Analysis of Fruit and Vegetable Pesticides by GC/MS/MS Using Agilent Inert Flow Path

Application Note

Food Testing and Agriculture

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Abstract

The Agilent Inert Flow Path (IFP) solution was evaluated and validated thoroughly for the analysis of trace pesticides in various fruits and vegetables by GC/MS/MS. The results showed that the IFP provides excellent surface inertness for the entire GC flowpath and thus reduces negative impacts on the target analytes caused by surface active sites. Additionally, the comparison results also showed that the Agilent IFP provides more benefits for some critical pesticides in certain matrices than those provided by corresponding deactivated components from a non-Agilent supplier.

Introduction

Flowpath inertness plays a critical role in the accuracy, precision, durability, and consistency in pesticide analysis in complicated sample matrices. The Agilent IFP solution, including Ultra Inert column, Ultra Inert inlet liner and gold seal, with UltiMetal Plus inert inlet, capillary flow technology (CFT) devices, and flexible metal ferrules, provides excellent surface inertness for the entire GC flowpath and reduces negative impacts on target analytes caused by surface active sites.

Multiresidue analysis of pesticides is always a challenge for GC and GC/MS detection. The required quantitation limits for many pesticides are at low ppb levels that demand more sophisticated analytical processes. Compared to widely used GC/MS, GC/MS/MS technique provides much better selectivity, thus significantly improve system detection limits.

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Pesticide compounds frequently contain functional groups such as hydroxyl (-OH) and amino (R-NH-) groups, imidazoles and benzimidazoles (-N=), carbamates (-O-CO-NH-), urea derivatives (-NH-CO-NH-), and organophosphate (-P=O) groups. These types of molecules are prone to interact with active sites on flowpath surfaces, resulting in compound adsorption or degradation. As a result, flowpath surface inertness is critical for trace pesticide analysis.

The QuEChERS sample preparation method was introduced for pesticide analysis in food by USDA scientists in 2003 [1]. It has been rapidly accepted worldwide for multiresidue pesticide analysis due to its special features, referred to as quick, easy, cheap, effective, rugged, and safe. QuEChERS extracts are concurrently analyzed by LC and GC combined with MS to determine a wide range of pesticide residues. However, these food extracts processed by the QuEChERS method are still complicated and contain impurities such as high-boiling indigenous compounds. When using GC/MS or GC/MS/MS as the instrument detector, the QuEChERS extracts can cause contamination and deterioration of the analytical column and MS source, thus resulting in inaccurate results due to the poor peak shape and intensity of active compounds. QuEChERS extracts also lead to shortened life times of analytical columns and frequent MS maintenance. Therefore, it is desirable to use technologies and supplies to achieve reliable results and, at the same time, maximize protection of the analytical column and MS source.

Agilent Ultra Inert components have been demonstrated to provide excellent surface inertness for trace pesticide analysis [2-6]. In this study, the entire Agilent IFP was evaluated for multiple pesticide analysis in various fruit and vegetable matrices. A non-Agilent flowpath was built with corresponding deactivated components and compared with the Agilent IFP. In addition, the impact of using analyte protectant, along with the Agilent IFP, was investigated. The results were evaluated and compared for calibration curve linearity in the matrix, spiked QC quantitation results for accuracy and precision, and durability for multiple injections over time. A sandwiched injection method was applied for online matrix spiking by either two-layer or three-layer injection. By using sandwiched injection, different matrix calibration curves were achieved by injecting one set of calibration standards together with sandwiched injection of matrix blank. This saved significant bench work to prepare the matrix-matched calibration standards, while increasing the consistency and decreasing the potential errors caused by preparation mistakes. Six different matrixes, including strawberry, orange, plum, onion, red pepper, and spinach, were used for system performance evaluation.

Experimental

All reagents and solvents were HPLC or analytical grade. Acetonitrile (ACN) was from Honeywell B&J (Muskegon, MI, USA). Ultra Resi-analyzed grade acetone was from J. T. Baker (Phillipsburg, NJ, USA). Acetic acid was from Sigma-Aldrich Corp. (St Louis, MO, USA). The pesticide standards and internal standard (triphenyl phosphate, TPP) were purchased from Sigma-Aldrich Corp, Chem Service (West Chester, PA, USA), or Ultra Scientific (North Kingstown, RI, USA). L-gulonic acid γ-lactone and D-sorbitol were from Sigma-Aldrich Corp.

Sample preparation

A 1% acetic acid solution in ACN was prepared by adding 1 mL glacial acetic acid to 100 mL ACN, and was used as reagent blank. This solution was also used as extraction solvent for the QuEChERS method and blank solvent to prepare neat pesticide standards. Standard and internal standard (IS) stock solutions (2 mg/mL) were made in acetone, individually, and stored at –20 °C. A 20 µg/mL mixed standard (32 pesticides) solution was made in acetone by proper dilution of individual pesticide stock solutions. A 20 µg/mL triphenyl phosphate solution made in ACN was used as internal standard (IS) spiking solution by appropriate dilution of individual pesticide stock solutions. Eight standard solutions of 2, 4, 10, 20, 40, 100, 150, and 200 ng/mL were prepared in reagent blank by appropriate dilution of 20 µg/mL mixed standard solution. Three QC levels of 20, 100, and 200 ng/mL were prepared in matrix blanks by diluting 20 µg/mL mixed standard solution as appropriate. IS solution was then spiked into samples to generate a concentration of 200 ng/mL in the sample.

L-gulonolactone stock solution (50 mg/mL) and D-sorbitol stock solution (50 mg/mL) were made in water. An analyte protectant solution (20 mg/mL L-gulonolactone and 10 mg/mL D-sorbitol) was then made by appropriate dilution of the stock solutions with acetonitrile. The solution (40 µL) was spiked into 500 µL matrix blank or reagent blank, for sandwiched injection together with standards or samples.

Six types of fruits and vegetables were used to prepare matrix blank samples, including strawberry, orange, plum, onion, red pepper, and spinach. The standard AOAC QuEChERS extraction procedure was followed to prepare matrix blank using Agilent Bond Elut QuEChERS AOAC Extraction kit and Dispersive SPE kit for general fruits and vegetables.

Instrumentation

All testing was done on an Agilent 7890A GC equipped with a 7693B Autosampler and 7000 Series GC/MS/MS system. Multiple-reaction-monitoring (MRM) conditions for 32 target analytes were listed in previous publications [3,5] and referenced exactly in this study, thus they are not repeated here. As mentioned previously, the MRM method optimization process can easily be achieved by using the Agilent Pesticides and Environmental Pollutants MRM Database (G9250AA), which contains MS/MS conditions and retention time information for over 1,070 compounds [7].

Backflushing was used, which is highly recommended for complicated sample matrices [8,9]. Retention time locking (RTL) was used to eliminate the need for recalibration of the individual retention times and timed events such as MRM groups [10]. The total run time for a sample spiked with standard was 23 minutes with 2 minutes for backflush. For matrix blanks run in the middle of the sequence, a fast oven gradient with 8 minutes and 2 minutes backflush were used to save time.

