SPECIAL GUEST EDITOR SECTION

Analysis of Pesticide Residues in Mixed Fruit and Vegetable Extracts by Direct Sample Introduction/ Gas Chromatography/Tandem Mass Spectrometry

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Direct sample introduction (DSI), or "dirty sample injection," was investigated in the determination of 22 diverse pesticide residues in mixed apple, green bean, and carrot extracts by benchtop gas chromatography/tandem mass spectrometry (DSI/GC/MS–MS). The targeted pesticides, some of which were incurred in the samples, included chlorpyrifos, azinphos-methyl, parathion-methyl, diazinon, terbufos, *p,p′*-DDE, endosulfan sulfate, carbofuran, carbaryl, propargite, bifenthrin, dacthal, trifluralin, metalaxyl, pendimethalin, atrazine, piperonyl butoxide, diphenylamine, vinclozolin, chlorothalonil, quintozene, and tetrahydrophthalimide (the breakdown product of captan). The analytical DSI method entailed the following steps: (*1*) blend 30 g sample with 60 mL acetonitrile for 1 min in a centrifuge bottle; (*2*) add 6 g NaCl and blend 30 s; (*3*) centrifuge for 1–2 min; (*4*) add 5 mL upper layer to 1 g anhydrous MgSO₄ in a vial; and (*5*) analyze 11 µL extract, using DSI/GC/MS–MS. Sample cleanup is not needed because GC/MS–MS is exceptionally selective for the targeted analytes, and nonvolatile coextracted matrix components do not contaminate the injector or the GC/MS–MS system. Average recoveries of the pesticides were 103 ± 7% with relative standard deviations of 14 ± 5% on average, and limits of detection were <2 ng/g for nearly all pesticides studied. The DSI/GC/MS–MS approach for targeted pesticides is quantitative, confirmatory, sensitive, selective, rugged, rapid, simple, and inexpensive.

Traditionally, multiclass pesticide residue analysis of foods is a time-consuming process that often entails several post-extraction cleanup steps before analysis (1–4). Conventional wisdom holds that ruggedness and reli-

ability of an approach suffer as shortcuts are taken in the sample preparation procedures (5). The use of organic solvents, such as acetone (1), acetonitrile (2–3), or ethyl acetate (4), for extraction provides high recoveries of pesticides over a wide range of polarity, but further cleanup is generally required before gas chromatographic analysis. These cleanup techniques, which often include solid-phase extraction (SPE), liquid–liquid partitioning, and/or gel permeation chromatography (GPC), make up the bulk of the cost, time, and labor in analytical methods.

In recent years, supercritical fluid extraction (SFE) has been found to be a viable alternative to organic solvents in the extraction of pesticide residues from nonfatty foods (6, 7). SFE conditions can be selected to provide a more selective extraction process that does not require post-extraction cleanup steps before analysis. Furthermore, the SFE approach is semiautomated, uncomplicated, waste-free, and inexpensive. However, a result of the greater selectivity is a narrower range of polarity for the pesticides that give complete recoveries in SFE. Two or more sets of conditions are needed in SFE to cover the wide range of polarity that liquid organic solvents can encompass (6).

The use of selective detectors in gas chromatography (GC) reduces the amount of cleanup necessary for the removal of interfering coextracted components in the analysis. A high degree of selectivity, commonly considered a strength of selective detectors, such as the electrolytic conductivity detector, flame photometric detector, nitrogen-phosphorus detector, and others, is also a weakness because of the relatively narrow range of analytes that each can detect. In multiclass, multiresidue methods, several GC injections are often required when these selective detectors are used to determine the gamut of GC-amenable pesticides. Furthermore, cleanup of extracts is integral for improving the ruggedness and reliability of the GC system. Nonvolatile matrix components build up in the injection liner and capillary column and reduce performance of the system until maintenance is performed.

The use of mass spectrometry (MS), a universally selective detection approach, in GC analysis permits the detection and confirmation of a wide range of pesticides in complex extracts (1, 3, 7, 8). Modern GC/MS instruments can achieve

detection limits that approach those possible with traditional selective detectors, and the use of large-volume injection (LVI) in GC can further help to reduce detection limits (9), but in these cases, cleanup of complex extracts becomes even more important to avoid contamination of the injection liner, capillary column, and MS source with nonvolatile matrix components.

In typical split/splitless injection, 1–3 μL of the final extract is injected into the gas chromatograph at ca 250°C, but in LVI, sample quantities of ≤100 μL may be injected if appropriate precautions are used (9, 10). In LVI, the injection port initially remains below or near the boiling point of the extract solvent until the solvent evaporates, and then the injector temperature is increased to introduce the semivolatile analytes. However, any nonvolatile materials, such as certain salts, carbohydrates, proteins, and lipids, will remain in or near the injector and/or slowly migrate through the GC column. These interferants can impair the performance of the GC analysis and lead to frequent system maintenance.

Recently, Amirav and coworkers (11–13) described a direct sample introduction (DSI) (or "dirty sample injection") device that enables direct sampling of solid materials or LVI of liquids into a GC instrument. Figure 1 is a drawing of a DSI device that has become commercially available as the ChromatoProbe. The device operates by placing a 40 μL microvial that contains the sample into the GC injection port. For liquid extracts, the injector is operated just as in LVI techniques in which initial temperature is kept low to gently evaporate the solvent (with the split vent open), and then the

Figure 1. Drawing of the ChromatoProbe used in DSI (reprinted from ref. 11 with permission from the authors).

temperature is rapidly increased to volatilize the semivolatile analytes (with the split vent closed). Unlike liquid injection techniques with syringes, an elegant aspect of this sample introduction technique is that the volatility range of compounds entering the GC liner and column matches the volatility range amenable to the GC system. The nonvolatile components remain in the microvial, which is discarded and replaced for the next injection. This approach is not currently automated, but there are no technical obstacles in doing so.

Previous reports of DSI presented various applications of the device (11) and qualitative aspects involving a few representative pesticides (12, 13). The use of pulsed flame photometric detection (PFPD), by itself (12) or in combination with simultaneous MS confirmation (13), was shown to provide the high degree of selectivity and sensitivity needed to detect exceptionally low levels of phosphorus- and sulfur-containing pesticides in the complex DSI extracts. However, investigation of the quantitative aspects of the DSI approach was not the goal of previous work. Furthermore, the DSI/GC/ MS approach lacked selectivity and sensitivity for low-level detection and confirmation without PFPD (13).

Tandem MS using benchtop ion-trap systems has been shown to be a powerful approach in pesticide residue analysis to both increasing the selectivity and decreasing the limits of detection (LODs) in the analysis of complex extracts (10, 14, 15). These previous reports of GC/MS–MS applications for pesticide analysis used 5–40 μL LVI techniques (10, 14, 15), one of which involved the injection of the extracts onto a packing bed material, known as Carbofrit, in the injector liner (15). The GC/MS–MS approach that did not use the packing material gave a relative standard deviation (RSD) of <10% (14), but it was used mainly for screening purposes only, despite the 3-cartridge SPE cleanup procedure conducted before injection (15).

In this study, the DSI approach was coupled with GC/MS– MS in the determination of 22 representative pesticides in fruits and vegetables. This approach takes advantage of the ease and ruggedness of the ChromatoProbe and the sensitivity and universal selectivity of MS–MS detection. The quantitative aspects of DSI/GC/MS–MS were studied in the analysis of samples prepared for an AOAC INTERNATIONAL collaborative study for the determination of pesticide residues in nonfatty foods by SFE and GC/MS.

Experimental

Apparatus

(a) *GC/MS–MS system.*—A Saturn 2000 (Varian, Walnut Creek, CA) GC/MS instrument equipped with a Model 3800 gas chromatograph, Model 1079 injection port, and Model 8200 autosampler (for split/splitless injections) were used in the analysis of the sample extracts. The ChromatoProbe (Varian) was used for DSI on the instrument. Saturn Workstation software version 5.2.1 was used for instrument control and analysis of results. A 30 m, 0.25 mm id, 0.25 μm film thickness, DB-5ms (J&W, Folsom, CA) capillary GC col-
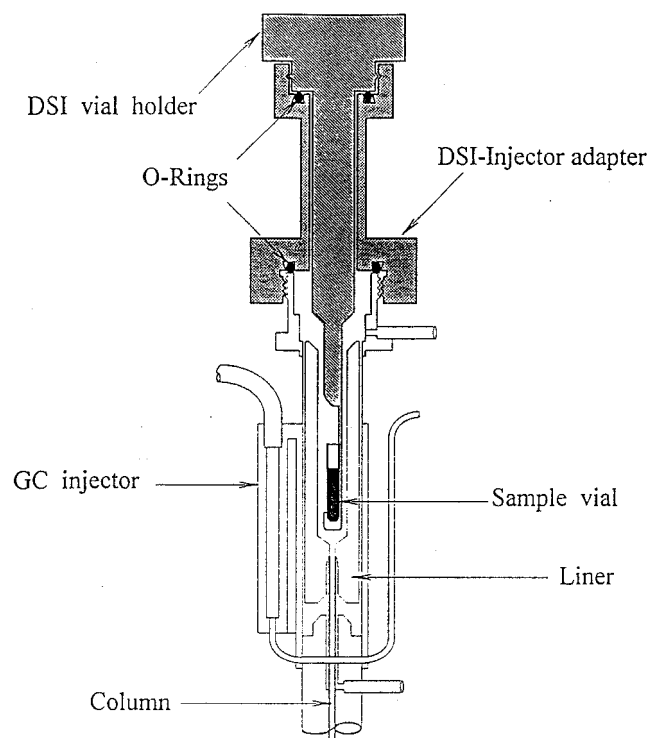
umn and electronic flow control of He at 1 mL/min were used in all experiments. The GC/MS scan range in selected-ion storage was set to 70–430 m/z at 1 s/scan spectral averaging. Electron ionization (EI) with a filament current of 50 µA and a multiplier voltage of 1500 V was used in all MS and MS–MS experiments. A full autotune of the mass spectrometer was performed before the analysis of every set of samples. Experiments were conducted to optimize conditions for the generation of MS–MS spectra, and Table 1 lists the final MS–MS conditions for the individual analytes. Table 2 lists the different GC conditions used in the experiments with the different injection techniques and final extract solvents. Transfer line temperature was 300°C, manifold temperature was 50°C, and ion-trap temperature was 220°C. A 4 mm id deactivated-glass straight liner packed with a 7 mm piece of Carbofrit (Restek, Bellefonte, PA) was used in the split/splitless injection of 3 µL SFE extracts in acetone (3.5 mg

sample equivalent). In the case of DSI, the liner was turned around so that the wide opening was at the top to accept the ChromatoProbe. For the SFE extracts in acetone, 3 µL extract (3.5 mg sample equivalent) was added to the microvial in the ChromatoProbe, which was introduced into the Model 1079 injection port. For the acetonitrile DSI extracts, 11 µL (5.5 mg sample equivalent) was injected. Other volumes were used in experiments to determine the effect of injection volume.

