAc is that some of the most polar pesticides do not readily partition into the EtAc phase. To increase recoveries of polar compounds, large amounts of Na_2SO_4 are usually added in MRM procedures, with EtAc used to bind the water. Polar co-solvents, such as methanol and ethanol, have been used to increase the polarity of the organic phase (31). Another disadvantage associated with the use of EtAc is the extraction of a large amount of nonpolar co-extractives, such as lipids and epicuticular wax material, which must be removed before the determinative step. This is commonly achieved by a time-consuming and wasteful gel-permeation chromatographic (GPC) cleanup step.

During the 1990s, increased urgency to further reduce solvent usage and manual labor in analytical laboratories led to the commercial introduction of several alternative extraction approaches, including supercritical fluid extraction (SFE; 33–36), matrix solid-phase dispersion (MSPD; 37–39), microwave-assisted extraction (MAE; 40), solid-phase microextraction (SPME; 41–43), and pressurized liquid extraction (PLE), also known commercially as accelerated solvent extraction (ASE; 44–46). Despite their advantages, none of the techniques have overcome critical flaws or practical limitations to enable their widespread implementation. For example, PLE and SFE, which are instrument-based techniques that perform extractions in sequential, semi-automated fashion, still require time-consuming manual steps and specialized items that require cleaning after each use; sample throughput is not optimal, and instruments and maintenance are costly. SFE, MSPD, and SPME do not provide a wide enough analytical scope within a single procedure, and MAE and PLE do not provide enough selectivity (extracts require more cleanup than liquid-based extractions at room temperature). Also, sample size in all of these approaches may be too small. These approaches are useful for certain applications but are not sufficiently simple or effective to provide the ideal MRM.

Despite the many different multiclass MRMs that have been described in the past 40 years, none has been truly streamlined to the minimum factors that achieve a fast and easy extraction while still maintaining high recoveries for a wide range of analytes and providing the selectivity and repeatability needed of a reliable procedure. Many of the advantages and opportunities provided by modern analytical techniques are not properly integrated into most current methods. The aim of this study was to develop a simple, rapid, and inexpensive multiclass MRM that provides high-quality results, but minimizes the number of analytical steps, uses few reagents in small quantities, and requires very little glassware. The main focus was to simplify the analytical process as much as possible during extraction and cleanup without sacrificing high recoveries even for the most difficult analytes.

Experimental

Apparatus

(**a**) *Gas chromatography/mass spectrometry (GC/MS) instrument.*—Extracts were analyzed with a Hewlett-Packard (Agilent, Little Falls, DE) Model 5890 Series II Plus GC coupled to a 5972 mass-selective detector (MSD). The system was equipped with a split/splitless injection inlet, electronic pressure control (EPC), and a 7673A autosampler. Chemstation software was used for instrument control and data analysis.

(**b**) *Nuclear magnetic resonance (NMR) spectrometer.*—NMR spectroscopy measurements to determine water

content in extracts were conducted with a Varian (Palo Alto, CA) Unity Plus 400 MHz instrument.

(**c**) *Chopper and mixers.*—A 1 L volume RSI 2Y1 Robotcoupe (Ridgeland, MS) chopper was used to comminute fruit and vegetable samples. A Vortex-Genie 2 apparatus from Scientific Industries (Bohemia, NY) was used for initial extraction and for cleanup by dispersive SPE. In a comparison experiment, a Tek-Mar (Cincinnati, OH) probe blender was also used to extract samples.

(**d**) *Centrifuges.*—A Sorvall RT6000B (Newtown, CT) was used for 40 mL centrifuge tubes when needed; a Hill Scientific mv13 (Derby, CT) mini-centrifuge was used for 1.5 mL micro-centrifuge tubes.

(**e**) *Liquid dispensers.*—An adjustable-volume solvent dispenser provided 10 mL MeCN from bottle to samples. An adjustable repeating pipet was used to transfer 1 m and 0.5 mL aliquots of extract.

(**f**) *Analytical balances.*—Top-loading balances with digital displays were used to weigh chopped samples, bulk salts, and smaller portions of SPE sorbents and $MgSO_4$. Scoops of 50 μL for primary secondary amine (PSA) and 0.77 mL for NaCl could be substituted for measurement of 25 mg and 1 g portions, respectively. Because the volume of $MgSO_4$ powder was not consistent, weighing was needed for reasonably accurate measurement of the 4 g portion; however, a scoop of 275 μL could be used to provide ≈ 150 mg.

(**g**) *Vials and vessels.*—For initial extraction, 40 mL Teflon centrifuge tubes (Nalgene, Rochester, NY) were used, and 1.5 mL flip-top microcentrifuge tubes were used for dispersive SPE. Whatman (Clifton, NJ) syringeless filtering vials (equipped with 0.45 μm Teflon filters) were also evaluated in experiments for use in the method. Otherwise, standard 1.8 mL glass autosampler vials for GC were used to contain the final extracts.

Reagents

(**a**) *MeCN, acetone, EtAc, petroleum ether, dichloromethane, and water.*—All organic solvents were of sufficient quality for pesticide residue analysis and were obtained from Fisher (Fair Lawn, NJ). Deionized water was used when needed in experiments.

(**b**) *Magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl).*—Reagent grade anhydrous $MgSO_4$ in powder form and ACS grade NaCl were obtained from Aldrich (Milwaukee, WI). The $MgSO_4$ was determined to be a source of contamination from phthalates, but the analysis was not affected adversely by their presence. Pretreatment of the powder for 5 h at 500°C in a muffle furnace removed the phthalates. Acceptably pure $MgCl_2$, $NaNO_3$, Na_2SO_4, LiCl, and fructose were also evaluated in salt-out experiments.

(**c**) *Pesticide standards.*—Pesticide reference standards were obtained from the National Pesticide Standard Repository of the U.S. Environmental Protection Agency (EPA; Fort Meade, MD), Dr. Ehrensdorfer GmbH (Augsburg, Germany), Ultra Scientific (North Kingstown, RI), and Chemservice (West Chester, PA). Stock solutions of 1000 μg/mL were prepared in various solvents, and working standard pesticide mixtures of 50 and 10 μg/mL were prepared in MeCN.

(**d**) *Internal standard (ISTD).*—Triphenylphosphate (TPP) was purchased from Sigma (St. Louis, MO). A solution of 20 μg/mL TPP in MeCN was used as the ISTD in experiments.

(**e**) *Analyte protectants.*—A mixture of 100 mg/mL 3-ethoxy-1,2-propandiol (3-*O*-ethylglycerol) and 5 mg/mL sorbitol (both from Sigma) was prepared in MeCN–water (7 + 3).

(**f**) *SPE sorbents.*—PSA and Nexus polymer-based sorbent obtained from Varian (Harbor City, CA), graphitized carbon black (GCB) from Supelco (Bellefonte, PA), alumina-neutral (alumina-N), strong-anion exchange (SAX), cyanopropyl (-CN), and aminopropyl (-NH2) from J.T. Baker (Phillipsburg, NJ), and octadecylsilane (ODS), also commonly known as C_{18}, from Applied Separations (Allentown, PA) were used in different experiments involving dispersive-SPE cleanup.

(**g**) *Internal standard for NMR spectroscopy.*—Trideuterated acetonitrile (CD_3CN) was obtained from Cambridge Isotope Laboratories (Woburn, MA).

Sample Comminution

Follow proper techniques (47–49) and use powerful chopping devices to achieve good sample homogeneity and to ensure that a 10 g subsample is representative for the analysis. The use of dry ice is highly recommended (and required to minimize pesticide losses).

Extraction/Partitioning

Weigh 10 g previously homogenized sample into 40 mL Teflon centrifuge tube. Add 10 mL MeCN with the dispenser, add screw cap, and shake sample vigorously for 1 min by using Vortex mixer at maximum speed. Add 4 g anhydrous $MgSO_4$ and 1 g NaCl, and mix on a Vortex mixer immediately for 1 min. *Note:* Perform this action immediately to prevent formation of $MgSO_4$ conglomerates. Add 50 μL ISTD solution, mix on a Vortex mixer for another 30 s, and centrifuge extract (or batch of extracts) for ≈ 5 min at 5000 rpm.

Dispersive-SPE Cleanup

Transfer 1 mL aliquot of upper MeCN layer into 1.5 mL microcentrifuge vial containing 25 mg PSA sorbent and 150 mg anhydrous $MgSO_4$; cap tightly. Shake by hand or with Vortex mixer for 30 s. Centrifuge extracts (or batch of extracts) for 1 min at 6000 rpm to separate solids from solution, and transfer 0.5 mL extract into autosampler vial for GC/MS analysis.

Preparation of Calibration Solutions

Quantitation was performed and compared by using calibration standards involving both matrix-matching (standards added to blank extracts) and non-matrix-matching (standards in solutions containing analyte protectants). For matrix-matching, the blank extract can be fortified with pesticide working standard and ISTD solutions either before or after dispersive-SPE cleanup (we tested both approaches with no differences in results). In the first approach, 1 mL blank extract is subjected to dispersive-SPE cleanup as described

above. Standards are then prepared by adding 50 µL ISTD solution diluted 20-fold (1 µg/mL in MeCN) and 50 µL pesticide standard of desired concentration to 0.5 mL final extracts from a blank. MeCN (100 µL) is added to each 0.5 mL sample extract to ensure similar matrix concentration in sample extracts and matrix-matched standards. In the second case, an appropriate volume of the initial extract is fortified before cleanup. For example, 5 mL is fortified with 50 µL ISTD solution diluted 2-fold (10 µg/mL in MeCN) and with pesticide working solution of desired concentration. Afterwards, 1 mL of this matrix-matched standard is subjected to dispersive-SPE cleanup as described above. No volume adjustment of the samples is necessary because differences between sample and standard in terms of matrix concentration are negligible. Also, the volume of pesticide working solution (which is used for the matrix-matched standard) must be accurate. The added volume of the ISTD, however, need only be consistent throughout the procedure. Use of the same pipet and volume is therefore recommended.

Alternately, calibration solutions can also be prepared in the absence of matrix by using analyte protecting agents. For example, 50 µL analyte protectant solution is added to each 0.5 mL final extract and calibration standard in MeCN already containing ISTD. This technique can be used to overcome matrix-induced effects in the analysis for laboratories that cannot or do not wish to use matrix-matched calibration standards. Because the ratios of ISTD to sample amount in the extract, and ISTD to analyte amount in the standards, are known, the analyte concentration, i.e., amount analyte per amount sample (e.g., ng/g) can be determined.

Fortifications

In recovery studies, 100 µL MeCN solution of pesticides at desired concentration was added to each 10 g blank sample. The tube containing fortified sample was vortexed for 30 s and left standing for ≈ 1 min to distribute pesticides evenly and give them time to interact with the matrix.

GC/MS Analysis

GC analysis was conducted on a DB-35ms (Agilent, Folsom, CA) capillary column of 30 m, 0.25 mm id, 0.25 µm film thickness, and the following conditions were used: He constant flow 1 mL/min, inlet temperature 250°C, injection volume 1.5 µL (splitless), MS transfer line temperature 290°C, temperature program 95°C for 1.5 min; then 20°C/min ramp to 190°C followed by 5°C/min ramp to 230°C and 25°C ramp to 290°C (held for 20 min). Total run time was 36.67 min. Full-scan analysis (50–450 *m/z*) was used to determine cleanup effects, and selected ion monitoring (SIM) mode was used for recovery experiments.

Determination of Water Content by NMR Spectroscopy

A 550 µL aliquot of MeCN/water solutions/extracts to be measured was transferred into an NMR tube, and 100 µL CD_3CN was added as internal standard and marker compound. For calibration, several aqueous MeCN solutions were prepared with water content ranging between 5 and 200 mg water/mL total volume. All calculations to determine water concentrations were made by using a calibration plot of spectral shift of the hydrogen peak (from water) with respect to the reference deuterium peak (from CD_3CN).