Column and sample prep

Column

Agilent J&W HP-5ms UI, 15 m × 0.25 mm, 0.25 µm (p/n 19091-431UI)

Sample prep

Agilent Bond Elut QuEChERS AOAC Extraction kit (p/n 5982-5755) and Dispersive SPE kit for general fruits and vegetables (p/n 5982-5022)

GC conditions

GC:	Agilent 7890A GC
Autosampler:	Agilent 7693 Autosampler and sample tray 5 µL syringe (p/n 5181-5246), 1 µL injection volume Three pre-injection solvent A (acetone) washes Three sample pumps Three post-injection solvent B (acetonitrile) washes
Sandwich injection:	Total volume 1.1 µL For clean standards not in matrix, the three-layer sandwich injection consisted of 0.5 µL clean standard + 0.5 µL matrix blank + 0.1 µL solvent blank. For samples already in matrix, the two-layer sandwich injection consisted of 0.5 µL matrix sample + 0.6 µL solvent blank.
Carrier:	Helium, constant pressure
Gas filter:	Gas Clean Kit for GC-MS, 1/8 inch (p/n CP17974)
Inlet:	UltiMetal split/splitless inlet (p/n G3970A) at pulsed splitless mode, 280 °C
Injection pulse pressure:	36 psi until 1 minute
Purge flow to split vent:	50 mL/min at 1 minute
Inlet pressure:	18.35 psi (RT locked) during run, and 1.0 psi during back flushing
RT locking:	Chlorpyrifos methyl at 8.298 minutes
Oven:	For sample run, 100 °C for 2 minutes, then to 150 °C at 50 °C/min, to 200 °C at 6 °C/min, to 300 °C at 16 °C/min and hold for 2 minutes For matrix blank run, 100 °C for 1 minute, then to 300 °C at 50 °C/min and hold for 1 minute
Post run:	2 minutes at 300 °C (for sample run), 4 minutes at 300 °C (for matrix blank run)
Capillary Flow Technology:	UltiMetal Plus Purged Ultimate Union (p/n G3182-61581) for backflushing the analytical column and inlet
Aux EPC gas:	Helium plumbed to Purge Ultimate Union
Bleed line:	0.0625-inch od × 0.010-inch id × 100 cm, 316SS tubing, on top of the oven
Aux pressure:	4 psi during run, 75 psi during backflushing
Connections:	Between inlet and Purged Ultimate Union
Restrictor:	Inert fused silica tubing, 0.65 m × 0.15 mm (p/n 160-7625-5)
Connections:	Between Purged Ultimate Union and the MSD

MSD conditions

MSD:	Agilent 7000 Triple Quadrupole GC/MS System, inert, with performance electronics
Vacuum pump:	Performance turbo
Mode:	MRM
Tune file:	Atune.u
Transfer line temperature:	280 °C
Source temperature:	300 °C
Quad temperature:	150 °C for Q1 and Q2
Solvent delay:	2.3 minutes
Collision gas flow:	He quench gas at 2.35 mL/min, N_2 collision gas at 1.5 mL/min
MS resolution:	MS1 and MS2 = 1.2u

Flow path supplies

Vials:	Amber screw-cap (p/n 5182-0716)
Vial caps:	Blue screw-cap (p/n 5182-0717)
Vial inserts:	150 µL Glass with polymer feet (p/n 5183-2088)
Septum:	Advanced Green, Non-Stick, 11 mm (p/n 5183-4759)
Ferrules:	0.4 mm id, 85/15 Vespel/graphite inlet ferrules (p/n 5181-3323), 0.4 mm id, 85/15 Vespel/graphite MS interface ferrule (p/n 5062-9526), UltiMetal Plus Flexible Metal ferrules (p/n G3188-27501)
Capillary flow technology:	Purged Ultimate Union (p/n G3182-61581)
Internal nut:	p/n G2855-20530
Bleed line:	0.0625-inch od × 0.010-inch id × 100 cm, 316SS tubing (p/n 0100-2354)
Tee:	1/16 inch Brass Swagelok (p/n 0100-0680)
Bleed tee ferrule:	1/16 inch Vespel, 10/pk (p/n 0100-1329)
Inlet seal:	Ultra Inert gold-plated inlet seal with washer (p/n 5190-6144)
Inlet liners:	Agilent Ultra Inert deactivated single taper splitless liner with wool with pre-installed nonstick O-ring (p/n 5190-2293)

Using comparable deactivated components from a non-Agilent supplier, the configurations for the Agilent IFP and the other inert flow components are listed in Table 1.

A split/splitless inlet was used in this study under hot splitless mode. Although a MultiMode Inlet (MMI) has been highly recommended for pesticide analysis [11], the split/splitless inlet remains the most common inlet used for such applications. The MMI can show improved performance for many active analytes as it can also be operated in cold splitless or LVI mode, where active analytes could behave better at low temperature.

Sample injection sequence

A typical sample batch contained a total of 72 injections, which included reagent blank, matrix blanks, six sets of matrix-spiked QCs at three levels, and a set of calibration standards at eight levels. One set of QCs were injected roughly every 10 injections, except the injections of 10th to 20th, which were taken by the calibration standards. Matrix blanks were injected between QCs and standards when needed. A fast oven temperature program was used for most of the matrix-blank runs to shorten the instrument running time for the entire sequence.

Results and Discussion

The purpose of these tests was to evaluate the Agilent IFP for the analysis of pesticides in fruit and vegetable matrices by GC/MS/MS. The evaluation and comparison were based on the calibration curve linearity (R^2) over the 2 to 200 ng/mL range. Matrix-spiked QC accuracy was based on recovery and precision was based on %RSD and 95% confidence interval (CI). For each sequence, the average QC recovery and %RSD or 95% CI were calculated based on n = 18 for each pesticide. Since three levels of QCs were included, and six sets of QCs were run over a total of 70 injections, the average QC accuracy and precision data reflected not only the repeatability of the method over multiple injections of matrix samples, but also the response linearity from low to high concentrations.

Table 1. GC flowpath deactivated components for comparison.

Flowpath	Split/splitless inlet	Inlet seal	Liner	Column	CFT device and ferrules
Agilent Inert Flow Path	UltiMetal Plus	Ultra Inert Gold Seal	Ultra Inert single taper splitless with wool	HP-5ms UI	UltiMetal Purged Ultimate Union UltiMetal Flexible Metal ferrules
Competitor's flowpath with deactivated components	Siltek-treated	Siltek-treated stainless steel seal	Sky single taper splitless with wool	Rxi-5ms	Siltek purged ultimate union Siltite metal ferrules

Sandwiched injection

Sandwiched injection is a simple concept but very useful for various sample matrix analyses. The use of matrix-matched calibration standards has always been widely accepted in pesticide analysis to reduce the impact of the matrix and achieve accurate and reliable quantitation results. However, the preparation of matrix-matched calibration standards is a tedious and time-consuming procedure, especially when multiple sample matrices are to be analyzed. This practice also can introduce human errors during preparation, and thus directly cause a failed run. The concept of sandwiched injection takes advantage of the 7693 autosampler's two-layer and three-layer injection functions, and uses these functions for online matrix spiking during sample injection. As a result, only one set of calibration standards in neat solvent is needed, and these standards can be used for the analysis of various sample matrices. For example, in this study, one set of calibration standards (eight) were prepared in reagent blank, and then used for six different matrix sample analyses by sandwich injecting matrix blanks during the sequence run. This significantly reduces the time and effort needed for sample preparation benchwork, makes quantitation more consistent across different matrix samples, and reduces unpredictable human error on calibration standard preparation and unwanted failed sequences.

