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Analysis of Pesticide Residues in Eggs by Direct Sample Introduction/Gas Chromatography/Tandem Mass Spectrometry

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Direct sample introduction (DSI) or "dirty sample injection" is a rapid, rugged, and inexpensive approach to large volume injection in gas chromatography (GC) for semivolatile analytes such as pesticides. DSI of complex samples such as eggs requires a very selective detection technique, such as tandem mass spectrometry (MS−MS), to determine the analytes among the many semivolatile matrix components that also appear. In DSI, the nonvolatile matrix components that normally would contaminate the GC system in traditional injection methods remain in a disposable microvial, which is removed after every injection. For example, 3 μg of nonvolatile residue typically remained in the microvial after an injection of egg extract using the DSI method. This analytical procedure involves the following: (i) weighing 10 g of egg in a centrifuge tube and adding 2 g of NaCl and 19.3 mL of acetonitrile (MeCN); (ii) blending for 1 min using a probe blender; (iii) centrifuging for 10 min; and (iv) analyzing 10 μL (5 mg of egg equivalent) of the extract using DSI/GC/MS−MS. No sample cleanup or solvent evaporation steps were required to achieve quantitative and confirmatory results with <10 ng/g detection limits for 25 of 43 tested pesticides from several chemical classes. The remaining pesticides gave higher detection limits due to poor fragmentation characteristics in electron impact ionization and/or degradation. Analysis of eggs incurred with chlorpyrifos-methyl showed a similar trend in the results as a more traditional approach.

Keywords: *Pesticide residue analysis; direct sample introduction; gas chromatography; tandem mass spectrometry; eggs*

INTRODUCTION

The current methods used in the analysis of pesticide residues in eggs are rather time-consuming, labor-intensive, costly, and/or do not detect a wide range of analytes. For example, the method used by the U.S. Department of Agriculture's Food Safety Inspection Service (FSIS), which is responsible for the monitoring of unshelled egg products in the United States, calls for multiple steps for extraction, solvent exchange, and cleanup with gel-permeation chromatography (GPC) prior to gas chromatographic/electron-capture detection (GC/ECD) analysis of primarily chlorinated compounds (*1*). The U.S. Food and Drug Administration (FDA) is responsible for the monitoring of shelled eggs in the United States, and although a wider analytical range is achieved, the methods require cleanup and solvent evaporation steps prior to analysis using selective GC detectors (*2*, *3*). Supercritical fluid extraction (SFE) has been demonstrated to more selectively extract pesticide residues from eggs and eliminate post-extraction cleanup steps, but the polarity range of the approach is compromised (*4*). In the case of egg matrix, SFE methods have been developed mainly for single classes of analytes (*5*, *6*).

Ideally, an analytical approach would eliminate the time-consuming, costly, and laborious cleanup, solvent exchanges, evaporation steps, and other inconveniences commonly involved in analytical methods and still provide confirmatory and quantitative capabilities for a wide range of analytes at low levels. Direct sample introduction (DSI) for GC injection is one technique that greatly minimizes sample preparation and yet still provides a rugged analytical approach for complex matrices (*7*−*9*). DSI (with a ChromatoProbe) involves the placement of a small amount of sample material or liquid extract into a 40-μL disposable microvial. The sample and microvial are manually placed into the temperature-programmable GC injector and heated gently at first to evaporate the solvent and then heated rapidly to thermally desorb semivolatile components in the sample, such as many pesticides. A major benefit in this DSI approach is that nonvolatile matrix components, which normally contaminate the GC liner and column in traditional injection approaches, remain in the microvial, which is disposed after every injection.

In complex extracts, DSI requires a very selective detection technique to determine the analytes among the many semivolatile matrix components. Tandem

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mass spectrometry (MS−MS) using an ion trap instrument has been previously demonstrated to detect many targeted pesticides in complex extracts (*9*−*12*). The use of DSI/GC/MS−MS was previously shown to provide excellent quantitative and confirmatory results for approximately 20 representative pesticides in fruit and vegetables (*9*). The main goal of this study was to evaluate the DSI/GC/MS−MS approach for approximately twice as many pesticides and to investigate the utility of the approach for a fatty matrix such as eggs.

MATERIALS AND METHODS

Chemicals. Pesticide standards were obtained from the U.S. Environmental Protection Agency's National Pesticide Standard Repository (Fort Meade, MD), Chemservice (West Chester, PA), Ultra Scientific (North Kingstown, RI), or Dr. Ehrenstorfer GmbH (Augsburg, Germany); phenylbutazone was obtained from Sigma (St. Louis, MO). Although phenylbutazone, a banned veterinary drug tranquilizer, has little chance of appearing in eggs, it was included in the study for determining its feasibility for DSI/GC/MS−MS analysis in a fatty matrix. Stock solutions of approximately 2000 ng/μL of each analyte were prepared in toluene, ethyl acetate (EtOAc), or acetone. A mixed spiking standard of 10 ng/μL in acetonitrile (MeCN) that contained all analytes (except terbufos, which was added separately as a quality control measure) was prepared from the individual stock solutions.