Results and Discussion

Sample Size and Comminution

Usually, the simplest way to improve efficiency of an analytical method is to reduce sample size to the minimum amount that will provide statistically reliable results, and scale the method accordingly. Most multiclass MRMs use a 50–100 g subsample taken from a bulk sample that has been comminuted with large chopping devices (6, 9, 20, 22–25, 30). Methods in which excessive sample sizes are used require larger solvent volumes and larger glassware items, thus leading to more waste, greater safety concerns, greater storage and bench space needs, more labor and time, and more expense than necessary. An efficient method uses the minimal size of the analytical sample to provide statistically reliable results while taking into account the degree of sample homogeneity. A small amount of extra time and effort spent conducting proper sampling and homogenization procedures saves time and resources overall by enabling use of smaller sample size.

The desire to miniaturize analytical methods, along with the introduction of extraction techniques such as SFE, MSPD, and PLE, has made sample comminution increasingly important. This step is key to achieving a representative sample with its integrity intact (no pesticide losses) so that meaningful results can be obtained. Unfortunately, laboratories rarely evaluate the quality of the sample comminution step in their quality control procedures, and the procedures used vary significantly from laboratory to laboratory. Appropriate devices and techniques must be used during the chopping procedure, which entails using the correct sample amount to volume of the chopper container, frozen conditions (via refrigeration, dry ice, or liquid N_2), and sufficient time (47–49). When these procedures are followed, a smaller subsample (e.g., 5–15 g) can provide the same quality of result as a larger subsample.

For example, the 15 g size sample used by the Dutch monitoring program has been proven to be sufficient in extensive validations and proficiency check sample programs (50). Using a common chopping device, Young et al. (47) determined that 10 g samples were acceptable, and Lehotay et al. (51) showed that as little as 2 g subsample was satisfactory for fortified pesticides in potato when frozen conditions and a dispersing agent were used. SFE analyses using well-homogenized 2–3 g subsamples dispersed in Hydromatrix often achieved equivalent results for pesticides in proficiency check samples with methods that used 50–100 g samples.

Based on evidence in the literature and our experience, we chose a subsample size of 10 g (33–35, 47–49). Sufficiently representative subsamples of this size can be generated even in mobile laboratories for which this method is also intended. Different representative sample size may be used if the method is scaled appropriately, but in any case, *samples should*

be comminuted thoroughly to maximize surface area and ensure better extraction efficiencies during shaking, especially in light that a blender/homogenizer is not used for extraction to break up the sample further in the presence of solvent.

Method Development Strategy

Once the sample size and comminution procedure have been set, conditions of extraction, partitioning, and cleanup must be assessed. The search for optimal conditions is challenging because decisions and compromises must be made to integrate simplicity and speed of the procedure with broad applicability, high recoveries, and adequate selectivity. Each of these analytical steps can be affected by a variety of factors, including 1) sample constitution (e.g., pH, content of water, lipids, sugars); 2) type of extraction solvent(s); 3) sample/solvent ratio; 4) extraction procedure (e.g., blending or shaking); 5) extraction temperature (pressure is another potential parameter, but only relevant when very high temperatures are applied); 6) principle of phase partitioning (e.g., addition or not of nonpolar co-solvents and/or salts); 7) time spent on blending and shaking steps and number of repetitions; and h) materials used for cleanup.

We sought to systematically compare and measure the effect of each of these adjustable parameters by using multiple tools of objective measurement. The following factors were measured or observed to assess the effectiveness of different extraction, partitioning, and cleanup approaches in the development of the final method: 1) pesticide recoveries; 2) amount of co-extracted sample material (gravimetric); 3) comparison of coloration of extracts (optical); 4) comparison of matrix background in SIM and full-scan MS chromatograms; 5) amount of water in extracts (NMR spectroscopy); and 6) analyte protection (matrix-induced chromatographic effects) before and after cleanup. Many experiments were conducted to isolate each parameter, and the results are reported and discussed in the following sections.

Advantages and Disadvantages of Different Extraction Solvents

The choice of the solvent(s) is one of the most crucial decisions to be made when developing a new MRM. Many aspects have to be considered, including: 1) ability to cover the desired analytical spectrum (in our case, ranging from methamidophos at the polar end to the pyrethroids and organochlorine pesticide at the nonpolar end); 2) selectivity that can be achieved during extraction, partitioning, and cleanup; 3) achieving a separation from water; 4) amenability to chromatographic separation techniques (e.g., SPE, GC, LC, GPC); 5) cost, safety, and environmental concerns; and f) handling aspects (e.g., ease of evaporation, volume transfers).

The solvents most commonly used for multiresidue analysis of pesticides are MeCN, acetone, and EtAc; each has been shown to give high recoveries of a wide range of pesticides (1–16, 20–32, 50, 52). Each solvent has some advantages and disadvantages in terms of selectivity and practical matters. The miscibility of MeCN and acetone with water leads to a single-phase solvent extraction of the sample matrix (except for samples with high sugar content, in which MeCN and water form 2 phases). Unlike acetone, which needs a nonpolar co-solvent to induce a well-defined phase separation with water, salts can be used with aqueous MeCN extracts to avoid the need for nonpolar solvents altogether. Compared with EtAc and acetone, MeCN does not extract as much lipophilic material, e.g., waxes, fat, and lipophilic pigments (27). Moreover, MeCN forms distinct partitioning phases with nonpolar solvents such as hexane, which provides the potential for another convenient cleanup step to remove co-extracted lipophilic components if needed (53). Another advantage of MeCN versus acetone is that residual water (after the partitioning step) can be removed better by drying agents such as $MgSO_4$ (17, 27). MeCN is not only compatible with GC applications, but because of its low viscosity and intermediate polarity, it is very useful in reversed-phase liquid chromatography (LC) and SPE applications.

EtAc is more compatible with common GPC methods, which should be avoided if possible, but neither EtAc nor acetone is useful in common LC applications. Another disadvantage of EtAc is the potential for build-up of acetic acid in the solvent during storage.

The disadvantages of MeCN versus acetone and EtAc in MRMs for pesticides include: 1) a larger solvent expansion volume during vaporization in GC; 2) MeCN's detrimental effect on nitrogen phosphorus (NPD) and electrolytic conductivity (ELCD) detectors (but some GC inlets can purge the solvent front from the system); 3) lower volatility (but comparable with EtAc, which makes evaporation times longer; 4) greater cost (2.2 U.S. cents/mL for pesticide grade MeCN versus 1.6 U.S. cents/mL for EtAc and 1.3 U.S. cents/mL for acetone of similar quality); and 5) higher toxicity, but not to the extent of chlorinated solvents.

Some chemists cite the greater toxicity of MeCN than acetone or EtAc as an important reason to avoid the use of MeCN-based MRMs (5–7). We believe that this is a rather poor argument considering the low chronic toxicity of MeCN and its comparatively low volatility; other common solvents in the laboratory also have potential acute and chronic intoxication risks (such as blindness in the case of methanol). MeCN is widely used in LC applications and other common extraction methods, e.g., biochemical and clinical assays, which also potentially expose laboratory workers to MeCN. Our use of a solvent dispenser, rather than graduated cylinders, and the use of sealed vials for the extraction step reduce the chance of spills and greatly minimize exposure of the worker to the solvent. The solvent dispenser additionally avoids extra glassware needs, removes a source of potential contamination, and provides better accuracy and precision of liquid transfers. There is no blending, no solvent evaporation, and minimal handling of samples in the procedure, which also greatly reduce risk to the analyst.

Selectivity of Extraction Solvents

MeCN, acetone, and EtAc were compared in a series of experiments in which the only parameter changed was the type of solvent. In the first experiment, mixed fruit and vegetable

samples were extracted with MeCN and acetone in 1:1 and 1:2 sample (g):solvent (mL) ratios. After extraction and centrifugation, triplicate 5 g equivalent portions of the extracts (7.0 or 14.0 mL acetone and 7.1 or 14.2 mL MeCN, accounting for water content and volume differences) were taken to dryness in preweighed test tubes, and the amount of coextracted material was determined by differences in weight. Table 1 gives the amount of matrix that was co-extracted and shows that approximately 2–8 times more material in fruits and vegetables was dissolved in acetone–water than in MeCN–water solutions.

The amounts of co-extractives from a mixed produce sample are compared in Figure 1. An MeCN–acetone solvent (1 + 1) mixture was also used for comparison. In this experiment, the final method was followed, and the type of solvent was the only variable altered. Figure 1 shows that EtAc gave the least amount of co-extractives and acetone gave the most in the final extracts without SPE cleanup. However, MeCN gave the least co-extractives after the dispersive-SPE cleanup step with PSA.

Similarly, extracts from this experiment were injected into the GC/MS instrument (full scan), and both the number and intensity of matrix peaks in the chromatograms were summed. In this measurement, MeCN had the fewest possible interfering peaks in GC/MS both before and after cleanup. For MeCN before cleanup, 130 peaks appeared, whereas 101 were counted after cleanup. For EtAc, acetone, and MeCN–acetone (1 + 1), there were 150, 155, and 151 peaks, respectively, before cleanup, and 128, 145, and 140 peaks, respectively, after cleanup. The sums of peak areas followed the same pattern. After these experiments (and others described below), it became clear that MeCN was the most selective of the different extraction solvents tested and the most advantageous overall.

Sample:Solvent Ratio and the Extraction/Partitioning Step

The lower volatility of MeCN may be a disadvantage in terms of solvent evaporation, but this was not relevant in our method because we chose to avoid solvent evaporation and reconstitution steps by using a small solvent-to-sample ratio for extraction. The lower volatility of MeCN served as an advantage during liquid transfers of small volumes because volume changes caused by solvent evaporation were less likely. For extraction, a 1:1 sample-to-solvent ratio (10 g sample + 10 mL MeCN), which gave a resulting concentration of ≈1 g sample/mL in the final extract, was satisfactory. With the use of modern instruments, this should be sufficient to achieve adequate limits of detection (LODs), about 10–100 ng/g, for most pesticide residue analyses. If lower LODs are needed and matrix is not the limiting factor, the use of large-volume injection (LVI), programmable temperature vaporization (PTV), and/or direct sample introduction (DSI) can help to achieve good detection limits for virtually all GC-amenable pesticides (13, 24, 26, 27). Of course, concentration of an aliquot after the cleanup step, by using a gentle nitrogen flow and moderate heat, is also possible. The formation of azeotropic mixtures with acetone or hexane may help to speed up evaporation if needed (20, 23).

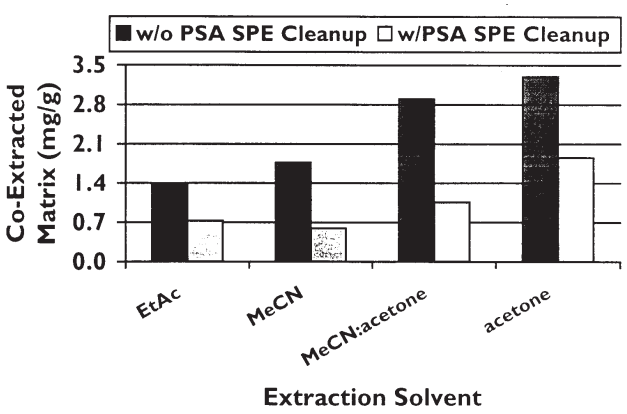

Figure 1. Comparison of different solvents in extraction of a mixture of fruits and vegetables in the final method with and without dispersive-SPE cleanup step using PSA sorbent. Co-extracted matrix is amount (mg) dissolved in extract vs original sample amount extracted (g). The ratio of MeCN:acetone was 1:1.

Table 1. Co-extracted matrix material (% of initial sample dissolved in extract) in MeCN or acetone extracts prior to the liquid–liquid partitioning step[a]

Conditions	MeCN, %	Acetone, %
1 g sample A per 2 mL solvent	2.1	4.0
1 g sample B per 2 mL solvent	1.6	7.4
1 g sample B per 1 mL solvent	0.7[a]	5.7

[a] A water–MeCN partition formed because of the high sugar content in the sample. Sample A consisted of 33% each of apple, carrot, and green bean; sample B consisted of 26% orange, 19% spinach, 16% apple, 15% lettuce, 14% grape, and 10% carrot.