(**b**) *SFE System.*—A Model 7680T (Hewlett-Packard, Little Falls, DE) was used for the extraction of 1 set of test samples from the collaborative study. The 10 g frozen test samples of apples, green beans, and carrots were mixed with 11 g Hydromatrix (Varian, Harbor City, CA) by using a mortar and pestle (frozen conditions were maintained by prechilling all materials used in the mixing procedure). When the mixed sample appeared homogeneous, 4.0 g was loaded into a 7 mL SFE vessel and placed in the sample turret for extrac-

Table 1. Pesticide analytes and MS–MS parameters used for their determination

Segment	Pesticide	Scan time, s	Parent ion, m/z	Storage level, m/z	Amplitude, V	Quantitation ions, m/z
				MS-MS parameters		
				Excitation		
1	Tetrahydrophthalimide	1	151	66	45	79+80+106+122
2	Diphenylamine	1	169	74	80	139+140+166+167
3	Trifluralin	1	306	116	80	160+188+206+264
4	Carbofuran	0.5	164	72	50	149
4	Atrazine	0.5	215	95	60	138+172+200
5	Terbufos	0.27	231	102	58	175+199+203
5	Quintozene	0.27	295	130	74	123+237+263+265
5	d_{10}-Anthracene	0.27	188	83	96	156+160+161+184
5	Diazinon	0.27	304	134	72	162+179
6	Chlorothalonil	1	266	91	94	133+168+264
7	Vinclozolin	0.26	212	93	90	109+145+172+177
7	Parathion-methyl	0.26	263	116	60	136+153+246
7	Carbaryl	0.26	144	63	60	115+116
7	Metalaxyl	0.26	206	91	65	121+132+162
8	Chlorpyrifos	0.5	314	102	50	258+286
8	Dacthal	0.5	301	100	87	271+273+299
9	Pendimethalin	1	252	111	62	162+191+208+212
10	*p,p′*-DDE	1	318	105	76	246+248+281+283
11	Ethion	1	231	102	56	175+203
12	Endosulfan sulfate	1	272	120	94	235+237+270
13	Propargite	0.5	135	59	48	107
13	Piperonyl butoxide	0.5	176	78	60	103+117+131+145
14	Bifenthrin	0.5	181	95	78	153+165+166
14	d_{12}-Chrysene[a]	0.5	240	106	0.8	237
15	Azinphos-methyl	1	132	65	63	77+104

[a] Resonant ionization; nonresonant ionization was used for the other analytes.

Table 2. GC conditions used in the determination of the pesticides

Parameter	Carbofrit (3 µL acetone)	ChromatoProbe (3 µL acetone)	ChromatoProbe (11 µL acetonitrile)
Injector temperature program	50°C for 20 s, to 325°C at 100°C/min, hold until 29 min	hold until 24 min	70°C for 30 s, to 275°C at 100°C/min, hold until 24 min
Split ratio and times	50:1 for 20 s, off until 3.5 min, 50:1 until end of run		50:1 for 30 s, off until 4 min, 50:1 until 25 min
Column temperature program	50°C for 3 min, to 125°C at 25°C/min, then to 275°C at 10°C/min, hold for 9 min	hold for 4 min	70°C for 3 min, to 125°C at 25°C/min, to 275° at 10°C/min, hold until 25 min
He flow rate, mL/min	1.0	1.0	1.0
Total run time, min	30	25	25

tion. A quality control (QC) spike to yield terbufos at 263 ng/g was added to the samples in the SFE vessels. SFE conditions were 329 bar (320 atm, 4700 psi), 60°C (0.85 g/mL, CO_2 density), 1 min static time, 2 mL/min dynamic flow rate for 21 min (42 mL CO_2), 50°C restrictor, and 15°C octadecylsilane trap (0.5 g in a 1 mL trap). The extracts were washed from the solid-phase trap into an autosampler vial with 1.5 mL acetone at 35°C and 2 mL/min; then the trap was rinsed with 2 mL acetone–water (4 + 1), followed by 2 mL acetone at 35°C and 2 mL/min.

Reagents

Pesticide-grade acetonitrile, (Fisher, Fair Lawn, NJ) was used for extraction of 1 set of test samples for DSI. SFE-grade CO_2 (Airgas; Linthicum, MD) was used in SFE to extract another set of samples, and liquid chromatography (LC)-grade acetone (Fisher) was used to rinse the solid-phase trap in SFE. Hydromatrix (Varian) is a diatomaceous-earth material used as a drying agent and dispersant in SFE. NaCl (ACS reagent grade) and anhydrous $MgSO_4$ were obtained from Aldrich (Milwaukee, WI). Pesticide standards were obtained from the U.S. Environmental Protection Agency (Fort Meade, MD) and Chem Service (West Chester, PA). The d_{10}-anthracene and d_{12}-chrysene, which were used for internal standards, were obtained from Cambridge Isotope Laboratories (Woburn, MA). All working standard solutions were prepared in acetone. Apples, green beans, and carrots were obtained from the U.S. Food and Drug Administration (FDA) pesticide surveillance laboratory in Baltimore, MD and from a local grocery store.

Sample Preparation

Mixed samples of apples, green beans, and carrots (1:1:1) were used in the study. The samples were prepared for the AOAC INTERNATIONAL collaborative study "Determina-

tion of Pesticide Residues in Nonfatty Foods by SFE and GC/MS" by individually blending 3 kg (in 1 kg portions) pre-chopped, frozen apples, carrots, or green beans in a Model RSI 6V chopper (Robot-Coupe, Ridgeville, MS). Small amounts of dry ice pellets were periodically added to help maintain frozen conditions. Piperonyl butoxide solution was added during the homogenization process to yield an expected concentration of 200 ng/g in each commodity. After repeated mixings, 10 g frozen portions of the chopped samples were placed in glass containers, and pendimethalin was added to each container to yield an expected concentration of 200 ng/g. Additionally, the 10 g samples in sets of 7 were spiked with pesticide solutions of varying concentrations, as described in the collaborative study protocol (16). The samples were stored at −40°C until extraction within a few days in the case of SFE and a month later in the case of DSI. The SFE extracts in acetone were stored for a month at −40°C before the analyses described in this study were performed.

In the case of DSI, a set of prethawed, similarly numbered 10 g test samples were combined in 250 mL Teflon centrifuge bottles. Terbufos was added to the mixed commodity to give a concentration of 200 ng/g just before extraction. Then 60 mL acetonitrile was added to the 30 g mixed commodity, and the mixture was blended by using a Tissumizer (Tekmar, Cincinnati, OH) on the high setting for 1 min; 6 g NaCl was added to the extract, which was blended for an additional 30 s. The extract was placed in a Model SC200 centrifuge (Savant, Farmingdale, NY) for 2 min. Afterwards, 5 mL of the upper layer was placed into a vial containing 1 g anhydrous $MgSO_4$, and the vial was capped and shaken. Anhydrous $MgSO_4$ is much better than Na_2SO_4 for removing water from acetonitrile. The hydration of $MgSO_4$ is highly exothermic, and the heat generated demonstrated that the water reacted with the drying agent in the extract. Internal standard solution was added to each extract to give an equivalent of 200 ng/g for d_{10}-anthracene and d_{12}-chrysene. The extracts were ana-

lyzed by DSI/GC/MS–MS when they reached room temperature.

The extraction procedure took ca 20 min for a batch of 6 samples. For analysis, an 11 μL aliquot of each extract (5.5 mg mixed sample) was added to a DSI microvial, which was placed in 1 of the 2 ChromatoProbes, which are provided as a pair. The ChromatoProbe was then placed in the injection port and the GC/MS–MS run was initiated. After the run, another extract was loaded, and the ChromatoProbes were exchanged. The used microvial, which contained a noticeable film of nonvolatile matrix coextractives, was discarded.

In the case of SFE, the samples were extracted individually by following the procedure of the collaborative study. After extraction by SFE, d_{10}-anthracene and d_{12}-chrysene were added to each extract to give an equivalent concentration of 200 ng/g. After the extracts were analyzed by the GC/MS system used in the collaborative study, equal portions (0.5 mL) of the extracts from the 3 different commodities were combined into single mixed extracts. The mixed extracts were spiked with ethion to give an equivalent concentration of 200 ng/g in the mixed commodity. Table 3 lists the final concentrations of the pesticides in each mixed extract for the 7 different test samples.

Calibration

Calibration standards were prepared in both acetone and mixed blank extracts for comparison purposes. Mixed pesticide standard solutions were added to 4 of the 5 blank extracts at different levels after SFE to generate the calibration standards in matrix. In the case of DSI, separate 5 mL portions were taken from the same 60 mL blank extract for preparation of the calibration standards. The use of matrix-matched standards in the GC determination of pesticides has previously been demonstrated to be a practical way to overcome the matrix-enhancement effect (7, 17–20). The incurred pesticides and QC spikes were already in the extracts; thus, the calibration plots did not pass through zero in these cases. The method of standard additions was used in which the negative of the x-intercept of the calibration plot determined average concentrations in the mixed test samples. The signal used for quantitation was the peak area of the analyte divided by the peak area of the internal standard (ethion for SFE extracts and d_{10}-anthracene for DSI). Signal/noise (S/N) ratios as reported by the instrument software were used to calculate the LODs, which were the average analyte concentrations that gave S/N = 3.