The two-layer injection mode allows the autosampler's syringe to withdraw an aliquot of samples from two different vials in one injection, while the three-layer injection mode allows the syringe to withdraw an aliquot of sample from three different vials in one injection. A default air gap is included to prevent cross contamination when withdrawing sample from another vial. The entire sample is then injected into the inlet liner for vaporization, mixing and injection onto the GC column. Since an Ultra Inert liner with wool was used, the highly deactivated wool inside the liner provided a large surface area that aided the vaporization of liquid samples and promoted homogenous sample mixing in the liner prior to samples entering the column. For the calibration standards prepared in reagent blank, an aliquot of matrix blank can be added, while for the matrix samples, an aliquot of reagent blank can be added. It is important to keep the ratio of solvent and matrix in the final injection volume consistent for different sample sandwich injections, otherwise, the matrix can be diluted differently and result in different matrix effects, or target analytes can be diluted differently. Either could deliver misleading quantitation results.

Since either matrix blank or reagent blank is added during injection, the actual sample is injected with less volume, which increases the method limit of quantitation. Fortunately, given the high sensitivity and selectivity provided by GC/MS/MS, the instrument detection limits and quantitation limits are usually far below pesticide MRLs in fruits and vegetables. Therefore, the sensitivity loss by a lower injection volume will not impact the pesticide analysis requirement. Given the obvious benefits provided by sandwiched injection, it is worth considering the use of this method in pesticide analysis in various sample matrices.

To validate sandwiched injection methodology, a series of calibration standards (1 to 100 ng/mL) was prepared in a strawberry matrix blank, and a method with normal 1 µL injection volume was used. Another set of calibration standards (2 to 200 ng/mL) was prepared in reagent blank, and another method with sandwiched injection of 0.5 µL of standards plus 0.5 µL of strawberry blank was used. The two sets of calibration curves were then used to quantitate the same strawberry QCs spiked at 20 and 100 ng/mL. The results demonstrated that the sandwiched injection method can provide very similar chromatography (Figure 1), excellent linearity (Figure 2), and accurate and precise quantitation results (Figure 3). It is thus fully validated for use in pesticide analysis in sample matrices.

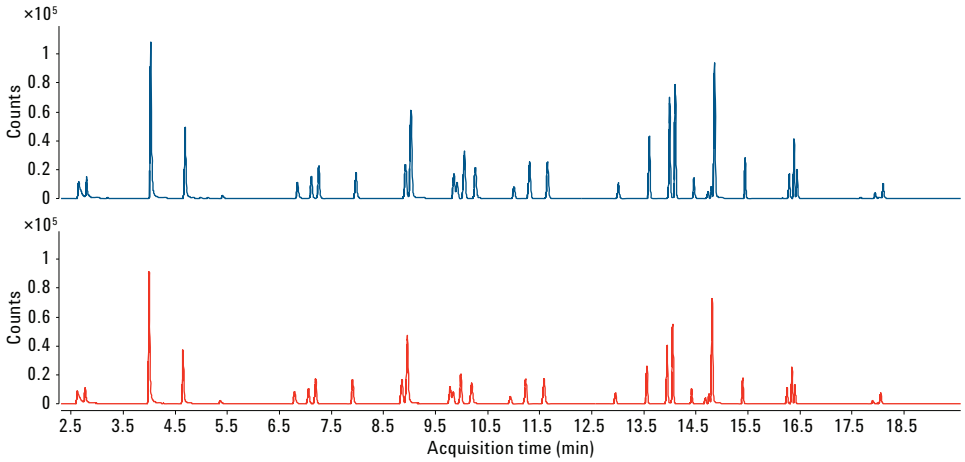

Figure 1. Chromatograms of 100 ng/mL standards in strawberry matrix blank using normal injection (blue) and sandwiched injection (red).

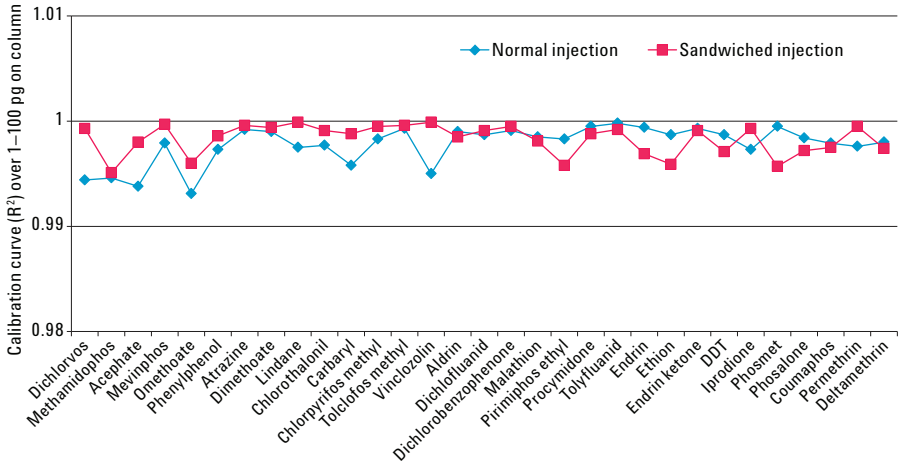

Figure 2. Calibration curve coefficients (R^2) comparison for normal injection and sandwiched injection method. Calibration range for all of pesticides 1 to 100 pg on column.

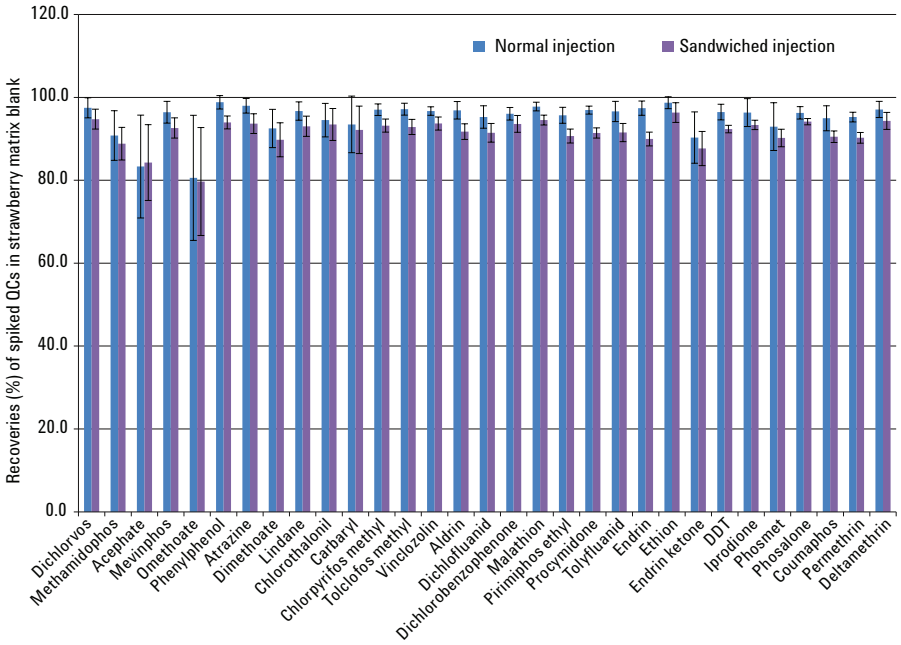

Figure 3. Strawberry matrix spiked QCs, 20 and 100 ng/mL, recovery comparison between normal injection and sandwiched injection, n = 12. Error bars = 95% CI.

