Advantages of Reversed Sandwich Injection for Pesticide Residue Analysis

Application Note

Author

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Abstract

This application note demonstrates the use of the Agilent 7693A ALS system's Reversed 3-Layer Switch sandwich injection functionality for the analysis of pesticide residues in food. For the best quantitative results, it is customary during pesticide residue analysis to perform matrix-matched calibration to overcome the challenges introduced by the various matrix types. Sandwich injection is a simple technique that is very useful in this case. Over 50 target selected pesticides were analyzed in four food commodity matrices. Using the Reversed 3-Layer Switch Sandwich Injection with the matrix as the bottom layer, provided the best responses for trace pesticide analysis. For each of the matrices, over 85 % of the target pesticides achieved a calibration curve with an R^2 ≥0.991 (1.25 ppb to 62.5 ppb). All analyzed pesticides obtained a %RSD for repeated measurements at 1.25 ppb of ≤30 %, and 85 % of the analyzed pesticides were found to have a Limit of Quantitation (LOQ) ≤0.1 ppb.

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Introduction

The use of matrix-matched calibration standards has always been widely accepted in pesticide residue analysis to ensure accurate and reliable quantitation results in different commodities. This is important because pesticide response is influenced by the various matrices, and may lead to biased quantitative results. Performing matrix-matched calibrations eliminates the response biases, and allows for more accurate identification and quantitation results. However, the preparation of matrix-matched calibration standards can be a tedious and time-consuming procedure, especially when multiple sample matrices are analyzed [1]. This practice also introduces the possibility of human errors during preparation, affecting the analytical results. Using a 2- or 3-layer sandwich injection establishes an automated, streamlined process, and eliminates the need to create unique calibration solutions for every commodity analyzed, reducing preparation time, cost, and potential errors.

Sandwich injection is an injection technique in which two or three aliquots are drawn into the autosampler syringe from multiple vials, and injected into the GC inlet. The result is that the aliquots are simultaneously vaporized, mixed in the liner, and transferred as a single sample onto the GC column. The Agilent 7693A Automatic Liquid Sampler (ALS) system provides several different layering injection functions. Having multiple layering options provides more user flexibility. This technique aids the addition of an internal standard, a derivatizing agent, or analyte protectants to the samples, and or also greatly simplifies the creation of matrix-matched calibration. The optimized order of the different aliquots in the syringe enhances system performance [2], therefore, flexibility is added to the basic sandwich injection technique, allowing simple and easy specification of the desired aliquots and their order.

Experimental

Sample preparation

Many laboratories that are focused on pesticide residue analysis in food commodities routinely use the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) extraction method [3,4]. This straightforward sample preparation permits analysis of hundreds of pesticides at low concentrations with a single extraction. Calibration standards were prepared in solvent for the use of matrix-matched calibrations for four different food commodity matrices. These matrices were extracted with a matrix-specific QuEChERS methodology, in which various dispersive SPEs (dSPE) were used for matrix cleanup (Table 1) [5].

Instrumentation

The Pesticides and Environmental Pollutants (P&EP) Analyzer M7412AA method is the optimal method of choice for the pesticide residue analyses. The 7693A Autosampler was connected to an Agilent 7890B GC and an Agilent 7010A Triple Quadrupole GC/MS. Tables 2 and 3 display the GC and the MS/MS method parameters, respectively. The 7890B GC was configured with a Multimode Inlet (MMI) equipped with a 4 mm ultra inert, splitless, single taper liner with glass wool (p/n 5190-2293). Two Agilent HP-5ms UI, 15 m × 0.25 mm, 0.25 µm columns (p/n 19091S-431 UI) were coupled to each other through a purged ultimate union (PUU) to facilitate midcolumn/post run backflushing (Figure 1).

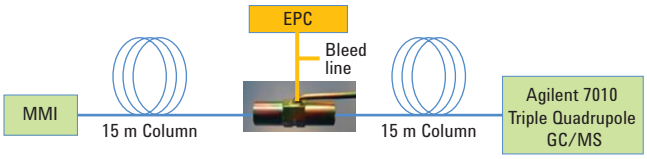

Figure 1. M7412AA Configuration for Optimal Pesticides MRM Residue Analysis.