All solvents used in the study were a grade suitable for pesticide residue analysis from Burdick and Jackson (Muskegon, MI). Analytical reagent grade NaCl was obtained from Mallinckrodt (Paris, KY), and ACS certified grade anhydrous $MgSO_4$ and Na_2SO_4 were from Fisher (Fair Lawn, NJ). Deuterated MeCN and deuterated anthracene and chrysene were obtained from Cambridge Isotope Laboratories (Woburn, MA). The molecular sieves (Davison Grade 564, 3-12 mesh beads, 3 D pore size) used in the experiment were obtained from Fisher. He gas for the GC/MS−MS was zero-grade quality from Air Products (Allentown, PA). Solid-phase extraction (SPE) cartridges (500 mg each) evaluated in experiments consisted of Applied Separations (Allentown, PA) C_{18}/18% loading, Varian (Harbor City, CA) primary−secondary amine (PSA), Applied Separations Alumina-neutral (Alumina-N), and J. T. Baker (Phillipsburg, NJ) aminopropyl (-NH_2) sorbents.

Samples. Eggs were purchased from a local supermarket or were provided by the FDA Center for Veterinary Medicine (Laurel, MD). In the preparation of eggs with incurred residues, white Leghorn laying hens were dosed in two separate ways. Initially, a hen was fed a capsule containing 2 mg of chlorpyrifos-methyl on two adjacent days. Eggs were collected for a period of 2 weeks from the hen and stored refrigerated. The eggs were extracted within 3 days of collection, and final extracts were stored in vials at −40 °C until analysis within a few weeks. Only a small amount of chlorpyrifos-methyl was found in 4 eggs, and the experiment was repeated at higher dosing levels for a longer period of time. In the second experiment, a hen was fed capsules containing 10 mg of chlorpyrifos-methyl for 9 days. Individual eggs (white + yolk) were homogenized soon after collection, but due to laboratory and personnel moves, the samples were stored at −20 °C for more than 8 months before they could be extracted and analyzed.

Apparatus. A Varian Saturn 2000 (Walnut Creek, CA) fitted with a 1079 injector and ChromatoProbe was used for DSI/GC/MS−MS. The metal surfaces on the ChromatoProbe, 1079 injector, and ion trap contained a SilcoSteel coating to minimize potential pesticide losses to active metal surfaces. The 40-μL disposable glass microvials used in DSI with the ChromatoProbe were obtained from Scientific Instrument Services (Ringoes, NJ). The following conditions were the final DSI/GC/MS−MS settings in the analysis: injection volume = 10 μL; 1.0 mL/min flow rate with electronic flow control (9.6 psig He head pressure at 85 °C column temperature); initial injector temp = 85 °C held for 6 s, ramped to 275 °C at 100 °C/min, and then held at 275 °C; splitless injection until 6 min and then the purge valve was opened with a 50:1 split ratio; column = 30 m, 0.25 mm i.d., 0.25 μm film thickness Rtx-5ms (Restek; Bellefonte, PA); 85 °C oven for 1 min, to 125 °C at 25 °C/min, then to 275 °C at 10 °C/min and held for 18 min, then ramped to 290 °C at 50 °C/min until 25 min; 275 °C transfer line; 200 °C ion trap temperature; 50 °C manifold temperature. The filament current was 50 μA, and typical multiplier voltage was 1550 V. An MS autotune was conducted prior to every sample set (approximately 16 samples) using the software default parameters.

Nuclear magnetic resonance (NMR) spectroscopy was conducted to determine water content of extracts using a Varian (Palo Alto, CA) Unity Plus 400 MHz instrument. For extraction, 40- or 250-mL Teflon centrifuge tubes (depending on sample size) were used to contain the egg samples, and a Tek-Mar (Cincinnati, OH) Tissumizer was used for blending. For ease of use and improved precision, a solvent dispenser was used to add the extraction solvent. Some experiments also required general laboratory equipment such as a centrifuge, top-loading balance, analytical balance, freezer, and pH meter.

Procedures. The final procedure for the extraction and analysis of the 40+ pesticides in eggs entailed four rapid and simple steps: (i) weighing 10 g of mixed egg into a 40-mL Teflon centrifuge tube and adding 2 g of NaCl and 19.3 mL of MeCN; (ii) blending on a medium setting for 1 min; (iii) centrifuging at 4000 rpm at room temperature for 10 min; (iv) removing an aliquot of the upper layer and injecting 10 μL for analysis in DSI/GC/MS−MS. For quantitation, calibration standards were prepared in blank matrix extracts that were treated exactly as the samples. Use of matrix-matched calibration has been shown to be an effective method to overcome matrix effects in pesticide analysis (*13*−*15*). To improve precision and ease of use, the aliquot taken for analysis was transferred to an autosampler vial by weight (e.g., 0.800 ± 0.005 g) using Pasteur pipets, and MeCN was added to all samples to compensate for the volume of spiking solution added in the preparation of the standards. No internal standard was needed when such precautions were taken, and even though 100 ng/g each of d_{10}-anthracene and d_{12}-chrysene were added to the final extracts, they were not used to normalize peak areas in the final method.

RESULTS AND DISCUSSION

Extraction. The first step in the development of the analytical method was the determination of the optimal solvent to minimize the co-extracted matrix components of eggs while still achieving high recoveries of the range of analytes. According to the Nutrient Composition Database (*16*), eggs on average consist of 75.3% water, 12.5% protein, 10.0% total lipid, 1.2% carbohydrate, and 0.9% ash. The lipids include 3.1% saturated fatty acids, 3.8% monounsaturated fatty acids, 1.4% polyunsaturated fatty acids, and 0.42% cholesterol, all of which can be especially troublesome in residue analysis. Acetone, EtOAc, methanol (MeOH), and MeCN are common extraction solvents that each give high recoveries in multiresidue pesticide methods, and each of these solvents were considered in this study. An experiment was conducted in which these four solvents were used to extract eggs + NaCl, and the extent of matrix co-extractives were measured by weight after centrifugation, addition of anhydrous $MgSO_4$ to the upper layer, and complete evaporation of the solvent.