Table 3. Concentrations of pesticides (ng/g) in the mixed apple, green bean, and carrot test samples

Pesticide	Mixed test sample						
	1	2	3	4	5	6	7
Atrazine	25	167	—	25	133	133	167
Azinphos-methyl	Unknown concentration (incurred in apples)						
Bifenthrin	80	—	200	80	30	30	200
Captan	Unknown concentration (incurred in apples)						
Carbaryl	Unknown concentration (incurred in apples)						
Carbofuran	—	25	25	67	67	167	167
Chlorothalonil	Unknown concentration (incurred in green beans)						
Chlorpyrifos	33	50	150	250	300	200	17
Dacthal	133	133	—	20	53	53	20
Diazinon	53	53	133	133	—	20	20
Diphenylamine	Unknown concentration (incurred in apples)						
Endosulfan sulfate	333	333	—	133	133	50	50
Metalaxyl	167	25	25	167	63	63	—
p,p'-DDE	40	40	100	15	—	100	15
Parathion-methyl	25	67	167	25	—	167	67
Pendimethalin	200	200	200	200	200	200	200
Piperonyl butoxide	200	200	200	200	200	200	200
Propargite	Unknown concentration (incurred in apples)						
Quintozene	133	—	133	53	20	20	53
Terbufos	200	200	200	200	200	200	200
Tetrahydrophthalimide	Unknown concentration (incurred in apples)						
Trifluralin	—	10	10	67	27	27	67
Vinclozolin	133	—	133	20	20	53	53

Results and Discussion

The samples were mixed in order to increase the challenge and better demonstrate the capabilities of DSI/GC/MS–MS. The mixing of the samples diluted most pesticide concentrations by 3, compared with the concentrations of those in the collaborative study, and introduced more matrix coextractives from the 3 commodities that could interfere in the determination of individual analytes. A number of experiments conducted before analysis of the samples by GC/MS–MS and DSI/GC/MS–MS are discussed below.

Choice of GC/MS–MS Conditions

Previously published papers concerning GC/MS–MS with ion traps have reported conditions used for the determination of some of the same pesticides targeted in this study (10, 14–15). However, these conditions were not the most appropriate for dissociation of the pesticides with the instrument and the GC conditions used in this study. Furthermore, the personal preference of the analyst and the desire to start afresh led to experiments in which GC/MS–MS parameters for the different pesticides were independently chosen for this study.

The relevant considerations in developing GC/MS–MS parameters include retention time, ionization mode, mass spectrum, scan time, parent ion, ion isolation window, excitation storage level, excitation amplitude, resonant or nonresonant MS–MS ionization, and quantitation masses. Another parameter, ion time, varies depending on targeted automatic gain control (AGC), prescan time settings, and the ion concentration in the ion trap during a particular scan. The instrument default settings of 25 ms maximum ion time, 1.5 ms prescan time, and 10 000 counts AGC target were used in MS–MS experiments, and the AGC target was 20 000 counts in MS experiments. EI was used in all analyses, and the default setting of 3.0 m/z was used as the ion isolation window for all of the analytes in MS–MS.

To develop the GC/MS–MS method, the flow chart or decision tree presented in Figure 2 was followed. The initial step was to choose GC parameters. The final GC conditions chosen were the same as the initial conditions, which were the generic parameters selected for the AOAC INTERNATIONAL collaborative study. These conditions were not designed for maximum speed, as suggested in Figure 2, but they were satisfactory for the purposes of this study.

In MS detection, it is not necessary to completely separate the targeted analytes unless coeluting components have key mass spectral ions that also overlap. There are only a few pesticides that have the potential to both coelute in the 30 m capillary GC column and produce interfering mass spectra (e.g., metribuzin and vinclozolin, and endosulfan I and *cis—* chlordane). Unlike full-scan MS, in which 1 set of conditions can detect all analytes in a GC run, MS–MS with an ion-trap instrument is much like selected-ion monitoring (SIM), in which specific conditions are used for targeted analytes within time segments during a GC run. The GC/MS–MS instrument used in this study was capable of employing 5 different sets of conditions within a single segment, and time delays between segments were ca 1 s. To overcome this time delay and take into account room for typical peak widths in GC, reproducibility of retention times, and the need to establish a baseline for integration functions, analytes should be ca 0.2 min (12 s) apart for their detection in separate segments. Depending on peak width and scan rate, precision and sensitivity of the analysis may be compromised by using multiple sets of conditions in a single segment. However, for each analyte to have its own segment would undesirably extend the analysis time and would be impractical in multiclass, multiresidue analysis. Others have resorted to multiple injections in GC/MS (1), GC/MS(SIM) (3), and GC/MS–MS (15) to detect a large number of pesticides, but this is less efficient than detecting all pesticides in a single run. Thus, in this study, the optimal separation of the analytes was not sought. Conditions would have been altered to increase the resolution between GC peaks only if >5 analytes occurred in a single segment. The generic GC parameters were satisfactory in this case, and no changes were necessary (although 2 segments consisted of 4 analytes each). Despite that only a few data points were collected across some peaks, the quantitative results were satisfactory in this study.

Once retention times were determined in GC/MS, and the analytes were divided into segments, the next steps involved the determination of the optimal MS–MS conditions for each analyte, as outlined in Figure 2. A mid-level standard mixture of pesticides in acetone should be used to evaluate mass spectra because high concentrations may give somewhat different spectra in ion traps. For example, Sheridan and Meola (15) apparently used an excessive concentration to prepare MS–MS conditions for atrazine and diazinon. The reported parent ion at m/z 216 for atrazine or at m/z 305 for diazinon should not have been a significant MS peak except at high concentrations in the ion trap (10). It is very important to base the choice of the parent ion on the proper spectrum, and spectral search libraries can be very helpful in this task. It is also imperative that the chosen parent ion originate only from the targeted analyte; otherwise a false MS–MS spectrum will be optimized and entered as the reference spectrum in the quantitation file. At this time, there is no MS–MS spectral library for identification of the secondary spectrum as in the case of MS, and different conditions yield different spectra; thus, precautions had to be taken. Even so, an interfering mass at m/z 149 was mistaken as captan during method development in this study and explains why MS–MS results are not reported for captan.

The choice of parent ion is often simply the base peak for many pesticides, in many cases, there is only a single choice, such as the single major ion in the EI mass spectra for ethion, terbufos, d_{10}-anthrancene, d_{12}-chrysene, piperonyl butoxide, pendimethalin, and other pesticides. In other cases, such as diazinon, chlorpyrifos, metalaxyl, endosulfan sulfate, quintozene, and several others, multiple ions could adequately serve as the parent ion. The most intense parent ion generally
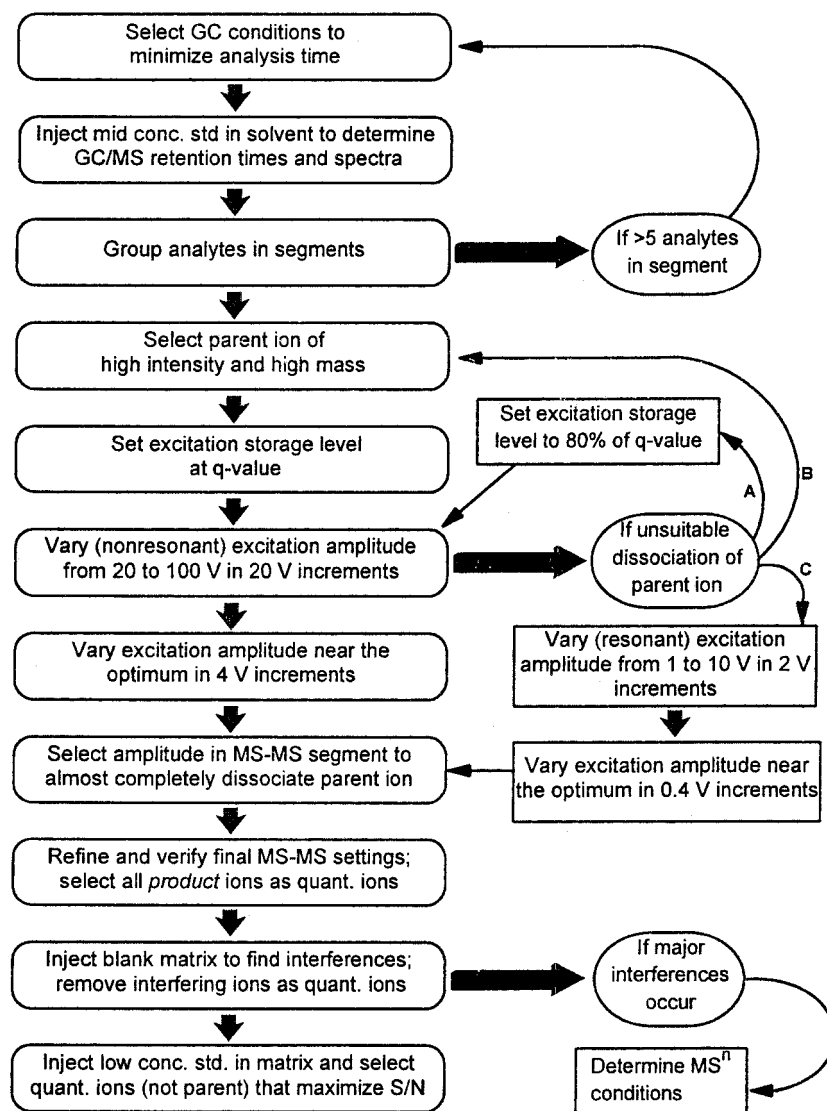
Figure 2. Flow chart outlining the process to determine desirable MS–MS parameters for the analytical method.

gives the largest MS–MS signal, but if the parent ion has a low mass, it may not yield many daughter ions for identification purposes or give the highest S/N ratio in a high background. Thus, it may be better to select a slightly less intense ion at a higher mass. For example, 304 m/z rather than the base peak of 179 m/z was chosen as the parent ion for diazinon in this study.

The next step in the process was to set the excitation storage level. This value, set in terms of m/z, relates to the applied voltage waveform that stores the ion(s) of interest in the ion trap (14). An excitation storage level setting of 40–44% of the parent ion m/z acts to position the parent ion in the center of the trap. This setting is known as the q-value. In cases in which the parent ion is >300, it is often necessary to choose a storage level of lower excitation because a high level often requires a high excitation amplitude to induce dissociation of the parent ion. The maximum excitation amplitude setting is 100 V; thus, a lower excitation storage level may be needed

when 100 V is not adequate to cause the parent ion to dissociate. The flow chart in Figure 2 shows this contingency.