Table 1. Matrix Selection and Sample Preparation Used for Pesticide Residue Analysis

Category	Matrix	Sample prep
High oil	Extra virgin olive oil	3 g oil/7 mL water, EN salts (5982-5650), EMR—L (5982-1010), Polish Pouch (5982-0102), Dry step
High difficultly	Black loose leaf tea	3 g tea/7 mL water, EN salts, EN dSPE pigment (5982-5256)
High pigment	Fresh leaf baby spinach	10 g, EN salts, EN dSPE pigment (5982-5356)
High sugar	Organic honey	5 g honey/5 mL water, EN salts, EN dSPE General (5982-5056)

Table 2. Agilent 7890B GC Method Conditions

Parameter	Value		
MMI Injection mode	Splitless		
Injection volume	1 µL (L3 volume)		
Injection type	Reversed 3-Layer Switch (L3,L1,L2)		
L1 Airgap	0.2 µL		
L1 Volume	1 µL		
L2 Airgap	0.2 µL		
L2 Volume	1 µL		
L3 Airgap	0.2 µL		
	Total volume = 3.6 µL		
Plunger speed	Slow		
Inlet temperature	280 °C		
Carrier gas	He, constant flow 1.00 mL/min (column 2 = 1.20 mL/min)		
MS transfer line temperature	280 °C		
Oven program	Ramp (°C/min)	Temp (°C)	Hold time (min)
		60	1
	40	170	0
	10	310	2.25
PUU Backflush settings*			
Timing	1.5 minutes duration during post-run		
Oven temperature	310 °C		
Aux EPC pressure	~50 psi		
Inlet pressure	~2 psi		

*Backflush conditions optimized for method.

Table 3. Agilent 7010A dynamic MRM (dMRM) Parameters

Parameter	Value
Electron energy	70 eV
Tune	atunes.eihs.tune.xml
EM gain	10*
MS1 and MS2 resolution	dMRM unit
Collision cell	1.5 mL/min N_2 and 2.25 mL/min He
Quant/Qual transitions	Matrix Optimized for M74122AA [5]
Dwell times	Optimized by dMRM**
Source temperature	280 °C
Quad temperature	150 °C

*Instrumental conditions increased the optimal EM gain for this experiment.
**All dwells were set to achieve a scan rate of ~5 scans/sec.

Sandwich injection

There are two ways that sandwich injections can be viewed:

- The order in which the aliquots are drawn up
- The order in which the aliquots are injected

Agilent MassHunter GC/MS Data Acquisition software defines the standard sandwich injection in the order the aliquot layers are drawn up into the syringe; this document follows this convention. This GC method focuses on the use of the Reversed 3-Layer Switch (L3,L1,L2) injection mode (Figure 2). Using this injection for this analysis permitted:

- Placement of the calibration standards or samples in order, starting with Tray 1: Vial 1
- Drawing up the ISTD first to avoid cross-contamination
- Preparation of matrix-matched calibration curves for various matrices with one set of calibration standards

To apply the ALS sandwich injection functionality correctly, the user must:

1. Set the injection type in the ALS parameters of the GC method (Figure 2).

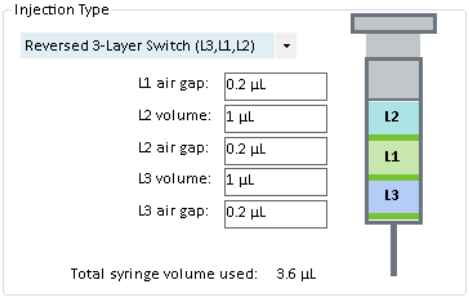

Figure 2. Reversed 3-Layer Switch (L3,L1,L2) injection method parameters. Note: there is a default air gap (0.2 µL) included to prevent cross contamination when withdrawing a sample from another vial.

2. Select the correct keyword in the sequence (Figure 3).

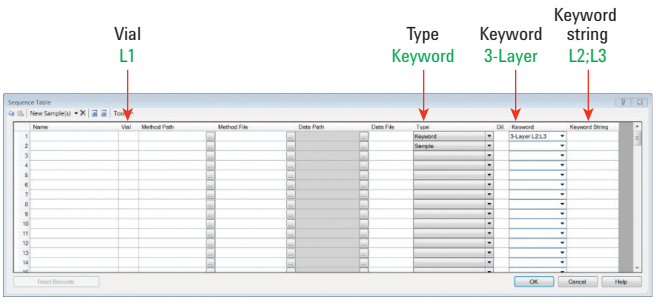

Figure 3. Blank sequence with sandwich injection prerequisites. Note: when running a 3-layer sandwich injection, the keyword string separates L2 and L3 by a semicolon (;).