Table 1 presents the results from this experiment, and as the table shows, MeCN is clearly the most selective extraction solvent tested. Without the use of a drying agent with eggs, EtOAc formed an emulsion to render its use impractical in this application. For the water-miscible solvents, MeCN was superior mainly because water could be better separated from MeCN than acetone and MeOH with NaCl and $MgSO_4$ (or other

Table 1. Comparison of Different Extraction Solvents in the Co-extraction of Matrix Components in Eggs

solvent	amt extracted from 5 g of egg (mg)	amt after heating to 100 °C (mg)	amt after heating to 275 °C (mg)
EtOAc		formed gel	
MeOH	720	630	360
acetone	63	37	23
MeCN	8.0	6.4	2.9
Results after SPE Cleanup of MeCN Extracts			
PSA	4.9	4.7	1.7
-NH_2	4.5	4.4	1.7
alumina-N	4.8	4.8	1.9
C_{18}	2.1	1.0	0.6

salts). The presence of water in the extracts leads to a higher percentage of polar co-extracted components, such as proteins and carbohydrates. Thus, MeCN minimized the extraction of lipids, proteins, and carbohydrates from the eggs and has been proven to be an effective solvent for multiresidue analysis of pesticides previously (*2*, *9*, *11*, *17*, *18*).

SPE. In traditional methods, MeCN extracts of food samples also require extensive cleanup (*2*, *11*, *18*). Schenck and Lehotay (*13*) compared different SPE sorbents to evaluate the effect of cleanup of food commodities extracted with acetone or MeCN. In this study, an experiment was conducted to measure the removal of matrix components in the SPE cleanup of eggs extracted with MeCN. Table 1 also presents the gravimetric results obtained after passing 10 mL (5 g of egg equivalent) of the MeCN extracts through 500 mg of alumina-N, C_{18}, PSA, or -NH_2 SPE cartridges in which 5 mL of MeCN was used for elution. The C_{18} sorbent removed nearly twice the amount of matrix co-extractives (6 mg vs 3.1−3.5 mg) among the tested SPE cartridges, and C_{18} was also the only one to remove the yellow color of the extracts. Thus, SPE with C_{18} was an effective way to clean up the egg extracts, and this aspect was evaluated in the DSI/GC/MS−MS approach, but after recovery studies, it was decided that SPE did not provide adequate benefit in the analysis to justify the additional cost in time, effort, extra dilution factor, and expense related to incorporating the C_{18} step into the final method.

pH. The effect of pH in extraction of eggs with MeCN was also investigated. Eggs mixed with distilled water had a pH of 7.6, and the addition of 2 g of NaCl/10 g of egg lowered the pH to 7.4 (and caused a darkening of the egg color). Addition of 2 M HCl or 1 M NaOH was used to alter the pH to 4.0, 7.0, and 10.0 prior to extraction with MeCN, but no measurable weight differences in the amount of co-extractives were found.

Volatility of Co-extractives. To mimic the thermal desorption in DSI, the extracted egg residue was heated to 100 °C for 20 min (to remove volatiles) and then 275 °C for 20 min (to remove semivolatiles). The difference in weight between the two temperatures provides an approximation of the amount of semivolatile co-extracted material that is introduced into the analytical GC column in DSI. In traditional injection techniques, the amount of nonvolatile residue that remains at the injection temperature (typically 250 °C) contaminates the GC system, but in DSI, it is removed from the GC system after every injection. The experiments showed that for a 10-μL injection of MeCN extract (representing 5 mg of egg), typically 8 μg of egg matrix residue is added to the microvial of which approximately 1 μg consists of volatiles, 4 μg consists of semivolatiles, and 3 μg consists of nonvolatiles.

Table 2. Comparison of Different Desiccants for the Removal of Water from MeCN Extracts

desiccant	amt added	starting % H_2O	final % H_2O
NaCl	2 g/27 mL	27	6.0 (in upper phase)
Na_2SO_4	0.8 g/4 mL	6.0	3.9
molecular sieves	0.8 g/4 mL	6.0	3.2
$MgSO_4$	0.8 g/4 mL	6.0	0.6

Removal of Water from Extracts. Even trace amounts of water may have strong effects in some analytical separations and methods. The addition of anhydrous Na_2SO_4 serves as a very common approach to remove water from liquids, but Na_2SO_4 is far from the strongest drying agent. To find a drying agent that would effectively and conveniently reduce water content in MeCN extracts, an experiment was conducted to compare different desiccants.

In the experiment, 100 μL of deuterated MeCN was added to fixed volumes (4 mL) of standard solutions containing known amounts of water in MeCN and to MeCN egg extracts after the addition of NaCl (to salt-out the water phase), $MgSO_4$, Na_2SO_4, and molecular sieves. NMR was used to measure water content; both spectral shift of the water band versus the deuterium and peak height were linear versus concentration. To begin, the MeCN extraction method of 10 g of egg (75% water) plus 20 mL of MeCN yielded a solution of approximately 27% water. To compensate for volume changes in mixed solvents and to provide an upper layer extract of 2 mL/g egg, it was found that 19.3 mL of MeCN should be added to 10 g of egg + 2 g of NaCl (the NaCl must be added prior to the blending step to minimize foaming). After the salting-out step, analysis by NMR determined that the upper layer of the MeCN extract contained 6.0% water. Table 2 gives the results of the experiment in which three other desiccants were added to 4-mL aliquots of this extract. Clearly, anhydrous $MgSO_4$ was better than the other desiccants tested for removing water from MeCN solutions. Of the 13 mmol of H_2O in solution, the 6.7 mmol of $MgSO_4$ (which can form a heptahydrate) removed 90% of the water, whereas in the case of 5.6 mmol of Na_2SO_4 (which can form a decahydrate), only 35% of the water was removed from the solution. The hydration of $MgSO_4$ is exothermic, and the generation of heat helped verify that water was being removed from solution.

