RESIDUES AND TRACE ELEMENTS

Development of a Method of Analysis for 46 Pesticides in Fruits and Vegetables by Supercritical Fluid Extraction and Gas Chromatography/Ion Trap Mass Spectrometry

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A multiresidue method using supercritical fluid extraction (SFE) and gas chromatography/ion trap mass spectrometry (GC/ITMS) was developed for analysis of 46 pesticides in fruits and vegetables. The SFE procedure used 2 commercial instruments that trapped the extracts on solid-phase material. Silica gel chemically bound to octadecylsilane (ODS) collected the extracted pesticides efficiently, and elution of the trap with acetonitrile gave high recoveries. Extracts thus obtained were sufficiently clean for subsequent GC/ITMS analysis. The SFE conditions were 320 atm and 60°C (0.85 g/mL CO_2 density) and 1.6 mL/min CO_2 flow rate for 6 extraction vessel volumes. Trapping on 1 mL ODS occurred at 10°C, and a 0.4 mL/min flow rate of acetonitrile at 40°–50°C was used to elute the pesticides. Quantitative and qualitative analyses of the 46 pesticides were performed simultaneously by GC/ITMS. Studies of fortified samples gave >80% recoveries for 39 pesticides, and recoveries of >50% for the other pesticides, except methamidophos and omethoate. Grapes, carrots, potatoes, and broccoli were used as samples during method development, and a blind experiment involving incurred and fortified samples was used to test the approach. Results of the blind study compared satisfactorily with results from 7 laboratories using traditional GC detectors and solvent-based extractions.

Currently, analyses of pesticides in food are commonly performed with organic solvent extraction methods (1–3), which can be expensive, time consuming, and labor intensive and require much space and glassware as well as generate a large amount of hazardous waste. The Environmental Protection Agency has directed government agencies to reduce consumption of solvents, especially chlorinated solvents, in laboratories (4). The most commonly used multiresidue method for analysis of pesticides in fruits and vegetables uses 800 mL organic solvent (including 300 mL methylene chloride) per 100 g sample (1). Furthermore, because of large sample size, nonselective extraction conditions, and concentration of matrix interferants and organic solvent impurities, organic solvent extracts require extensive cleanup before analysis. The progress in chromatographic separation and detection of pesticides was not accompanied by an adequate improvement in sample preparation techniques (5). With technological advances in extraction methods and instrumentation, and heightened awareness of environmental and fiscal responsibilities, more efficient multiresidue methods for analysis of pesticides in produce are required.

Supercritical fluid extraction (SFE) offers an alternative to solvent-based extractions. It poses little threat to the environment, improves extraction selectivity, saves time and laboratory space, and lends itself to automation (6–9). Despite the great interest in SFE, however, not many studies describing applications of SFE in multiresidue analysis of pesticides in food have been published (10–23).

Gas chromatography/ion trap mass spectrometry (GC/ITMS) was selected as the universal method of detection because of its ability to perform simultaneous quantitative and qualitative analyses of different classes of pesticides at ultratrace concentrations. Reports have been published (23–26) describing the use of GC/ITMS in determining various components in complex matrixes.

This work combines commercialized SFE technologies with GC/ITMS to develop a method that will simultaneously monitor various pesticides in fruits and vegetables. The 46 pesticides chosen for analysis were based on compounds included in the Pesticide Data Program (27) that could be analyzed by GC. Many of these pesticides are commonly found at very low levels in produce (27). The 4 commodities tested–potatoes, carrots, broccoli, and grapes–had incurred pesticides or were fortified with pesticides. Also, the SFE and GC/ITMS procedure was used to analyze check samples as a quality assurance measure. Samples fortified with pesticides at concentrations unknown to the analysts were analyzed, and results were compared with those from 7 other laboratories that analyzed the same samples by traditional methods.

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Experimental

Apparatus

(a) *Supercritical fluid extractors.*—A Model 7680T (Hewlett-Packard, Little Falls, DE) and a Prepmaster (Suprex, Pittsburgh, PA), both equipped with automated variable restrictors, solvent modifier pumps, and solid sorbent collection systems, were used. For the 7680T, optimal instrumental parameters were: 320 atm extraction pressure and 60°C temperature (CO_2 density, 0.85 g/mL); 7 mL extraction vessel; 2 min static extraction followed by 42 mL CO_2 at a flow rate of 1.6 mL/min; 50°C restrictor temperature; collection on octadecylsilane (ODS) sorbent trap (1 mL) at 9°C; and elution with 1.5 mL acetonitrile at 0.4 mL/min and 50°C. The trap was rinsed to waste with 2 mL ethyl acetate followed by 2 mL acetonitrile at 2 mL/min to clean and regenerate the ODS between extractions. For the Prepmaster, instrument settings were the same except for the following: 10 mL vessel size and 60 mL CO_2 extraction volume; trap elution at 40°C with 6 mL acetonitrile; N_2 gas at 80 psi to blow trap dry between flushes. Total time for extraction–elution per sample was 36 min with the 7680T and 54 min with the Prepmaster. For experiments conducted under conditions other than those described, the settings are specified in the discussion of those experiments.

(b) *Gas chromatograph.*—A Model ITS40 GC/ITMS system (Finnigan MAT, San Jose, CA), consisting of a Varian 3300/3400 gas chromatograph and a CTC A200S autosampler, was used. Operating conditions for the GC/ITMS were: 1 µL injection volume into a Model 1093 (Varian, Walnut Creek, CA) septum-programmable injector (SPI); 3 s needle hold time in port before injection; 55°C injection port for 30 s followed by ramping to 250°C at 250°C/min; 5 psig He column head pressure; 55°C initial oven temperature for 30 s, ramped to 130°C at 50°C/min, then to 165°C at 1.5°C/min and to 250°C at 4°C/min, and held at 250°C until a total time of 65 min elapsed; 240°C transfer line temperature; and 215°C detector manifold temperature. Typical ITMS operating conditions (autotune calibration was performed before each injection sequence) were as follows: electron impact mode; 10 µA filament current; 1850 V electron multiplier tube; 1 ms ion time; and automatic gain control at 20 000.