Considering that the water content of most fruits and vegetables is between 80 and 95%, the water content of the initial sample–MeCN (1 + 1) extract is 40–46%. This is a higher percentage than in the Mills method (29–32% water), which has been shown to effectively extract nonpolar residues (1, 54). For this reason, we were initially concerned about whether the method would extract incurred nonpolar pesticides, which are sometimes incorporated in lipid and wax particles in the sample. However, in contrast to the Mills method, the matrix is not separated after the initial extraction step in our method, but remains in the system after the formation of 2 phases (the extraction/partitioning step). During this second mixing step in the same tube, the separated MeCN is a much stronger solvent for extraction of nonpolar pesticides than the aqueous MeCN of the initial single-phase extraction. During this extraction/partitioning step, the sample is exposed to ≈ 92/8 MeCN–water solvent (as determined in NMR experiments), which retains the polar pesticides during partitioning and extracts nonpolar pesticides that were not dissolved in the initial solution con-

taining 54–60% MeCN. The heat generated by the hydration of $MgSO_4$ further aids and speeds extraction during this step.

In a further attempt to provide a more concentrated extract while still avoiding solvent evaporation, we also tested a sample:solvent ratio of 2:1 (10 g sample + 5 mL MeCN). Table 2 compares the recoveries of selected pesticides with 1:1 and 2:1 sample:solvent ratios in a spiked fruit sample. The most polar pesticides did not partition into the upper MeCN phase as completely in the 2:1 ratio as in the 1:1 ratio, but recoveries of all pesticides tested were still >75%, which is acceptable for most purposes. This experiment was not repeated because we were not willing to sacrifice this much recovery of the most polar GC-amenable pesticides (methamidophos, acephate, omethoate, and thiabendazole) in order to double the concentration factor. This is an option for others to try if they wish, however, comparisons should be made with incurred samples and not merely fortified samples.

Comparison of Shaking Versus Blending of an Incurred Sample

We assessed the extractability of incurred polar and nonpolar residues and compared shaking versus blending as the approach for the initial extraction step. We compared our method with a 10-fold scaled-down version of the AOAC Official Method **985.22** (10, 11), including the 3 partitioning steps (commonly known as the Luke method), for the same mixture of incurred samples provided by the Florida Department of Agriculture and Consumer Services. The sample was a mixture of green beans and Romaine lettuce (2 + 1), which was previously shown to contain incurred residues of acephate, methamidophos, and permethrin.

We also compared extraction of the incurred sample by the vortexing mixer, which is widely applied in residue analyses of animal tissues, with a traditional blending approach (2 min extraction with a probe blender). Most MRMs for pesticides in foods use a blender during extraction, but Cook et al. (25) validated and implemented a shaking procedure. Although shaking should work as well as blending for fortified samples, we were not sure whether the approach would be adequate for incurred residues that might not be easily accessible to the solvent. The results of proficiency test samples (some of which contain incurred residues) by shaking have been acceptable compared with blending-based methods; thus, we investigated the shaking procedure in our streamlined method for extraction of fruits and vegetables.

The advantages of shaking over blending include:1) the sample is not exposed to the active metal surfaces of the blender; 2) shaking can be performed by hand if needed (in the field or laboratory); 3) no cleaning of the blender jar/probe is needed between samples; 4) extraction is conducted in a closed vessel, thus it is safer; 5) chance of carry-over from one sample to another is eliminated; 6) no extra solvent from rinsings is added to the sample and no extract or sample is removed by the mixing approach, unlike the case with blenders; 7) only a single container is used for each extraction; 8) a batch of samples can be extracted more easily in parallel rather than sequentially as with blenders; 9) cost for Vortex mixers/shakers is less than blenders and need less maintenance; 10) vortexers are much less noisy than blenders; and 11) no frictional heat is generated during blending (especially when solids are added as in our method).

Table 3 presents results of the comparison of different extraction approaches. With the present method, results with the vortexing procedure were similar to those with the blender approach for all incurred pesticides. Note that the blender was used only for the initial extraction step but not for the extraction/partitioning step where vortexing was used to avoid friction and damage of the blender in the presence of salts. A probe blender was also used in the mini-Luke procedure and gave results similar to those with the MeCN vortexing method. Similar results were also obtained by the Florida laboratory with its MeCN-based shaking extraction method (25).

From this experiment, it appears that shaking is acceptable for extraction of relatively polar and nonpolar pesticides in fresh fruits and vegetables. To form more definite conclusions about shaking vs blending, this type of experiment should be repeated in a routine monitoring laboratory in side-by-side analyses of a greater variety of samples and incurred pesticides. However, we were satisfied that the comparison results were close enough to the reference methods that we continued using the vortexing approach in this new method. For good measure, an intensive agitation of the sample should be utilized and samples should be comminuted thoroughly as previously described.

Table 2. Average recoveries of selected pesticides from 10 g fruit sample[a]

Pesticide	MeCN, 5 mL	MeCN, 10 mL
Dichlorvos	95	96
Methamidophos	**76**[b]	95
Mevinphos	96	100
Acephate	**84**[b]	99
o-Phenylphenol	94	94
Omethoate	**85**[b]	100
Diazinon	95	99
Chlorothalonil	94	95
Metalaxyl	94	100
Carbaryl	93	99
Dichlofluanid	97	97
Captan	97	100
Thiabendazole	**88**[b]	99
Folpet	92	94
Imazalil	92	102

[a] Ratios of 2:1 and 1:1 sample:MeCN (g:mL) and 1 g NaCl + 4 g $MgSO_4$ were used to induce partitioning.

[b] Pesticides with lower recoveries using 5 mL MeCN.

Table 3. Comparison of the analytical results obtained by using different extraction methods for a real sample containing residues of methamidophos, acephate, and permethrin

Method[a]	Acephate concn, ng/g	Methamidophos concn, ng/g	Permethrin concn, ng/g
Florida[b]	67	167	247
Mini-Luke	67	175	261
Method A	64	170	240
Method B	66	169	237

[a] The Florida method (25) used a shaking procedure with 1:2 sample:MeCN ratio; the "mini-Luke" method involved a blending procedure with 1:2 sample:acetone ratio and 3 liquid–liquid partitioning steps using NaCl; our Method A used Vortex mixing of 1:1 sample:MeCN; and our Method B used a probe blender with all other conditions being the same as Method A with 1 g NaCl + 5 g $MgSO_4$ to induce partitioning.

[b] Result from analysis by the Florida Dept. of Agriculture and Consumer Services.

Comparison of Various Salts and Fructose To Induce Phase Separation

After the initial single-phase extraction with MeCN, salts ($MgSO_4$ and NaCl) were added to induce phase separation. The second extraction step is therefore a combination of extraction and liquid–liquid partitioning. The addition of NaCl to initiate or influence liquid–liquid partitioning has been used in many MRMs (6, 7, 10–13, 16, 17, 19–27). The salting-out effect resulting from addition of NaCl usually leads to increased recoveries of polar compounds, but this also depends on the nature of the solvents involved in the partitioning step. The addition of the proper amounts and combination of salts can be used to control the percentage of water in the organic phase (and vice versa for organic solvent in the water phase), thus enabling a certain degree of adjustment in the polarity of the phases.

In most MRMs involving extraction with acetone (6–13, 16, 17, 50), the partitioning behavior is controlled by addition of a combination of NaCl and nonpolar co-solvents, but the main disadvantages of this approach are that: 1) the co-solvents dilute the extract; and 2) the co-solvents typically make the extracts too nonpolar.

In most MRMs using MeCN (19–28), extraction/partitioning is achieved without addition of nonpolar co-solvents. The addition of salts is not only conveniently fast, cheap, and easy, but it also has the advantages of not increasing the volume of the extracts (thereby reducing or avoiding the need for post-extraction solvent evaporation steps) and not instilling excessively nonpolar solvent properties (thereby reducing the need for post-extraction cleanup).

In developing our method, we decided to use any co-solvents to facilitate phase separation or modify the properties of MeCN. Traditional MeCN-based MRMs simply saturate the water phase with NaCl, but we compared the use of different amounts of fructose, $MgSO_4$, $MgCl_2$, $NaNO_3$, Na_2SO_4, LiCl, and NaCl to induce the phase separation. In addition to determining pesticide recoveries, we used NMR spectroscopy as in other studies (17, 27, 34) to measure the water content of the MeCN phase after the salting-out step. We dissolved different types and amounts of salts (or fructose) into 10 g (10 mL) water, which (after reaching room temperature) was mixed on a Vortex mixer with 10 mL (7.86 g) MeCN fortified with pesticides (0.5 μg/mL). The volumes of the upper layers and water content were measured by NMR, and the amount of MeCN in the lower aqueous layer was approximated assuming 1 mL water + 1 mL MeCN = 2 mL solution (the small bias in the calculation did not affect relative values and the results were acceptable to form conclusions). Analyte protectants were used to improve peak shapes in the absence of a food matrix, and recoveries of the most polar pesticide, methamidophos, were determined in the samples versus calibration standards in which signals were normalized to fenthion (Table 4).

Obviously, the partitioning of pesticides between the 2 phases depends on the polarity differences of the upper and lower phases. We expected that determining the water and MeCN concentrations in each phase would provide evidence to support our original hypothesis that recoveries of methamidophos and other polar pesticides would primarily correlate with the water content in the MeCN phase. However, when all results from the different compounds were compiled, we concluded that both composition and volumes of the 2 layers (organic and aqueous) were very important. As Table 4 indicates, MeCN/water partitioning with $MgSO_4$ yielded: 1) the highest recoveries of methamidophos; 2) a significantly larger volume of the upper layer than was the case with the other compounds; 3) the lowest concentrations of MeCN in the lower phase; 4) the highest concentrations of the water in the upper phase; and 5) the greatest differences between the concentrations (and amounts) of water in the upper phase and MeCN in the lower phase.

The use of drying salts to improve recoveries of polar compounds is not new. In the Swedish multiresidue method, Andersson and Palsheden (30) used Na_2SO_4 to remove water from samples and achieved high recoveries of polar compounds with EtAc as the extracting solvent. Using SFE with CO_2, Valverde-García et al. (36) dramatically increased recoveries of methamidophos and acephate by mixing samples with $MgSO_4$. Eller and Lehotay (34) used a similar approach by combining $MgSO_4$ with Hydromatrix.

We added $MgSO_4$ because of its ability to bind large amounts of water and thus significantly reduce the water phase, which would promote partitioning of pesticides into the organic layer. To bind a significant fraction of water, $MgSO_4$ should be added at amounts well exceeding its saturation in water. $MgSO_4$, however, exhibits a considerable water solubility of 337 g/L water at 20°C (55), which indicates that the amount of water bound by $MgSO_4$ in our method is rather insignificant (4 g was used for 10 g sample). Nevertheless, we did not use more $MgSO_4$ in our method because recoveries are excellent even with less $MgSO_4$, and the use of more $MgSO_4$ makes vortexing difficult due to the formation of conglomer-

Table 4. Influence of different salts and fructose on water content in the MeCN phase and MeCN content in the water phase after liquid–liquid-partitioning

Compound	Solubility in H_2O at 20°C, g/L	Amount added, g	Volume of upper (MeCN) layer, mL	Concn H_2O in MeCN layer, mg/mL	Concn MeCN in H_2O layer, mg/mL	Methamidophos recovery, %
LiCl	835	0.5	4.3	220	338	17
		1	6.4	144	267	23
		2	7.5	76	199	21
		3	7.4	55	194	17
		4	7.2	31	192	12
		6	6.4	17	220	5
		8	5.0	18	272	2
$MgCl_2$	546	1	7.5	144	232	19
		2	8.0	86	183	23
		3	8.3	57	153	21
		4	8.3	37	142	18
		5	8.3	20	132	15
NaCl	359	0.5	5.0	233	328	25
		1	6.9	157	257	34
		2	8.0	105	193	41
		3	8.3	74	162	47
		4	8.4	70	155	48
$NaNO_3$	876	4	3.5	112	333	—
		6	6.0	88	260	—
		8	6.4	84	245	—
$MgSO_4$	337	0.75	15.0	364	88	67
		1	14.2	357	131	**95**
		2	11.9	231	92	**88**
		3	11.2	174	76	**91**
		4	10.6	136	79	**97**
		5[a]	10.7	185	117	**98**
Na_2SO_4	195	1	10.3	257	199	69
Fructose	Very soluble	2	5.0	251	333	30
		3	6.5	197	284	31
		4	7.0	178	263	34
		6	6.9	174	264	35

[a] $MgSO_4$ precipitated due to oversaturation.

ates. Furthermore, the hydration of $MgSO_4$ is a highly exothermic process, causing the sample extract to get hot during the extraction/partitioning step, reaching temperatures as high as 40–45°C. We also believe that heat is beneficial for extraction, especially in the case of nonpolar pesticides.