Setting of DSI Parameters. Essentially, DSI begins with solvent evaporation in the injection port. Experiments were conducted with MeCN to determine conditions for injection volume, initial injector temperature, He head pressure, and split flow ratios that would reasonably minimize the solvent evaporation time. Each of the parameters listed above was varied in controlled experiments to determine their effects on evaporation time of MeCN in the microvial. The time was measured by monitoring 41 *m*/*z* (molecular ion of MeCN) with the MS detector set at low filament current and multiplier voltage.

Figure 1 provides a summation of the experiments for 10-μL MeCN injections with the ChromatoProbe at 80, 85, and 90 °C injector temperatures and different He column head pressures. Split flow ratios of 100:1 and 200:1 were found to have a much smaller effect in evaporation time than temperature and pressure. The boiling point of MeCN is 81.8 °C at atmospheric pres-

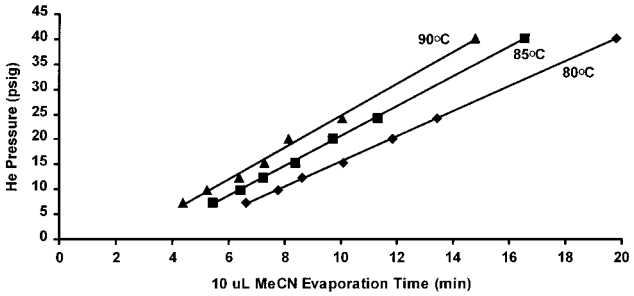

Figure 1. Evaporation time of 10 μL of MeCN in DSI with the ChromatoProbe with respect to injector temperature and He pressure.

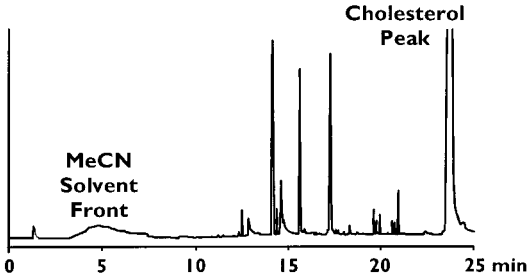

Figure 2. DSI/GC/MS total ion current chromatogram of egg extract showing the evaporation of MeCN and semivolatile egg matrix components (including large cholesterol peak).

sure, and it would take very little time for 10 μL of MeCN to evaporate at this temperature in an open container with flowing gas, but in DSI, the narrow opening (0.2 mm) of the microvial, higher pressure in the injector port, and extra time needed for the metal ChromatoProbe to reach the injector temperature extends the MeCN evaporation time. At 90 °C port temperature and 11.6 psig (1.2 mL/min He flow through the column), it took 5.8 min for 10 μL of MeCN to evaporate. This was too long in practice, thus in the final method, the injection port temperature was simply raised from the initial temperature of 85 to 275 °C at 100 °C/min before the MeCN evaporated in the final method. All of the MeCN entered the GC column, but this had no adverse consequences on the chromatography of most semivolatile pesticides (however, the more volatile dichlorvos could not be analyzed at these conditions). Figure 2 shows a DSI/GC/MS total ion chromatogram that shows the evaporation of the MeCN in the approach.

Setting of GC Parameters. MS−MS analysis works similarly as single ion monitoring (SIM) with quadrupole instruments in that analytes are targeted for detection in retention time windows. The general advantages of MS−MS over SIM include a higher selectivity associated with the further dissociation of the precursor ion(s), improved confirmation capability, and potentially lower detection limits. Quantitation in ion trap instruments is more greatly affected by coelution of high background components (from matrix components and/or excessive column bleed), and the shorter ion dwell times possible in SIM can give more data points across a chromatographic peak. Thus, SIM with quadrupole instruments may provide better precision for quantitation in some situations. However, detection with distinctive GC/MS instruments is often difficult to compare because injection, GC column condition, and instrument maintenance are also significant features in GC analysis. Oftentimes, the comparison of results between quadrupole and ion trap instruments is a reflection of injection and GC factors rather than detection, especially for more problematic analytes.

In the DSI/GC/MS−MS analysis of eggs, little effort was given to maximize separation between analytes, and a simple GC oven temperature program for a standard GC/MS column was chosen to provide a reasonable separation of the targeted pesticides. A higher priority was placed on a shorter run time than separation of all analytes. In the 25-min final DSI/GC/MS−MS method, 45 targeted compounds were monitored in 23 MS−MS segments, 7 of which used two distinct MS−MS conditions, and 5 segments used three or four separate conditions. MS−MS scan time was minimized in these situations. The instrument software permitted as many as five sets of MS−MS conditions within a retention time segment, but it may be helpful for quantitation, but not necessarily, to have fewer compounds per segment (*9*, *11*).