(c) *Chromatographic columns.*—A DB-1701 capillary column (14%-cyanopropylphenyl)methylpolysiloxane, 30 m, 0.32 mm id, 0.25 µm film thickness (J&W Scientific, Folsom, CA), and 5 m phenylmethyl deactivated guard column (0.32 mm id) (Restek Corp., Bellefonte, PA).

(d) *Data collection.*—Data acquisition for mass spectra (70–425 *m/z*), was obtained from 5 to 65 min of the chromatogram. The GC/ITMS system had a Magnum version 2.4 software package loaded into a Gateway 2000 computer for data acquisition and processing and instrument control.

Reagents

(a) *Gases.*—SFC/SFE grade CO_2 (Air Products, Allentown, PA), with a He headspace of 1800 psi (for Prepmaster) or without He headspace (for 7680T), was used. Bone-dry grade CO_2 (for both instruments) and N_2 (for Prepmaster) were required for cryogenic cooling and drying of the trap, respectively. The septum-programmable injector on the GC/ITMS system also used CO_2 with dip-tube for cooling.

(b) *Solvents.*—Acetonitrile, methanol, ethyl acetate, acetone, and isooctane were pesticide grade (Fisher, Fair Lawn, NJ).

(c) *Solids.*—Hydromatrix (Varian, Harbor City, CA), a pelletized diatomaceous earth, was sieved (325 mesh) and washed with acetone before use to remove fine particles and contaminants. Its use in SFE has been described previously (11, 15, 17). The prepacked 30 µm Hypersil ODS (Hewlett-Packard) traps were provided with the 7680T; for the Prepmaster, 55–105 µm C_{18} (Waters, Milford, MA) and 80/100-mesh silanized glass beads (Suprex) were packed into the trap manually.

(d) *Pesticide standards.*—Pesticides were obtained from the U.S. Environmental Protection Agency (Research Park, NC, or Beltsville, MD), except for a duplicate standard of ethion (Niagara Chemical, Middleport, NY) used to double check accuracy of ethion results. Table 1 lists the pesticides, arranged by classification, included in this study. Chrysene-d_{12} and phenanthrene-d_{10} (Cambridge Isotope Laboratories, Woburn, MA) were used as internal standards. Individual stock solutions were prepared by weighing 10–12 mg amounts of standards, dissolving the pesticide with acetone and/or isooc-

Table 1. Pesticides included in the study and their chemical classes

Organochlorine (11)	Organophosphate (21)
Chlorothalonil	Azinphos-methyl
Dacthal	Chlorpyrifos
DDE	Diazinon
DDT	Dichlorvos
Endosulfan I	Dimethoate
Endosulfan II	Disulfoton
Hexachlorobenzene (HCB)	Ethion
Lindane	Ethoprop
Methoxychlor	Fenamiphos
Pentachlorobenzene (PCB)	Malathion
Pentachloronitrobenzene (PCNB)	Methamidophos
	Methidathion
Carbamate (3)	Mevinphos
Carbaryl	Omethoate
Carbofuran	Parathion
Chlorpropham	Parathion-methyl
	Phorate
Other (8)	Phosalone
Atrazine	Phosmet
Captan	Phosphamidon
Dicloran	Terbufos
Diphenylamine	
Iprodione	Pyrethroid (3)
Myclobutanil	Esfenvalerate
Propargite	Fenvalerate
Vinclozolin	*cis*-Permethrin

tane, and making up to 100 mL in volumetric flasks. Concentrations were corrected for the stated purities (typically >98%) of the standards. Working standard mixtures in acetone, containing 20 μg/mL for each pesticide, were used for spiking samples and preparing calibration standards.

Sample Preparation and Analysis

Commercially purchased potatoes, grapes, broccoli, and carrots served as blank or fortified samples. Incurred (contaminated) grape and carrot samples were provided by the State of Michigan Department of Agriculture, and fortified samples (containing unknown pesticides at concentrations unknown to the authors) of potato and broccoli were provided by the California Department of Food and Agriculture. The potato and broccoli samples were also analyzed 7 times by traditional methods (1, 2) by 6 state laboratories (California, Texas, Florida, New York, Michigan, and Washington) participating in the Pesticide Data Program (27), and the grape and carrot samples were analyzed solely by the Michigan laboratory.

For the store-bought samples, a 50 g portion of vegetables was shredded and mixed in a food processor, and a 3 g subsample was weighed into a tared beaker. Hydromatrix (2 g) was added to the beaker; a glass rod was used for mixing. The mixed samples were packed into the extraction vessels, and for fortified samples, the 20 μg/mL spiking solution was added to the sample in the middle of the vessel. A few minutes were allowed for the solvent to evaporate. Spiking levels varied from 0.1 to 1 μg/g in the samples; triplicate samples were extracted for analysis. For incurred grapes and carrots, which arrived precut and frozen, 3 g portions of frozen sample were mixed with 2 g Hydromatrix and packed into the vessels. The 100 g each of potato and broccoli check samples were mixed with 66.7 g Hydromatrix in the sample container, because chopped samples may not have been mixed thoroughly after fortification. In those cases, 5 g mixed sample–Hydromatrix was loaded into the vessels. To ensure instrument performance, a control spike of pentachlorobenzene at 0.2 μg/g was added to the vessels before extraction. The samples were extracted as described earlier. An internal standard (chrysene-d_{12} or phenanthrene-d_{10}) was added to the extracts before injection for quantitation by GC/ITMS.

For GC/ITMS calibration, the spiking solutions were diluted to make the calibration standards, and internal standard solution was added to the calibration standards in the same ratio as the extracts. For best quantitation, the calibration standards were prepared in SFE extracts from sample blanks of the same matrix. For samples of known fortification levels, 4 calibration concentrations varying from 4 times lower to 2 times higher than the fortification level were used. For samples of unknown concentration, 4–5 calibration standards ranging from 0.025 to 1.5 μg/g were used.