3. Specify the vial locations in the keyword string of the sequence (Figure 4).

These selections allow the ALS to select the correct vials for the specified order of the sandwich injection.

After each of the 3 aliquot layers have been drawn up by the syringe, the entire sample is injected into the inlet liner for vaporization, mixing, and transfer onto the GC column. The highly deactivated wool inside the Ultra Inert liner provided a large surface area to aid the vaporization of the liquid samples, and promote homogenous sample mixing in the liner prior to entering the column. In the final injection volume, it is important to keep the ratio of solvent and matrix consistent for different sample sandwich injections, otherwise the matrix or the target analytes can be diluted differently, and result in different matrix effects. This could deliver misleading quantitation results [1].

Sandwich injection sequence

The sandwich injection software identifies the aliquot layers by designating L1, L2, and L3 to specific locations within the sequence. These identifiers are specified as vial locations in the sequence for the ALS syringe. Figure 3 shows a blank sequence table with the prerequisites for a 3-layer sandwich injection.

The sequence table is modified by a sequence line with a keyword string. This keyword string allows the user to define the specified vials to be used for each layer. Figure 4 provides a section of the organic honey calibration sequence.

	Name	Vial	Method Path	Method File	Data Path	Data File	Type	Dil.	Keyword	Keyword String
1							Keyword		3-Layer L2;L3	31;41
2	ACN Blank001	51	C:\MassHunter\...\methods	M7412AA_20..._checkout.M	D:\MassHu..\20OCT2016	ACN Blank001	Sample			
3	ACN Blank002	52	C:\MassHunter\...\methods	M7412AA_20..._checkout.M	D:\MassHu..\20OCT2016	ACN Blank002	Sample			
4							Keyword		3-Layer L2;L3	31;41
5	Honey_CAL01_Rep 01	1	C:\MassHunter\...\methods	M7412AA_20..ey_3Layer.M	D:\MassHu..\20OCT2016	Honey_CAL01_Rep 01	Sample			
6	Honey_CAL01_Rep 02	1	C:\MassHunter\...\methods	M7412AA_20..ey_3Layer.M	D:\MassHu..\20OCT2016	Honey_CAL01_Rep 02	Sample			
7	Honey_CAL01_Rep 03	1	C:\MassHunter\...\methods	M7412AA_20..ey_3Layer.M	D:\MassHu..\20OCT2016	Honey_CAL01_Rep 03	Sample			
8							Keyword		3-Layer L2;L3	32;42
9	Honey_CAL02_Rep 01	2	C:\MassHunter\...\methods	M7412AA_20..ey_3Layer.M	D:\MassHu..\20OCT2016	Honey_CAL02_Rep 01	Sample			
10	Honey_CAL02_Rep 02	2	C:\MassHunter\...\methods	M7412AA_20..ey_3Layer.M	D:\MassHu..\20OCT2016	Honey_CAL02_Rep 02	Sample			
11	Honey_CAL02_Rep 03	2	C:\MassHunter\...\methods	M7412AA_20..ey_3Layer.M	D:\MassHu..\20OCT2016	Honey_CAL02_Rep 03	Sample			
12							Keyword		3-Layer L2;L3	32;42
13	Honey_CAL03_Rep 01	3	C:\MassHunter\...\methods	M7412AA_20..ey_3Layer.M	D:\MassHu..\20OCT2016	Honey_CAL03_Rep 01	Sample			
14	Honey_CAL03_Rep 02	3	C:\MassHunter\...\methods	M7412AA_20..ey_3Layer.M	D:\MassHu..\20OCT2016	Honey_CAL03_Rep 02	Sample			
15	Honey_CAL03_Rep 03	3	C:\MassHunter\...\methods	M7412AA_20..ey_3Layer.M	D:\MassHu..\20OCT2016	Honey_CAL03_Rep 03	Sample			
16							Keyword		3-Layer L2;L3	33;43
17	Honey_CAL04_Rep 01	4	C:\MassHunter\...\methods	M7412AA_20..ey_3Layer.M	D:\MassHu..\20OCT2016	Honey_CAL04_Rep 01	Sample			

Figure 4. Selection of organic honey matrix-matched calibration curve. Note that when a vial change is made, a new keyword line is required.