Note that Na_2SO_4, the other salt tested that forms a hydrate, also gave relatively higher recovery of methamidophos than the other salts did. Perhaps adding more Na_2SO_4 to the solution would have yielded higher recovery of methamidophos, but the lower solubility of Na_2SO_4 in water limited this possibility. Ultimately, experiments could be conducted to better explain the partitioning process with respect to pesticide recoveries, but these experiments fell outside the scope of our explorations.

We also evaluated the effects of $MgSO_4$ and NaCl (both separately and in combination) in the extraction of fortified fruit and vegetable samples. Table 5 gives the results of experiments designed to determine the effect of different amounts of these 2 salts on the partitioning of the most polar pesticides into the upper layer (all other GC-amenable pesticides tested gave high recoveries in the experiment). Table 5 shows that $MgSO_4$ alone gave the highest recoveries for the polar pesticides, and even 2 g $MgSO_4$ in the extraction of tomato induced

the partitioning of nearly 80% of methamidophos into the upper phase. However, the recoveries were not satisfactory when NaCl was used alone, as in traditional MeCN-based methods. Combinations of $MgSO_4$ with NaCl also worked very well, but recoveries decreased slightly when a greater amount of NaCl was added. The ideal amount of $MgSO_4$ added to a sample was 4–5 g. We used the lesser amount in the final method.

We found that the more NaCl is added to the system, the more complete the phase separation becomes and less water remains in the MeCN phase. The organic phase thus becomes less polar and therefore less receptive for polar compounds such as methamidophos. The amount of NaCl added to this system had a strong influence on the phase separation between water and MeCN, and by varying the amount of NaCl added to the extract, we could control the polarity range (selectivity) of the partitioning step, as discussed in the next section.

Selectivity of the Extraction/Partitioning Step

Based on recoveries, $MgSO_4$ was the best choice as the salt used in the method, but selectivity of the extraction process must also be considered. In our final method, we therefore recommend the combination of $MgSO_4$ and NaCl. The salt concentration in the water phase not only affects recoveries of the polar pesticides but also the partitioning of polar matrix compounds into the organic phase. Figure 2 shows the full-scan GC/MS chromatograms of the apple extracts from Table 5, where different amounts of NaCl were used in combination with 5 g $MgSO_4$ during liquid–liquid partitioning. All extracts

Table 5. Influence of NaCl and/or $MgSO_4$ on recoveries of methamidophos, acephate, and omethoate from different matrices. Recoveries >80% are shown in bold.

			Recovery, %		
Matrix	Amount $MgSO_4$, g	Amount NaCl, g	Methamidophos	Acephate	Omethoate
Tomato	0	0.25	32	42	42
	0	0.5	36	39	39
	0	1	41	44	44
	0	2	43	43	45
	0	3	53	53	64
Tomato	2	0	79	**97**	**96**
	3	0	**92**	**98**	**93**
	4	0	**98**	**102**	**103**
	5	0	**101**	**95**	**91**
	6	0	**94**	**99**	**106**
Apple	5	0	**100**	**98**	**100**
	5	0.125	**95**	**96**	**99**
	5	0.25	**89**	**98**	**98**
	5	0.5	**89**	**93**	**87**
	5	1	**85**	**91**	**93**
	5	2	**80**	**87**	**90**
Zucchini	0	1	42	33	30
	1	1	63	61	72
	2	1	76	77	77
	3	1	78	77	**81**
	4	1	**83**	**87**	**87**
	5	1	**84**	**84**	**85**
Tomato	0	2	42	34	34
	1	2	51	57	67
	2	2	69	70	74
	3	2	79	**88**	**92**
	4	2	**81**	**82**	**92**
	5	2	**88**	**85**	**87**

were dried with $MgSO_4$ before injection to eliminate possible differences in the amount of water in each extract.

Many matrix components that elute in the first 10 min of the chromatogram gradually disappear from the extracts as more NaCl is used. Characteristic is the presence of 5-hydroxymethyl furfural (HMF), as noted in the chromatograms. HMF is mainly formed from fructose, which is a main component of the sugar fraction of many fruits. During the transformation to HMF, fructose successively loses 3 water molecules. The reaction is acid-catalyzed and accelerated by heat; thus it readily occurs in the GC injector. The amount of HMF detected reflects the amount of fructose present in the extract and provides a useful tool to assess the polar end of the polarity range spectrum of the extraction/partitioning step. The effect of NaCl on the amount of polar matrix-components present in the extracts was observed for fructose and for glucose, which forms a tailing anhydro-glucopyranose peak, and organic acids (fatty and nonfatty), such as vanillic, hydroxycoumaric, and cinnamic acid. Differences in the color of extracts indicated that various fractions of pigments were extracted differently in each case.

By varying the amount of NaCl added to the sample during partitioning with $MgSO_4$, it is possible to control the polarity range of the method and thus the amount of matrix-components (degree of cleanup) in the extract. Interestingly, the amount of NaCl used during the partitioning also had a great influence on the peak shapes and areas of several pesticides. This effect is related to the amount and nature of the co-extracted matrix components and is discussed later (*see Analyte Protectants*).

Table 6 summarizes the different effects observed when the amount of NaCl is varied during liquid–liquid partitioning in combination with $MgSO_4$. It is clear that the NaCl concentration should be chosen to make a compromise among the different effects. In the final method, we chose 4 g $MgSO_4$ + 1 g NaCl as the extraction/partitioning conditions, which was a compromise to avoid co-extraction of polar matrix components but still achieve high recoveries of polar pesticides, albeit slightly lower than recoveries possible by use of $MgSO_4$ alone.

Effect of pH

The natural pH values occurring in fruits and vegetables can vary considerably, ranging between 2.5 and 6.5. Many common pesticides used in agriculture are sensitive to pH (e.g., thiabendazole, imazalil, carbendazim, captan, folpet, dichlofluanind, chlorothalonil), and special analytical conditions are required to monitor for these pesticides in multiclass MRMs.

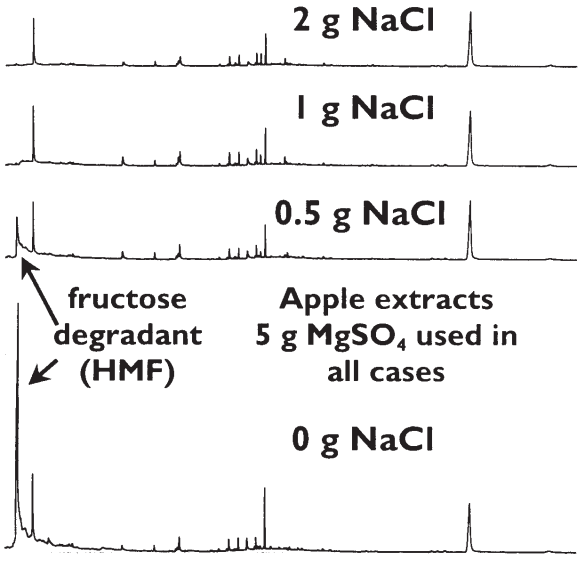

Figure 2. GC/MS chromatograms (full-scan mode) of apple extracts obtained by using different amounts of NaCl with 5 g $MgSO_4$ in the phase separation between water and MeCN.

In general, pesticides are more stable at lower pH. Several pesticides widely applied in agriculture (such as chlorothalonil, captan, folpet, and dichlofluanid) are known to degrade rapidly at higher pH, and an adjustment of the pH to lower values is therefore advisable in certain commodities. However, basic fungicides such as imazalil and thiabendazole, which are also widely applied, are poorly recovered from samples with low pH in typical MRMs because they get protonated in the water phase at low pH and do not partition into the relatively nonpolar organic phase. Thus, there is an inherent conflict related to pH in typical multiclass MRMs among these groups of analytes (35).

In our method, the MeCN/water partitioning system differs from partitioning systems where nonpolar solvents are involved because a significant amount of water still remains in the MeCN after phase separation. We hypothesized that pH would not have such a strong effect on the recoveries of basic fungicides with our method because they would still partition into the semipolar upper phase. We performed several recovery experiments using apple juice, which was adjusted to pH of 2.5, 3, 4, 5, 6, and 7, thus covering the natural pH range of

Table 6. Impact of amount of NaCl used in combination with $MgSO_4$ in the method on various parameters

	Recoveries of polar analytes	Amt of polar matrix co-extractives	Water content in extract	Matrix-induced peak enhancement
More NaCl	Lower	Lower	Lower	Lower
Less NaCl	Higher	Higher	Higher	Higher

fruits and vegetables. We repeated the same experiment by using EtAc instead of MeCN to observe the differences (Table 7). As the results indicate, the influence of pH for MeCN extraction was negligible, but strong effects were observed for EtAc at low pH. Even imazalil, which is the most basic pesticide (pK_a of 6.53), gave high recoveries at low pH in the MeCN approach. This finding demonstrates that the analytical scope of our method is very broad and covers a wide spectrum of pesticides and pH values of matrices.

This experiment demonstrated that there is no need to increase the pH of samples with low pH in order to achieve good recoveries for basic pesticides. However, when analyzing samples with high pH values, such as various vegetables, there is still a risk of losing some of the alkaline-sensitive pesticides. It is therefore advisable to adjust the pH of such samples to a level below 4 to minimize degradation of these pesticides. We did not include acidic pesticides (such as chlorophenoxyacetic acids) in our study, but we expect good recoveries for these pesticides, even without lowering the pH below 2 as other methods usually require.

Not only can pH have a strong influence on the recoveries of basic or acidic pesticides (as well as the degradation rate of certain compounds), but also on the co-extraction of matrix components. To assess this aspect, we analyzed the extracts of the previous experiment in the full-scan mode. Figure 3 shows that the co-extraction of fatty and other acids increased as the pH decreased. Lee et al. (20) made a similar observation and chose to adjust pH to 7 in their method. According to our experiments, Lee et al. (20) could have increased the amount of salt added (they used about 0.5 g NaCl/10 g sample) in the partitioning step to reduce the co-extraction of these matrix components rather than increasing the pH, which negatively affects the stability of alkaline-sensitive pesticides.

The risk of losing alkaline-sensitive pesticides, such as captan, captafol, folpet, chlorothalonil, and dichlofluanid, occurs not only in homogenized samples, but also in the final extracts. Sample extracts with pH >5 lead to losses of these pesticides, but losses also can occur in extracts from acidic samples after cleanup with PSA, and were observed even when pesticides were fortified in blank extracts after PSA cleanup. PSA contains primary and secondary amino groups that act to remove acidity from the extracts, thus increasing the risk of base-catalyzed degradation. We demonstrated that alkaline-sensitive pesticides were only briefly stable in MeCN extracts of samples with a pH of 6–7, but were stable for more than a day in MeCN extracts containing 0.05–0.1% acetic acid. It is therefore advisable to maintain sufficiently acidic conditions for greater stability and higher recoveries of these pesticides. In the future, we shall investigate the usage of a small amount of acetic acid in MeCN for the entire extraction process to achieve greater stability and higher recoveries of alkaline-sensitive pesticides.