The next step in the process involved determining the optimal excitation amplitude in the nonresonant ionization mode. A procedure known as automated methods development (AMD), which is provided in the instrument software, can help perform this task with only a few injections of the mid-level standard. Because of the generally narrow peak width in GC, only 5 excitation amplitude settings (20, 40, 60, 80, and 100 V) were used rather than the possible 10 that the instrument allows. Typically, a peak takes fewer than 10 scans to rise and fall; thus, some of the settings will miss the peak even if the shortest possible scan time is used. By using only 5 scans with 20 V intervals, the entire range of possible excitation amplitude settings can be evaluated in a single peak.

Ideally, the MS–MS spectrum should be optimized to maximize the signal of the highest mass daughter ion while

obtaining at least 2 daughter ions for confirmation purposes. In the author's opinion, an easy procedure to achieve an adequate MS–MS spectrum is the one that shows nearly complete dissociation of the parent ion (indicated by a small peak that aids in analyte identification and helps the analyst verify proper settings in subsequent analyses). An example of a desirable MS–MS spectrum for metalaxyl appears in Figure 3. When using AMD, the lowest excitation amplitude setting in which the spectrum did not contain the parent ion became the focus of the next injection. For example, if the parent ion still appeared at 60 V, but did not appear at 80 V, the next experimental conditions used would have been AMD settings of 62, 66, 70, 74, and 78 V. In some cases, the MS–MS spectrum at 1 of the initial AMD settings was ideal, and another injection in the AMD mode was not necessary.

Once the optimum excitation amplitude setting was determined, this value would be set in the MS–MS mode, or in the multiple-reaction monitoring (MRM) mode in the case of >1 analyte in the segment. The scan time was set to 1 s/scan (which was the average of 7 or 8 scans) in the MS–MS mode for a single analyte in a segment, or 0.5 s/scan (ca 3 scans/each data point) in MRM for 2 analytes, or 0.33 s/scan (ca 2 scans/data point) for 3 analytes, or at the fastest rate of 1 scan/data point (0.2–0.3 s/scan, depending on the scan ranges) for 4 or 5 compounds in a segment. To slightly increase the scan rate, the narrowest scan range (bound by 5 m/z < lowest quantitation ion and 5 amu > parent ion) was set within a segment to scan the product ions of interest. Further refinement of the amplitude setting was often necessary in the MS–MS or MRM mode to achieve the optimal setting. Generally, a 2–5 V higher excitation amplitude setting was needed to give the same secondary mass spectrum in the MS–MS or MRM mode as in AMD.

In some cases, the 100 V maximum excitation amplitude did not adequately dissociate the parent ion. In these cases, there were 3 options which entailed a choice of the following (in order of preference): (*1*) a lower excitation storage level; (*2*) a different parent ion; or (*3*) resonant ionization. The previous optimization steps using AMD were repeated in each option until the conditions for a suitable MS–MS spectrum were determined. Only d_{12}-chrysene required resonant ionization in this study, even though Schachterle and Feigel (14) found that nonresonant ionization satisfactorily induced the dissociation of d_{12}-chrysene. In resonant ionization, the initial AMD settings were set between 0 and 10 V in 2 V increments rather than between 0–100 V in 20 V increments as used in nonresonant ionization. Otherwise, the MS–MS optimization process was the same.

Once the final MS–MS and MRM settings were refined and verified, the final step in the process was to choose the
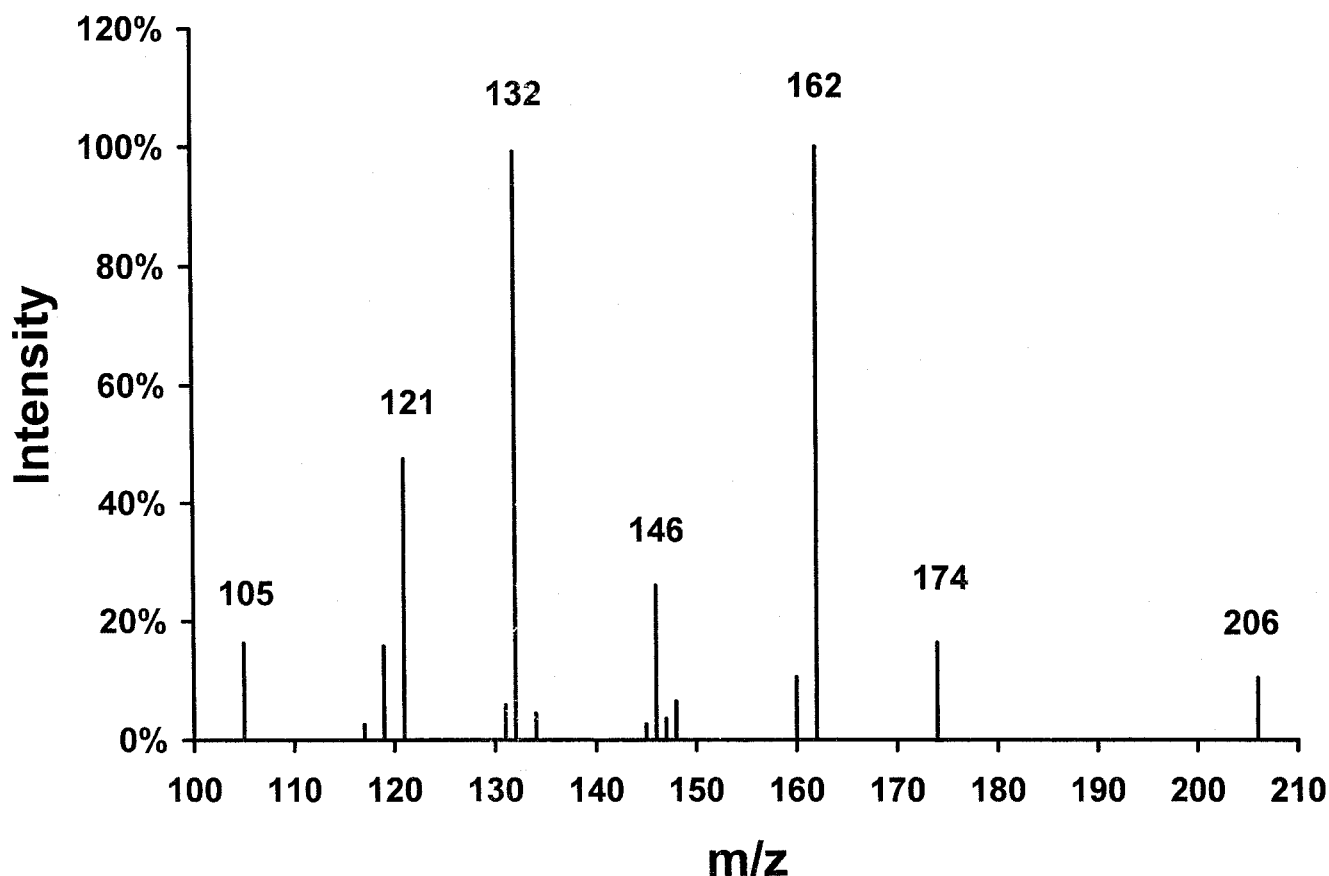
Figure 3.   Desirable MS–MS spectrum of metalaxyl with parent ion at m/z 206 (m/z 91 excitation storage level, 65 V excitation amplitude, and nonresonant ionization).

quantitation ions. Essentially all product ions in the MS–MS spectrum were initially chosen (but not the parent ion because of the higher possibility of an interference). Then a blank matrix extract was injected, and the mass spectrum was scrutinized for possible mass spectral interferences within the individual segments. Observed interfering ions were removed from consideration as quantitation ions (this only occurred in the cases of 121 and 122 m/z in carbofuran and 212 m/z in d_{12}-chrysene). Then, a low-level pesticide standard in matrix was injected, and quantitation masses were chosen that maximized the S/N of the analyte peaks. In nearly all cases, the ions initially selected were those ultimately used.

Table 1 presents the final MS–MS conditions used in the study. Despite the seemingly long decision tree in Figure 2 and optimization of several variables, the MS–MS optimization experiments took only a few days to determine the final conditions for the 25 compounds. With practice and experience, and with greater reliance on previous studies, MS–MS optimization times can be further reduced or eliminated. Furthermore, any number of settings will give high S/N values and reproducible spectra for confirmation of targeted analytes. However, MS–MS in this type of ion-trap approach cannot identify unknown compounds as in the case of full-scan MS or MS combined with other types of detection (13).

Reproducibility of Retention Times

Reproducible retention times are important in helping to identify an analyte in GC/MS and GC/MS–MS. Confirmation criteria in GC/MS generally set a retention-time window, based on the reference retention time, within which the analyte peak for a real sample must occur (21). Because of the larger injection volume and the manual-injection approach when the ChromatoProbe is used (the GC program is initiated by pressing a button), the reproducibility of the pesticide retention times was of greater concern than in automated approaches.

Table 4 provides the resulting average GC/MS–MS retention times and RSD values for each compound injected by using the different techniques. Electronic flow control was employed with He at a flow rate of 1.0 mL/min in each set of GC conditions. No significant difference in the reproducibilities was observed between automated split/splitless injection onto Carbofrit and manual DSI using the ChromatoProbe for 3 µL injections of SFE extracts in acetone. However, a slightly higher overall RSD resulted when DSI was used for 11 µL injections of acetonitrile extracts. This is understandable because of the larger volume, higher boiling solvent, dirtier extract, and higher initial column temperature used in the latter analysis.

Overall, retention times were very reproducible with each injection technique. For example, an RSD of 0.040% for a 15 min retention time corresponds to a standard deviation of only 360 ms, and similarly, an RSD of 0.034% corresponds to a standard deviation of 306 ms. This 54 ms difference be-

tween the reproducibility achieved with DSI/GC/MS–MS and that achieved with split/splitless injection with an autosampler has a minimal effect in broadening the retention-time window for confirmation. This high degree of reproducibility was attained despite the manual operation of the ChromatoProbe for LVI of mixed-commodity acetonitrile extracts that did not undergo cleanup. Furthermore, long-term retention time reproducibility is maintained in DSI because the front of the GC column does not become contaminated and need to be cut off periodically.