Results and Discussion

Area response comparison

Some applications showed better results based on the injection order, such as injecting APs or matrix into the system before a solvent standard [2]. Therefore, an experiment was conducted for this application to compare the injection of the matrix (M) as the top layer (drawn up by the syringe first; ahead of the sample (S), and internal standard (I) in Figure 5) and as the bottom layer (drawn up by the syringe last; after the I and S; Figure 6).

Area counts were compared at a midcalibration level (~12–25 pg/µL, compound dependent). Figures 7–9 display selected pesticides and their area counts for organic honey, fresh leaf baby spinach, and black loose leaf tea, respectively. Each figure displays the comparison of area counts for the pesticides when the matrix was injected as the bottom layer (drawn up last by the syringe; blue bars), and when the matrix was injected as the top layer (drawn up first by the syringe; orange bars).

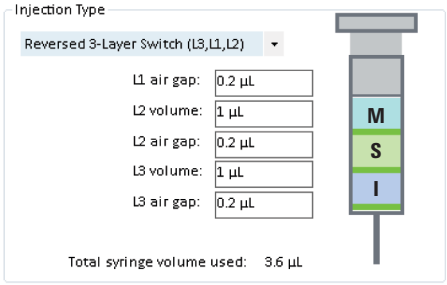

Figure 5. Reversed 3-Layer Switch Injection (L3,L1,L2), with the matrix drawn up first by the syringe. Matrix (M), sample (s), and ISTD (I).

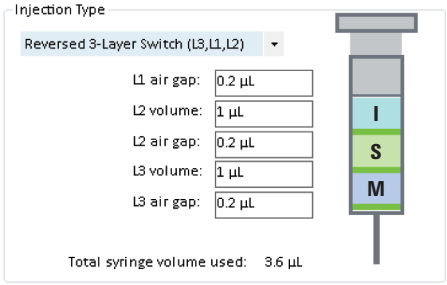

Figure 6. Reversed 3-Layer Switch Injection (L3,L1,L2), with the matrix drawn up last. Matrix (M), sample (s), and ISTD (I).

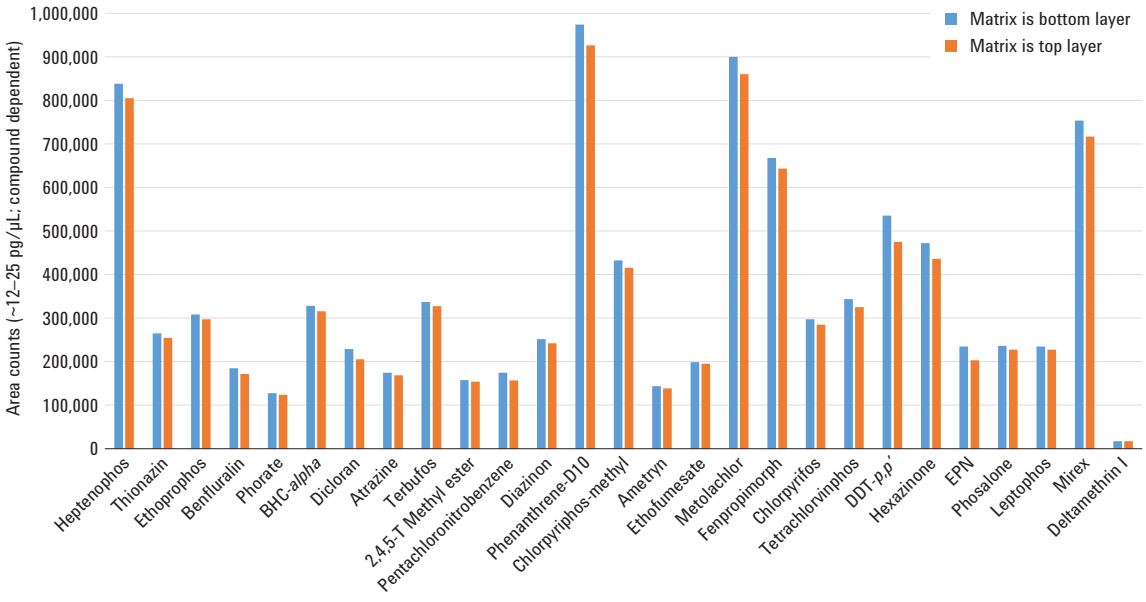

Figure 7. Area comparison for selected pesticides in organic honey extract.

