Rugged GC/MS/MS Pesticide Residue Analysis Fulfilling the USDA Pesticide Data Program (PDP) Requirements

Application Note

Food Safety

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Abstract

A GC/MS/MS method for the analysis of pesticide residues in fruits and vegetables was developed, optimized, and successfully implemented for routine use in a PDP laboratory. The method used the Multi-Mode Inlet in PTV solvent vent mode and concurrent backflushing for time-effective elimination of less volatile matrix components from the GC column, which reduced the cycle time and also prevented contamination of the MS ion source. Suitable analyte protectants and internal standards were added to the sample extracts and calibration standards prior to the GC/MS/MS analysis to improve mainly ruggedness and precision. Over 70 pesticides were analyzed in a 20-minute run and excellent method performance, fulfilling the PDP quality control requirements, was achieved throughout the tested sequences of PDP samples.

Introduction

The Pesticide Data Program (PDP) is a national pesticide residue database program administered by the United States Department of Agriculture (USDA), Agricultural Marketing Service since 1991. The program collects and reports pesticide residue data on agricultural commodities in the U.S. food supply, with an emphasis on foods highly consumed by infants and children as directed by the 1996 Food Quality Protection Act. A list of tested commodities is created for each testing period and distributed among the participating Federal and State government laboratories. PDP specifies priority pesticides but collects data for any additional pesticides tested by the laboratories. Any analytical method can be used for the PDP sample analysis as long as it fulfills the PDP validation and quality control (QC) requirements [1].

For the analysis of GC-amenable pesticides, most PDP and other modern pesticide residue testing laboratories have replaced GCs with element-selective detectors, such as ECD, ELCD, FPD or NPD, by GC/MS instruments. GC/MS provides many benefits, including analysis of a wide-range of GC-amenable analytes independent of their elemental composition; simultaneous quantification and structure-based identification; and spectrometric resolution of compounds coeluting in GC, enabling faster GC separations.

In terms of selectivity, tandem quadrupole mass spectrometry (MS/MS) is very helpful in separating analyte signals from coeluting matrix interferences [2] but other matrix-related issues can still be detrimental to the analysis. One problem is a potential contamination of the column by less volatile matrix components that can greatly affect method ruggedness by causing gradual decrease in analyte signals, peak broadening, and retention time shifts [3]. This can be prevented by using column backflushing, which can eliminate the less volatile matrix components from the GC column by reversing the column flow [4, 5].

This application note provides information about a GC/MS/MS method using column backflushing and other procedures that were successfully implemented for the routine analysis of PDP samples at the California Department of Food and Agriculture (CDFA).

Experimental

Sample Preparation

Preparation of fruit and vegetable sample extracts was based on the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method [6], resulting in the equivalent of 1 g sample per mL of acetonitrile extract. Internal standards and analyte protectants were added to the extract prior to the GC/MS/MS analysis. Blank matrix extracts were used for preparation of matrix-matched calibration standards, which were employed for quantification.

GC/MS/MS Conditions

GC/MS/MS analyses were performed using an Agilent 7890A GC combined with an Agilent 7000B Triple Quadrupole GC/MS System with an electron ionization (EI) source. The GC system was equipped with Electronic Pneumatics Control (EPC), a Multi-Mode Inlet (MMI) with air cooling, an Agilent 7693A Automatic Liquid Sampler (ALS), and a backflushing system based on a purged ultimate union controlled by either a Pneumatics Control Module (PCM) or AUX EPC module [7]. MassHunter software was used for instrument control, and for qualitative and quantitative data analysis.

The GC and MS conditions are summarized as follows:

GC oven conditions	
Oven temperature program	60 °C for 1.5 minutes; then 50 °C/min to 160 °C; then 8 °C/min to 240 °C; then 50 °C/min to 280 °C (2.5-minute hold); then 100 °C/min to 290 °C (3.1-minute hold)
Run time	20 minutes
GC injection conditions	
Inlet type	Multi-Mode Inlet (MMI)
Liner	2 mm id dimpled liner (p/n 5190-2296)
Mode	PTV solvent vent
Injection volume	2 µL (syringe size: 5 µL)
Inlet temperature program	60 °C for 0.35 minutes; then 900 °C/min to 280 °C (15-minute hold); then 900 °C/min to 300 °C (until the end of the analysis)
Purge flow to split vent	50 mL/min at 1.5 minutes
Vent flow	25 mL/min
Vent pressure	5 psi until 0.3 minutes
Gas saver	20 mL/min at 5 minutes
Septum purge flow	3 mL/min
Air cooling	On at 100 °C
GC column flow conditions	
Carrier gas	Helium
Column 1	HP-5MS UI; 5 m × 250 µm, 0.25 µm (cut from a 15-m or 30-m column, p/n 19091S-431 UI or 19091S-433 UI, respectively) (configured from the MMI to AUX EPC or PCM)
Column 2	HP-5MS UI; 15 m × 250 µm, 0.25 µm (p/n 19091S-431 UI) (configured from the AUX EPC or PCM to vacuum)
Column 1 flow program	1.1 mL/min for 16.024 minutes; then 100 mL/min to –2.5 mL/min (2.1-minute hold); then 100 mL/min to –5 mL/min (until the end of the analysis)
Column 2 flow program	1.2 mL/min for 18.16 minutes; then 100 mL/min to 2.4 mL/min (until the end of the analysis)
MS conditions	
MS source	EI –70 eV
Source temperature	280 °C
Quadrupoles temperature	150 °C
Transfer line temperature	280 °C
Solvent delay	4.4 minutes
He quench gas	2.25 mL/min
N_2 collision gas	1.5 mL/min
Gain setting	10
Acquisition mode	Multiple Reaction Monitoring (MRM)
MS1/MS2 resolution	Wide
Dwell time	10 ms