In the analysis of eggs, the most important aspect was to permit the necessary time and temperature for an extremely large cholesterol peak to elute from the column prior to the next injection or ghost peaks appeared in subsequent chromatograms. Figure 2 is an DSI/GC/MS total ion chromatogram of the separation that shows how this cholesterol peak was by far the largest component in the thermally desorbed egg MeCN extract. No problems with interference were posed by the cholesterol because no analytes coeluted with the relatively sharp peak.

Setting of MS−MS Parameters. Lehotay (*9*) describes the procedure, which was also followed in this study, to attain the final GC separation and MS−MS conditions. Essentially, the goal of the MS−MS optimization process is to maximize S/N ratio of the sum of at least two products ions in the MS−MS spectrum (two products ions are generally acceptable for confirmation in MS−MS). The MS−MS spectra were varied mainly by adjusting the excitation storage level and excitation amplitude (energy for collision-induced dissociation). Table 3 provides the final MS−MS conditions chosen in the study, but it was possible to achieve similar spectra and sensitivities in different combinations of these parameters and/or altering of ion trap temperature.

The most desirable MS−MS spectrum had two product ions with relative abundances >50% and a small (5−10%) amount of precursor ion evident, but in some cases, such as permethrins and methoxychlors, many product ions were produced, and it was not necessary to dissociate the precursor ion so much to yield high S/N ratio of the product ions. Furthermore, a great advantage of MS−MS in these cases when the MS spectrum only gave a single strong ion, the additional information provided by MS−MS was exceptional for confirmation and improved analysis. However, there were also a few cases, such as dimethoate, when only a single product ion could be produced in the nonresonant mode using electron impact (EI) ionization. These cases were marked by use of a relatively low *m/z* fragment ion in MS as the precursor ion in MS−MS. Figure 3 compares EI MS and MS−MS spectra of methoxychlors and dimethoate in which the advantages and disadvantages become apparent.

Gamón et al. (*12*) demonstrated that chemical ionization (CI) can improve MS−MS results by increasing the molecular ion abundance in MS in instances such as dimethoate, but for practical purposes in multiresidue

Table 1. List of Pesticide Analytes and MS−MS Parameters Used for Their Analysis[a]

segment	start time (min)	ret time (min)	analyte	excitation: precursor ion (*m/z*)	excitation: isolation window (*m/z*)	excitation: storage level (*m/z*)	excitation: ampl (V)	scan range (*m/z*)	scan time (s/scan)	quantitation ions (*m/z*)
1	8.00	8.394	1-naphthol	144[d,e]	3	63	60	110−149	0.50	115+116
2	9.00	9.341	omethoate	156[d]	3	68.6	50	75−161	0.50	110+141+79
3	9.75	9.957	CEAT[a] (174 *m/z*[d,e])	173	5	76	74	85−311	0.27	89+131+158+104+136
3	9.75	10.006	CIAT[b] (172 *m/z*[d])	173	5	76	74	85−311	0.27	104+136
4	9.75	10.020	trifluralin	306[d]	3	125	80	85−311	0.26	264+206
4	10.50	10.808	dimethoate	125	3	55	53	75−205	0.26	79
4	10.50	10.866	carbofuran	164[d]	3	72	53	75−205	0.26	149+121+122
4	10.50	10.944	atrazine	200[d]	3	88	80	75−205	0.26	104+136+172+164+158
5	11.05	11.121	diazinon oxon	273[d]	3	120	85	135−278	0.26	217+147+245
5	11.05	11.203	terbufos	231[d]	3	102	63	135−278	0.26	175+203
5	11.05	11.208	lindane	182	5	80	70	135−278	0.26	146+148+147
6	11.34	11.409	diazinon	179[d]	3	78	75	115−193	0.25	137+121+163
6	11.34	11.500	d_{10}-anthracene	188[d,e]	3	83	98	115−193	0.25	160+156+184
7	12.00	12.450	chlorpyrifos-methyl	287[d]	5	110	100	111−292	0.27	208+241+243+210+224+226
7	12.00	12.480	parathion-methyl	263[d,e]	3	116	62	111−292	0.27	246+153+136
7	12.00	12.647	carbaryl	144[d]	3	63	62	111−292	0.27	116+115
8	13.00	13.144	malathion	173[d]	3	76	50	95−178	0.50	99+117
9	13.30	13.363	fenthion	278[d,e]	3	110	80	110−320	0.27	245+213+135
9	13.30	13.385	chlorpyrifos	315[d]	5	110	58	110−320	0.27	258+286
9	13.30	13.414	parathion	291[d,e]	3	110	56	110−320	0.27	261+263+142+114+235
9	13.30	13.492	isofenphos oxon	229[d]	3	100	59	110−320	0.27	201+199+121
10	13.90	14.133	isofenphos	213[d]	3	94	49	117−218	0.50	185+121+167
11	14.50	14.570	methidathion	145[d]	3	64	44	80−150	0.50	85
11	14.67	14.750	tetrachlorvinphos	331[d]	3	110	95	128−336	0.27	199+201
12	14.67	14.833	endosulfan I	241	3	110	100	128−336	0.27	204+206+170
12	14.67	14.945	fenamiphos	303[d,e]	3	110	85	128−336	0.27	180+132+225
13	15.05	15.122	profenofos	338[d]	5	149	80	241−343	0.25	267+269+309+311
13	15.05	15.203	*p,p′*-DDE	317[d,e]	5	110	88	241−343	0.25	248+246+281+283
14	15.50	15.931	endosulfan II	241	3	100	100	125−246	0.25	170+204+206+136
14	15.50	16.017	ethion	231[d]	3	102	58	125−246	0.25	175+203
15	16.15	16.267	sulprofos	322[d,e]	3	110	99	137−327	0.50	141+156
16	16.50	16.677	*p,p′*-DDT	235[d]	3	103	87	160−392	0.27	165+199
16	16.50	16.699	endosulfan sulfate	387	3	100	70	160−392	0.27	287+289+251+253+217
17	16.85	16.923	*o,p′*-methoxychlor	227[d]	3	100	90	137−232	0.50	181+153+169+141+195+197
18	17.10	17.181	phenylbutazone	308[e]	3	110	52	180−313	0.50	184+252
19	17.50	17.656	phosmet	160[d]	3	71	70	71−245	0.26	133+77+102
19	17.50	17.702	*p,p′*-methoxychlor	227[d]	3	100	90	71−245	0.26	181+141+153+169+195+197
19	17.50	17.688	d_{12}-chrysene	240[d,e]	3	105	1	71−245	0.26	212+236+237
20	18.25	18.362	azinphos-methyl	132[d]	3	58	59	74−137	0.50	104
20	18.47	18.530	mirex	272[d]	3	110	90	232−277	0.50	237+235
21	19.00	19.196	*cis*-permethrin	183[d]	3	81	77	149−188	0.50	153+166+168+165
21	19.00	19.220	*trans*-permethrin	183[d]	3	81	77	149−188	0.50	153+166+168+165
22	19.45	19.497	coumaphos	362[d,e]	3	110	0.6	200−367	0.50	226+210+221+334+306
23	21.00	21.420	fenvalerate	225	3	99	85	115−230	0.50	119+181+169+142
23	21.00	21.752	esfenvalerate	225	3	99	85	115−230	0.50	119+181+169+142