Calculations

(a) *Limits of detection.*—The ratio $3\sigma_{blank/sensitivity}$ was used to calculate limits of detection (LODs) where σ_{blank} is the standard deviation of blank measurements (or noise) and sensitivity is the slope of the linear calibration plot for each analyte. The average noise in the 60–100 s retention windows of the quantitation masses for each pesticide was calculated from the software-reported signal-to-noise (S/N) ratios for the calibration standards in potato. LODs were calculated by multiplying these noise levels by 3 and then dividing by the slope of a linear calibration curve generated from peak height data (both noise and signal were divided by the internal standard signal).

(b) *Pesticide concentrations.*—Integrated peak area data of selected masses versus the internal standard were used for quantitation. Table 2 lists masses chosen for quantitation of each pesticide. Calculations were done with a spreadsheet program or the instrument's software program. In most cases, the calibration curves were linear, and the linear least-squares calibration line was used for quantitation. In some cases, however, especially for the organophosphates, the calibration curve formed a distinctly quadratic relationship. In those cases, the best-fit quadratic curve was used for quantitation. A method of standard addition was also used to determine pesticide concentrations in the potato check sample.

(c) *Confirmation of pesticides.*—With GC/ITMS, the following criteria had to be met to confirm presence of a pesticide in the sample: retention time (t_r) difference of less than 10 s, S/N ratio >3, and mass spectrum match >90% versus the spectrum library for the pesticide (generated from pesticide standards). Only results for confirmed pesticides are presented in this paper.

Results and Discussion

Sampling

The 3 g sample size for SFE was much smaller than the 50–100 g sample sizes used in traditional methods of multiresidue analysis of pesticides in produce (1–3). For carrot and potato, reproducible results of several 3 g subsamples from a 50 g blended sample indicated that 3 g is sufficient to represent a larger sample (23).

For wet samples such as fruits and vegetables (80–95% water), moisture must be removed or absorbed before SFE. Addition of Hydromatrix to the sample (water–Hydromatrix, approximately 1 + 1) is an effective way to absorb water (11, 15, 23). For the potato and broccoli check samples, Hydromatrix was added to the entire 100 g sample and then homogenized because the fortified sample was not assumed to be homogeneous.

Development of SFE Procedure

In general, a new SFE method should be developed in 4 steps: (*a*) confirm quantitation accuracy and precision of the detection method, (*b*) ensure 100% collection and elution of analytes from SFE trap, (*c*) determine SFE conditions for highest recoveries, and (*d*) optimize the method for analysis of real samples. Each step may affect another, and care must be taken to maintain instrumentation in optimal operating conditions. This general outline was followed during development of the SFE procedure.

(a) *Elution of pesticides from SFE trap.*—Experiments to compare recoveries of pesticides from 3 sorbents (glass beads,

Table 2. Pesticide retention times (t_r), quantitation masses, and limits of detection (LODs) for potato SFE extracts analyzed by GC/ITMS

No.	Pesticide	t_r, min	Masses,[a] *m/z*	LOD,[b] ng/g	No.	Pesticide	t_r, min	Masses,[a] *m/z*	LOD,[b] ng/g
1	Dichlorvos	5.8	109* + 127 + 185	6	24	Phosphamidon	32.8	72 + 127* + 264	27
2	Methamidophos	7.7	94* + 95 + 141	14	25	Dacthal	33.0	299 + 301* + 303	0.4
3	PCB	9.9	248 + 250* + 252	2	26	Carbaryl	33.6	115 + 116 + 144*	5
4	Mevinphos	10.9	127* + 164 + 192	2	27	Malathion	33.8	125 + 127 + 173*	6
5	HCB	16.7	282 + 284* + 286	2	28	Parathion	34.8	97 + 109* + 291	18
6	Ethoprop	17.1	97 + 158* + 243	6	29	Endosulfan I	35.5	195 + 241* + 339	7
7	Diphenylamine	17.2	167 + 168 + 169*	3	30	DDE	37.1	246 + 316* + 318	17
8	Phorate	19.3	75* + 121 + 260	2	31	Captan	37.7	79*	10
9	Chlorpropham	20.0	127* + 171 + 213	5	32	Methidathion	38.1	85 + 93 + 145	9
10	PCNB	21.9	295 + 297 + 299	3	33	DDT	39.6	165 + 235* + 237	1
11	Omethoate	22.3	110* + 156 + 214	20	34	Fenamiphos	39.8	260 + 288 + 303	5
12	Terbufos	23.0	231*	3	35	Endosulfan II	41.2	195 + 241* + 339	8
13	Diazinon	24.3	137 + 179* + 304	2	36	Ethion	41.5	97 + 153 + 231*	6
14	Lindane	24.6	181* + 183 + 219	4	37	Myclobutanil	42.4	150 + 179* + 181	48
15	Disulfoton	25.4	88* + 89 + 97	4	38	Propargite	43.4	135* + 335 + 350	9
16	Dicloran	25.6	124* + 176 + 206	18	39	Methoxychlor	45.3	227*	3
17	Carbofuran	26.1	149 + 164*	2	40	Iprodione	46.9	314* + 316	5
18	Atrazine	26.1	200* + 215 + 216	4	41	Phosmet	47.1	160*	12
19	Dimethoate	28.6	87* + 93 + 125	4	42	Phosalone	48.8	182* + 184 + 367	17
20	Chorothalonil	30.6	264 + 266* + 268	2	43	Azinphos-methyl	49.2	132* + 160	150
21	Vinclozolin	31.3	198 + 212* + 285	4	44	*cis*-Permethrin	49.3	127 + 163 + 183*	13
22	Parathion-methyl	32.1	109 + 125 + 263*	6	45	Fenvalerate	61.3	125 + 225* + 419	29
23	Chlorpyrifos[c]	32.4	197 + 199 + 314	20	46	Esfenvalerate	63.2	125 + 225* + 419	13
IS[d]	Phenanthrene-d_{10}	22.7	188*		IS	Chrysene-d_{12}	46.7	240*	