Method validation in six sample matrixes

The established GC/MS/MS method using Agilent IFP was validated in six different sample matrices, namely strawberry, orange, plum, onion, red pepper, and spinach. The 2 ng/mL limit of quantitation (LOQ) was achieved for all pesticides in all six matrices. A typical MRM chromatogram of LOQ in red pepper is shown in Figure 4 as an example for peak identification. The stability of three base-sensitive pesticides, chlorothalonil, dichlofluanid and tolyfluanid, are worthy of being mentioned. These three pesticides are very labile in onion matrix, and can degrade quickly when spiked into matrix. For the onion samples, the calibration curves for these three compounds were still acceptable, because sandwiched injection prevented their degradation due to a very short interaction time between the compounds and the onion matrix. However, even when preparing QC samples just before

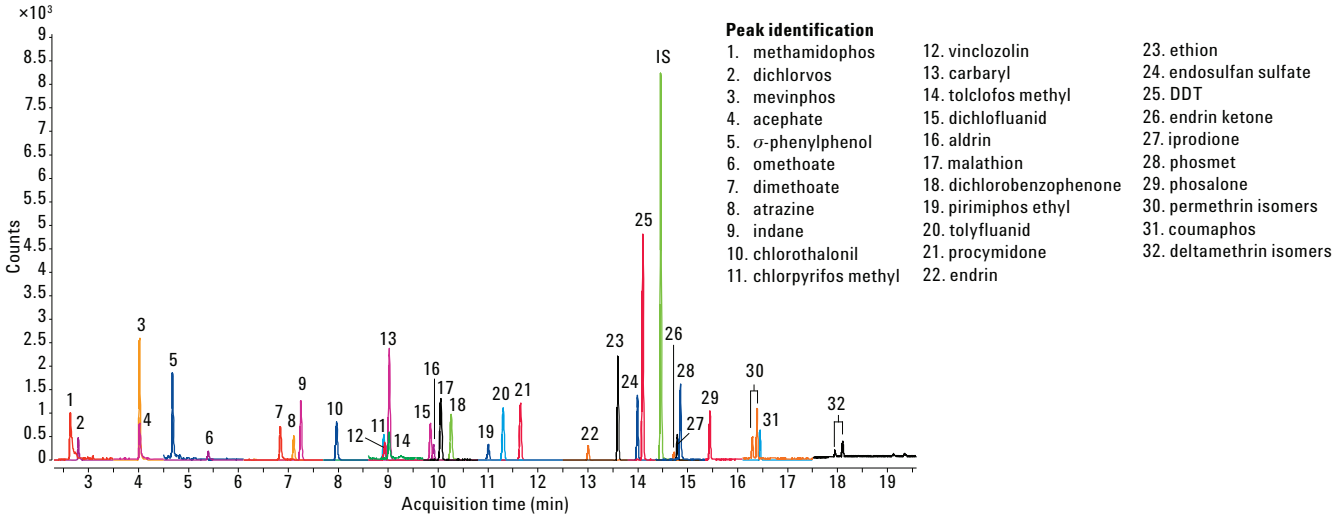

Figure 4. GC/MS/MS chromatogram (MRM) for 20 ng/mL spiked orange sample using Agilent Inert Flow Path.

running samples, the recovery and precision results were poor due to significant signal drop for the later injected samples. When QCs were not freshly spiked, these compounds were lost completely and not observed in the chromatogram. This observation indicated that further sample preparation modifications are needed to better preserve these base-sensitive pesticides in onion matrix.

Figure 5 shows the calibration curve coefficient (R^2) for all pesticides in the six matrices. Excellent linearity ($R^2 > 0.99$) was achieved in all cases, even for very difficult pesticides such as acephate, omethoate, etc. Figure 6 shows the average recoveries with 95% CI precision for the matrix-spiked QC quantitation. In general, satisfactory recoveries and precision were achieved for most pesticides in the six sample matrices. Poor quantitation results for the three base-sensitive pesticides in onion were due to poor stability of these compounds in onion matrices. Endosulfun sulfate was found in strawberry blank samples, thus it was not quantifiable with the current methodology in strawberry extract. Lower average recoveries (< 80%) with higher 95% CI for acephate and omethoate were observed in several matrices; however, these were attributed to signal drop over multiple injections. Acephate and omethoate are very difficult pesticides for GC or GC/MS analysis. It was demonstrated that the use of IFP can provide significantly higher responses and better peak shapes than a traditional noninert GC flowpath [4,6]. However, signal loss for these two compounds over multiple injections still resulted in unacceptable recoveries after about 40 to 50 injections, especially in certain matrices such as orange, onion, spinach, etc. The use of analyte protectant could make signals more consistent in some matrices, but not in all of the tested matrices. Many labs doing pesticide analysis actually move these difficult pesticides from GC/MS to LC/MS/MS for more reliable and durable analysis.

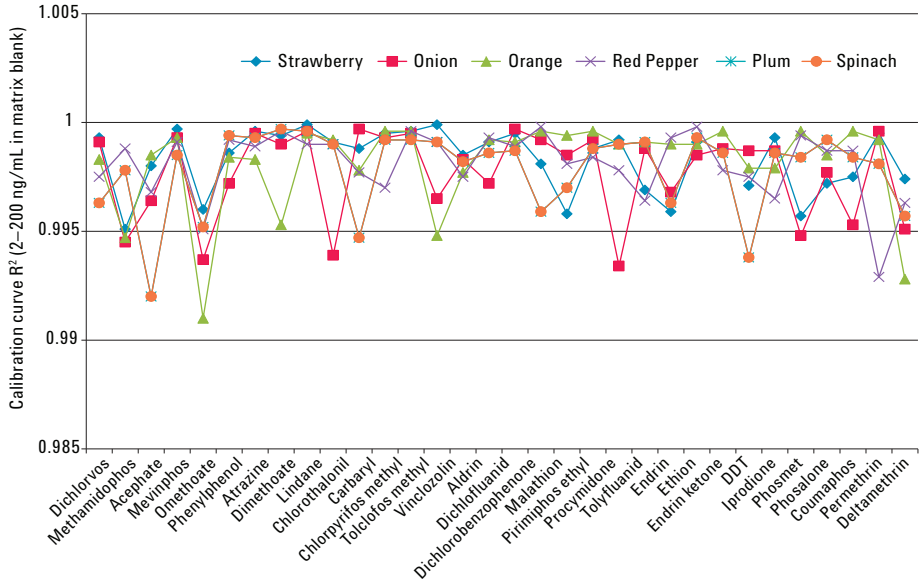

Figure 5. Calibration curve coefficients (R^2) for 32 pesticides in six different matrixes on GC/MS/MS using Agilent Inert Flow Path. Calibration range for all of pesticides 2 to 200 ng/mL.