[a] Nonresonant ionization used in MS−MS in all cases except d_{12}-chrysene and coumaphos in which resonant ionization was applied. When isolation window = 5 *m/z*, actually two precursor ions are taken 1 *m/z* on both sides of the given ion (e.g., 314 + 316 *m/z* for chlorpyrifos). [b] 2-Chloro-4-(ethylamino)-6-amino-1,3,5-triazine. [c] 2-Chloro-4-isopropylamino-6-amino-1,3,5-triazine. [d] Base peak in MS spectrum. [e] Molecular ion in MS spectrum.

analysis, this approach works better when either a long retention time difference occurs between analytes or when CI is used for all analytes. EI was better for many pesticides in this study, and sacrifices in sensitivity and confirmation capability of several pesticides were made to maintain a single injection for all targeted analytes within a reasonable chromatographic run time. In the future, it will probably be necessary to make two injections and separately target those pesticides better suited for CI from those better suited for EI. Resorting to multiple injections is a common sacrifice that is made in the analysis of many targeted pesticides using GC/MS(SIM) and GC/MS−MS (*11*, *12*, *18*).

Recovery Studies. Recovery experiments were conducted in fortified eggs on several occasions at concentrations from 5 to 100 ng/g. These experiments involved the use of no cleanup or cleanup using C_{18} or PSA SPE cartridges, addition of $MgSO_4$ or not, use of internal standards or not, and minor alteration of instrument conditions. The results for the majority of the targeted pesticides were acceptable in all experiments, but certain pesticides gave irreproducible results independent of the changes made to the method. Ultimately, the final method was streamlined to provide the fastest and easiest overall procedure by eliminating SPE cleanup, extended solvent evaporation time, addition of $MgSO_4$, and use of internal standards. Good results were still obtained for ≈25 pesticides and inadequate sensitivity was achieved for the others.

Table 4 lists the results from the DSI/GC/MS−MS analysis of the targeted pesticides fortified in triplicate at three different levels (25, 50, and 100 ng/g) on three consecutive days ($n = 27$). The pesticides are generally ordered in terms of quality in the results. For the first several pesticides, the 10−20% RSD indicated that results were similar from day to day and that no significant differences were observed versus concentration up to 100 ng/g. Some pesticides toward the middle

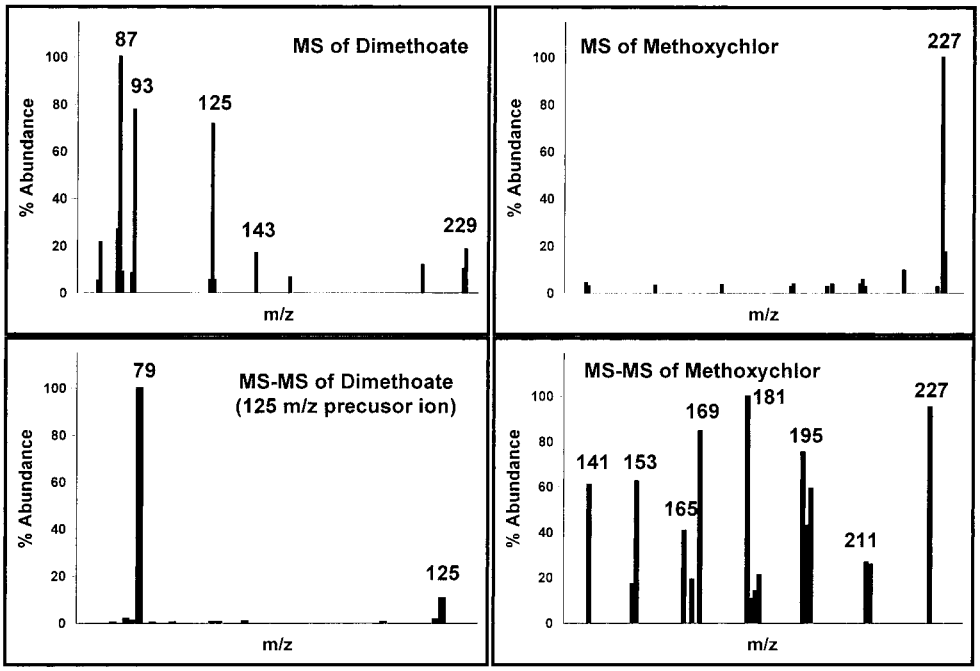

Figure 3. Comparison of MS and MS−MS spectra in EI mode of methoxychlor and dimethoate.