[a] *, base peak.
[b] For the SFE method with the 7680T (1.5 mL final volume); LODs with the Prepmaster were 4 times higher (6 mL final volume).
[c] Potato matrix interfered spectrally.
[d] IS, internal standard.

alumina, and ODS) were planned. The first step was to ensure 100% elution from the traps. The trap materials were spiked with pesticide mixtures and rinsed with solvents to determine elution volumes. The pesticides studied completely eluted with 1–2 mL solvent from glass beads and ODS for all solvents tested (methanol, acetonitrile, acetone, and ethyl acetate), but many pesticides did not elute from alumina even with 10 mL solvent. A previous study found alumina useful for complete trapping, elution, and cleanup of organochlorine pesticides in vegetables (23), but alumina has limited use in a method involving a diverse mixture of pesticides.

(b) *Collection of pesticides on SFE trap.*—General extraction conditions were known for several analytes from previous studies (23), and were used to test the efficiency of collection of pesticides on the glass bead and ODS traps. In this study, pesticides were spiked onto Hydromatrix, CO_2 extraction density was 0.9 g/mL, and flow rate was 2.5 mL/min for 20 min. For both traps, collection temperatures were 10° and 25°C, and 1.5 mL methanol was used for elution at 0.5 mL/min and 25°C. Figure 1 compares recoveries of several pesticides (numbers refer to pesticides in Table 2) listed in order of increasing GC retention time (a trend of decreasing volatility). ODS trapped 100% of the pesticides tested, but no pesticide was trapped at 100% by the glass beads. For glass beads, the trend of increasing loss versus pesticide volatility indicated the importance of using a trap material that interacts with the analyte in the rapid stream of CO_2 and not to simply create a surface for analyte precipitation from the supercritical fluid. Slightly higher recoveries were obtained at 10°C than at 25°C in both cases, but the difference was not significant when considering the precision of the measurement. A lower temperature may improve recovery for glass beads, but a trap temperature below 0°C increases the likelihood of problems due to ice formation. Another advantage of ODS over glass beads is its potential for additional cleanup of SFE extracts.

(c) *Extraction conditions.*—In most cases, increasing CO_2 density increases SFE extraction capability (6–9). Experiments were performed to determine the effect of different CO_2 density on pesticide recovery: 0.3 g/mL (100 atm, 60°C), 0.5 g/mL (130 atm, 60°C), and 0.85 g/mL (320 atm, 60°C). SFE at a CO_2 density of 0.3 g/mL gave maximum recoveries for all pesticides tested except dimethoate, carbaryl, mevinphos, atrazine, dicloran, captan, and iprodione. Only a CO_2 density of 0.85 g/mL gave maximum recovery for those pesticides. A CO_2 density of 0.95 g/mL improved recovery slightly for a few analytes, but the benefits of slightly higher recoveries for only a few pesticides did not compensate for the cost of higher matrix interferences. An extraction pressure of 320 atm and a tempera-

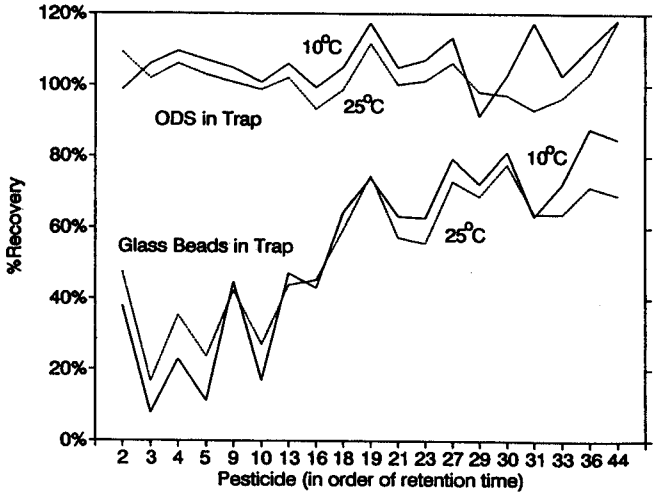

Figure 1. Comparison of SFE collection of pesticides on glass bead or ODS material in trap at 10° and 25°C. Pesticides are numbered as presented in Table 2 and ranked according to increasing retention time (a trend of decreasing volatility).

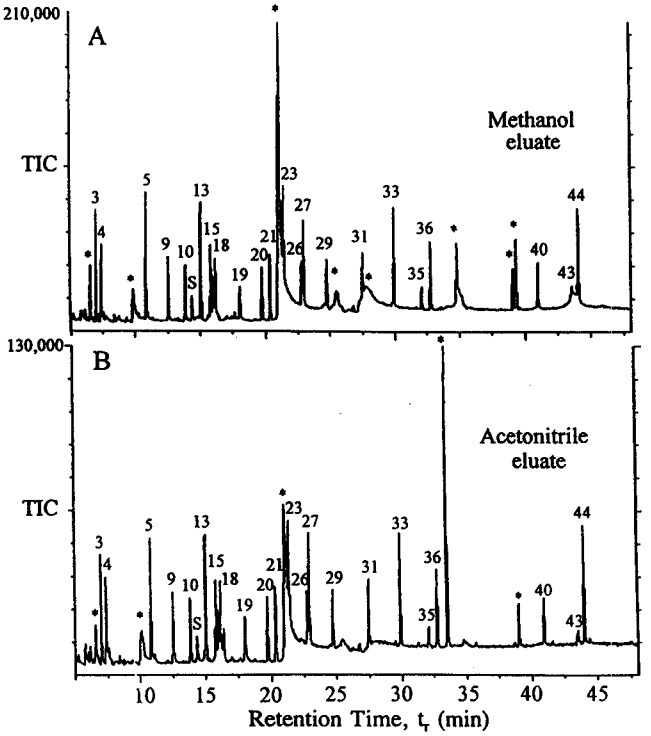

Figure 2. Comparison of GC/ITMS total-ion chromatograms of SFE potato extracts eluted from ODS with (A) methanol and (B) acetonitrile. Numbered peaks refer to the pesticides listed in Table 2; peaks designated with an asterisk were matrix components, and the peak labeled S was the internal standard, phenanthrene-d_{10}.

ture of 60°C (a CO_2 density of 0.85 g/mL) were chosen for subsequent extractions.