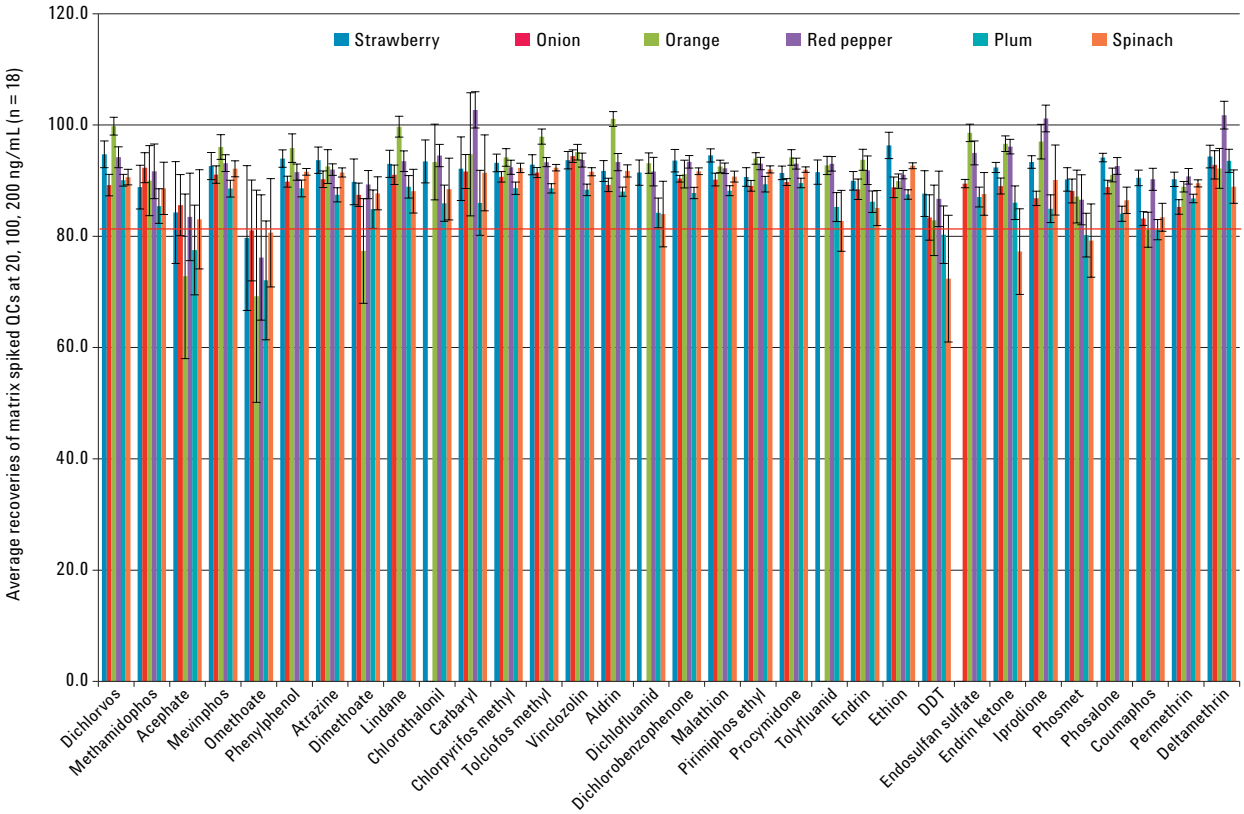

Figure 6. Six types of matrix-spiked QC quantitation results by GC/MS/MS using Agilent Inert Flow Path. Three levels of QCs at 20, 100, and 200 ng/mL spiked in matrix blank extract. n = 18. Error bars = 95% CI.

Agilent Inert Flow Path versus non-Agilent deactivated components

The Agilent IFP was compared with corresponding deactivated components from another supplier. The deactivated components are listed in Table 1. The two GC flowpaths were installed on the same GC/MS/MS. The Agilent IFP was installed using the front inlet, connected to the MS and tested first. Then the non-Agilent deactivated components were installed using the back inlet, connected to the MS, and tested. The same samples were used for comparison tests on both flowpaths. The MS was tuned every time the GC channel (inlet and column) entering the MS was switched.

Overall, the Agilent IFP provided higher responses than the non-Agilent deactivated component flowpath. Figure 7 shows chromatograms of 20 ng/mL spiked QC in six different matrices using Agilent IFP and the non-Agilent deactivated components. Different sample matrices showed specific differences. In matrices such as red pepper, orange, and onion, the differences were more obvious, while in other matrices such as plum and spinach, the overall responses were more comparable. This overall response increase with the Agilent IFP was reported previously [12]. However, the overall response increases cannot be completely attributed to an improvement on surface inertness, because the increases were noticeable for both active and many stable analytes, and the peak-area ratios of analytes/IS for most analytes were comparable between the two versions of inert flowpath.

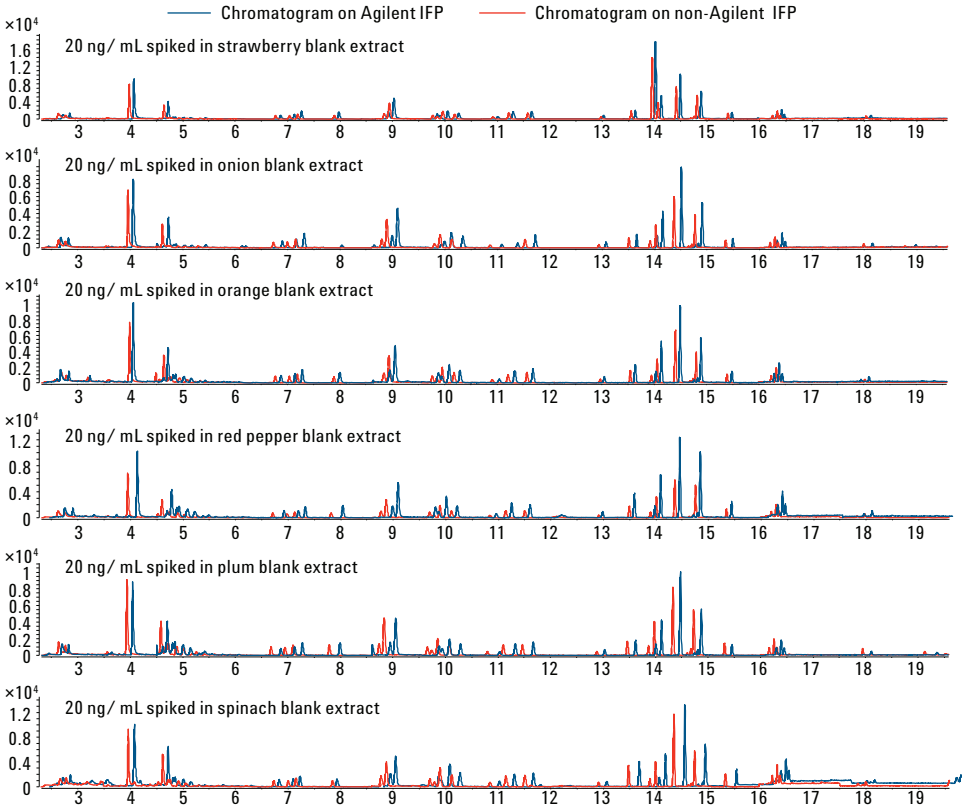

Figure 7. Chromatograms of 20 ng/mL standards in six matrixes using Agilent Inert Flow Path (blue) and non-Agilent deactivated components (red).