Sample Injection Sequence

A typical sample extraction and analysis batch of PDP samples at the CDFA contains 31 samples of the same matrix type, matrix blank, matrix spike, and a reagent blank. Matrix-matched standards are prepared in the matrix blank extract at levels corresponding to the limit of detection (LOD), limit of quantification (LOQ), and additional concentration levels ranging typically to 10 times the LOQ levels established for individual analytes.

Performance Test Sequence

To test the calibration integrity, one of the PDP QC requirements, a sample injection sequence was designed to include multiple QC checks and to mimic a typical sequence used for the PDP sample analysis. The sequence had 56 injections, including three sets of acetonitrile blanks, matrix blank, LOD, 1 × LOQ, 2 × LOQ, 4 × LOQ, 6 × LOQ, and 10 × LOQ matrix-matched standards injected at the beginning, in the middle (after 16 samples), and at the end (after another set of the same 16 samples) of the sequence. The two identical sets of 16 samples included alternated injections of eight unknown samples and eight QC samples (post-extraction spikes at 2 × and 6 × LOQ levels) of the same commodity.

Results and Discussion

GC/MS/MS Method Optimization

Table 1 shows the MS/MS conditions optimized to obtain 2–3 MRM transitions for each analyte. The optimization included analyses in full scan and product ion scan MS modes to determine suitable precursor and product ions, respectively. Then, the optimum collision energies were determined for each MRM. This tedious and time-consuming optimization process can be reduced by using the Pesticides and Environmental Pollutants MRM Database (G9250AA), which contains MS/MS conditions (on average eight MRMs per analyte) and retention time information (obtained under different conditions) for over 1,070 compounds [8].

The MMI was used for the sample introduction into the GC. This inlet can be employed in multiple different modes, including hot or cold, split or splitless (without or with a pressure pulse), and a PTV solvent vent. The PTV solvent vent mode enables solvent elimination from the inlet prior to the analyte transfer to the column, thus, larger volumes can be injected to increase sensitivity. The optimized method used the PTV solvent vent mode to inject 2 µL of the QuEChERS extract in acetonitrile. The majority of this solvent was eliminated to obtain optimum peak shapes especially for the early eluting analytes, for which focusing can be negatively affected by excessive amount of acetonitrile in the GC column [9].

Use of Column Backflushing and Analyte Protectants

Without the use of column backflushing, less volatile, late eluting matrix components have to be baked out at a high temperature after the analyte elution. This common GC practice increases the analysis time, reduces column life time, and leads to potential contamination of the MS ion source. Column backflushing is a technique that can eliminate less volatile compounds from the GC column by reversing the column flow at a pressure junction point [4, 5], such as by employing a purged ultimate union. If the column flow is reversed before the late eluting compounds start to move or get too far down the column, it will take less time (a shorter distance) and a lower oven temperature to remove them from the system through a split vent in the inlet.

The column flow can be reversed after (post-run) or during the analytical run [7, 9]. The latter case is called concurrent backflushing, which can start as soon as the last analyte of interest gets safely from a first column to a second column connected by the purged ultimate union [9]. This method used concurrent backflushing. As opposed to post-run backflushing, concurrent backflushing is more difficult to optimize but provides even more time- and cost-effective elimination of less volatile compounds and protection of the MS source and second column against contamination. As a result, the need for MS source maintenance is greatly reduced and its cleaning should be typically performed only as part of preventive maintenance (about every 6 months) of instruments analyzing fruit and vegetable extracts on a routine basis.

Backflushing can only eliminate matrix components that can move through the column. It is important to prevent deposits of nonvolatile matrix components in the column. This can be done with well optimized injection and the use of a suitable liner, such as the dimpled liner, that can protect the column against nonvolatile matrix deposits [9].