Extractions were conducted to optimize other SFE parameters. The length of static extraction steps made no difference. A flow rate of 1.6 mL/min gave a higher recovery of dichlorvos (72% versus 24%), the most volatile component, than did extraction at 2.5 mL/min. An amount of CO_2 equal to 6 empty vessel volumes gave slightly higher recoveries than if 5 vessel volumes were swept. Solvent modifiers were unnecessary, because satisfactory recoveries were achieved without them. However, modifier tests with acetone, methanol, and ethyl acetate were made; recoveries were not substantially different from results without modifiers, but matrix effects worsened.

(d) *Extract elution and cleanup.*—Elution of the ODS trap with methanol was compared with elution with acetonitrile. Both solvents gave similar recoveries, but acetonitrile extracts gave fewer matrix peaks and lower background levels during GC/ITMS than the methanol extracts. Figure 2 presents total ion chromatograms of SFE extracts of potato containing several pesticides at 5 μg/g eluted with methanol and acetonitrile. The methanol eluate gave a maximum background peak with total ion current (TIC) of 210 000, whereas the maximum background peak for the acetonitrile eluate was 130 000 under the same conditions. The peaks identified in chromatograms refer to the pesticides listed by number in Table 2, and peaks marked with an asterisk signify matrix components. Broad matrix peaks at ca 28, 35, and 44 min in the methanol eluate were not present in the acetonitrile eluate. Also, the higher boiling point of acetonitrile made it more compatible for septum-programmable injection. For the 7680T, 1.5 mL acetonitrile at 0.4 mL/min and 50°C was sufficient to remove all pesticides from the trap; no analytes were found in the ethyl acetate rinse afterwards. The C_{18} trap used with the Prepmaster required a larger volume of acetonitrile to elute the pesticides, but this was probably due to differences in sorbent material, elution temperature of 40°C (instrument maximum), and manner of packing the trap.

GC/ITMS Analysis

Chromatographic separation of the 46 pesticides by GC/ITMS and analysis of SFE results in the presence of matrix interferences required careful attention to many details.

(a) *Injector.*—Initial experiments performed with a splitless injector gave good results for many stable pesticides, but many organophosphates, as well as captan, carbaryl, iprodione, and chlorothalonil, gave poor peak shapes and/or reduced responses. Injector temperature was varied from 100° to 250°C, but no significant difference in results was observed. For this reason, on-column injection with an SPI was investigated. The initial injector temperature was kept at 55°C (below the boiling point of the final extract solvent), and after 30 s, the temperature was ramped rapidly to 250°C. In this manner, the SPI rapidly transported the pesticides into the column at mild conditions, thereby minimizing losses to the walls of the glass injector liner. The peak shapes of many pesticides improved with the SPI, and losses of captan, chlorothalonil, iprodione, and carbaryl were reduced significantly.

(b) *Quantitation.*—In a complex sample matrix, GC/ITMS quantitation overestimated the concentrations of several pesticides when calibration curves were based on standards in pure solvent. Possible reasons for this systematic error in quantitation are: differences in injection conditions for samples containing matrix components versus calibration standards without matrix components and mass spectral overlaps due to coeluting matrix components. Mass spectral overlaps were noticeable in chromatograms. If overlap occurred, different quantitation masses were chosen to eliminate or reduce this source of error. However, in many instances, no spectral interferences were present but calculated concentrations of pesticides were still higher than the true concentrations. In those cases, it was believed that matrix components in the extracts filled active sites on the glass injection liner and analyte losses were reduced (28). But with standards, no matrix components were present and the incidence of analyte loss was higher. Use of the SPI reduced this error, but overestimation still occurred for several pesticides.

This source of quantitation error was virtually eliminated by preparing the calibration standards in blank sample extracts rather than in pure solvent. Table 3 compares the differences in quantitation of several pesticides with calibration standards in pure solvent or in blank potato SFE extracts. Most organochlorine pesticides, such as hexachlorobenzene (HCB), pentachlorobenzene (PCB), DDT, and DDE, were not affected by matrix components, but matrix effects were considerable for several other pesticides such as iprodione, captan, and carbaryl, and organophosphates such as dimethoate and mevinphos.

Table 3. Quantitation of pesticides fortified in potato (3 replicates), extracted by SFE, and analyzed by GC/ITMS versus calibration standards prepared in pure solvent or in blank potato extracts

Pesticide	Recovery, %	
	Calibration in pure solvent	Calibration in blank sample extracts
Mevinphos	161	88 ± 2
HCB	83	86 ± 7
Chlorpropham	145	97 ± 2
PCNB	90	101 ± 6
Diazinon	90	91 ± 2
Disulfoton	81	85 ± 6
Dicloran	98	90 ± 5
Atrazine	98	96 ± 3
Dimethoate	239	94 ± 6
Vinclozolin	110	86 ± 6
Chlorpyrifos	145	90 ± 3
Carbaryl	277	81 ± 2
Malathion	169	85 ± 2
Endosulfan I	87	88 ± 2
DDE	90	90 ± 2
Captan	207	38 ± 10
DDT	102	90 ± 4
Ethion	144	98 ± 4
Iprodione	230	94 ± 5
cis-Permethrin	93	93 ± 5

(c) *Method of standard addition.*—In SFE recovery studies, the same batch of fruit or vegetable served as the blank and fortified sample, and by controlling matrix effects, results were precise and accurate. However, in analyses of samples originating from a different source than the source of the control samples, results were satisfactory, but small differences in matrix effects were thought to affect results to a small extent. In general, the best way to control matrix effects is by the method of standard addition. In the analysis of a potato check sample, the check sample (3 g subsamples) was extracted by SFE 4 times. Then internal standard was added to each extract, followed by addition of 0.1, 0.2, or 0.5 μg/g of the 46 pesticides into 3 of the extracts. Figure 3 presents calibration curves obtained by the method of standard addition for iprodione and ethion (all other calibration curves passed through the zero point within the error in slope and y intercept). Three other subsamples of the potato were spiked with pesticides at 0.5 μg/g and analyzed. Figure 3 also includes analytical results for iprodione (recovery, 102 ± 28%) and ethion (recovery, 97 ± 16%) in the fortified samples.