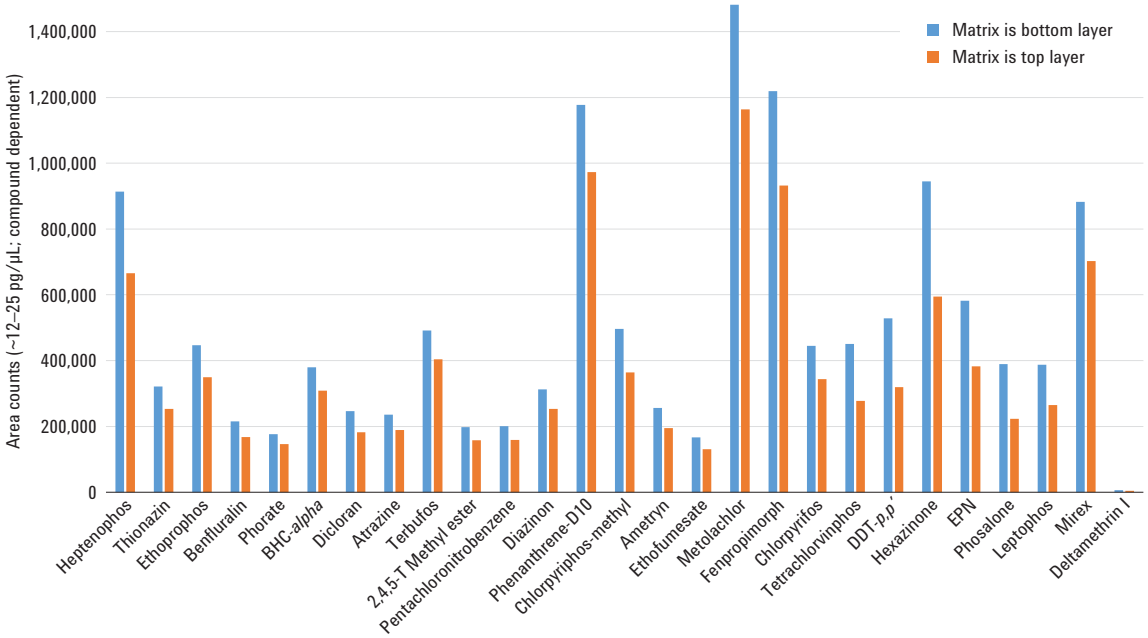

Figure 8. Area comparison for selected pesticides in fresh leaf baby spinach extract.