Table 7. Influence of pH on recoveries of fungicides with basic properties[a]

	Thiabendazole		Imazalil	
pH	EtAc	MeCN	EtAc	MeCN
2.5	57	92	58	101
3	54	90	51	92
4	85	90	73	94
5	96	84	84	86
6	104	90	94	90
7	104	94	94	89

[a] A 10 g apple juice sample was adjusted to the desired pH by using H_2SO_4 or K_2CO_3 solutions, and 10 mL MeCN + 5 g $MgSO_4$ and 1 g NaCl was used for extraction.

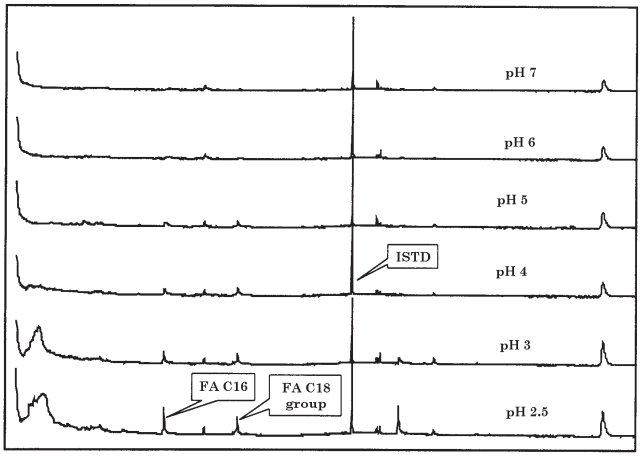

Figure 3. Effect of pH on presence of matrix co-extractives in GC/MS (full scan) chromatograms of MeCN apple juice extracts obtained by using 1 g NaCl + 5 g $MgSO_4$ to induce partitioning. ISTD = internal standard; FA = fatty acid.

Drying of MeCN Phase

Dispersive-SPE cleanup and drying are performed simultaneously in our method. Drying is thought to be beneficial because residual water can potentially affect SPE and GC separation (56). We examined the extent that residual water can be removed from the final extract by different amounts of $MgSO_4$. $MgSO_4$ was preferred over Na_2SO_4, which has been widely used in other methods, because it is much more effective in removing water (17, 19, 26, 27). We used the apple extracts of a previous experiment (Table 5) containing different amounts of residual water. The amount of water in each 1 mL extract after drying with different amounts of anhydrous $MgSO_4$ was determined by NMR spectroscopy (Figure 4). When 1 g NaCl was used in combination with 5 g $MgSO_4$ to partition the 10 g apple sample extracted with 10 mL MeCN, 150 mg $MgSO_4$ in 1 mL of the upper layer reduced the water content from about 70 to about 20 mg/mL, which was deemed sufficient for the SPE cleanup step.

Drying with $MgSO_4$ has another beneficial effect. The removal of water makes the final MeCN extracts less polar and causes precipitation of certain polar co-extractives. We observed visible precipitation of red strawberry pigments (anthocyanines) to some extent during and after drying ex-

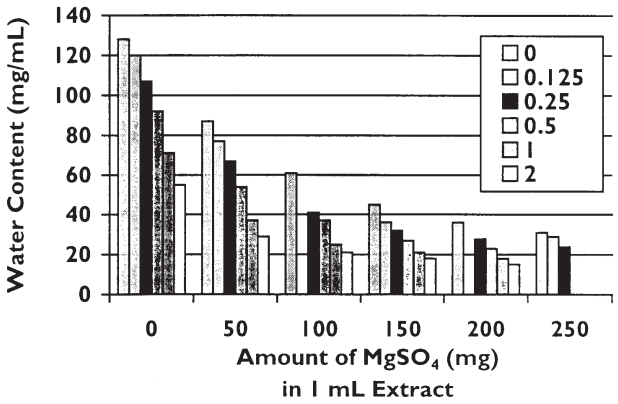

Figure 4. Residual water content of apple extracts (1 mL portion of upper layer) dried with different amounts of anhydrous $MgSO_4$ (NMR used to determine water content). Table legend gives the amount of NaCl used in combination with 5 g $MgSO_4$ to cause partitioning of 10 g + 10 mL MeCN apple extract.

tracts with $MgSO_4$. In several cases, we also observed that peaks belonging to polar matrix components were partly removed from GC/MS chromatograms after the extracts were dried with $MgSO_4$.

Dispersive-SPE Cleanup

Traditionally, SPE cleanup uses plastic cartridges containing 250–1000 mg sorbent material, vacuum manifolds, column preconditioning, solvent waste fractions, collection fractions, solvent evaporation steps, manual operation, and multiple solvents. Proper performance of SPE requires knowledge of the practice and theory of the technique, and requires training and careful attention on the part of the analyst. Common factors affecting reproducibility in SPE are difficulties with adjustment of the vacuum (and thus the flow rate), channeling, and column going dry. SPE does not lend itself easily to automation, and automated SPE devices are quite expensive, require maintenance, and lack versatility for nonroutine applications. Certainly, column-based SPE has its advantages over alternative approaches, but it is still far from ideal in practice.

In this study, we used a very simple cleanup approach that we call "dispersive-SPE." A 1 mL aliquot of the sample extract is added to a vial containing a small amount of SPE sorbent (25 mg PSA), and the mixture is shaken or mixed on a Vortex mixer briefly to evenly distribute the SPE material and thus facilitate the cleanup process. The sorbent is then separated by centrifugation or filtering, and an aliquot of the final extract is taken for analysis. This approach is most convenient when the SPE sorbent acts as a "chemical filter" to remove matrix components (and analytes are unretained), but, conceivably, dispersive-SPE can be used, albeit less conveniently, in retention/elution applications involving different solvents.

Dispersive-SPE is similar in some respects to MSPD (37–39), but the sorbent is added to an aliquot of the extract rather than to the original sample as in MSPD. The potentially high cost of the sorbent limits the sample size that can be used in MSPD. This leads to concerns about sample representation and homogeneity, but dispersive-SPE relies on the extraction process to provide a homogeneous aliquot from an original sample of any size and only a small amount of sorbent is used.

By using a much smaller quantity of sorbent and avoiding the cartridge format, dispersive-SPE saves time, labor, money, and solvent compared with the traditional SPE approach. No preconditioning of cartridges is needed, minimal analyst training or attention is necessary, and the sorbent bed cannot dry out. Unlike column-based formats, all of the sorbent interacts equally with the matrix in dispersive-SPE, and a greater capacity per milligram sorbent is achieved (no breakthrough effects due to channeling).

The use of stacked SPE cartridges is common (22–24) but inefficient. Manufacturers have devised mixed-bed SPE formats to address this inefficiency, but unless special orders are made or the users prepare their own cartridges, manufacturers dictate the types of sorbents that will be provided. Dispersive-SPE permits the user to prepare whatever combination of sorbents in any amounts to meet their needs.

Just as a drying agent is often added to the top of an SPE cartridge, $MgSO_4$ is added simultaneously as the SPE sorbent in our method to remove much of the excess water and to provide better cleanup. We compared filtration with centrifugation to separate the solid materials from the final extract after the dispersive-SPE step, and ultimately chose centrifugation. We would have preferred filtering with a convenient autosampler vial that forces the extracts through a filter from an outer open vessel into an inner container that is already capped (Whatman syringeless filtering vials), but the amount of solid material and extract volume was too much for the vials and we did not wish to reduce volume further. Also, the relative cost of the vials was higher than desirable for the method. It is conceivable that simultaneous filtering/cleanup could be used with this method in the future, or SPE could be conducted in a pipet tip, which would further streamline the method to involve only a single transfer of the extract.

Comparison of Different SPE Sorbents by Gravimetric Analysis

A number of multiclass MRMs use SPE for cleanup of extracts (14, 15, 22–25), and the most commonly used sorbents include weak ion exchange (PSA or $-NH_2$), GCB, SAX, and/or ODS SPE cartridges. In this study using the dispersive-SPE approach, we compared PSA, $-NH_2$, alumina-N, GCB, polymer, -CN, SAX, and ODS. Figure 5 summarizes the final results of these experiments in terms of weights of co-extracted material removed by the different sorbents and their combinations in MeCN extracts of mixed fruits and vegetables, as well as mixed calf, pork, and chicken livers, and ground beef for further interest to possibly extend the method to fatty tissues.

For points of reference, experimental controls in the method, which was scaled up 7-fold to provide measurable differences in weights, gave the following amounts of co-extracted matrix material: mixed fruits and vegetables = 0.17%

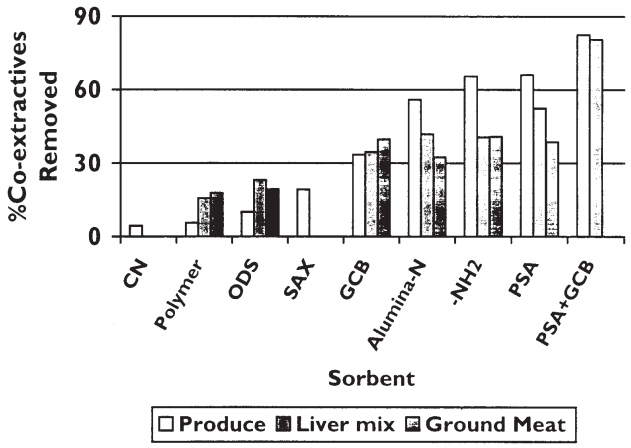

Figure 5. Cleanup capabilities of different sorbents using dispersive-SPE of 1 g/mL MeCN extracts of mixed fruit and vegetable samples (lettuce, tomato, apple, strawberry, orange, spinach, grapes, and carrot), mixed liver samples (calf, pork, and chicken), and ground beef in which 1 g NaCl + 4 g $MgSO_4$ was used to induce partitioning.

on average (or 1.7 mg/g sample); mixed livers = 0.40%; and ground beef = 0.19%. Thus, PSA+GCB (25 mg of each sorbent per gram equivalent sample) removed about 1.4 mg co-extracted matrix material per gram fruit and vegetable sample (or 3.2 mg/g liver). Therefore, with respect to the 1.5 μL GC/MS injection of fruit and vegetable extract, the dispersive-SPE cleanup with PSA avoided the introduction of about 1.6 μg matrix material per injection, leaving about 0.9 μg. The use of PSA+GCB would remove about 0.5 μg more matrix material (leaving only about 0.4 μg) to further avoid potential matrix interferants, but as discussed in the following section, GCB also retains certain pesticides.

We also determined that the combination of different sorbents does not necessarily provide additional cleanup because some sorbents remove the same matrix components as other sorbents (data not shown). For example, the same 20% of mixed food matrix co-extractives removed by ODS were also removed by PSA and GCB. In additional experimental results not shown in Figure 5, no additional benefit was gained by their combination with ODS in an MeCN extract (the situation could be different in a more aqueous extract). GCB and PSA also had considerable overlap in the amount and type of matrix components retained, but additional cleanup was provided by their combination.

Some MRMs call for the initial single-phase MeCN–water or acetone–water extract to be passed through an ODS SPE cartridge to provide cleanup before the liquid–liquid partitioning step is conducted (25). We performed an experiment in which 500 mg ODS sorbent was added to a mixed fruit and vegetable sample at the same time as the extraction solvent (both MeCN and acetone were compared). Some chlorophyll was removed by the ODS, as noted by a reduction in the green color of the extract, but weighing of the dried extracts showed no change in the amount of co-extracted matrix material whether the ODS was added or not.

Comparison of Different SPE Sorbents by GC/MS Analysis

In addition to the use of gravimetry to determine the bulk removal of matrix co-extractives, GC/MS was used to determine the types of chemicals in the extracts retained by the different sorbents. GC/MS verified the conclusion from gravimetric experiments that ODS provided virtually no cleanup. PSA, -NH_2, and alumina-N interact with chemicals by hydrogen bonding, and removed similar types of compounds, including fatty acids, other organic acids, and to some extent various sugars and pigments such as anthocyanidines from strawberry, grape, and raspberry extracts. As gravimetric analysis indicated (Figure 5), PSA removed more matrix co-extractives than -NH_2 and alumina-N per given quantity, which makes sense because PSA has higher capacity due to the presence of the secondary as well as the primary amine. A greater amount of -NH_2 in the experiment would likely have given the same result as PSA.