When it comes to GC system activity, the addition of analyte protectants to the sample extracts and calibration standards can help deactivate the liner and column in every injection. Analyte protectants are compounds that strongly interact with the active sites (free silanol groups and active sites created by nonvolatile matrix deposits) in the inlet and column, thus protecting susceptible analytes against adsorption or degradation on the active sites [10]. A suitable combination of analyte protectants should be selected for a given set of analytes to cover their volatility range, decrease matrix effects, and improve method ruggedness [11]. For the analytes included in this method (see Table 1), a mixture of L-gulonic acid γ-lactone and D-sorbitol was used for effective analyte coverage and protection. These analyte protectants are inexpensive, nontoxic, and safe to use routinely in the GC/MS system.

Table 1. *List of Analytes (Individual Isomers Listed), Their Retention Times (RT), and MRM Transitions, Precursor Ion > Product Ion (Collision Energy, V), Used for Quantitative and Qualitative Purposes*

RT (min)	Analyte	Quant	Qual 1	Qual 2
4.71	Dichlobenil	170.9 > 136.0 (15)	170.9 > 100.0 (25)	
5.22	Propham	179.1 > 137.1 (5)	179.1 > 93.1 (20)	
5.42	Tetrahydrophthalimide (THPI)	151.1 > 80.1 (5)	151.1 > 106.0 (5)	
5.60	1-Naphthol	144.1 > 116.0 (10)	144.1 > 115.1 (20)	
5.68	*o*-Phenylphenol	170.1 > 141.1 (25)	170.1 > 115.1 (40)	
5.77	Pentachlorobenzene (PCB)	249.8 > 179.9 (40)	249.8 > 141.9 (50)	
6.34	Demeton-O	171.0 > 115.0 (10)	171.0 > 97.0 (25)	
6.44	Chlorethoxyfos	299.0 > 97.0 (20)	301.0 > 125.0 (7)	
6.45	Diphenylamine	169.1 > 167.1 (30)	169.1 > 77.1 (35)	
6.63	Chlorpropham	212.9 > 171.1 (5)	212.9 > 127.1 (15)	
6.70	Ethalfluralin	276.0 > 202.2 (20)	276.0 > 105.2 (40)	
6.83	Trifluralin	306.1 > 264.1 (15)	306.1 > 206.1 (15)	
7.04	Phorate	260.0 > 75.0 (5)	260.0 > 231.0 (1)	
7.14	BHC, *alpha-*	218.8 > 183.0 (5)	218.8 > 145.0 (20)	
7.28	Hexachlorobenzene (HCB)	283.8 > 248.8 (25)	283.8 > 213.9 (40)	
7.31	Demeton-S	169.9 > 126.0 (1)	169.9 > 93.0 (5)	169.9 > 142.0 (1)
7.33	Dicloran	205.9 > 148.0 (25)	205.9 > 124.0 (30)	
7.58	Clomazone	204.1 > 107.2 (20)	204.1 > 78.2 (40)	
7.71	Lindane (*gamma*-BHC)	218.8 > 183.0 (5)	218.8 > 145.0 (20)	
7.79	Terbufos	231.0 > 175.0 (15)	231.0 > 129.0 (30)	
7.80	Pentachloronitrobenzene (PCNB)	295.0 > 236.8 (20)	295.0> 142.9 (45)	
7.83	Pronamide	172.9 > 109.1 (30)	172.9 > 145.1 (15)	
7.86	Fonofos	246.0 > 137.1 (3)	246.0 > 109.1 (15)	
8.09	Disulfoton	274.0 > 88.0 (1)	274.0 > 60.0 (25)	
8.15	Terbacil	160.9 > 144.1 (15)	160.9 > 88.1 (15)	
8.19	Telfluthrin	197.1 > 141.0 (15)	197.1 > 161.1 (5)	
8.26	Triallate	267.9 > 184.1 (20)	267.9 > 226.1 (15)	
8.91	Vinclozolin	284.9 > 212.0 (15)	284.9 > 178.1 (15)	
8.91	Parathion-methyl	263.0 > 109.0 (10)	263.0 > 79.0 (30)	
8.92	Chlorpyrifos-methyl	286.0 > 93.0 (20)	286.0 > 270.9 (20)	
9.06	Heptachlor	272.1 > 236.9 (15)	272.1 > 143.0 (50)	
9.13	Ametryn	227.1 > 58.3 (10)	227.1 > 155.2 (20)	
9.20	Fenchlorphos	284.9 > 270.0 (15)	284.9 > 93.0 (20)	
9.20	Prometryn	241.1 > 184.2 (10)	241.1 > 58.2 (15)	
9.46	Fenitrothion	277.0 > 260.1 (3)	277.0 > 109.0 (20)	
9.56	Bromacil	207.1 > 134.1 (30)	207.1 > 54.1 (35)	
9.75	Aldrin	262.8 > 193.1 (40)	262.8 > 191.1 (40)	
9.81	Metolachlor	238.1 > 162.2 (10)	238.1 > 133.2 (30)	
9.84	Parathion-d_{10} (ISTD)	301.0 > 115.0 (10)	301.0 > 147.0 (4)	
9.86	Fenthion	278.1 > 109.1 (20)	278.1 > 169.0 (20)	
9.92	Parathion-ethyl	291.1 > 109.1 (10)	291.1 > 81.1 (30)	
9.94	Dicofol (degr. product)	249.9 > 139.1 (10)	249.9 > 215.1 (5)	
10.00	Dacthal	300.8 > 222.9 (30)	300.8 > 257.8 (25)	
10.22	MGK-264 I	164.1 > 98.1 (15)	164.1 > 80.1 (30)	
10.45	Cyprodinyl	223.9 > 208.2 (25)	223.9 > 222.3 (25)	