(d) *Quadratic calibration curves.*—For analyses using standard addition, calibration curves for GC/ITMS were linear, but in other cases, calibration curves were quadratic. In such cases, the quadratic relation was used for quantitation. Figure 4 shows an example of how a quadratic calibration curve more closely fits the calibration of ethion (Figure 3 illustrates when a calibration curve was linear). Organochlorine pesticides nearly always followed linear slopes, and organophosphate pesticides sometimes presented quadratic relationships. The cause of this effect is unknown, but it is possibly due to partial losses of trace amounts of pesticide at particular GC conditions that become more significant at the picogram injection level.

(e) *Limits of detection.*—Table 2 lists the retention times (t_r), quantitation masses, and limits of detection (LODs) for the 46 pesticides analyzed by GC/ITMS. LODs are reported in ng/g for potato analyzed by the 7680T method (final volume, 1.5 mL). For the Prepmaster, the final volume was 6 mL, and LODs were 4 times higher. The reported values were typical of the GC/ITMS method, but S/N ratios fluctuated approximately 15%, depending on instrumental performance and matrix effects. Despite the 17-fold smaller sample size used in SFE, the LODs for the GC/ITMS method generally matched the method detection limits reported by regulatory laboratories using selective detection, such as electron capture, electrolytic conductivity (Hall), flame photometric and nitrogen–phosphorus detectors (27).

Sample Results

Table 4 presents recoveries of 46 pesticides fortified in potatoes at 0.5 μg/g. Recoveries were >80% for 39 pesticides and >55% for 44 pesticides; only omethoate and methamidophos gave recoveries of <50%. These results were typical of pesticide recoveries with either SFE instrument. The recovery of chlorpyrifos was probably higher than presented because, as shown in Figure 2, a potato matrix component chroma-

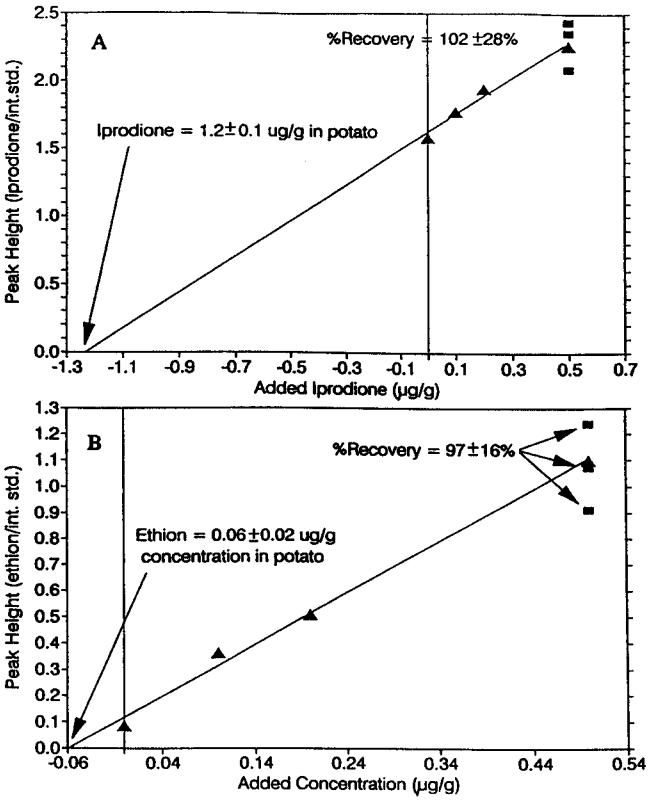

Figure 3. Results of method of standard addition for (A) iprodione and (B) ethion in potato check sample. Actual iprodione and ethion concentrations were 1.6 and 0.12 µg/g, respectively. Legend: (■), check samples fortified with pesticides at 0.5 µg/g before SFE, and (▲), fortified extracts used for calibration. Recoveries were 100% for the pesticides. The reason for the differences in experimental and expected ethion concentrations is unknown.

tographically overlapped with chlorpyrifos and affected quantitation (the background peak also coeluted with parathion-methyl, but less overlap of the chosen quantitation masses occurred). The lower recovery of dichlorvos was likely due to its high volatility. Decreasing trap temperature and/or CO_2 flow rate during SFE probably would have increased its recovery.

(a) *Troublesome pesticides.*—Of the 46 pesticides, disulfoton, captan, propargite, and especially omethoate and methamidophos, consistently gave lower recoveries compared with the others. These pesticides have unique traits. For example, an existing analytical method converts disulfoton to the more stable sulfone and sulfoxide forms before analysis (29). Captan, *N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide, contains reactive structural moieties distinct from those in other pesticides. Propargite was the only pesticide analyzed containing an alkyne group. Omethoate and methamidophos, metabolic products of dimethoate and acephate, respectively, gave severely tailing peak shapes that made peak integration and quantitation difficult. Methamidophos was the only compound tested containing an unprotected phosphoramide, which also likely made SFE and elution from the ODS trap more difficult.