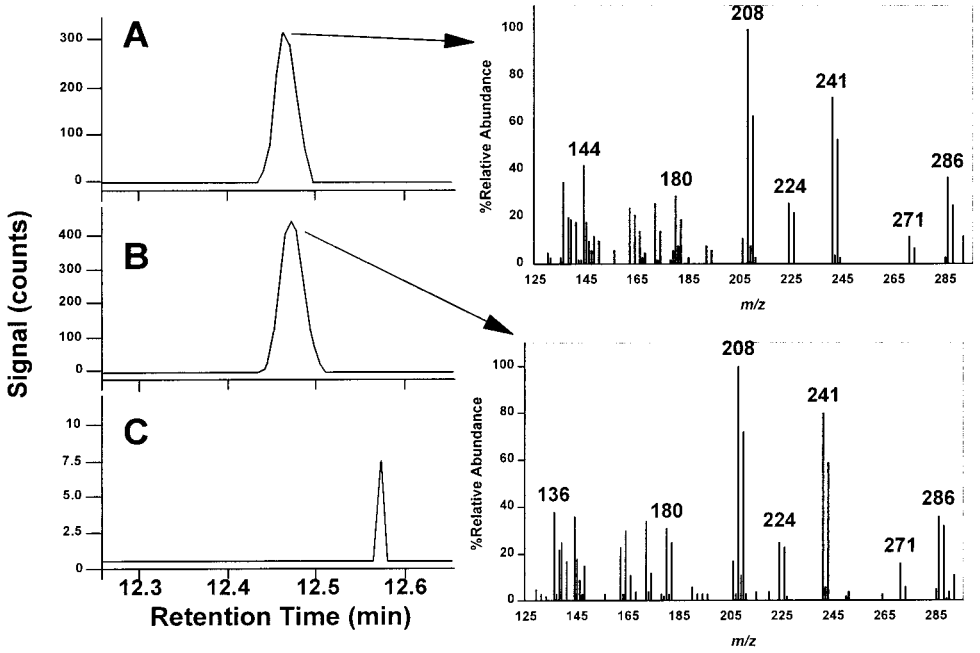

Figure 4. DSI/GC/MS−MS analysis of chlorpyrifos-methyl: (A) 10 ng/g standard in egg; (B) egg collected the first day after dosing the hen with chlorpyrifos-methyl; (C) blank egg. Unambiguous MS−MS confirmation of the pesticide is shown (the blank gave a null spectrum at the expected retention time).

of the table had more variable results possibly due to indirect effects from coeluting matrix peaks. Those pesticides at the bottom were not detected <100 ng/g and thus could not be analyzed in the fortified samples. The lowest calibrated level (LCL) is provided in the table in lieu of the limits of detection (LOD). LOD were difficult to estimate using the software because noise was absent in many cases and the signal/noise ratios given were inaccurate. For pesticides with LCL of 10 ng/g (the lowest level used in the experiments), LOD were often <1 ng/g. For example, this is demonstrated by Figure 4 in the case of chlorpyrifos-methyl.

Table 4 also gives the highest calibration level in which linearity of the calibration plot was maintained. Matrix-matched calibration standards, consisting of 0, 10, 20, 50, 100, 150, 200, 500, 1000, and 1500 ng/g of each pesticide, were conducted in replicate and averaged together over the three days. In some cases, such as permethrins, the best-fit line with correlation coefficient of >0.99 was achieved from 0 to 1500 ng/g, while in other cases, the linear dynamic range only stretched to 200 ng/g. The pesticides that gave reproducibly high recoveries also gave good calibration plots, and bad actors in the spiked samples often also gave relatively poor calibration curves.

Analysis of Incurred Samples. As described in Materials and Methods and in a previous study (*2*), chlorpyrifos-methyl (and other pesticides separately)

Table 4. Results from Recovery Experiments Using the Final DSI/GC/MS−MS Method[a]