Table 1. List of Analytes (Individual Isomers Listed), Their Retention Times (RT), and MRM Transitions, Precursor Ion > Product Ion (Collision Energy, V), Used for Quantitative and Qualitative Purposes (Continued)

RT (min)	Analyte	Quant	Qual 1	Qual 2
10.47	MGK-264 II	164.1 > 80.1 (30)	164.1 > 98.1 (15)	
10.58	Heptachlor epoxide	352.8 > 262.9 (20)	352.8 > 282.0 (20)	
10.58	Pendimethalin	252.1 > 162.1 (15)	252.1 > 146.1 (15)	
11.07	Chlordane, *trans*-	372.8 > 265.8 (25)	372.8 > 263.8 (25)	
11.33	Endosulfan I (*alpha*-)	241.0 > 206.0 (15)	207.0 > 172.0 (15)	339.0 > 267.0 (2)
11.39	Chlordane, *cis*-	372.8 > 265.8 (25)	372.8 > 263.8 (25)	
11.62	Napropamide	271.1 > 72.1 (15)	271.1 > 128.1 (5)	
11.86	DDE, *p,p'*-	318.0 > 248.0 (30)	318.0 > 246.0 (30)	
11.88	Dieldrin	262.7 > 193.1 (40)	262.7 > 191.1 (40)	
12.11	Oxyfluorfen	317.0 > 300.0 (7)	361.0 > 300.0 (10)	
12.34	Endrin	262.7 > 193.1 (40)	262.7 > 191.1 (40)	281.0 > 245.0 (7)
12.54	Endosulfan II (*beta*-)	207.0 > 172.0 (15)	241.0 > 206.0 (15)	339.0 > 267.0 (2)
12.76	DDD, *p,p'*-	234.9 > 165.1 (20)	234.9 > 199.1 (15)	
12.88	Oxadixyl	163.1 > 132.2 (5)	233.0 > 146.0 (5)	163.1 > 117.1 (30)
13.33	Carbophenothion-ethyl	342.1 > 157.1 (10)	342.1 > 143 (25)	
13.47	Endosulfan sulfate	271.8 > 237.0 (20)	271.8 > 235.0 (20)	387.0 > 253.0 (10)
13.56	DDT, *p,p'*-	234.9 > 165.1 (20)	234.9 > 199.1 (15)	
13.56	DDT, *p,p'*-$^{13}C_{12}$ (ISTD)	247.0 > 211.0 (15)	249.0 > 211 .0(15)	
13.93	TPP (ISTD)	326.0 > 233.0 (10)	325.0 > 169.0 (20)	
13.98	Piperonyl butoxide	176.1 > 103.1 (30)	176.1 > 117.1 (25)	338.0 > 176.0 (2)
14.22	Iprodione	313.9 > 245.1 (15)	313.9 > 56.2 (30)	
14.43	Methoxychlor, *p,p'*-	227.1 > 169.2 (30)	227.1 > 141.2 (40)	
14.65	Tetradifon	228.8 > 79.0 (30)	228.8 > 145.0 (40)	
14.91	Cyhalothrin, *lambda*- epimer	208.0 > 181.0 (7)	197.0 > 141.0 (10)	197.0 > 161.0 (5)
15.04	Cyhalothrin, *lambda*-	208.0 > 181.0 (7)	197.0 > 141.0 (10)	197.0 > 161.0 (5)
15.12	Fenarimol	219.1 > 107.2 (10)	219.1 > 79.2 (30)	
15.56	Permethrin I	163.0 > 127.0 (5)	165.0 > 127.0 (5)	183.0> 77.0 (25)
15.65	Permethrin II	163.0 > 127.0 (5)	165.0 > 127.0 (5)	183.0> 77.0 (25)
16.05	Cyfluthrin I	226.1 > 206.1 (15)	226.1 > 199.0 (10)	
16.13	Cyfluthrin II	226.1 > 206.1 (15)	226.1 > 199.0 (10)	
16.21	Cyfluthrin III	226.1 > 206.1 (15)	226.1 > 199.0 (10)	
16.24	Cyfluthrin IV	226.1 > 206.1 (15)	226.1 > 199.0 (10)	
16.34	Cypermethrin I	209.0 > 116.0 (15)	209.0 > 103.0 (15)	
16.43	Cypermethrin II	209.0 > 116.0 (15)	209.0 > 103.0 (15)	
16.51	Cypermethrin III	209.0 > 116.0 (15)	209.0 > 103.0 (15)	
16.54	Cypermethrin IV	209.0 > 116.0 (15)	209.0 > 103.0 (15)	
17.24	Fenvalerate [RS,SR]	167.1 > 125.1 (5)	225.0 > 119.0 (15)	419.0 > 167.0 (10)
17.43	Fluvalinate, *tau*- I	250.0 > 55 .0(15)	250.0 > 200.0 (15)	252.0 > 55.0 (15)
17.43	Fenvalerate [RR,SS]	167.1 > 125.1 (5)	225.0 > 119.0 (15)	419.0 > 167.0 (10)
17.48	Fluvalinate, *tau*- II	250.0 > 55 .0(15)	250.0 > 200.0 (15)	252.0 > 55.0 (15)
17.77	Deltamethrin isomer	253.0 > 172.0 (5)	253.0 > 174.0 (5)	253.0 > 93.0 (20)
17.99	Deltamethrin	253.0 > 172.0 (5)	253.0 > 174.0 (5)	253.0 > 93.0 (20)