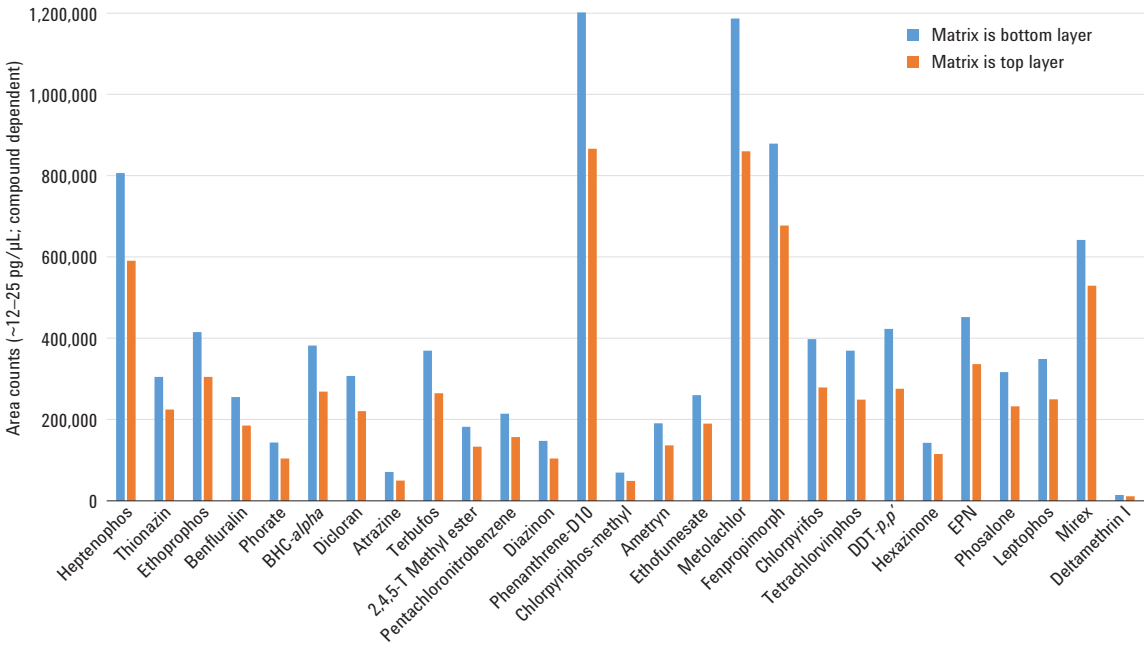

Figure 9. Area comparison for selected pesticides in black loose leaf tea extract.

The area responses for the overwhelming majority of pesticides in each of the matrices were found to be larger when the matrix was the bottom layer of the Reversed 3-Layer Switch sandwich injection. The percent difference in the responses between the matrix as the top layer or bottom layer varied by matrix. With the increase in matrix complexity, the percent difference between the pesticides response would also increase. A reason for this could be the result of analyte loss to active sites (uncoated Si-OH, metal surfaces, nonvolatile residues, and so forth) in the injection port. Many pesticides are sensitive to active sites and, as such, will have a lower response because residue is lost at the active sites. By introducing the matrix first with the Reversed 3-Layer Switch injection, the matrix would encounter the active sites just before the target pesticides. The matrix competes with the pesticides for the active sites, and because it gets there first, it will adsorb onto the active sites preferentially. This will result in more linear calibration curves, less peak tailing, better %RSDs (see Table 4), and lower detection limits of trace pesticides.

Table 4. %RSDs for Matrix Layer in Sandwich Injection for Two Difficult Matrices: Olive Oil and Black Tea at ~12.5 ppb

Compound	**% RSD Olive oil**		**% RSD Black tea**	
	Matrix = bottom layer	**Matrix = top layer**	**Matrix = bottom layer**	**Matrix = top layer**
Heptenophos	3.02	7.44	3.27	10.44
Thionazin	2.99	8.25	3.05	11.36
Ethoprophos	2.66	7.17	2.89	9.82
Benfluralin	3.20	5.72	2.50	11.83
Phorate	3.07	5.61	2.62	10.61
BHC-*alpha*	4.78	5.91	2.73	9.08
Dicloran	2.73	3.24	2.19	11.63
Atrazine	1.90	3.29	4.18	15.89
Terbufos	0.91	6.06	1.12	13.74
2,4,5-T methyl ester	4.59	6.49	3.25	11.27
Pentachloronitrobenzene	1.90	9.02	2.89	10.16
Diazinon	2.01	5.64	1.79	10.59
Phenanthrene-D10	4.62	7.47	3.44	11.68
Chlorpyrifos-methyl	1.56	5.84	0.92	11.01
Ametryn	4.90	5.88	0.53	12.25
Ethofumesate	1.13	2.58	1.74	13.73
Metolachlor	1.34	4.91	2.02	11.55
Fenpropimorph	5.95	7.12	0.62	11.20
Chlorpyrifos	2.76	3.16	0.97	13.30
Tetrachlorvinphos	3.25	9.02	5.15	13.97
DDT-*p,p´*	1.59	6.63	10.35	26.08
Hexazinone	3.01	13.14	1.79	2.28
EPN	1.45	9.25	3.23	14.00
Phosalone	2.43	10.09	3.96	13.83
Leptophos	2.11	7.02	1.89	14.08
Mirex	1.44	2.21	0.71	13.80
Deltamethrin I	0.56	9.74	5.00	8.81