A more convincing proof for the surface inertness improvement of the Agilent IFP over the non-Agilent deactivated components includes better calibration curve linearity and longer durability, demonstrated by slower and reduced signal drop over multiple injections for critical active pesticides. As shown in Figure 8, for the critical pesticide omethoate, better calibration curve linearity ($R^2 > 0.99$) was achieved in the six tested sample matrices. However, when the non-Agilent deactivated components were used, five of six omethoate calibration curves in matrices produced unacceptable $R^2 < 0.99$ curve linearity. Figure 9 shows the system durability comparison for sensitive pesticides. As demonstrated, the use of Agilent IFP provided more consistent responses of active pesticides, and thus supported more sample runs with acceptable results.

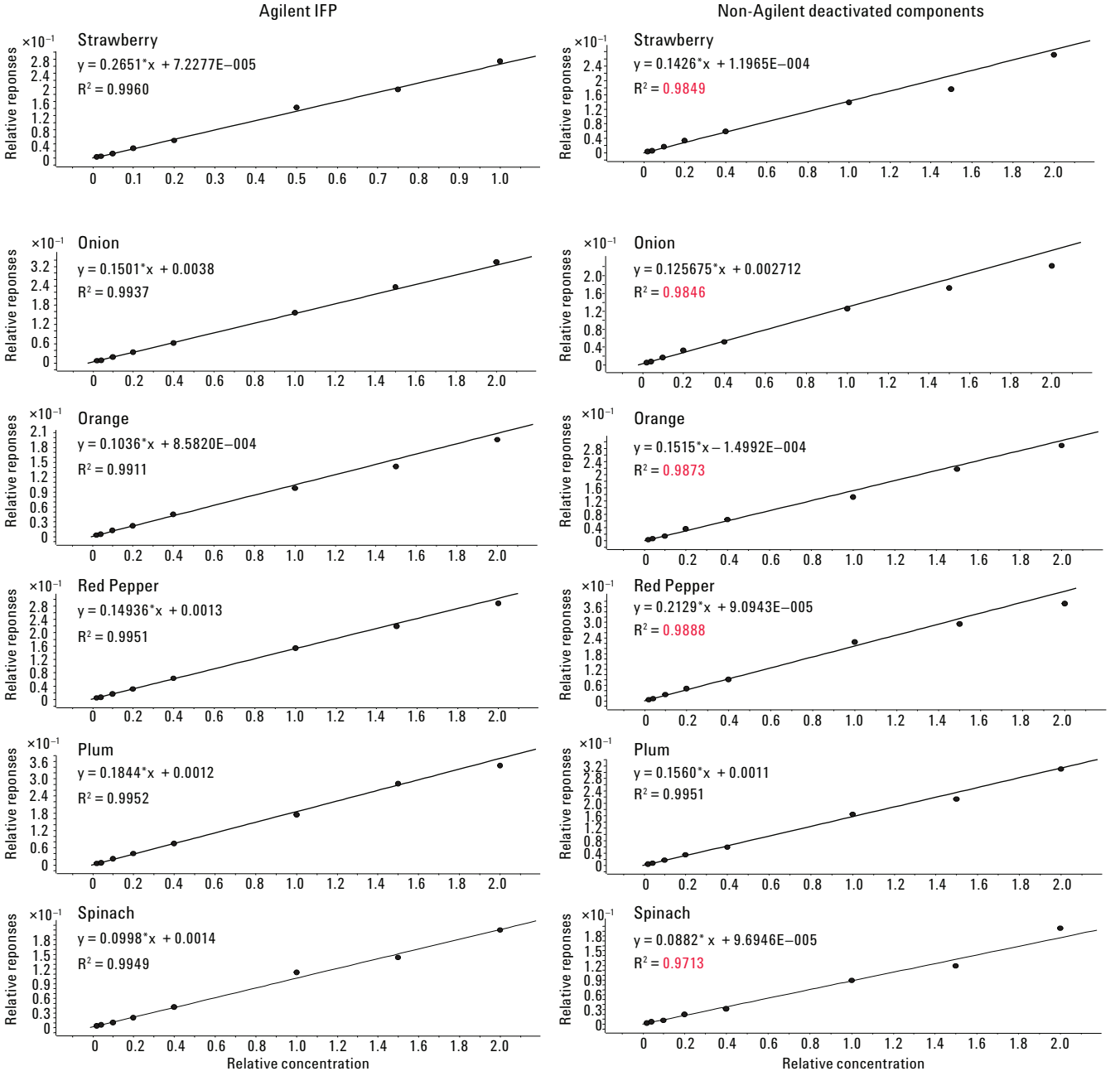

Figure 8. Omethoate calibration curves in various matrices using Agilent Inert Flow Path (left) and non-Agilent deactivated components (right). Calibration range 2 to 200 ng/mL in matrix blank.