Use of Internal Standards

Using internal standards (ISTDs) is a good analytical practice to mainly improve precision. In the QuEChERS method, ISTDs can be added to the sample prior to the extraction (to control the entire analytical process) or to the final extract prior to the instrumental analysis. In the latter case, which was preferred by the CDFA, using ISTDs can correct potential volumetric issues caused during the preparation of final extract or during sample injection, such as potential presence of small bubbles in the syringe. Specific issues, such as compound losses or signal variability due to degradation in the GC inlet or column, can be addressed when a suitable, compound-specific ISTD is used for signal normalization.

Figure 1 shows structures of two similar pesticides, *p,p′*-DDT and *p,p′*-methoxychlor, that are known to degrade in the GC inlet. Table 2 compares mean accuracies (relative ratios of calculated versus theoretical/expected concentration) obtained for *p,p′*-DDT and *p,p′*-methoxychlor in QC samples and all calibration standards in plum matrix: (i) without the use of any ISTD, (ii) using triphenyl phosphate (TPP) as a generic ISTD for pesticide residue analysis, and (iii) using labeled $^{13}C_{12}$-*p,p′*-DDT as the ISTD for both *p,p′*-DDT and *p,p′*-methoxychlor. The results in Table 2 show that even the use of a generic ISTD, such as TPP, can improve precision as compared to the situation when no ISTD is employed. This is demonstrated by the almost 50% reduction in the RSD values when TPP was used as the ISTD. Even more dramatic reduction in the RSD values, thus improvement in precision, was obtained when employing $^{13}C_{12}$-*p,p′*-DDT as the ISTD for both *p,p′*-DDT and *p,p′*-methoxychlor.

The general use of labeled ISTDs in pesticide multiresidue methods is problematic because of their availability and cost. In specific cases, such as the case of *p,p′*-DDT, for which a labeled standard is commercially available and the issues are mainly GC-related, the post-extraction addition represents a more cost-effective use of this ISTD than if it was added prior to the extraction (for example, if the final extract is 0.25 mL while the initial acetonitrile extract is 10 mL, then only 1/40 of the ISTD is required). Moreover, compounds with similar properties can share the same ISTD, addressing similar behavior, as demonstrated by the use of $^{13}C_{12}$-*p,p′*-DDT as the ISTD for *p,p′*-methoxychlor.

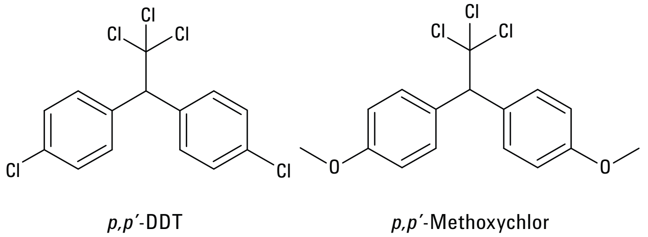

Figure 1. Structures of p,p′-DDT and p,p′-methoxychlor.

Table 2. Comparison of Mean Accuracies and Their Relative Standard Deviations (RSDs) Obtained for p,p′-DDT and p,p′-Methoxychlor in the QC Samples and all Calibration Standards in Plum Matrix: (i) Without the Use of any ISTD, (ii) using TPP as a Generic ISTD for Pesticide Residue Analysis, and (iii) using $^{13}C_{12}$- p,p′-DDT as the ISTD for both p,p′-DDT and p,p′-Methoxychlor

	***p,p′*-DDT**		***p,p′*-Methoxychlor**	
ISTD	**Mean accuracy (%)**	**RSD (%), *n* = 31**	**Mean accuracy (%)**	**RSD (%), *n* = 31**
None (i)	95.5	14	94.3	13
TPP (ii)	100	7.8	98.0	6.9
$^{13}C_{12-}$ *p,p′*-DDT (iii)	100	1.5	98.3	2.0