Effect of Injection Volume in DSI

An experiment was conducted to determine the effect of volume of the extracts in acetone under the DSI conditions used in this study. The DSI conditions were not studied in an effort to increase the injection volume, but instead the experiment was designed to determine empirically the largest injection volume that the chosen conditions could accommodate. Increasing the injector temperature, split vent ratio, and/or time allowed for solvent evaporation had the effect of permitting larger injection volumes than were used in this study.

Figure 4 shows the effect of increasing the injection volume from 3 to 15 µL (equivalent to an increase from 3.5 to 17.5 mg sample) in the DSI/GC/MS–MS determination of 0.39 ng/g quintozene in a mixed-commodity SFE acetone extract. A 25 µL injection of the extract was also attempted, but the solvent volume was too great and overloaded the capillary to the extent that no pesticides were detected. As Figure 4 shows, the signal increased as the injection volume increased, but the S/N ratio did not necessarily show a corresponding increase. Table 5 provides the S/N ratios for the pesticides that were detected at the low spiking levels in the experiment. For example, only small gains were made by increasing the injection volume from 8 to 15 µL in all reported cases except p,p'-DDE. The S/N ratio increased substantially, however, in most cases between the 3 and 8 µL injection volumes. These differences indicate that 15 µL began to saturate the capillary column, as evidenced by the higher baseline (Figure 4) and S/N ratios that were lower than expected. The baseline probably rose because of chromatographic band broadening, and not as a result of interferences.

This experiment demonstrates that increasing injection volume does not always increase S/N. Even in cases such as this when matrix interferences are not limiting, other factors may counteract the gains made by the injection of more analyte. In the case of the acetonitrile extracts, an injection volume of 11 µL (equivalent to 5.5 mg mixed commodity) was deemed satisfactory for DSI. The conditions were not optimized to increase this injection volume. An injection volume of as much as 35 µL is realistically possible with the 40 µL microvial in the ChromatoProbe, but a gentle, time-consuming evaporation of the solvent in the injection port would be necessary in that situation.

Table 4. Average retention times (t_r) and their reproducibility in GC/MS–MS, using split/splitless injection on Carbofrit and DSI of mixed-matrix extracts[a]

Analyte	Carbofrit (3 µL acetone)		ChromatoProbe (3 µL acetone)		ChromatoProbe (11 µL acetonitrile)	
	Av t_r, min	RSD, %	Av t_r, min	RSD, %	Av t_r, min	RSD, %
Tetrahydrophthalimide	11.174	0.038	11.321	0.040	10.492	0.072
Diphenylamine	12.694	0.034	12.815	0.038	11.986	0.033
Trifluralin	13.158	0.025	13.246	0.039	12.421	0.038
Carbofuran	13.967	0.042	14.079	0.023	13.257	0.043
Atrazine	14.029	0.056	14.183	0.065	13.369	0.079
Terbufos	14.306	0.043	14.399	0.036	13.576	0.029
Quintozene	14.392	0.024	14.506	0.020	13.681	0.049
Diazinon	14.507	0.031	14.590	0.045	13.769	0.043
d_{10}-anthracene	14.568	0.040	14.707	0.028	13.880	0.030
Chlorothalonil	14.890	0.039	15.042	0.042	14.223	0.043
Vinclozolin	15.495	0.037	15.617	0.025	14.795	0.035
Parathion-methyl	15.536	0.037	15.655	0.020	14.833	0.039
Carbaryl	15.660	0.037	15.802	0.032	14.983	0.033
Metalaxyl	15.761	0.036	15.858	0.027	15.041	0.043
Chlorpyrifos	16.444	0.038	16.550	0.020	15.731	0.039
Dacthal	16.558	0.022	16.668	0.031	15.849	0.041
Pendimethalin	17.072	0.034	17.180	0.021	16.361	0.034
p,p'-DDE	18.239	0.033	18.358	0.036	17.540	0.032
Ethion	19.059	0.033	19.167	0.019	NA[b]	NA
Endosulfan sulfate	19.727	0.034	19.862	0.029	19.044	0.034
Propargite	19.958	0.039	20.069	0.044	19.257	0.040
Piperonyl butoxide	20.016	0.024	20.125	0.025	19.308	0.030
Bifenthrin	20.544	0.027	20.648	0.019	19.829	0.029
d_{12}-Chrysene	20.697	0.022	20.867	0.022	20.046	0.031
Azinphos-methyl	21.363	0.015	21.517	0.021	20.695	0.030
Overall av. RSD		0.034		0.031		0.040

[a] n = 17.
[b] NA = not applicable.

Matrix Effects and Calibration

Matrix effects in the GC determination of pesticides have been the subject of several studies (7, 17, 18, 22). The major source of the effects involves active sites in the glass injector liners (17, 22). Pesticides that contain amide and phosphate groups often strongly interact with hydroxyl groups on the glass surfaces. When only pesticides at low concentrations are injected, the active sites are filled by pesticides, and this interaction decreases the efficiency of analyte introduction into the GC column. In the presence of matrix, the active sites in the glass are filled by matrix components, and this interaction increases the efficiency of the analyte introduction.

As expected, the effect of matrix enhancement of the signal was observed for several of the analytes in this study, when both Carbofrit and DSI were used. The split/splitless injection approach on Carbofrit showed a greater effect of matrix enhancement than did DSI with the ChromatoProbe. This result indicated that Carbofrit has many active sites, further evidenced by the need to increase the final injection temperature to 325°C, which was needed to release certain analytes from this material (most notably d_{12}-chrysene). Carbofrit has been advertised as an alternative insert packing material that can replace glass wool in GC injection methods; Sheridan and Meola (15) used Carbofrit in their GC/MS–MS studies to enable 10 µL injections.

In DSI, the glass microvial also has active sites, but apparently they are fewer than, or not as strongly interactive, those in Carbofrit. For example, Figure 5 shows the differences in the calibration plots prepared for carbofuran by 3 µL injections in the split/splitless mode onto Carbofrit and by 3 µL DSI, with calibration standards in both blank matrix ex-
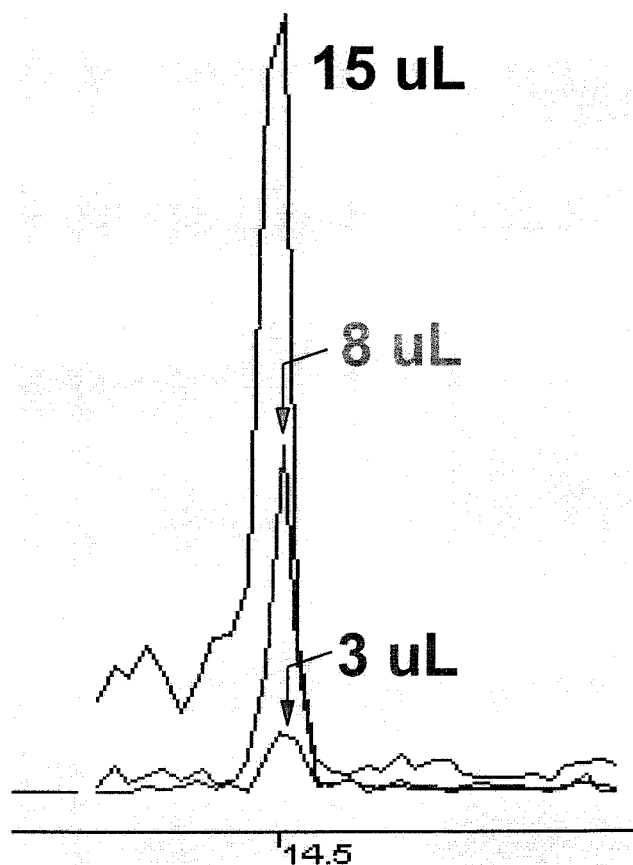
Figure 4. Effect of increasing injection volume in the DSI/GC/MS–MS analysis of an SFE acetone extract spiked with quintozene at 0.39 ng/g.

tract and acetone. The decreased sensitivity of the calibration curves in both cases involving the standards in acetone indicates losses of carbofuran during the injections; however, the effects were worse on Carbofrit than in DSI.

Other analytes that gave quadratic calibration curves in acetone (injections onto Carbofrit) were chlorothalonil, para-thion-methyl, carbaryl, metalaxyl, and bifenthrin. In the case of DSI, chlorothalonil, parathion-methyl, carbaryl, and azin-phos-methyl gave quadratic relationships. Quadratic calibration curves have been obtained before in GC/MS with ion-trap instruments (7), and it was thought that because of AGC functions, differing scan times within segments, and changes in the ion time, the ion trap may have been the source of the complication; however, careful review of the data did not indicate a correlation between these factors and the linear or quadratic plots. For example, carbofuran gave a quadratic curve in acetone and showed sizable matrix enhancement, but its partner in the same MS–MS segment, atrazine, exhibited no effects. Each concentration of the atrazine and carbofuran standards in acetone permitted the maximum ion time of 25 ms in their detection. Similar situations occurred with vinclozolin (linear in acetone) and its segment partners, parathion-methyl, metalaxyl, and carbaryl (quadratic in acetone). Because the ion trap may have been partially responsible for the quadratic results, an autotune of the instrument was per-

formed before every set as a precaution, but the more likely cause was the active sites in the Carbofrit and glass surfaces. Previous studies obtained linear correlation coefficients of ≥ 0.995 for determination of pesticides by GC/MS–MS (10, 14). In this study, all matrix-matched standards in either injection approach (Carbofrit or DSI) yielded linear calibration plots with correlation coefficients of ≥ 0.99.

Another factor that was considered in DSI was the day-to-day variability of results. Because the ChromatoProbe was not automated, it was necessary to continue a set of injections ≈ 16 h later on the following day. Figure 6 shows the day-to-day variability in the calibration results for diazinon in the DSI/GC/MS–MS approach for 11 μL injections of acetonitrile extracts. The correlation coefficient of the combined calibration curve was 0.993. Of course, automated injections would be preferable because they would increase sample throughput by extending the working day, and this would perhaps lead to improved precision.