GCB has a strong affinity toward planar molecules and thus effectively removes pigments (e.g., chlorophyll, carotinoids) as well as sterols that are commonly present in foods (57). GC/MS analysis showed that the 2 sorbents behave complementarily by removing different kinds of matrix compounds. The combination of PSA and GCB is an excellent cleanup for removal of a variety of matrix materials, but unfortunately, GCB also retained structurally planar pesticides, such as thiabendazole, coumaphos, cyprodinil, and chlorothalonil, and our ISTD, TPP.

In an experiment, MeCN extracts were dried with $MgSO_4$ and fortified with pesticides. Aliquots were then subjected to dispersive-SPE, and calibration standards were prepared by fortifying extracts that had been previously cleaned up under the same conditions. When GCB was applied in dispersive-SPE, the recoveries for thiabendazole ranged from 17 to 44%, and chlorothalonil ranged between 4 and 30%, depending strongly on the type of the matrix used and the amount of GCB added. Cyprodinil and coumaphos gave between 7 and 48% recoveries in these experiments. The losses from GCB were less for matrices with high chlorophyll content, but recoveries also depended on the concentration of matrix and the amount of GCB. Although the addition of toluene to the extract would increase recoveries somewhat (22, 23), this would lead to dilution of the sample, less effective cleanup, and still incomplete recoveries.

Most of the components removed by GCB do not interfere in GC analysis (pigments give no visible peaks and sterols usually elute later than pyrethroids). Short-term benefits from GCB are therefore not substantial, but because of our concern about pigment components and nonvolatiles that could contaminate the GC system, we tested whether the use of GCB provides a long-term benefit.

An extract of a mixed matrix (consisting of one part each of green pepper, zucchini, strawberry, and peach, and half part each of carrot and lettuce) was prepared, and dispersive-SPE

was performed using: 1) no sorbent; 2) PSA; 3) GCB; and d) PSA+GCB. Each final extract was injected more than 30 times in a sequence. Matrix-free standards were injected every 6th injection to check GC performance and to check for ghost peaks. After each sequence, the GC-liner was changed to restore the initial conditions of the system, and peak areas and shapes for the matrix-free standards during each sequence were compared. Afterwards, the 4 liners with different histories were compared by injecting the same sample in each of them. The liner used for the "no cleanup" extract showed the highest activity, while the liner used for the PSA+GCB extract showed the least activity. However, the differences were not very significant, and therefore PSA alone was chosen over the other sorbents and possible combinations of sorbents in our final method.

Capacity of PSA in Dispersive-SPE

Experiments showed that 25 mg PSA was enough to completely remove the fatty acids in almost all matrices tested for a 1 mL aliquot of 1 g sample equivalent. *Note*: If the partitioning is performed without addition of NaCl, the extracts contain more polar components such as sugars; thus PSA can be overloaded from sugars. For strawberry extracts, which were rich in anthocyanidines, >25 mg PSA was necessary to avoid saturation of the PSA. Figure 6 shows the effect of different amounts of PSA in dispersive-SPE in the cleanup of concentrated asparagus extracts (5 g sample equivalents in 1 mL aliquots). GC with flame photometric detection (FPD) was used in this experiment, and very low pesticide concentrations in an especially complicated matrix were tested to demonstrate the utility of the approach. In this experiment, 50 mg PSA was satisfactory to remove nearly all background interfences in the GC–FPD chromatogram. This corresponds to 10 mg PSA sorbent per gram equivalent of asparagus, which is typical of most vegetables.

None of the pesticides tested, even *o*-phenylphenol, which has high potential for hydrogen bonding, showed any losses during the dispersive-SPE cleanup with PSA, even when the amount of PSA was increased 4-fold per gram equivalent sample. Captan and folpet gave inconsistent recoveries, but this was believed to be due to pH issues rather than cleanup

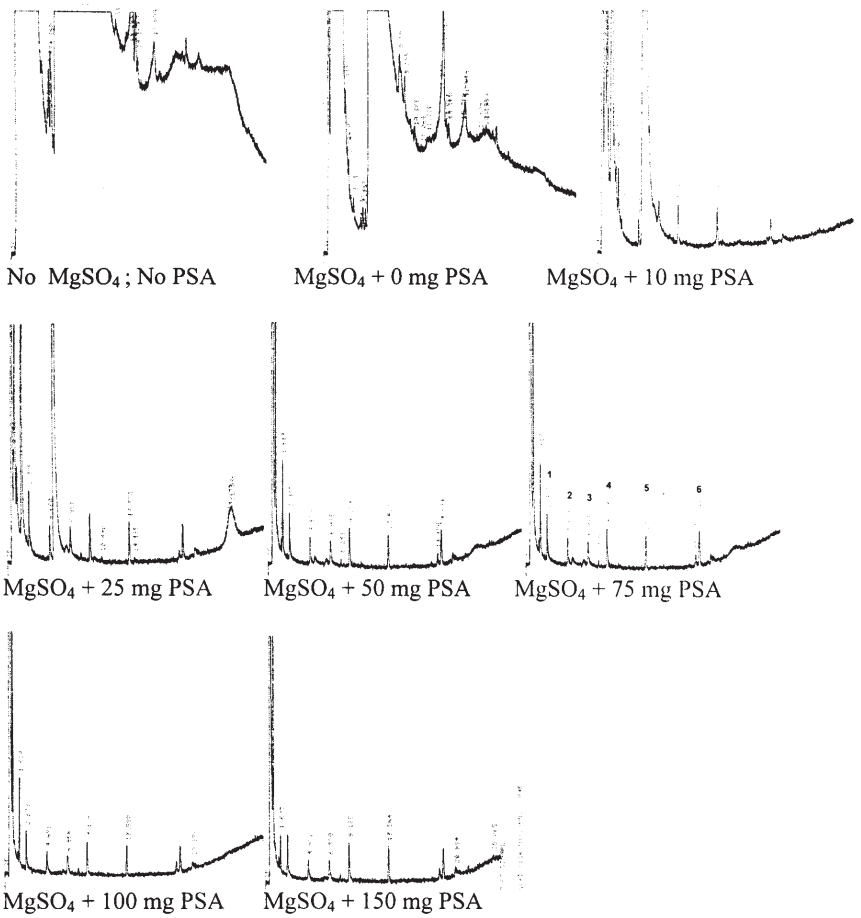

Figure 6. GC–FPD analysis of asparagus spiked at 2.0 ng/g using the final method with 5 mL aliquots of extract (equivalent to 5 g sample) evaporated to 1.0 mL and subjected to dispersive SPE cleanup with different amounts of PSA (with or without 150 mg $MgSO_4$). Peak identities are methamidophos, acephate, omethoate, dimethoate, chlorpyriphos, and azinphos methyl.

with PSA. The interaction between PSA and the retained compounds was essentially immediate because time of contact between the extract and the PSA did not affect recoveries. Because the fast formation of hydrogen bonds, cleanup was complete after samples were shaken for a few seconds. Exposure times as long as 30 min did not lead to any loss of the analytes tested.

Quantitation and Error

Any analysis is prone to errors arising at each stage of the procedure from sampling and subsampling through the sample preparation (extraction and cleanup), instrumental analysis, and quantitation. The following discussion focuses on errors occurring during sample preparation while errors occurring in GC analysis (related to matrix induced peak enhancement) will be addressed in the following section.

A degree of random error is inherent in any analysis, but a significant amount of systematic bias can also occur. Typical MRMs have many analytical steps and thus possess a high potential for errors. The chance for mistakes by analysts also increases as more steps are used in a method. Considering that biases are multiplicative, not additive (58), the number of error sources in analytical methods should be kept to a minimum. *The streamlining of methods not only leads to greater efficiency in the laboratory, but also reduces sources of error in the results*. With this in mind, we have kept the analytical steps as few and simple as possible in the development of this method.

The accuracy of analytical results depends on knowing the correct amount of sample represented by the final extract and the exact concentration of the reference standards used for calibration. An error in these factors will result in a systematic bias in the results. In many cases, such errors arise when volumes are wrongly adjusted, measured, or transferred. This is often the case when volumes are small or when miscalibrated pipets are used, e.g., when pipets calibrated for water at 20°C are used for organic solvents, which have significantly different viscosities and volatilities. Systematic errors also arise when the assumed volumes are different from the true ones, e.g., in the case of evaporative solvent losses or when aliquots are taken after liquid–liquid partitioning without knowing the true volume of the organic layer. Results with high bias are easily noted if recoveries are >100%, but systematic bias that occurs when recoveries are <100% are insidious because the analyst is fooled into believing either that recoveries are high when in fact they are low, or that recoveries are low when in fact they are high. Unfortunately, these biases in pesticide residue analysis are apparent in publications and are common occurrences in practice even when matrix-matched standards are used. Excellent discussions of sources of uncertainty in analytical methods appear elsewhere (58–61), but a very simple and time-honored approach in analytical chemistry can be used to overcome all systematic bias in liquid transfers: the use of an internal standard.

Our method simply entails 2 transfers of the MeCN extract, the taking of which constitutes the greatest potential source of error in the method. In most MRMs using MeCN extraction, an aliquot is taken after the phase-separation step, which is either performed directly or after drying with Na_2SO_4 (albeit a poor drying agent for MeCN; 17, 27). It is assumed in the method protocols that the organic phase will have the same volume as the amount of MeCN originally added to the sample. However, separation between the organic and the aqueous phases depends on a combination of factors such as water, sugar, and lipid content of the sample and the amount and types of salt dissolved in the aqueous phase. Other parameters include volume contraction phenomena and temperature effects: Samples can have very different initial temperatures; mixing MeCN with water causes a temperature drop; and addition of $MgSO_4$ causes heating. All of these factors affect the total volume of the organic phase and can therefore introduce systematic and/or random errors in the procedure. Even if a correct aliquot is taken at this stage, its effective volume will shrink after water is removed in the drying step, and a new source of error is introduced when aliquots of this solution are taken.

We used TPP as an ISTD to eliminate the sources of error inherent in the 2 liquid transfers in the method. Once the ISTD is added, transferred volumes are no longer critical because they are normalized to the ISTD. In both transfers, a repeating pipet of small volume is used. Although this normally could lead to a significant relative error, it is not a concern in our case because the ISTD is added to the extract before the first of these transfers is made. Provided that an appropriate ISTD is chosen, the earlier it is added the better it accounts for volume fluctuations that may follow (60). By using the ISTD before aliquots are taken, all liquid transfers become less important in terms of accuracy (even the initial 10 mL addition of MeCN). Even if all transfers are made accurately and precisely, the ISTD will serve to demonstrate this aspect and will do no harm. By knowing the amount of sample per amount of ISTD in the extract and the amount of pesticide per amount of ISTD in the calibration standards, the volume is taken out of the equation to calculate pesticide concentrations in the sample. The procedure to use the ISTD is very easy and straightforward, and it is unjustifiable for an ISTD not to be used in many common MRMs.

A key aspect in the use of the ISTD in this method is that the TPP must not partition into the aqueous phase or be retained by the sorbent during the dispersive-SPE step, and, indeed, experiments to assess these potential losses showed that they are negligible. In a system consisting of MeCN, water, and salts, 98% of the TPP added was recovered in the organic phase, and 99% was recovered in the cleanup step with PSA sorbent. However, losses were severe when GCB was used because of that sorbent's strong affinity for aromatic structures. Another advantage of TPP is that it can also serve as an excellent internal standard in LC/MS because it gives sharp peaks and intense signals both in the electrospray and atmospheric pressure chemical ionization positive modes, which are widely used in pesticide analysis (35).