PDP Requirements and GC/MS/MS Method Performance

PDP issues standard operating procedures (SOPs) that provide procedures and requirements for method validation and QC [1]. Calibration criteria, including calibration integrity and calibration curve fit, are most important for instrument method performance. Calibration integrity is defined as steady instrument response to a given amount of the analyte over the duration of the analytical sequence. Calibration integrity can be calculated as percent difference (%D) using the following equation:

$$\%D = \frac{C_1 - C_2}{C_1} \times 100$$

where C_1 is the known concentration of the analyte in a calibration standard and C_2 is the concentration of that standard calculated using the calibration curve. PDP specifies that %D should be less than or equal to 20%, thus, the relative back-calculated concentrations (% accuracies) in all calibration standards and post-extraction QCs should be within 80–120% of the theoretical (known) values.

If calibration curves are used for quantification of PDP samples, they should be constructed using matrix-matched standards, which bracket the expected range of residue concentration. A suggested concentration range is 1 × LOQ to 10 × LOQ. Second-order curves (that is, quadratic) may be used, providing that a sufficient number of points (a minimum of five points) are used to define the curve. The fitness of the curve should be demonstrated in the same injection sequence used to report the data by the correlation coefficient (where $R > 0.995/R^2 > 0.990$), percent relative standard deviation (where $\%RSD \leq 20$), or percent difference of calculated versus known standard concentration in the curve (where the difference is within 20%).

Table 3 provides correlation coefficients and calibration integrity data obtained in a sequence of plum matrix samples described in Experimental. The plum matrix was selected for this demonstration because it was causing the most variability and calibration integrity issues in the CDFA laboratory prior the implementation of this new method, which was successfully tested and implemented for other PDP matrices analyzed at the CDFA. Table 3 shows that very good calibration fit ($R \geq 0.997$ for all analytes), calibration integrity (individual accuracies in the range of 83–119%), and overall accuracy and precision (mean accuracies and related RSDs in the range of 95–102% and 1.5–8.3%, respectively) were obtained for all tested analytes at all calibration levels ranging from 1 × LOQ to 10 × LOQ throughout the entire analytical sequence.

Table 3. Calibration Curve Correlation Coefficients (R) and Calibration Integrity (Minimum, Maximum, and Mean Accuracy Results and RSDs) Obtained for the Tested Analytes in the Calibration Standards and QC Samples Injected in a Plum Matrix Sequence Described in Experimental

		Accuracy (%)			
Analyte	**R**	**Min**	**Max**	**Mean**	**RSD (%), *n* = 31**
1-Naphthol	1.000	90.6	114	101	4.9
Aldrin	0.999	91.0	113	101	4.6
Ametryn	1.000	97.5	105	101	1.9
BHC, *alpha*-	0.999	93.0	109	100	3.7
Bromacil	1.000	96.4	104	101	2.1
Carbophenothion-ethyl	1.000	89.3	105	99.0	3.4
Chlordane, *cis*-	1.000	94.6	108	100	3.2
Chlordane, *trans*-	1.000	94.9	105	101	2.5
Chlorethoxyfos	1.000	90.6	111	99.0	4.8
Chlorpropham	0.999	96.7	107	101	2.5
Chlorpyrifos-methyl	0.999	94.1	116	102	4.8
Clomazone	1.000	94.8	107	100	2.9
Cyfluthrin I-IV	1.000	87.1	103	97.0	4.0
Cyhalothrin, *lambda*-	0.999	90.5	106	101	4.9
Cyhalothrin, *lambda*- epimer	0.999	90.4	103	102	3.7
Cypermethrin I-IV	1.000	92.3	102	99.0	4.8
Cyprodinyl	1.000	94.0	102	99.0	1.7
Dacthal	1.000	94.3	106	100	2.7
DDD, *p,p'*-	0.999	89.4	107	98.0	5.1
DDE, p,p'-	1.000	94.9	105	100	2.5
DDT, *p,p'*-	1.000	97.2	103	100	1.5
Deltamethrin	1.000	83.2	104	99.0	4.8
Demeton-O	0.999	90.2	111	99.0	4.8
Demeton-S	1.000	92.9	106	100	3.0
Dichlobenil	0.997	89.3	117	101	7.0
Dicloran	1.000	92.3	110	99.0	4.5
Dicofol (degr. product)	1.000	96.5	104	100	1.8
Dieldrin	1.000	96.5	108	101	2.7
Diphenylamine	0.999	91.4	105	98.0	3.8
Disulfoton	0.999	94.1	105	100	3.0
Endosulfan I (*alpha*-)	1.000	95.3	110	101	3.1
Endosulfan II (*beta*-)	1.000	93.6	110	102	3.1
Endosulfan sulfate	1.000	96.1	109	102	2.9
Endrin	1.000	97.2	109	102	2.8
Ethalfluralin	1.000	87.7	104	98.0	3.9
Fenarimol	1.000	95.8	102	99.0	1.6
Fenchlorphos	1.000	95.8	107	101	3.0
Fenitrothion	1.000	87.4	109	99.0	5.0
Fenthion	1.000	96.3	104	100	1.9