The mixed commodities used in this study contained both fortified and incurred pesticides. For fortified pesticides, calibration curves generated from matrix-matched standards and forced through zero were used for quantitation. However, matrix blanks were not available in the determination of incurred pesticides. The need for matrix blanks can be a drawback in the use of matrix-matched standards, but a possible way to overcome this problem is to use the method of standard additions.

Figure 7 shows the use of the method of standard additions in the Carbofrit GC/MS–MS determination of tetrahydro-phthalimide in the mixed commodity after SFE. In this case, there was no significant difference between the average analyte concentrations in the test samples, whether calculated by the method of standard additions or by calibration with standards in acetone. The reproducibility (RSD) of the tetrahydrophthalimide signals in the blank matrix and test samples was only 9.9% ($n = 8$), and the calibration curves were both linear and had similar slopes. This was an ideal situation, but unfortunately a few of the other pesticides had less reproducible signals, quadratic responses in acetone, and/or much different sensitivities. The response of diphenylamine and propargite in the SFE extracts were highly variable. The variability of this baseline in subsequent analyte additions to other blank extracts led to poor calibration curves for these pesticides. Although diphenylamine and propargite in the SFE extracts were confirmed by GC/MS(-MS), the high variability of the results made quantitation imprecise. Compared with the SFE extracts, the acetonitrile extracts gave a much improved precision for diphenylamine and propargite.

Analytical Results

The analytical results from the study were divided into 2 categories: (*1*) incurred and QC pesticides at the same concentration in the blank and test samples; and (*2*) pesticides at different concentrations for fortification of the test samples.

Table 5. Effect of increasing injection volume in DSI/GC/MS–MS on the S/N of analytes from mixed-commodity SFE acetone extracts spiked at low levels

Pesticide	Spiking level, ng/g	S/N ratio		
		3 μL	8 μL	15 μL
Trifluralin	0.20	66	126	147
Carbofuran	0.98	4	6	19
Quintozene	0.39	8	39	41
Metalaxyl	0.49	7	10	14
Chlorpyrifos	1.0	105	296	63
Dacthal	0.39	12	35	47
p,p'-DDE	0.29	3	10	22

Table 6 provides the results from analyses using the method of standard additions for the incurred pesticides and QC spikes. The expected concentration of the spikes was 200 ng/g for all pesticides except terbufos, which was expected to be 263 ng/g in the SFE extracts. The recoveries of the QC spikes ranged from 80 to 117%, depending on the extraction, injection, and analytical method used. The reproducibilities (RSDs), which ranged from 3 to15 %, in the analyses of the QC spikes, demonstrated that no severe problems occurred during the sample preparation and analytical steps.

Agreement between the SFE and acetonitrile extracts was satisfactory for the QC spikes, azinphos-methyl, carbaryl, and chlorothalonil. The lack of consistency in the diphenylamine and propargite results was not unexpected (the acetonitrile extraction was likely to give the more accurate results). However, the differing results for tetrahydrophthalimide with the different methods was surprising; it is unknown why the same extracts could give results ranging from 134 to 222 ng/g with the different analytical methods. The calibration curves for the different approaches were satisfactory (e.g., Figure 7), and no interferences were observed in the chromatograms or spectra. The lack of a true blank was a possible source of the problem in this circumstance, or perhaps the variable results related to the possible continuing degradation of captan over time. Although captan was not detected by GC/MS, nor correctly targeted in GC/MS–MS, it may have been present at concentrations below its LOD in GC/MS. Approximately 2 g tetrahydrophthalimide is produced from 1 g captan, assuming 100% conversion.

In the case the pesticides used for fortification, Table 7 provides a comparison between the SFE + Carbofrit + GC/MS results and the acetonitrile extraction + DSI/GC/MS–MS
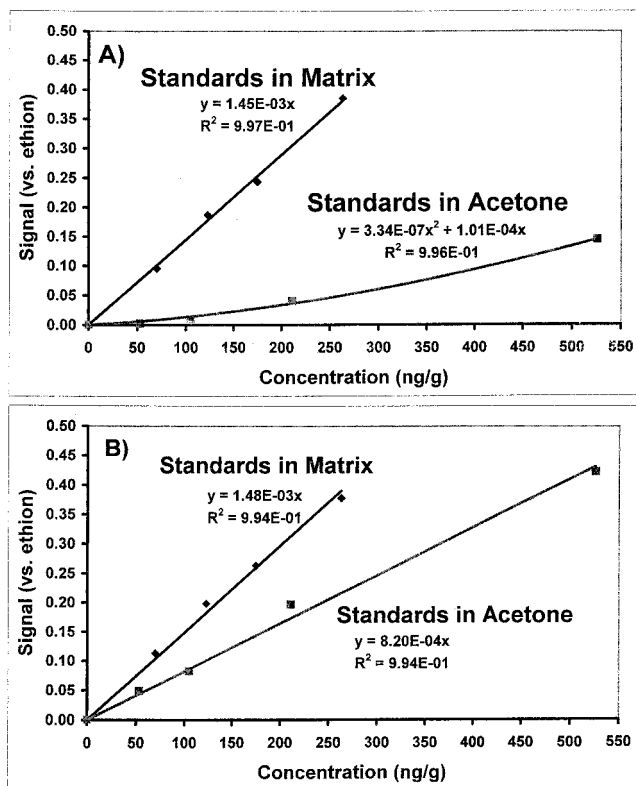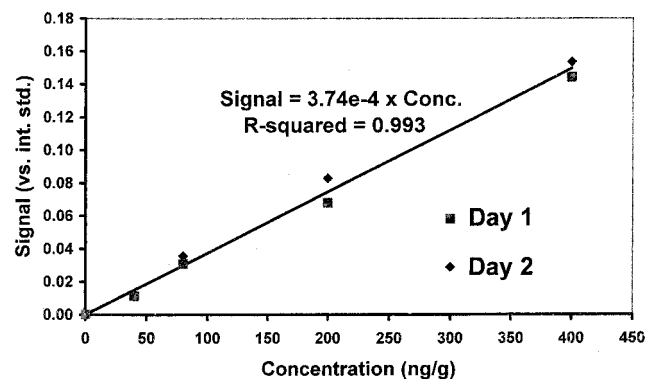
Figure 5. GC/MS–MS calibration plots for carbofuran in acetone and in SFE acetone extracts, prepared by using (A) 3 μL split/splitless injection on Carbofrit and (B) 3 μL DSI.

Figure 6. DSI/GC/MS–MS calibration plot for diazinon in the mixed commodity extracted with acetonitrile (11 μL injection volume), showing the day-to-day variability in the determination.
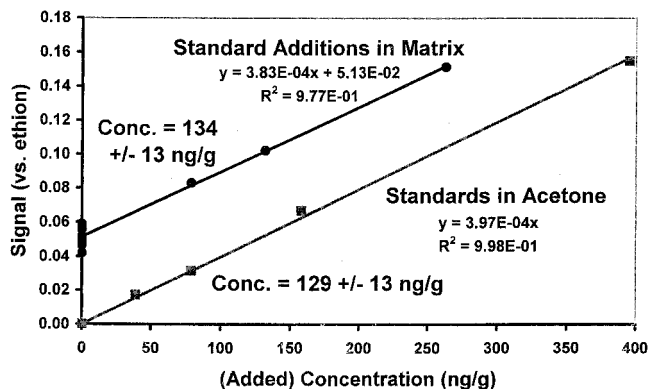
Figure 7. Determination of tetrahydrophthalimide (captan metabolite) by the method of standard additions and by the use of calibration standards in acetone.

results for the 2 sets of test samples. Table 8 provides the overall results, which also incorporate a mid-level QC spike in the average recoveries and RSD values. The recoveries and RSD values were very good with an overall average recovery of $88 \pm 10\%$ and an overall RSD of $12 \pm 8\%$ for the 13 pesticides in the SFE extracts, and an overall average recovery of $103 \pm 7\%$ recovery and an overall RSD of $14 \pm 5\%$ for the acetonitrile extracts. Slightly lower recoveries and higher RSD values were expected for the pesticides in the SFE extracts because of the recoveries of <100% typically associated with the selective extraction process and the smaller size of the subsamples taken for extraction. Indeed, the recoveries were lower in SFE for the most nonpolar pesticides, trifluralin, p,p'-DDE, and bifenthrin, but the overall precision was about the same for the different approaches. Only a few pes-

ticides had an RSD of >20% (atrazine in DSI/GC/MS–MS after acetonitrile extraction and metalaxyl and endosulfan sulfate in GC/MS after SFE); the individual results for these pesticides are shown in Table 7.

Also, the average results for the different pesticides in the individual test samples appear at the bottom of Table 7. The compilation of the recoveries in this manner provides a simple way to look for discrepancies in the results among the test samples. As the table shows, none of the overall average recoveries for the SFE test samples were much different from each other, but in the case of test sample 7 in DSI/GC/MS–MS, the 121% overall average recovery was consistently higher than the others (as evidenced by a similar overall average RSD). Although the difference in recovery did not meet the conditions for rejection of the value in the Q-test, it is likely that a systemic error occurred that acted to increase the calculated recoveries for test sample 7 and led to the higher variability for the DSI/GC/MS–MS approach (Table 8).