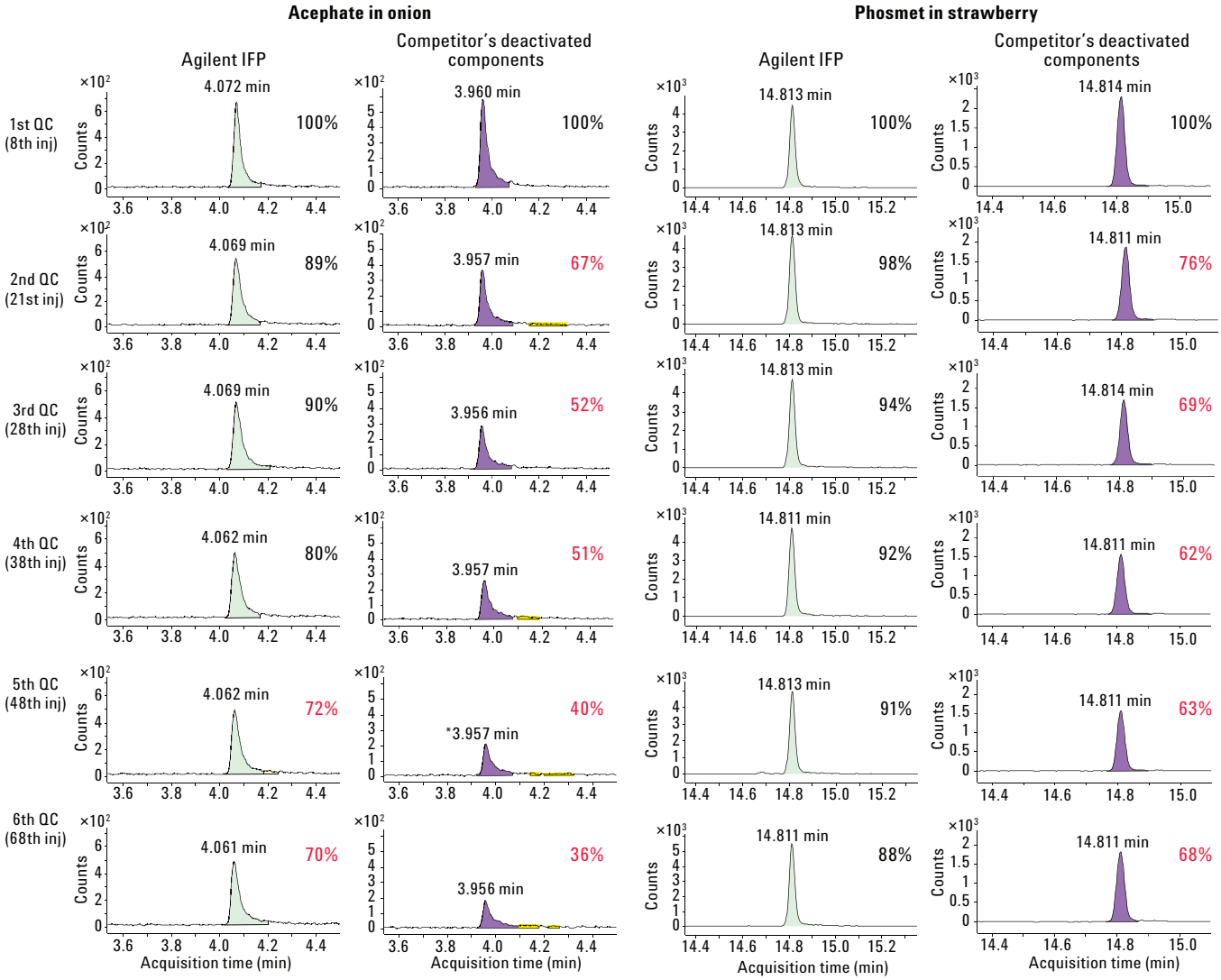

Figure 9. Example of system durability comparison between Agilent Inert Flow Path and non-Agilent deactivated components for critical pesticides in matrix. Matrix spiked QC 20 ng/mL in matrix blank.

Analyte protectants

Analyte protectants (APs) are compounds added simultaneously with each injection to strongly interact with the active sites in the GC system, and thus reduce degradation or adsorption of sensitive target analytes, or both [11,13]. This approach takes advantage of the matrix-induced enhancement effect to protect analyte losses in GC systems. The use of APs actually provides online deactivation for the entire system with each injection. It was demonstrated to be an effective way to improve sensitive pesticide responses, peak shapes and entire method ruggedness, and therefore has been highly recommended for pesticide analysis by GC/MS and GC/MS/MS.

Since previous studies were done using either traditional noninert or partially inert GC flowpath components, the impact of using APs with the Agilent IFP was investigated. A comparison of use and nonuse of APs was done on the Agilent IFP with strawberry (a weaker matrix) and spinach (a strong matrix) samples. Following the instruction on AP use [11], a mixture of gulonolactone and sorbitol solution was prepared. This AP mixture was then spiked into either a matrix-control blank or a reagent blank. By taking advantage of sandwiched injection, APs can be added automatically while making injections on the instrument. This is another benefit of using sandwiched injection. Instead of manually spiking AP solution in each sample during sample preparation, the autosampler served as a partial work bench

adding AP solution to each sample during sample injection. Again, the same samples were used for the sequence without AP addition. The results were evaluated by chromatogram comparison, and then overall quantitation results.

Figure 10 shows the overlaid chromatograms generated by using the Agilent IFP with and without adding analyte protectant. The sample chromatograms were collected after 70 injections in the respective sequence. Three representative compounds mentioned in the literature [11] were used to demonstrate the improvement of using analyte protectant, with lindane usually not susceptible, phosalone moderately susceptible, and o-phenylphenol very susceptible. These compounds are highlighted in Figure 10. The quantitation results are listed in Table 2, including the calibration curve R^2, average matrix spiked QC recovery and %RSD (three levels, n = 6 for each level).

The results demonstrate the benefit of using the Agilent IFP

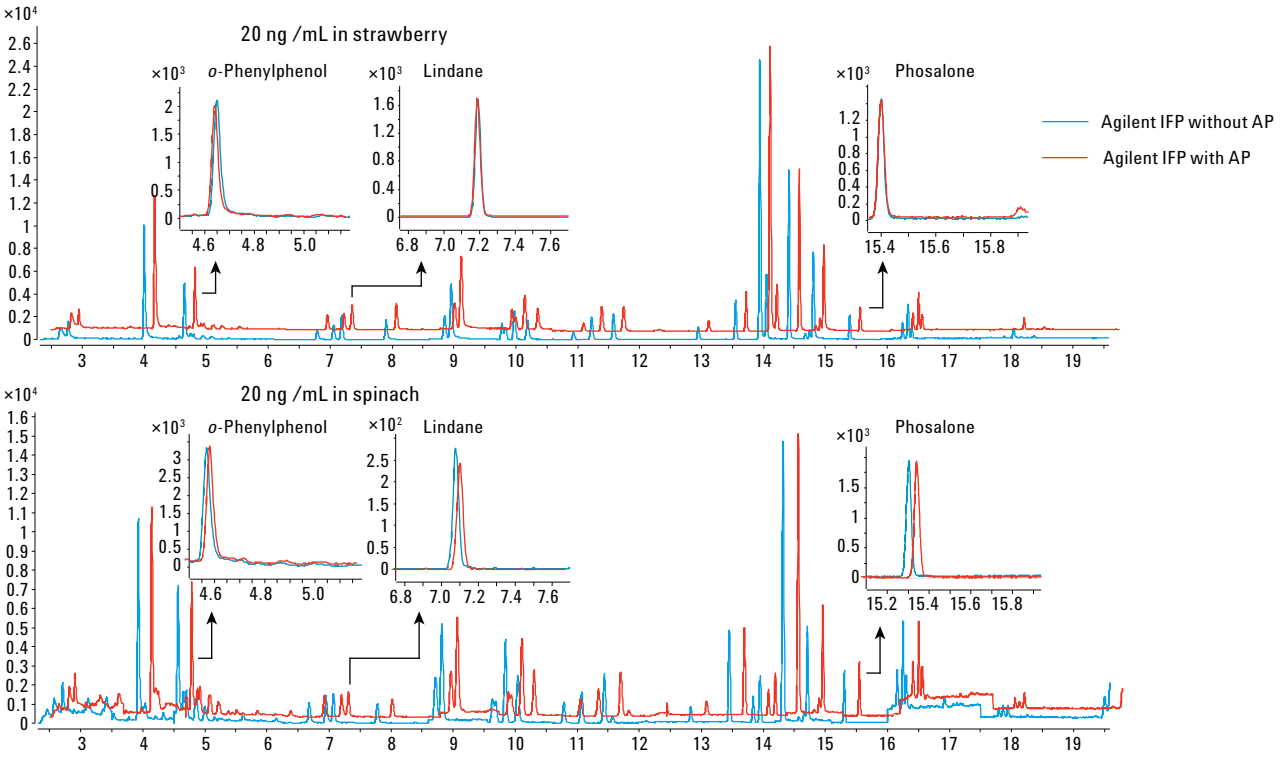

Figure 10. Chromatogram comparison of matrix spiked 20 ng/mL QCs in strawberry (top) and spinach (bottom) obtained by using Agilent Inert Flow Path without (blue) and with addition of analyte protectant (red). Chromatograms were collected after 70 injections of matrix samples in the sequence, respectively. Three compounds for enlarged peak overlapping were selected based on reference 11.

Table 2. Quantitation results comparison for samples spiked in strawberry and spinach using Agilent IFP with and without adding analyte protectant (AP).