Table 3. Calibration Curve Correlation Coefficients (R) and Calibration Integrity (Minimum, Maximum, and Mean Accuracy Results and RSDs) Obtained for the Tested Analytes in the Calibration Standards and QC Samples Injected in a Plum Matrix Sequence Described in Experimental (Continued)

		Accuracy (%)			
Analyte	**R**	**Min**	**Max**	**Mean**	**RSD (%), *n* = 31**
Fenvalerate [RR,SS]	1.000	84.8	104	95.0	5.0
Fenvalerate [RS,SR]	1.000	83.6	103	96.0	4.8
Fluvalinate, *tau*- I+II	1.000	82.9	106	98.0	3.8
Fonofos	0.999	94.0	108	100	3.4
Heptachlor	1.000	91.9	107	98.0	3.9
Heptachlor epoxide	1.000	94.0	105	100	3.1
Hexachlorobenzene (HCB)	0.998	91.4	115	101	6.1
Iprodione	1.000	95.3	106	101	2.6
Lindane (*gamma*-BHC)	1.000	94.8	105	100	2.6
Methoxychlor, *p,p'*-	1.000	95.6	103	98.3	2.0
Metolachlor	1.000	96.7	106	101	2.4
MGK-264 I	1.000	95.2	105	100	2.5
MGK-264 II	1.000	94.4	108	101	2.9
Napropamide	1.000	97.2	105	101	1.8
o-Phenylphenol	0.999	91.5	111	101	4.7
Oxadixyl	1.000	96.8	104	100	2.2
Oxyfluorfen	1.000	83.4	104	96.0	5.1
Parathion-ethyl	1.000	95.3	103	99.0	1.8
Parathion-methyl	1.000	90.2	109	100	5.0
Pendimethalin	1.000	89.5	104	99.0	3.6
Pentachlorobenzene (PCB)	0.999	87.1	119	100	8.3
Pentachloronitrobenzene (PCNB)	0.999	91.0	108	99.0	4.4
Permethrin I	1.000	96.3	105	101	4.7
Permethrin II	1.000	95.5	103	100	8.3
Phorate	1.000	93.9	106	100	3.3
Piperonyl butoxide	1.000	93.0	102	98.0	2.2
Prometryn	1.000	95.9	104	100	1.8
Pronamide	1.000	94.6	104	100	2.4
Propham	0.999	90.7	111	100	4.8
Telfluthrin	1.000	95.6	104	100	2.4
Terbacil	1.000	95.9	104	101	1.9
Terbufos	1.000	92.8	105	99.0	3.1
Tetradifon	1.000	95.8	107	101	2.1
Tetrahydrophthalimide (THPI)	0.999	94.3	110	102	3.7
Triallate	0.999	95.0	107	100	3.5
Trifluralin	1.000	90.4	104	98.0	3.5
Vinclozolin	1.000	95.0	104	100	2.2

Figure 2 illustrates this further by showing accuracy results obtained for all analytes in calibration standards and QC samples at the 2 × LOQ level, which is the concentration level recommended for routine recovery check in the PDP sample analysis. Figure 3 shows examples of calibration curves (calibration points shown as black dots) for representative analytes, which were constructed using the matrix-matched standard set injected in the middle of the sequence (see Experimental). The QC results (depicted as blue triangles in the charts) are analyte responses obtained in the QC samples injected throughout the sequence and in calibration standards analyzed at the beginning and end of the sequence.

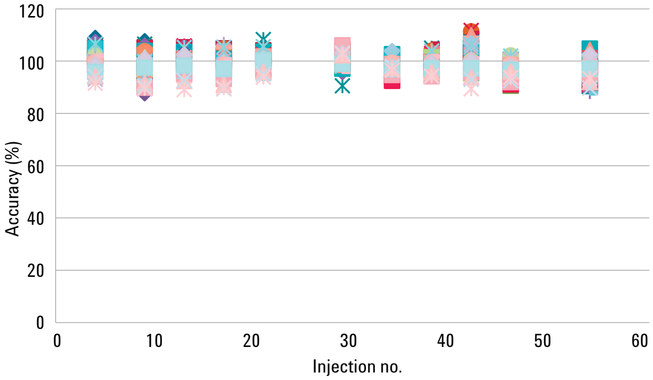

Figure 2. *Accuracy (%) obtained for all tested analytes at the 2 × LOQ concentration level in calibration standards and QC samples injected throughout the sequence of samples described in Experimental.*

Conclusions

The Agilent 7000B Triple Quadrupole GC/MS System allows for sensitive, selective, and reliable analysis of pesticide residues in various matrices, including PDP fruit and vegetable samples. Excellent results, fulfilling the PDP quality control requirements, have been achieved using well-optimized method conditions and procedures. Using the Multi-Mode Inlet in PTV solvent vent mode enables injection of larger volumes of QuEChERS extracts in acetonitrile without affecting analyte peak shapes. Column backflushing prevents contamination of the MS ion source and offers time-effective elimination of less-volatile matrix components from the system, especially when concurrent backflushing is employed in the method. The addition of suitable analyte protectants and internal standards can improve overall method performance, mainly when it comes to analytes that are susceptible to losses (due to degradation and/or adsorption) in the GC inlet or column.