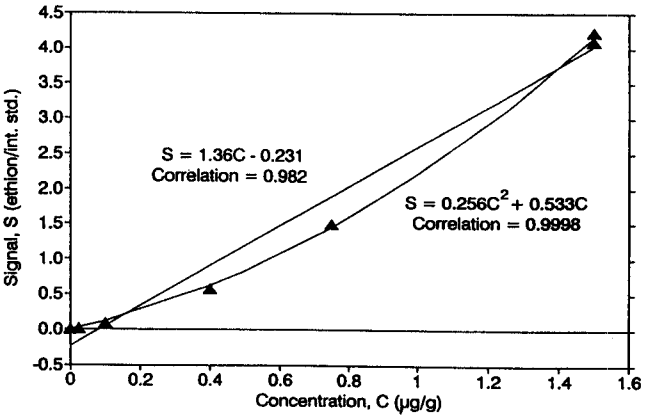

Figure 4. Calibration curve for ethion when quadratic relation was used for quantitation. Equations are the linear and quadratic best-fit functions for the data (and correlation coefficients), where S is peak area of the analyte divided by peak area of the chrysene-d_{12} internal standard, and C is analyte concentration.

(b) *Analyses of produce with incurred pesticides.*—An incurred carrot sample, previously analyzed by an SFE method developed for organochlorine pesticides (23), was extracted and reanalyzed by the multiresidue SFE and GC/ITMS procedure. HCB again was confirmed to be present at 8 ± 4 ng/g; additionally, DDE and iprodione were identified in the sample. Concentrations were calculated as 0.18 ± 0.01 µg/g for DDE and 0.19 ± 0.02 µg/g for iprodione, but comparison of results with those of a traditional approach was not possible because concentrations were below the LODs of a method performed by a regulatory laboratory.

(c) *Check sample results.*—A more detailed study to compare the results of the SFE and GC/ITMS method and traditional approaches was performed. Check samples of potato and broccoli, containing incurred and fortified pesticides at concentrations unknown to the analysts, were sent to 7 regulatory laboratories for analysis by validated traditional approaches (1, 2). Table 5 presents the results of interlaboratory and method comparisons for the potato check sample. The potato was analyzed in triplicate, 6 times on different days, with the 7680T and Prepmaster instruments. SFE and GC/ITMS results for iprodione compare favorably with results from the 7 laboratories. Average iprodione concentrations were 1.21 µg/g (RSD, 19%) by the SFE method and 1.27 µg/g (RSD, 20%) by the regulatory laboratories; the fortification level was 1.6 µg/g.

For ethion, the fortification level was 0.12 µg/g. The regulatory laboratories found 0.117 µg/g (RSD, 11%), whereas the SFE and GC/ITMS method determined only 0.057 µg/g (RSD, 22%). In all our previous SFE studies involving ethion, recoveries from fortified samples were consistently about 100%, and GC/ITMS results were accurate when appropriate calibration methods were used (as discussed previously). As Figures 3 and 4 show, extraction and quantitation results for ethion presented no indications of large error. Accuracy of the standard solutions was confirmed by comparison with a duplicate ethion standard.

Table 4. Recoveries of pesticides spiked at 0.5 μg/g in potatoes, by SFE and GC/ITMS method[a]

No.	Pesticide	Recovery, %	No.	Pesticide	Recovery, %
1	Dichlorvos	72 ± 9	24	Phosphamidon	91 ± 4
2	Methamidophos	0 ± 0	25	Dacthal	85 ± 5
3	PCB	91 ± 6	26	Carbaryl	91 ± 1
4	Mevinphos	92 ± 2	27	Malathion	87 ± 4
5	HCB	93 ± 2	28	Parathion	91 ± 3
6	Ethoprop	84 ± 3	29	Endosulfan I	93 ± 2
7	Diphenylamine	87 ± 2	30	DDE	91 ± 1
8	Phorate	82 ± 4	31	Captan	66 ± 4
9	Chlorpropham	91 ± 2	32	Methidathion	90 ± 4
10	PCNB	90 ± 4	33	DDT	93 ± 2
11	Omethoate	5 ± 8	34	Fenamiphos	83 ± 2
12	Terbufos	83 ± 5	35	Endosulfan II	114 ± 9
13	Diazinon	86 ± 5	36	Ethion	97 ± 16
14	Lindane	89 ± 2	37	Myclobutanil	83 ± 10
15	Disulfoton	78 ± 5	38	Propargite	57 ± 22
16	Dicloran	91 ± 4	39	Methoxychlor	90 ± 1
17	Carbofuran	90 ± 2	40	Iprodione	102 ± 28
18	Atrazine	92 ± 2	41	Phosmet	88 ± 4
19	Dimethoate	83 ± 8	42	Phosalone	86 ± 5
20	Chorothalonil	93 ± 2	43	Azinphos-methyl	94 ± 6
21	Vinclozolin	91 ± 2	44	*cis*-Permethrin	93 ± 3
22	Parathion-methyl	85 ± 6	45	Fenvalerate	93 ± 2
23	Chlorpyrifos	72 ± 5	46	Esfenvalerate	88 ± 2

[a] Data are means ± standard deviations of 3 replicate extractions.

The lower concentration of ethion obtained by the SFE and GC/ITMS method was probably due to degradation of ethion in the check sample. In Table 5, the SFE results are presented in the order that the experiments were performed. Twelve days elapsed from the first experiment (ethion at 0.077 μg/g), when the sample was first thawed and mixed in its entirety with Hydromatrix, to the 6th and final set of extractions (ethion at 0.04 μg/g). Table 5 shows a trend of lower result for each subsequent extraction and analysis (experiments 2 and 3 were performed on the same day, as were experiments 4 and 5). Another evidence to support sample degradation was that the sample was analyzed in this laboratory more than a month after analysis in the regulatory laboratories. The sample was stored at –40°C before shipment to this laboratory, where the sample was stored at –20°C before and after experiments. As the results indicate, the most degradation (from <0.12 to 0.077 μg/g) occurred when the sample was brought initially to room temperature and mixed thoroughly with Hydromatrix. In 12 days at –20°C, ethion concentration declined from 0.077 to 0.04 μg/g.