Analyte Protectants

Analytes injected into a GC system interact with the coating material of the separation column and with several other surfaces. Undesirable peak tailing and degradative effects can occur in the column, but most problematic are interactions in

the injector area (liner and entrance to the column or precolumn). For example, the fresh cut at the top of the column can have very strong interactions. The surfaces in exposed areas usually increase activity because they are covered with a film of nonvolatile compounds originating from previous injections. These interactions cause peak tailing and degrade certain types of analytes (e.g., phosphoramides, carbamates), which are further affected by the high temperatures involved (13, 35, 56, 62–65). These undesired effects are significantly reduced by the presence of other molecules in the injected solution that can mask the active sites. Usually, matrix components in the extracts serve as masking agents to improve the analyte introduction efficiency into the column. This well-known effect among residue chemists is described as the "matrix-induced signal enhancement effect" (63). To overcome this effect and improve peak shapes, increase signal, and lower LODs, many laboratories use matrix-matched standards for calibration in pesticide analysis (13, 22–27, 35).

Interestingly, in the GC/MS experiment comparing different sorbents, the peak shape of many pesticides contained in the extracts subjected to PSA and PSA+GCB cleanup tailed more than when no PSA was used. Although the PSA removed several interfering matrix components in the GC/MS analysis, it also removed the polar matrix components that masked active sites on glass surfaces. This led to broader peaks and significant degradation of certain analytes during injection. We realized that if the types of compounds that protected the analytes from degradative interactions could be added to the solution, and if these "analyte protectants" did not interfere in the analysis, then the approach could be very useful in our method and other GC methods that analyze susceptible analytes. Thus, we sought to find chemicals that would meet this need.

The starting point in our search arose from the knowledge that PSA selectively removes the type of chemicals that act as good protecting agents. PSA forms hydrogen bonds with compounds containing hydroxy or carboxy groups, and during our experiments with PSA, we frequently observed the removal of various acids and carbohydrates with hydrogen bonding properties. Previous studies of the matrix-induced enhancement effect also indicated that pesticides containing groups that have potential for hydrogen bond formation, such as basic nitrogens (-NH-, =N-), hydroxy compounds (-OH), organophosphates (P=O), carbamates (R-O-CO-NHR-), and urea derivatives (-NH-CO-NH-), are affected most strongly by these effects (13, 35, 56, 63–65). In addition, the peak shapes of susceptible pesticides improved dramatically when less or no NaCl was used during the liquid–liquid partitioning step in our method. These extracts contained more polar components, such as acids and sugars in the extracts (Figure 2). Based on this evidence, we narrowed our search to compounds with multiple hydroxy, amino, and/or carboxyl groups that possess functional groups capable of forming hydrogen bonds. A large number of substances from different classes were evaluated for their ability to protect susceptible analytes during GC injection and these experiments are reported in a separate publication (66).

None of the compounds tested protected all analytes. Factors such as molecular structure, concentration, and volatility of the protecting agents played significant roles. In general, agents possessing high volatility better protected early eluting analytes, whereas less volatile agents were better in protecting late eluting analytes. A very good protection over a broad analyte spectrum was achieved with sugars, such as glucose and fructose, and sugar-alcohols, such as sorbitol. An adequate protection of early eluting polar analytes, such as methamidophos and acephate, was achieved by using 3-*O*-ethylglycerol.

Thus, we used the combination of sorbitol and 3-*O*-ethylglycerol to cover the entire range of pesticides in this study, but other analyte protectants may prove more useful after future investigations of this approach (66). Both agents contain hydroxy groups that interact with active sites on the surface of the injector and with the analytes. In many cases, the signal enhancement achieved with the help of protecting agents was greater than that achieved in the presence of matrix components (matrix extracts). Figure 7 shows the effect of the analyte protectants on the GC/MS peaks of methamidophos and acephate. The examples given clearly demonstrate how the use of analyte protectants can give higher quality GC results. By improving response and shape of analyte peaks, even in a very dirty system as used in our experiments, interferences are minimized and identification and integration become easier and more accurate, allowing greater selectivity, lower LODs, and greater confidence in the results.

Quantitation with Analyte Protectants

Another major advantage in the use of analyte protectants is the possibility to avoid matrix-related errors in GC analysis. Ideally, analyte protectants should provide the same degree of protection (i.e., signal enhancement) regardless of whether the solution contains matrix components or not. To determine

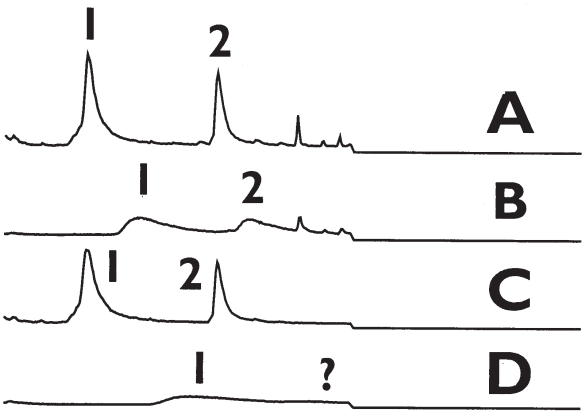

Figure 7. Improvement of peak shapes and peak areas with help of 1% ethylglycerol and 500 ppm sorbitol (analyte protectants). GC/MS (SIM) chromatograms of (1) methamidophos and (2) acephate (*m/z* = 94) at the same concentration in A = matrix extracts + analyte protectants; B = matrix extracts; C = solvent + analyte protectants; and D = pesticides in solvent only.

whether this was the case, we fortified pure solvent and several blank sample extracts (tomato, peach, strawberry, and orange, each with and without previous PSA cleanup) with a mixture of pesticides, and performed several injections. Figure 8 gives results for the most susceptible analytes.

The results demonstrate that the errors caused by matrix effects are reduced dramatically with the help of analyte protectants, making them a good alternative for dealing with the problem of matrix-induced signal enhancements. Other approaches proposed to counteract matrix effects include: 1) extensive cleanup of extracts; 2) matrix-matched calibrations; 3) method of standard additions (13, 35); 4) pressure-pulsed injection to minimize the time of interactions in the injector (64, 65); 5) on-column injection; 6) normalization versus isotopically labeled internal standards; and 7) priming or preconditioning the GC inlet. However, all these approaches have distinct disadvantages or lack effectiveness.

According to European guidelines, matrix-matched standards must be used unless it is shown that the matrix does not have an effect in the analysis (67). In the U.S., regulatory policies of the FDA and EPA do not permit the use of matrix-matched calibration standards for pesticide enforcement purposes, even though numerous studies have demonstrated that significant errors can occur when matrix effects are not taken into account. In any event, the use of matrix-matched standards in analysis is inconvenient for a variety of reasons: 1) blank matrices and extra freezer storage space are required; 2) additional extractions have to be performed; 3) more matrix material is injected in the GC system during a sequence, thus leading to more maintenance; and 4) matrices of the same type of sample can be significantly different from each other.

The principle of using analyte protectants may be a promising alternative, and EPA and FDA guidelines do not prohibit the use of such agents. However, more research is necessary to evaluate the approach and determine its long-term effect on instruments.

Pesticide Recoveries and Repeatabilites

For this study, we did not perform extensive validation studies, such as those described in the literature (68). However, we conducted recovery studies for many pesticides fortified at different concentrations in several different matrices. Table 8 shows the results of such an experiment for many pesticides on apple and lettuce samples fortified at a level of 250 ng/g. The pesticides chosen include some of the most difficult in multiresidue MRMs, covering a wide range of polarity and volatility. A great number of other GC-amenable pesticides with physicochemical properties that fall within the range defined by these pesticides will also give complete recoveries in the method.

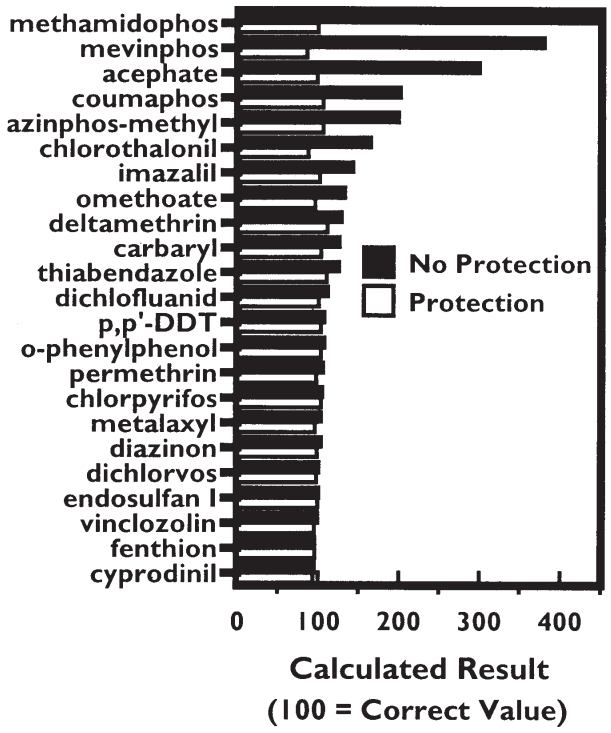

Figure 8. Effect of using analyte protectants (ethylglycerol and sorbitol) in calculated result of chosen pesticides spiked into a fruit matrix (average of peach and orange results).

Table 8. Percentage of recoveries and RSDs for representative pesticides fortified at 250 ng/g in lettuce and strawberry by final method (*n* = 5)

Pesticide	Lettuce	Strawberry
Dichlorvos	98 (0.6)	99 (3.0)
Methamidophos	86 (2.7)	87 (3.0)
Mevinphos	98 (1.2)	100 (1.7)
Acephate	92 (4.1)	95 (3.5)
o-Phenylphenol	100 (1.2)	98 (3.2)
Diazinon	100 (1.9)	96 (3.8)
Omethoate	95 (2.1)	99 (2.8)
Vinclozolin	101 (1.3)	98 (3.2)
Chlorothalonil	96 (3.1)	98 (3.8)
Metalaxyl	101 (1.1)	99 (2.7)
Carbaryl	95 (2.0)	99 (4.5)
Dichlofluanid	97 (13)	102 (3.4)
Fenthion	100 (1.9)	98 (2.6)
Cyprodinil	102 (2.0)	97 (2.9)
Thiabendazole	99 (0.9)	91 (5.6)
Endosulfan I	99 (1.4)	101 (4.1)
Imazalil	102 (2.8)	95 (2.7)
p,p′-DDT	98 (5.6)	96 (2.0)
Iprodione	97 (3.2)	99 (3.7)
Coumaphos	95 (5.4)	101 (4.1)
Permethrin	100 (3.5)	99 (3.4)
Deltamethrin	97 (2.9)	94 (3.1)

Comparable results from the analysis of incurred polar and nonpolar pesticides demonstrate the validity and feasibility of the method. Overall, we characterize the quality of results and practical advantages of the method as excellent compared to our experiences with other methods we have used. Future work in collaboration with routine monitoring laboratories will address more extensive validation of this method. Interlaboratory collaborative studies will be pursued if the single-laboratory validation and implementation are successful.

Conclusions

We feel that we have successfully fulfilled our aim to develop a method that is rapid, simple, inexpensive, effective, safe, potentially rugged, uses minimal amounts of solvents, needs no special equipment, avoids glassware (and cleaning/storage thereof), and still provides high quality results for a wide range of pesticides in foods. The method was streamlined extensively by avoiding or redesigning various inconvenient analytical steps that complicate traditional MRMs. Through the use of mixing on a Vortex mixer rather than blending, the extraction/partitioning procedure is performed in a sealed Teflon vessel (which is the only item to be cleaned or reused). Inconvenient separations of organic layers are avoided by performing a single liquid–liquid partitioning step. Unlike various other methods, the bulk matrix is not filtered after the initial extraction step, and evaporation/solvent exchanging steps that often lose volatile analytes are avoided. By adding the ISTD before the first liquid transfer, sample handling is more precise and convenient, and systematic and random errors related to liquid transfers are minimized.