Based on the area response study, the quantitative analysis was conducted with the matrix being drawn up last (bottom layer) using a Reversed 3-Layer Switch injection. A selection of 50+ target pesticides were selected for analysis in the four specified matrices. For each of the four matrices, over 85 % of the target pesticides achieved a calibration curve with R^2 ≥0.991 (1.25 ppb to 62.5 ppb). Table 5 provides method detection and quantitation limits for selected pesticides in organic honey and black loose leaf tea. All analyzed pesticides obtained a %RSD of repeated measurements at 1.25 ppb of ≤30 %, and 85 % of the analyzed pesticides were found to have a Limit of Quantitation (LOQ) ≤0.1 ppb.

Table 5. Method Detection and Quantitation Limits for Selected Pesticides

	Organic honey		Black tea	
Compound	**MDL (ppt)**	**LOQ (ppt)**	**MDL (ppt)**	**LOQ (ppt)**
Heptenophos	2.42	8.92	1.42	5.23
Thionazin	0.81	2.98	0.48	1.78
Ethoprophos	0.92	3.4	0.79	2.91
Benfluralin	0.65	2.38	0.38	1.41
Phorate	3.18	11.7	0.11	0.41
BHC-*alpha*	0.92	3.37	0.48	1.77
Dicloran	0.99	3.66	0.5	1.84
Atrazine	0.56	2.05	0.15	0.55
Terbufos	1.12	4.11	0.65	2.39
2,4,5-T methyl ester	0.7	2.59	0.2	0.73
Pentachloronitrobenzene	0.5	1.84	0.22	0.81
Diazinon	1.27	4.67	0.2	0.72
Phenanthrene-D10	3.1	11.4	1.7	6.27
Chlorpyrifos-methyl	15.06	50.24	0.16	0.57
Ametryn	0.83	3.04	0.39	1.44
Ethofumesate	4.23	15.56	0.51	1.87
Metolachlor	3.47	12.76	1.98	7.28
Fenpropimorph	2.19	8.08	2.68	9.84
Chlorpyrifos	1.4	5.14	0.74	2.71
Tetrachlorvinphos	0.78	2.86	0.29	1.07
DDT-*p,p´*	2.55	9.38	0.39	1.44
Hexazinone	0.75	2.78	0.26	0.96
EPN	0.78	2.88	0.63	2.32
Phosalone	0.5	1.84	0.29	1.05
Leptophos	0.32	1.19	0.28	1.03
Mirex	3.12	11.47	0.19	0.7
Deltamethrin I	26.98	85.83	0.03	0.1

Conclusions

The Agilent 7693A Automatic Liquid Sampler (ALS) Reversed 3-Layer Switch sandwich injection allowed for:

- The placement of the calibration standards or samples in order starting with Tray 1: Vial 1
- Drawing up the ISTD first to avoid cross-contamination
- Preparation of matrix-matched calibration curves for various matrices with one set of calibration standards

Introducing the matrix first using the Reversed 3-Layer Switch injection meant that the matrix encountered the active sites before the pesticides, which greatly improved the recovery of the pesticides. This added flexibility of multiple layering options eliminates the need to prepare matrix-matched calibrations standards that can be tedious and time-consuming. Also, by including the internal standard in the sandwich injection, there is no need to spike each vial. The use of sandwich injections with the P&EP M7412AA method delivers an optimal analysis for trace pesticide residues.

References

1. L. Zhao, M. Szelewski, Analysis of Fruit and Vegetable Pesticides by GC/MS/MS Using Agilent Inert Flow Path, *Agilent Technologies Application Note*, publication number 5991-3234EN (**2013**).
2. P. Wylie, Using Sandwich Injections to add Matrix, Internal Standards and/or Analyte Protectants for the GC/Q-TOF Analysis of Pesticide Residues, *Agilent Presentation* (O-29) at NACRW (**2016**).
3. M. Anastassiades, *et al. AOAC Int.* **86**, 412-431 (2003).
4. S. J. Lehotay, K. Mastovská, A. R. Lightfield. *J. AOAC Int.* **88**, 615-629 (2005).
5. J. Westland, J. Stevens, An Optimal Method for the Analysis of Pesticides in a Variety of Matrices, *Agilent Technologies Application Note*, publication number 5991-7303EN (**2016**).

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