Table 6 presents results for the broccoli check sample. The fortification levels were 0.14 μg/g for dimethoate and 0.47 μg/g for propargite. The sample was analyzed only once (triplicate subsamples) with the 7680T. The SFE and GC/ITMS method found 0.102 ± 0.005 μg/g for dimethoate and 0.28 ± 0.01 μg/g for propargite. In previous studies, SFE recoveries (as shown in Table 4) were 83% for dimethoate and 57% for propargite. When the broccoli check sample results were corrected for the known recovery factors, the calculated concentrations (0.12 μg/g for dimethoate and 0.50 μg/g for propargite) closely agreed with the actual concentrations. The regulatory laboratories obtained results of 0.13 μg/g (RSD, 18%) for dimethoate and 0.50 μg/g (RSD, 29%) for propargite. None of the laboratories detected dacthal incurred in the sample, which was confirmed to be present and quantitated at 0.0011 μg/g by the SFE and GC/ITMS method.

(**d**) *SFE instruments.*—Similar recoveries and concentrations for incurred and fortified pesticides were obtained with both the 7680T and Prepmaster SFE instruments (as presented in Table 5). The SFE instruments were used interchangeably; the only difference was the 6 mL final extract volume for the Prepmaster versus the 1.5 mL volume for the 7680T. This difference was most likely due to the dissimilar ODS sorbents used in the traps and the manner in which they were packed. Research comparing SFE results obtained with different commercial SFE instruments (10) and a product review of different instruments (30) have been published.

Conclusions

This work's goal was to develop a method for multiresidue analysis of pesticides in fruits and vegetables by SFE and GC/ITMS. The method gave recoveries >80% for most pesticides in produce; methamidophos was the only pesticide of the 46 tested that was not recovered at all. Although the SFE and GC/ITMS method requires more study before implementation in regulatory laboratories, the results compared satisfactorily

Table 5. Results of interlaboratory comparison of analysis of potato check sample

Lab. No.	Iprodione, µg/g		Ethion, µg/g	
	Solvent[a]	SFE[b]	Solvent[a]	SFE[b]
1	1.1	1.3 ± 0.1[c]	0.13	0.077 ± 0.006[c]
2	0.87	1.2 ± 0.1[c,d]	0.11	0.06 ± 0.02[c,d]
3	1.4	1.6 ± 0.2[e]	0.11	0.06 ± 0.02[e]
4	1.4	0.98 ± 0.04[c]	0.11	0.057 ± 0.002[c]
5	1.4	1.16 ± 0.05[e]	0.099	0.049 ± 0.004[e]
6	1.6	1.0 ± 0.1[c]	0.13	0.040 ± 0.008[c]
7	1.1	—	0.13	—
Average	1.27	1.21	0.117	0.057
Standard deviation	0.25	0.23	0.013	0.012
RSD,[f] %	20	19	11	22
Actual concentration	1.6 µg/g		0.12 µg/g	

[a] Regulatory solvent-based extraction method (1, 2).
[b] SFE using 2 different instruments; analyses were performed in triplicate on different days, and results are means ± standard deviations.
[c] SFE with 7680T.
[d] Result of method of standard addition.
[e] SFE with Prepmaster.
[f] Relative standard deviation.

with results of obtained using traditional approaches. LODs for the method were 20 ng/g or lower for 40 of the 46 pesticides, and in several instances, the SFE and GC/ITMS procedure confirmed the presence of pesticides not detected in samples analyzed by traditional approaches. The number of pesticides studied was limited to 46, but because several different classes of pesticides were represented, many other pesticides in the same classes could likely by simultaneously analyzed with only minor modifications.

The SFE and GC/ITMS approach has many advantages over the solvent-based extraction and GC/selective-detector methods currently used by regulatory laboratories. Speed of analysis is greatly increased. In approximately 2 h, a produce sample can be extracted by SFE and analyzed by GC/ITMS to simultaneously confirm the presence of and quantitate multiple pesticide residues at ultratrace levels. Using the automated instrumental techniques also reduces the amount of manual labor and laboratory space needed. SFE requires only small amounts of solvent and glassware, thereby reducing hazards to workers and amount of waste generated. SFE allows for a higher degree of selectivity in extraction compared with solvent-based methods, and use of solid-sorbent traps for SFE collection affords a rapid, single-step extraction and cleanup. Finally, the GC/ITMS method detects analytes in SFE extracts at ultratrace levels with a high degree of selectivity even in the presence of matrix components. With further research, the combination of SFE and GC/ITMS technologies may be able to supplant current inefficient approaches to multiresidue analysis of pesticides in food.

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Table 6. Results of interlaboratory comparison of analysis of broccoli check sample

Pesticide	Actual conc	Concentration, µg/g					
		Regulatory lab. results[a]			SFE and GC/ITMS result[b]		
		Av. ± SD	Low	High	Av. ± SD	Recovery, %	Corrected result
Dacthal	?[c]	ND[d]	—	—	0.0011 ± 0.0001	85	0.0013
Dimethoate	0.14	0.130 ± 0.024[e]	0.10	0.16	0.102 ± 0.005	83	0.12
Propargite	0.47	0.50 ± 0.14[f]	0.29	0.67	0.284 ± 0.012	57	0.50

[a] Regulatory solvent-based extraction method (1, 2).
[b] The 7680T was used; analyses were performed in triplicate on the same day.
[c] Unknown.
[d] Not detected.
[e] Results from 6 regulatory laboratories, one laboratory obtained 0.29 µg/g for dimethoate, which was not included.
[f] Results from 5 regulatory laboratories, 2 of which did not detect propargite.

ington for analyzing the samples; the State of Michigan Department of Agriculture for providing the incurred carrot and grape samples; and Hewlett-Packard for use of the 7680T SPE.

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