	Strawberry						Spinach					
	Agilent IFP			Agilent IFP with AP			Agilent IFP			Agilent IFP with AP		
Pesticide	R^2	Average recovery (%)	%RSD n = 18	R^2	Average recovery (%)	%RSD n = 18	R^2	Average recovery (%)	%RSD n = 18	R^2	Average recovery (%)	%RSD n = 18
Dichlorvos	0.9993	95.6	5.4	0.9996	96.3	2.8	0.9963	90.6	3.4	0.9971	91.2	2.4
Methamidophos	0.9951	88.8	9.6	0.9992	94.2	3.9	0.9978	88.6	11.5	0.9969	86.1	9.4
Acephate	0.9980	84.3	23.5	0.9997	94.9	8.8	0.9920	83.0	23.2	0.9976	84.7	22.6
Mevinphos	0.9997	92.6	5.7	0.9988	95.7	2.5	0.9985	92.1	3.3	0.9996	93.0	2.5
Omethoate	0.9960	79.7	35.3	0.9974	96.1	13.0	0.9952	80.6	26.2	0.9925	79.9	28.1
o-Phenylphenol	0.9986	94.0	3.6	0.9913	93.1	1.7	0.9994	91.6	1.4	0.9993	91.5	3.1
Atrazine	0.9996	93.7	5.5	0.9990	94.1	2.7	0.9993	91.5	1.9	0.9993	94.6	1.7
Dimethoate	0.9994	89.8	9.9	0.9991	96.2	2.1	0.9997	87.7	7.3	0.9985	87.6	10.1
Lindane	0.9999	93.0	5.7	0.9990	93.9	1.9	0.9996	88.1	9.7	0.9986	86.3	8.8
Chlorothalonil	0.9991	93.5	8.9	0.9989	95.5	4.2	0.9990	88.5	13.6	0.9984	85.2	20.1
Carbaryl	0.9988	92.2	13.4	0.9964	96.4	6.2	0.9947	91.4	16.2	0.9944	91.4	24.3
Chlorpyrifos methyl	0.9995	93.2	3.7	0.9944	94.9	2.0	0.9992	92.3	1.9	0.9992	92.6	2.5
Tolclofos methyl	0.9996	92.9	4.2	0.9993	95.4	1.5	0.9992	92.3	1.4	0.9994	93.9	0.7
Vinclozolin	0.9999	93.7	3.6	0.9987	95.7	2.0	0.9991	91.6	1.7	0.9992	95.7	2.3
Aldrin	0.9985	91.7	4.4	0.9988	93.5	2.6	0.9982	91.8	2.6	0.9974	96.0	2.3
Dichlofluanid	0.9991	91.4	5.4	0.9987	92.3	2.6	0.9986	84.0	15.2	0.9985	80.9	16.5
Dichlorobenzophenone	0.9995	93.6	4.7	0.9976	95.5	2.0	0.9987	91.7	1.4	0.9997	93.7	1.2
Malathion	0.9981	94.5	2.7	0.9988	95.6	1.3	0.9959	90.7	2.4	0.9995	92.0	3.4
Pirimiphos ethyl	0.9958	90.7	4.0	0.9985	94.7	1.6	0.9970	92.0	1.5	0.9973	93.4	3.5
Procymidone	0.9988	91.4	2.9	0.9993	94.3	1.5	0.9988	92.0	1.1	0.9996	93.6	3.5
Tolyfluanid	0.9992	91.5	5.2	0.9991	93.9	2.9	0.9990	82.8	14.3	0.9994	80.9	1.7
Endrin	0.9969	90.0	4.0	0.9961	93.3	4.1	0.9991	85.1	8.0	0.9965	87.8	8.4
Ethion	0.9959	96.3	5.4	0.9967	95.1	1.6	0.9963	92.7	1.3	0.9991	92.4	1.0
DDT	0.9991	87.7	10.2	0.9954	92.3	13.9	0.9993	72.4	34.1	0.9983	72.5	33.1
Endosulfan sulfate*	N/A	N/A	N/A	N/A	N/A	N/A	0.9986	87.6	9.5	0.9989	87.7	10.2
Endrin ketone	0.9971	92.4	2.1	0.9886	92.0	3.1	0.9938	77.3	21.6	0.9883	73.5	23.7
Iprodione	0.9993	93.4	2.6	0.9975	94.3	2.8	0.9986	90.1	15.0	0.9981	89.4	17.0
Phosmet	0.9957	90.2	5.1	0.9956	92.5	3.8	0.9984	79.2	18.0	0.9977	80.2	23.2
Phosalone	0.9972	94.2	1.8	0.9969	93.7	1.9	0.9992	86.5	5.9	0.9972	87.3	9.7
Coumaphos	0.9905	90.5	3.4	0.9980	88.4	3.7	0.9984	83.4	6.5	0.9993	85.3	10.6
Permethrin	0.9995	90.2	3.1	0.9980	90.5	1.9	0.9981	89.5	1.5	0.9948	94.3	1.9
Deltamethrin	0.9974	94.3	4.7	0.9986	97.9	4.2	0.9957	88.9	7.3	0.9960	89.6	15.0

* Endosulfan sulfate was detected in strawberry matrix blank, thus was not quantifiable.

for pesticide analysis in sample matrix by exhibiting excellent peak response, peak shape, and consistent analyte response over multiple matrix injections. The quantitation results achieved by using just the Agilent IFP were quite comparable to those obtained with the addition of analyte protectant. Even for the very susceptible compound o-phenylphenol, the peak shape and response were kept consistent after 70 injections using just IFP without analyte protectant. Similar results were obtained with lindane and phosalone in both sample matrices. However, it has to be acknowledged that signal drop was still observed for very critical pesticides such as acephate and omethoate. These relatively polar and volatile analytes should be moved to LC/MS for analysis. In a strawberry matrix, the use of analyte protectant significantly improved the consistency of response of those two analytes. However, in a spinach matrix, the improvement by using AP was not quite effective and unacceptable results were obtained in both cases. Spinach is a very complicated matrix, with more failures on other pesticides observed regardless of analyte protectant use.

Signal drop of critical analytes is probably linked to the matrix-induced response diminishment effect, which is caused by gradual accumulation of less or nonvolatile matrix interferences in the GC system, especially in the inlet liner, seal, and column head. These accumulated components can form new active sites and thus gradually cause signal drop. By using a liner with wool and backflushing, the introduction and accumulation of matrix residue was reduced yet not completely avoided. Therefore, even with the completely inert flowpath, the newly formed active sites still can cause problems. In such cases, the 'online' deactivation provided by using analyte protectants with each injection can dramatically improve the recovery performance of very sensitive compounds in many matrices. However, analyte protectant is not the 'magic' solution for every case. In spinach matrix, the use of analyte protectant did not help very much for critical analytes. Problematic pesticides still gave unacceptable results even with the addition of analyte protectant. For compounds that are difficult to analyze with GC/MS, it has been recommended to move some ones for LC/MS analysis [11,13], or use additional sample preparation processes to reduce the matrix effect.

Conclusions

The Agilent Inert Flow Path was evaluated thoroughly for the analysis of pesticide residue in six sample matrices extracted by the QuEChERS technique. A sandwiched injection method was employed for adding matrix blank during injection, which allowed the use of standards prepared in reagent blank to various matrix sample calibrations. The method was validated in six matrices on GC/MS/MS using Agilent IFP, which provided superior sensitivity (2 ng/mL of LOQ), excellent calibration curve linearity over 2 to 200 ng/mL ($R^2 > 0.99$), and acceptable quantitation accuracy and precision. The results demonstrated that the Agilent IFP provides excellent surface inertness for the entire GC flowpath and thus dramatically reduces negative impacts on target analytes caused by surface active sites. When compared to non-Agilent deactivated components, the Agilent IFP provided higher overall responses, better linearity for critical pesticides, and longer durability for sensitive pesticides in fruit and vegetable matrices.

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