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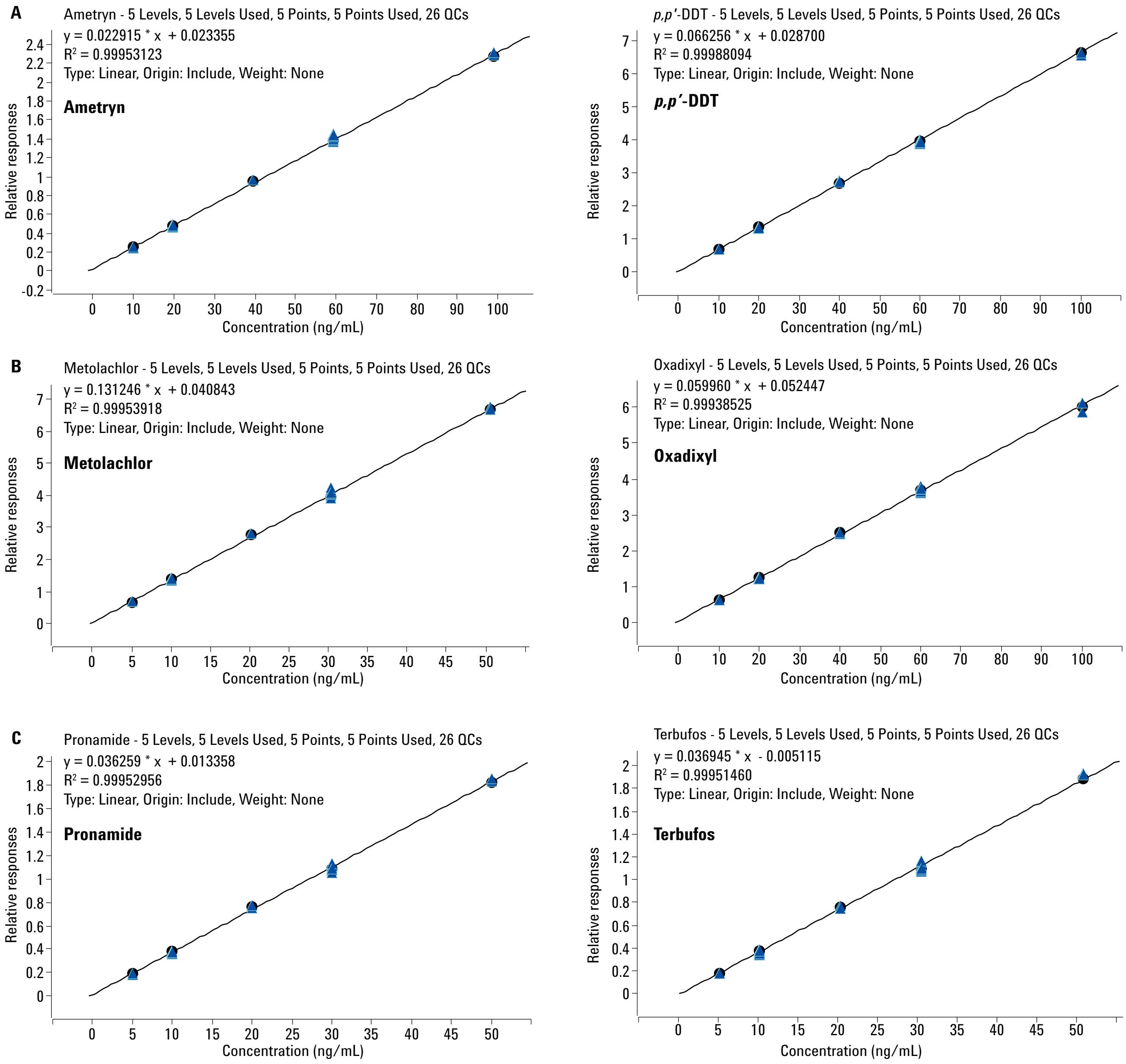

Figure 3. Calibration curves (calibration points shown as black dots) and QC results (depicted as blue triangles in the charts) obtained in plum matrix for representative pesticides (ametryn, p,p′-DDT, metolachlor, oxadixyl, pronamide, and terbufos).

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