analyte	*n*	% recovery	% RSD	LCL[b] (ng/g)	linear range (ng/g)
trifluralin	27	99	12	10	>1500
cis-permethrin	27	98	16	10	>1500
trans-permethrin	27	101	14	10	>1500
o,p′-methoxychlor	27	107	13	10	>1500
p,p′-DDE	27	103	13	10	>1500
atrazine	27	117	15	10	>1500
endosulfan I	27	95	12	10	1000
endosulfan II	27	103	14	10	1000
1-naphthol[c]	27	108	8	10	1000
diazinon	27	105	10	10	1000
diazinon oxon	27	96	17	10	1000
parathion	27	92	16	10	1000
chlorpyrifos	27	107	11	10	500
chlorpyrifos-methyl	27	112	16	10	200
fenthion	27	114	12	10	200
sulprofos	27	100	16	10	200
phenylbutazone	27	104	22	10	200
terbufos[d]	27	86	16	20	>100
profenofos	27	107	23	10	500
CIAT	27	136	15	20	>1500
isofenphos	27	74	33	10	500
parathion-methyl	26	123	17	10	200
ethion	27	82	27	10	200
lindane	27	104	22	10	200
carbofuran	27	97	21	20	500
mirex	27	59	20	10	500
p,p′-DDT	27	95	40	20	200
fenamiphos	24	53	39	10	200
coumaphos	16	79	25	50	500
p,p′-methoxychlor	16	90	27	50	200
tetrachlorvinphos	7	113	27	100	200
isofenphos oxon				200	>1500
carbaryl[c]				200	>1500
endosulfan sulfate				200	>1500
omethoate				200	>1500
dimethoate				500	>1500
malathion				500	>1500
methidathion				500	>1500
phosmet				500	>1500
CEAT				500	>1500
fenvalerate				500	>1500
esfenvalerate				500	>1500
azinphos-methyl				1000	>1500

[a] Average of 25, 50, and 100 ng/g spiking levels each in triplicate on 3 consecutive days. [b] LCL, lowest calibrated level. [c] Carbaryl converted to 1-naphthol. [d] Terbufos added at 50 ng/g in all samples.

was fed to laying hens, and eggs were collected for analysis. In the initial dosing experiment of 2 mg for 2 days, the chlorpyrifos-methyl levels were 1.5, 6.7, 5.1, and 0.2 ng/g in the collected eggs after 1, 2, 3, and 6 days, respectively (the hen did not lay an egg on days 4 and 5). In the second study in which 10 mg was administered each day for 9 days, chlorpyrifos-methyl was detected as high as 96 ng/g. Figure 4 presents the DSI/GC/MS−MS analysis of the day 1 egg from the chicken dosed with chlorpyrifos-methyl in the second study. The unambiguous GC/MS−MS confirmation of chlorpyrifos-methyl in the sample is also presented (the blank gave a null spectrum at the expected retention time of chlorpyrifos-methyl).

Figure 5 presents the overall results from this experiment and displays a comparison of results for a few of the samples analyzed separately as reported by Schenck and Donoghue (*2*) using an SPE method. The ratio between and pattern produced by the two analyses is consistent, and it is likely that the concentration increased (possible loss of water) over the long storage

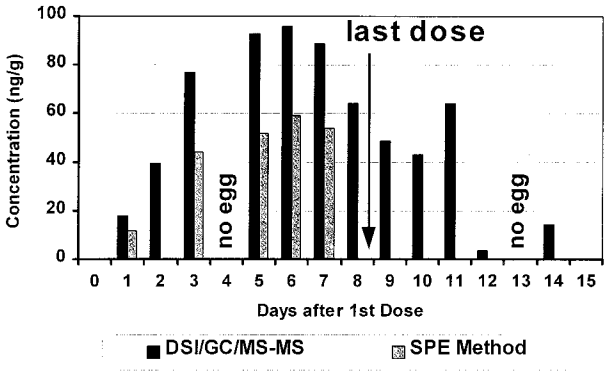

Figure 5. Analysis of incurred eggs containing chlorpyrifos-methyl. The hen was administered by capsule 10 mg of chlorpyrifos-methyl for 9 consecutive days. The SPE method of selected samples was performed separately as described in ref *2*.

time between the analyses of the samples in the different labs. It is also possible that random and/or systematic errors occurred in one or both of the analyses. Such events are not unusual in the pesticide residue analysis of foods and does not reflect negatively on the quality of either method (*19*).

Although this study was not intended to make conclusions about the depletion of pesticides in eggs, the results are consistent with the conclusions from previous studies (*2*, *5*, *6*, *20*, *21*) that many pesticides are not appreciably transported into eggs and/or readily degrade in eggs. For the hen dosed in this study, chlorpyrifos-methyl in its feed would have to be more than 90 mg/kg (with consumption of 110 g of feed/day) for several days for the concentration of the pesticide to exceed 100 ng/g in its eggs.

CONCLUSIONS

In this study, the feasibility of DSI/GC/MS−MS was evaluated for 43 diverse pesticides in a fat-containing matrix, eggs. Approximately 25 analytes gave acceptable results using the rapid, inexpensive, simple, confirmatory, and quantitative approach. No cleanup or solvent evaporation steps were included in the method, and in each step, the simplest approach was utilized, usually because the extra time and effort of cleanup, complete removal of water, longer separations, solvent evaporation, and use of internal standards did not provide an observable benefit for problematic pesticides.

The possible causes of problems for those analytes include the following: (i) a weak precursor ion and product ions in MS−MS; (ii) indirect effects of coeluting matrix interferants; (iii) insufficient number of points for analysis across a peak; (iv) excessive MeCN initially entering the chromatographic column; (v) nonvolatile matrix components in the microvial formed a layer on the glass surface that impeded thermal desorption of the analytes. The evidence gathered in this study and previously (*9*) indicates that points iii and v are less likely to be the cause of the problems for difficult analytes because other analytes gave excellent results within the same segments as bad actors and SPE cleanup did not improve those results. As demonstrated by Gamón et al. (*12*), the use of CI may provide better results for those pesticides that did not have a strong precursor ion (see Table 3). Future instrument improvements and further investigations of DSI/GC/MS−MS

may resolve or minimize the current limitations of the approach in complicated matrices such as eggs.

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