As shown in Table 7, there were 4 instances when the automated quantitation software associated with the GC/MS method gave false negatives (p,p'-DDE and endosulfan sulfate in test sample 4, bifenthrin in test sample 5, and diazinon in test sample 7). Furthermore, 2 cases of false positives occurred (quintozene in test sample 2 and endosulfan sulfate in test sample 3). All of these cases were found upon review of the GC/MS results, but they were included as false positives and negatives in this study to demonstrate the greater difficulty involved in the GC/MS analysis when compared with the GC/MS–MS analysis. The automated software presented no false positives or false negatives in GC/MS–MS independent of the different extraction and injection approaches. Furthermore, the GC/MS approach required many re-

Table 6. Average results (ng/g)[a] for QC spikes and incurred pesticides in mixed commodities analyzed by the method of standard additions

| | SFE acetone extracts | | | |
| | Carbofrit | | | |
Pesticide	GC/MS	GC/MS–MS	DSI/ GC/MS–MS	Acetonitrile extracts, DSI/GC/MS–MS
Terbufos[b]	224 ± 20	251 ± 16	283 ± 27	234 ± 35
Pendimethalin[c]	174 ± 12	178 ± 11	214 ± 13	206 ± 25
Piperonyl butoxide[c]	160 ± 7	225 ± 32	190 ± 5	229 ± 18
Azinphos-methyl	25 ± 6	37 ± 17	36 ± 9	37 ± 16
Carbaryl	135 ± 19	128 ± 22	186 ± 20	152 ± 23
Chlorothalonil	17 ± 2	19 ± 2	22 ± 7	30 ± 4
Diphenylamine	195 ± 95	179 ± 88	291 ± 66	99 ± 25
Propargite	94 ± 77	137 ± 133	28 ± 16	40 ± 3
Tetrahydrophthalimide	202 ± 26	134 ± 13	222 ± 6	131 ± 43

[a] Each value ± standard deviation.
[b] Terbufos was added just before extraction (263 ng/g in SFE, 200 ng/g in acetonitrile extraction).
[c] Pendimethalin and piperonyl butoxide (200 ng/g) were added before the homogenization steps.

Table 7. Recoveries (%) of pesticides from fortified test samples[a] analyzed by DSI/GC/MS–MS (acetonitrile extracts) and Carbofrit GC/MS (SFE extracts)

| Pesticide | Mixed test sample | | | | | | | Method |
	1	2	3	4	5	6	7	
Atrazine	115	90	—	97	86	82	94	GC/MS (SFE)
	82	119	—	60	96	93	141	DSI/GC/MS–MS
Bifenthrin	106	—	79	81	neg.[b]	59	78	GC/MS (SFE)
	101	—	108	90	98	98	126	DSI/GC/MS–MS
Carbofuran	—	102	76	90	83	105	90	GC/MS (SFE)
	—	120	114	93	102	100	134	DSI/GC/MS–MS
Chlorpyrifos	92	105	98	98	98	95	65	GC/MS (SFE)
	110	118	139	97	121	112	95	DSI/GC/MS–MS
Dacthal	83	90	—	90	86	86	99	GC/MS (SFE)
	101	101	—	86	92	83	123	DSI/GC/MS–MS
Diazinon	91	106	90	105	—	84	neg.	GC/MS (SFE)
	81	108	110	89	—	74	108	DSI/GC/MS–MS
Endosulfan sulfate	88	102	pos.[c]	neg.	96	134	146	GC/MS (SFE)
	67	97	—	79	82	103	125	DSI/GC/MS–MS
Metalaxyl	104	80	127	84	72	98	—	GC/MS (SFE)
	101	116	100	84	113	88	—	DSI/GC/MS–MS
p,p′-DDE	88	72	75	neg.	—	68	70	GC/MS (SFE)
	106	111	106	113	—	89	126	DSI/GC/MS–MS
Parathion-methyl	103	76	92	94	—	76	83	GC/MS (SFE)
	96	101	102	77	—	84	93	DSI/GC/MS–MS
Quintozene	79	pos.	79	90	68	79	82	GC/MS (SFE)
	116	—	113	92	98	114	124	DSI/GC/MS–MS
Trifluralin	—	74	67	68	68	65	61	GC/MS (SFE)
	—	91	126	84	97	101	131	DSI/GC/MS–MS
Vinclozolin	98	—	93	129	91	109	85	GC/MS (SFE)
	107	—	109	115	95	98	129	DSI/GC/MS–MS
Av. ± SD[d]	95 ± 11	90 ± 14	88 ± 17	93 ± 14	83 ± 11	88 ± 20	87 ± 23	GC/MS (SFE)
	97 ± 15	108 ± 10	113 ± 12	89 ± 14	99 ± 11	95 ± 12	121 ± 15	DSI/GC/MS–MS

[a] Refer to Table 3 for expected concentrations.
[b] neg. = false negative obtained by using automated quantitation software.
[c] pos. = false positive obtained by using automated quantitation software.
[d] SD = standard deviation.

integrations of the data, using cumbersome manual integration procedures in the software. Because of the consistent GC retention times, lack of background noise in the chromatograms, and clarity in the MS–MS analyte confirmation, analyses using GC/MS–MS needed few re-integrations; this advantage was a great time saver and simplified the analytical process.

Figure 8 shows an example of the advantages of GC/MS–MS over GC/MS in the detection of bifenthrin at 30 ng/g in test sample 5 (the lowest bifenthrin concentration used in the SFE and GC/MS collaborative study involving individual commodities was 90 ng/g). The upper chromatogram trace and mass spectrum is from the SFE + Carbofrit + GC/MS

analysis of the mixed extracts, and the lower trace and spectrum is from the GC/MS–MS analysis of the same extract. The GC/MS–MS analytical method was able to clearly identify the presence of the trace amount of bifenthrin in the complex extract, whereas the GC/MS method would have led the analyst to suspect the presence of bifenthrin, but confirmation could not be made without further information.

Limits of Detection

Table 9 provides the LODs calculated from the software-reported S/N ratios for the pesticide calibration standards in sample extracts or acetone solutions. The more highly con-

Table 8. Overall recoveries and relative standard deviations of the pesticides in the fortified test samples analyzed by the different methods

Pesticide	SFE acetone extracts Carbofrit, 3 μL (3.5 mg)				Acetonitrile extracts, DSI/GC/MS–MS 11 μL (5.5 mg)	
	GC/MS		GC/MS–MS			
	Recovery, %	RSD, %	Recovery, %	RSD, %	Recovery, %	RSD, %
Atrazine	92	12	100	9	98	26
Bifenthrin	80	18	82	19	103	11
Carbofuran	90	10	94	10	110	12
Chlorpyrifos	91	14	101	15	113	12
Dacthal	90	6	91	6	98	13
p,p'-DDE	72	10	78	15	109	10
Diazinon	94	9	91	9	95	15
Endosulfan sulfate	105	26	100	12	92	20
Metalaxyl	90	21	86	15	100	12
Parathion-methyl	87	11	85	16	92	10
Quintozene	80	8	82	18	110	10
Trifluralin	67	6	70	7	105	16
Vinclozolin	100	14	96	8	109	10
Overall av. rec. ± SD	88 ± 11	13 ± 6	89 ± 9	12 ± 4	103 ± 7	14 ± 5

centrated pesticides incurred or added as QC spikes were left out of the table because of the great differences between their concentrations in the samples and the calculated LODs. The LODs were not empirically verified by injecting low-level standards, although an experiment entailing injection of low-level pesticides to determine the effect of injection volume was conducted as discussed previously. The 3 μL injections,

which gave the S/N values reported in Table 5, were included in the LOD calculations corresponding to SFE + DSI/GC/MS–MS. In these cases, except for metalaxyl, the calculated LOD generally agreed with the injected LOD. The simple procedure used here was designed to estimate and compare the LODs (assuming 100% recovery) of the pesticides determined by the different methods in the matrix extracts and
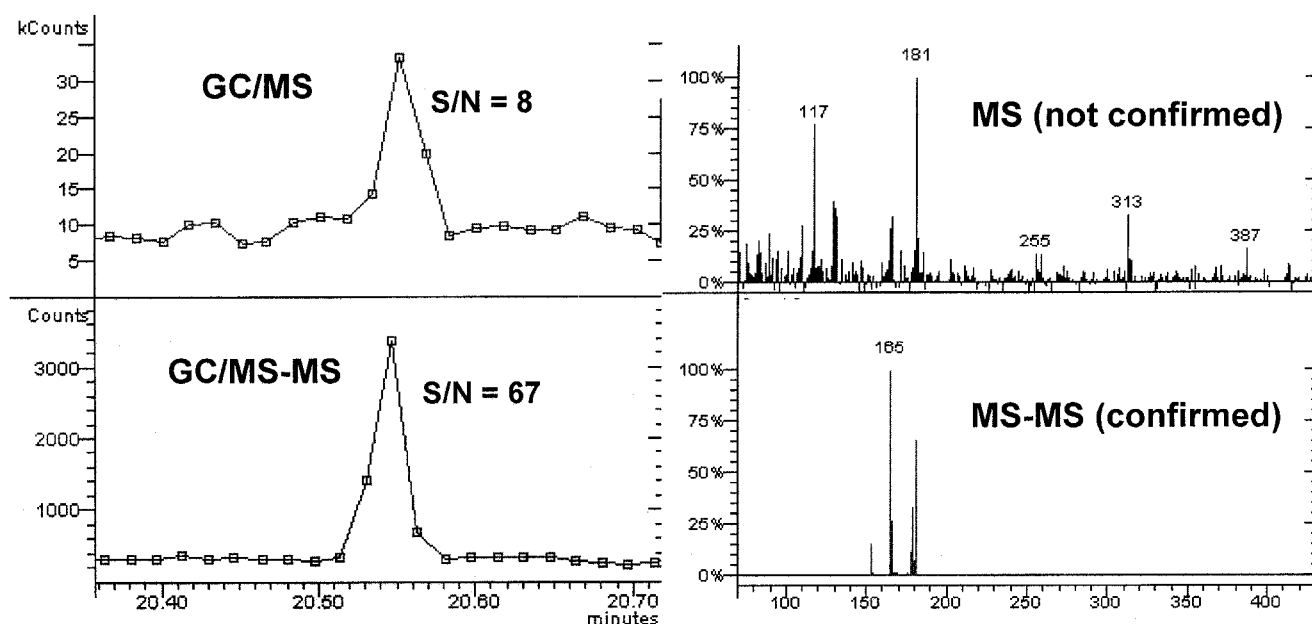

Figure 8. Comparison of GC/MS (after SFE and injection onto Carbofrit) and DSI/GC/MS–MS (after extraction with acetonitrile) in the detection and confirmation of bifenthrin at 30 ng/g in mixed test sample 5.