The selectivity of the partitioning step has been optimized by the addition of salts, and further selectivity is achieved with cleanup using PSA sorbent. The cleanup step has been considerably simplified by introducing the dispersive-SPE approach, in which the SPE material is simply mixed with a portion of the extract. Thus, much time, labor, and expense are saved, no dilution of the extract occurs, and better cleanup is provided. The simplicity of the method and the removal of manual analytical steps also greatly reduce the potential for errors. Furthermore, the method uses less than $1 (U.S.) of materials for a 10 g sample, and with practice and planning, a single chemist can prepare a batch of 6–12 prechopped samples in only 30–45 min. The method is also easy to perform by facilities that are poorly equipped or that have little space, such as mobile or field laboratories.

Pesticide monitoring programs are often criticized for their high costs and inability to produce rapid regulatory answers of high quality for a broad spectrum of pesticides in a variety of sample types. In part, this problem arises from the long time and much labor needed by current methods to produce the extracts. The delays resulting from time-consuming analyses limit the effectiveness of enforcement actions, and the common need of multiple methods to monitor for certain pesticides is very inefficient. This inevitably has an impact on the trade of products and negatively affects the abilities of regulatory agencies to ensure food safety.

This situation could be improved by applying this method Once it has been further evaluated in a routine laboratory, we propose that this method be called QuEChERS, which stands for quick, easy, cheap, effective, rugged, and safe. Further research will focus on the expansion of this method to LC-type pesticides, fatty matrices, large volume injection, and faster chromatographic techniques.

Acknowledgments

We thank Joanne Cook and Pat Beckett of the Florida Department of Agriculture and Consumer Services for the shared samples and their analytical results; Janine Brouillette for performing the NMR analyses; and Susan Braden for help in conducting some experiments. This research was supported by Research Grant Award No. IS-3022-98 from BARD, the United States–Israel Binational Agricultural Research and Development Fund.

References

(1) Mills, P.A., Onley, J.H., & Guither, R.A. (1963) *J. Assoc. Off. Anal. Chem.* **46**, 186–191

(2) Thier, H.P., & Bergner, K.G. (1966) *Dtsch. Lebensm. Rundsch.* **62**, 399–402

(3) Pfeilsticker, K. (1971) in *Lebensmittelchemie und Gerichtliche Chemie,*Vol. 25, VCH, Weinheim, Germany, pp 129–164

(4) Storherr, R.W., Ott, P., & Watts, R.R. (1971) *J. Assoc. Off. Anal. Chem.* **54**, 513–516

(5) Becker, G. (1971) *Dtsch. Lebensm. Rundsch.* **67**, 125–126

(6) Luke, M., Froberg, J.E., & Masumoto, H.T. (1975) *J. Assoc. Off. Anal. Chem.* **58**, 1020–1026

(7) Specht, W., & Tilkes, M. (1980) *Fresenius Z. Anal. Chem.* **301**, 300–307

(8) *DFG Rückstandsanalytik von Pflanzenschutzmitteln*, VCH, Weinheim, Germany, S8, DFG-S19

(9) U.S. Food and Drug Administration (1994) *Pesticide Analytical Manual*, Vol. I, *Multiresidue Methods*, 3rd Ed., FDA, Washington, DC

(10) *Official Methods of Analysis* (2000) 17th Ed., AOAC INTERNATIONAL, Gaithersburg, MD

(11) Sawyer, L.D. (1985) *J. Assoc. Off. Anal. Chem.* **68**, 64–71

(12) Specht, W., Pelz, S., & Gilsbach, W. (1995) *Fresenius J. Anal. Chem.* **353**, 183–190

(13) Anastassiades, M., & Scherbaum, E. (1997) *Dtsch. Lebensm. Rundsch.* **93**, 316–327

(14) Casanova, J. (1996) *J. AOAC Int.* **79**, 936–940

(15) Nordenmeyer, K., & Thier, H.P. (1999) *Z. Lebensm. Unters. Forsch. A* **208**, 259–263

(16) Luke, M., Cassias, I., & Yee, S. (1999) *Lab. Inform. Bull. No. 4178,* Office of Regulatory Affairs, U.S. Food and Drug Administration, Rockville, MD

(17) Schenck, F.J., Callery, P., Gannett, P.M., Daft, J.R., & Lehotay, S.J. (2002) *J. AOAC Int.* **85**, 1177–1180

(18) Parfitt, C.H., Jr (1991) *Lab. Inform. Bull. No. 3616,* Office of Regulatory Affairs, U.S. Food and Drug Administration, Rockville, MD

(19) Steinwandter, H. (1992) in *Emerging Strategies for Pesticide Analysis*, T. Cairns & J. Sherma (Eds), CRC Press, Boca Raton, FL, pp 3–38

(20) Lee, S.M., Papathakis, M.L., Hsiao-Ming, C.F., & Carr, J.E. (1991) *Fresenius J. Anal. Chem.* **339**, 376–383

(21) Liao, W., Joe, T., & Cusick, W.G. (1991) *J. AOAC Int.* **74**, 554–565

(22) Fillion, J., Hindle, R., Lacroix, M., & Selwyn, J. (1995) *J. AOAC Int.* **78**, 1352–1366

(23) Fillion, J., Sauvé, F., & Selwyn, J. (2000) *J. AOAC Int.* **83**, 698–713

(24) Sheridan, R.S., & Meola, J.R. (1999) *J. AOAC Int.* **82**, 982–990

(25) Cook, J., Beckett, M.P., Reliford, B., Hammock, W., & Engel, M. (1999) *J. AOAC Int.* **82**, 1419–1435

(26) Lehotay, S.J. (2000) *J. AOAC Int.* **83**, 680–697

(27) Lehotay, S.J., Lightfield, A.R., Harman-Fetcho, J.A., & Donoghue, D.A. (2001) *J. Agric. Food Chem*. **49**, 4589–4596

(28) *Official Methods of the AOAC* (1968) *J. Assoc. Off. Anal. Chem*. **51**, 482–485

(29) Krijgsmann, W., & van de Kamp, G.C. (1976) *J. Chromatogr*. **177**, 201–205

(30) Andersson, A., & Palsheden, H. (1991) *Fresenius J. Anal. Chem.* **339**, 365–367

(31) Holstege, D.M., Scharberg, D.L., Tor, E.R., Hart, L.C., & Galey, F.D. (1994) *J. AOAC Int.* **77**, 1263–1274

(32) Fernández-Alba, A.R., Valverde, A., Agüera, A., & Contreras, M. (1993) *J. Chromatogr.* **686**, 263–271

(33) Lehotay, S.J. (1997) *J. Chromatogr. A* **785**, 289–312

(34) Eller, K.I., & Lehotay, S.J. (1997) *Analyst* **122**, 429–435

(35) Anastassiades, M. (2001) *Entwicklung von schnellen Verfahren zur Bestimmung von Pestizidrückständen in Obst und Gemüsse mit Hilfe der SFE – Ein Beitrag zur Beseitigung analytischer Defizide*, Shaker Verlag, Aachen ISBN: 3-8265-9618-8

(36) Valverde-García, A., Fernández-Alba, A.R., Agüera, A., & Contreras, M. (1995) *J. AOAC Int.* **78**, 867–873

(37) Barker, S.A. (2000) *J. Chromatogr. A* **880**, 63–68

(38) Barker, S.A. (2000) *J. Chromatogr. A* **885**, 115–127

(39) Schenck, F.J., & Wagner, R. (1995) *Food Addit. Contam.* **12**, 535–541

(40) Pylypiw, H.M., Jr, Arsenault, T.L., Thetford, C.M., & Mattina, M.J.I. (1997) *J. Agric. Food Chem.* **45**, 3522–3528

(41) Pawliszyn, J. (1997) *Solid Phase Microextraction Theory and Practice*, Wiley-VCH, New York, NY

(42) Kataoka, H., Lord, H.L., & Pawliszyn, J. (2000) *J. Chromatogr. A* **880**, 35–62

(43) Mills, G.A., & Walker, V. (2000) *J. Chromatogr. A* **902**, 267–287

(44) Richter, B.E., Jones, B.A., Ezzell, J.L., Porter, N.L., Avdalovic, N., & Pohl, C. (1996) *Anal. Chem.* **68**, 1033–1039

(45) Lehotay, S.J., & Lee, C.H. (1997) *J. Chromatogr. A* **785**, 313–327

(46) Obana, H., Kikuchi, K., Okihashi, M., & Hori, S. (1997) *Analyst* **122**, 217–220

(47) Young, S.J., Parfitt, C.H., Jr, Newell, R.F., & Spittler, T.D. (1996) *J. AOAC Int*. **79**, 976–980

(48) Hemingway, R.J., Aharonson, N., Greve, P.A., Roberts, T.R., & Thier, H.P. (1984) *Pure Appl. Chem.* **56**, 1131–1152

(49) Hill, A.R.C., Harris, C.A., & Warburton, A.G. (2000) in *Principles and Practices of Method Validation*, A. Fajgelj, & Á. Ambrus (Eds), Royal Society of Chemistry, Cambridge, UK, pp 41–48

(50) General Inspectorate for Health Protection (1996) *Analytical Methods for Pesticide Residues in Foodstuffs*, 6th Ed., Ministry of Health Welfare and Sport, The Hague, The Netherlands

(51) Lehotay, S.J., Aharonson, N., Pfeil, E., & Ibrahim, M.A. (1995) *J. AOAC Int.* **78**, 831–840

(52) Reynolds, S.L., Fussel, R., Caldow, M., James, R., Nawaz, S., Ebden, C., Pendlington, D., Stijve, T., Lovell, S., & Diserens, H. (1997, 1998, 2000, 2001) '*Intercomparison Study of Two Multi-Residue Methods for the Enforcement of EU MRLs for Pesticides in Fruits, Vegetables and Grain*,' European Commission, Luxembourg

(53) Argauer, R.J., Lehotay, S.J., & Brown, R.T. (1997) *J. Agric. Food Chem.* **45**, 3936–3939

(54) Burke, J.A. (1971) *Residue Rev.* **34**, 59–90

(55) *Lange's Handbook of Chemistry* (1985) J.A. Dean (Ed.), McGraw-Hill, New York, NY, pp 10–14

(56) Schenck, F.J., & Lehotay, S.J. (2000) *J. Chromatogr. A* **868**, 51–61

(57) U.S. Department of Agriculture (2001) *USDA Nutrient Database for Standard Reference, Release 14*, Agricultural Research Service, Beltsville, MD, www.nal.usda.gov/fnic/foodcomp/Data/index.html

(58) Hill, A.R.C., & von Holst, C. (2001) *Analyst* **126**, 2044–2052

(59) Horwitz, W. (1998) *J. AOAC Int.* **81**, 785–794

(60) Meyer, V.R. (2002) *LC-GC N. Am.* **20**, 106–112

(61) Alder, L., Korth, W., Patey, A.L., van der Schee, H., & Schoenweiss, S. (2001) *J. AOAC Int.* **84**, 1569–1578

(62) Podhorniak, L.V., Negron, J.F., & Griffith, F.D., Jr (2001) *J. AOAC Int.* **84**, 873–890

(63) Erney, D.R., Gillespie, A.M., Gilvydis, D.M., & Poole, C.F. (1993) *J. Chromatogr*. **638**, 57–63

(64) Godula, M., Hajšlová, J., & Alterová, K. (1999) *J. High Resolut. Chromatogr*. **22**, 395–402

(65) Wylie, P.L., & Uchiyama, K. (1996) *J. AOAC Int.* **79**, 571–577

(66) Anastassiades, M., Maštovská, K., & Lehotay, S.J. *J. Chromatogr. A* (submitted)

(67) Hill, A. (1997) *Quality Control Procedures for Pesticide Residues Analysis—Guidelines for Residues Monitoring in the European Union*, Document 7826/VI/97, European Commission, Brussels, Belgium

(68) *Principles and Practices of Method Validation* (2000) A. Fajgelj & Á. Ambrus (Eds), Royal Society of Chemistry, Cambridge, UK, pp 179–295