Table 9. Comparison of the LODs (ng/g) calculated from S/N ratios for the matrix-matched and solvent-based calibration standards by using the different methods

	SFE acetone extracts						DSI/GC/ MS–MS 11 µL (5.5 mg)
	Carbofrit, 3 µL (3.5 mg)				DSI/GC/MS–MS 3 µL (3.5 mg)		
	GC/MS		GC/MS–MS				
Pesticide	Acetone (*n* = 5)	Matrix (*n* = 4)	Acetone (*n* = 5)	Matrix (*n* = 4)	Acetone (*n* = 5)	Matrix (*n* = 4)	Matrix (*n* = 8)
Trifluralin	0.3	0.005	0.07	0.03	0.03	0.02	0.03
Carbofuran	14	4	2	2	2	2	2
Atrazine	2	4	0.3	0.6	2	2	2
Quintozene	2	3	0.2	0.1	1	2	2
Diazinon	1	1	0.2	0.1	0.2	0.07	0.5
Chlorothalonil	5	4	1	0.7	3	0.9	0.6
Vinclozolin	2	4	0.1	0.06	0.6	0.8	0.8
Parathion-methyl	5	3	0.8	0.09	1	1	0.6
Metalaxyl	4	13	1	2	2	4	2
Chlorpyrifos	4	8	0.1	0.07	0.04	0.2	0.3
Dacthal	3	3	0.1	0.2	0.1	0.2	0.2
p,p′-DDE	1	4	0.2	0.4	0.3	0.4	0.2
Endosulfan sulfate	3	33	1	2	4	2	1
Propargite	34	Variable	12	Variable	40	Variable	27
Bifenthrin	2	8	0.8	0.9	2	2	0.9
Azinphos-methyl	Variable	Variable	82	3	55	36	21

acetone. However, the estimation appeared to provide reasonably accurate and precise results in most cases, especially in the few instances when the reproducibilities of the LODs were exceptional (RSD of <10%). Higher RSD values were more typical, however. For example, the average RSD of the LODs for the 22 pesticides determined by DSI/GC/MS–MS of the acetonitrile extracts (*n* = 8) was 35 ± 17%. This is actually very good, considering that an RSD of 35% for an LOD of 1 ng/g means that a result in a range of 0.65–1.35 ng/g falls within the standard deviation. However, as shown in Table 9, a couple of the incurred pesticides in the SFE methods gave such variable results for the calculated LOD that no value was listed. Also, a few values were oddly different from the other values in the series, such as those for the GC/MS determination of trifluralin in matrix, but when the calculations were double checked, the values were found to be correct.

For some pesticides, such as carbofuran, metalaxyl, and bifenthrin, seemingly modest gains were made by using GC/MS–MS rather than GC/MS for analysis, but as Figure 8 and Table 5 demonstrate, the ability to better confirm the presence of an analyte at low levels in a complex matrix extends beyond the numbers reported in Table 9. For most pesticides, however, the LODs in GC/MS–MS were 5–20 times lower than those in GC/MS with the same instrument. The effects of matrix enhancement can be observed for some of the

pesticides by comparing the LODs in matrix vs acetone. Furthermore, if the LODs in the different solutions were similiar, the absence of interferences was indicated. This is exceptional, considering that no cleanup of the mixed extracts was used in the DSI approach. However, the higher LODs for atrazine and quintozene in DSI/GC/MS–MS, compared with those for those pesticides in Carbofrit + GC/MS–MS, may have been due to the coelution of an interfering compound.

Advantages of DSI/GC/MS–MS

No cleanup or solvent evaporation steps.—Cleanup may be useful in removing unwanted matrix components, but it often leads to small losses of analytes as well. Besides the time, costs, and labor associated with cleanup, each additional step introduces its own recovery factor that cannot exceed 100%. The additional steps also lead to additional sources of error and the potential for introducing contaminants. Cleanup also frequently entails the need for several specialized pieces of glassware, which must be cleaned and stored.

Multiclass, multiresidue methods for pesticides often involve trade-offs and compromises between achieving high recoveries for a wide range of analytes and improving the selectivity of the method. By using a carbon-based SPE

cleanup procedure, Fillion et al. (3) obtained lower recoveries of hexachlorobenzene and a few other pesticides. DSI/GC/MS–MS uses the same initial extraction procedure as that method, but it eliminates the cleanup step in which the losses occur. Thus, the DSI/GC/MS–MS approach should give high recoveries of those pesticides. The commonly problematic pesticides, methamidophos and acephate, were not tested with this DSI/GC/MS–MS approach, but they are expected to remain problematic as they are in other GC/MS(-MS) approaches (15).

An inconvenience associated with traditional liquid-based methods is the need to concentrate sample extracts by evaporating the solvents. In many methods, the most time is spent during the evaporation steps. Also, large amounts of organic solvents released into the atmosphere are not beneficial to the environment, and safety hazards may result if the solvents are released into the laboratory. Collection of the evaporated solvent through condensation is the environmentally and occupationally safer alternative, but this leads to higher waste disposal costs. In DSI, LVI can be used in which the evaporation of a small amount of solvent (no more than what is needed for analysis) occurs during the injection process. In this study, an 11 µL injection of dilute extract in DSI introduced more sample (5.5 mg) than did 3 µL (3.5 mg) of the the more concentrated SFE extract. The large volume aspects of DSI were not optimized in this study, but this feature may be better used in the future.

Rapid, inexpensive, and simple procedure.—Only 20 min was needed to extract a batch of 6 samples when the DSI/GC/MS–MS method was used, and analysis took 25 min/sample. In routine applications, a single analyst could prepare, inject, and analyze a sample in 30 min and have the next sample ready for injection by the end of the previous run. In an 8 h day, an individual could analyze 16 samples in this manner with one instrument (including calibration standards). The chromatographic conditions in this study were not optimized for speed, and future studies will attempt to decrease the analysis time. Furthermore, automation of the ChromatoProbe would increase sample throughput by extending the length of a workday.

In the case of the 30 g sample, the cost of materials for the method was $1.62 (60 mL acetonitrile, $1.01; 6 g NaCl, $0.15; 1 g MgSO$_4$, $0.06; and DSI microvial, $0.40). The use of a smaller sample size and the elimination of the NaCl, centrifugation, and MgSO$_4$ steps were not investigated, and it may be possible to further reduce costs and increase speed after future investigations are conducted. Figure 9 is a material cost comparison of the Canadian Pesticide Management Regulatory Agency (PMRA) method (3) with the DSI approach used in this study. The SPE cleanup steps consume ≈75% of the material costs in the Canadian method and ≈40% of the time (the 2 evaporation steps require >50% of the total time). The savings in material costs, although substantial, are insignificant when compared with the savings of DSI/GC/MS–MS over many traditional methods in terms of labor, time, space, and other factors. The prices of GC/MS–MS iontrap instruments are comparable to those of some GC/MS
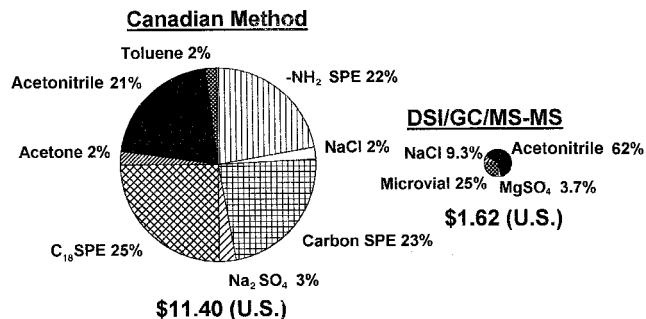

Figure 9. Comparison of material costs of the Canadian PMRA method and the DSI/GC/MS–MS methods which use the same initial extraction approach; however, the Canadian method includes SPE cleanup.

quadrupole instruments, and the ChromatoProbe itself is rather inexpensive, costing only ≈1/5 of the price of the autosampler used in this study.

Ruggedness.—The ruggedness of the DSI/GC/MS–MS approach was not sufficiently determined in the analyses of the few samples in this study. However, in theory and in long-term practice by others (A. Amirav, Tel Aviv University, personal communication, 1999), the DSI approach does not introduce nonvolatile contaminants into the gas chromatograph and, thus, requires less maintenance. In this study, a film of matrix components was observed on the microvials after the injection of dirty samples. In other approaches, these components would remain on the liner surface and/or make their way into the GC column. The common appearance of ghost peaks in chromatograms is often attributable to nonvolatile components that slowly migrate through the capillary column. In GC/MS, these components coat the surfaces in the MS source and degrade its performance.

Sensitive, quantitative, and confirmatory analysis.—The low LODs, acceptable quantitative aspects, and excellent confirmation abilities of DSI/GC/MS–MS were demonstrated in this study involving a variety of pesticides in nonfatty foods. Identification of targeted analytes is better in MS–MS than in MS alone. In the case of analytes such as ethion, terbufos, piperonyl butoxide, methoxychlor, diphenylamine, and many others that give only single ions or clusters in their MS spectra, typical confirmation criteria requiring 3 ions (21) are not met with the use of MS alone. Furthermore, the combination of retention times, parent ion, and MS–MS parameters, to give the resulting spectrum, and the ions produced in the MS–MS spectrum provide a high level of selectivity that verifies the identity of a targeted analyte.

Conclusions

DSI is a valuable approach to GC sample introduction that leads to advantageous analytical methods. The traditional liquid-based extraction methods have long been known to achieve high recoveries for a wide range of analytes, but the

extracts have had to undergo cleanup steps before GC analysis using traditional injection methods. SFE could be considered the next evolutionary step in that it provides a means to avoid the post-extraction cleanup step, but because of the greater selectivity of the extraction process, recoveries of the most polar and nonpolar pesticides are compromised. With the development and commercialization of GC/MS–MS, a very selective, sensitive, and universal detection method, one of the reasons for performing cleanup of dirty extracts, to avoid detection interferences, could be eliminated. However, the lack of ruggedness remained a problem in the case of dirty extracts because they contaminated the GC system with matrix components. With the advent of DSI and its combination with GC/MS–MS analysis, it becomes possible to use an exhaustive extraction approach without cleanup and still achieve high quality results while *increasing* the ruggedness of the system. By addressing the determination of pesticides as an example, this paper has demonstrated the feasibility of the DSI/GC/MS–MS approach and has shown it to be quantitative, confirmatory, sensitive, rugged, simple, inexpensive, rapid, and applicable to a wide range of GC amenable analytes. A limitation of the current GC/MS–MS approach relates to the detection of targeted analytes only; analysis for a large number of compounds in a single injection is difficult. Also, the DSI approach is not automated at this time.

Acknowledgments

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