

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 the preparation, affecting the analytical results. The Agilent 7693A ALS system's Reversed 3-Layer Switch sandwich injection functionality allows for the trouble-free addition of the internal standard to the calibration standards (Figure 1), and simultaneous injection for each of four different food commodities to simplify the creation of matrix-matched calibration standards. Over 50 target selected pesticides were selected and compared throughout the different matrices. While maintaining the matrix as the bottom layer in the 3-layer sandwich injection, over 85 % of the target pesticides achieved a calibration curve with an $R^2 \geq 0.991$ (1.25 ppb to 62.5 ppb). All analyzed pesticides obtained a %RSD for repeated measurements at 1.25 ppb of $\leq 30\%$, and 85 % of the analyzed pesticides were found to have a Limit of Quantitation (LOQ) ≤ 0.1 ppb.

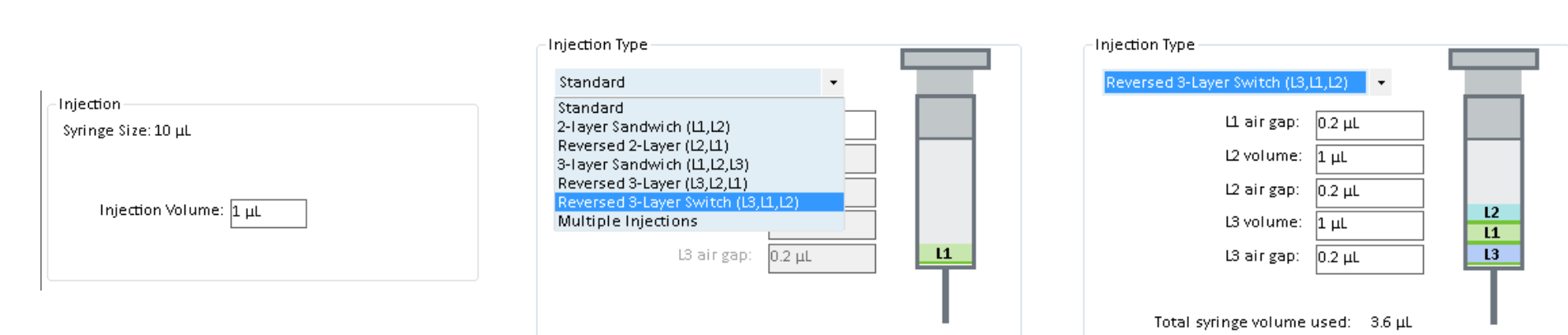


Figure 1. Injection volume and type selections. This application focused on utilizing the Reversed 3-Layer Switch (L3,L1,L2) injection type.

Experimental

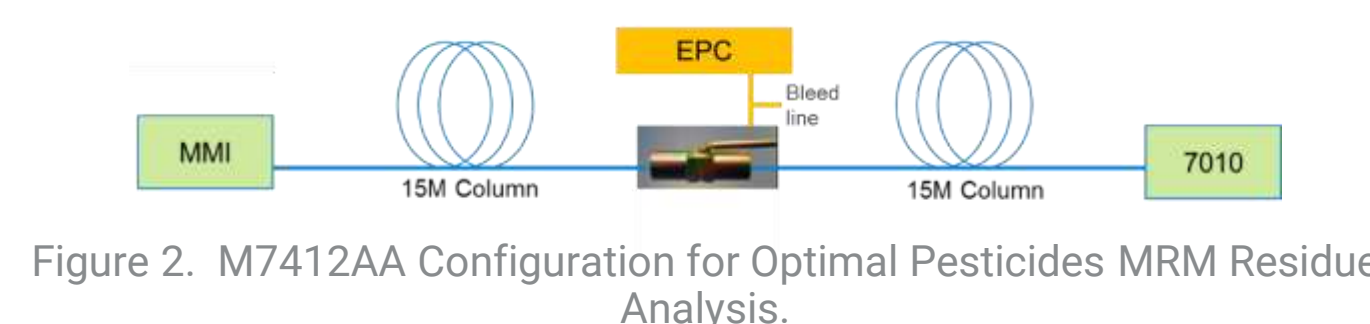
Sample Prep

Many laboratories that are focused on pesticide residue analysis in food commodities routinely use the QuEChERS extraction method [2,3]. 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) [4].

Method Parameters

The Pesticides and Environmental Pollutants (P&E) 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 method parameters and the PUU backflush settings for the application. Table 4 displays the MS/MS method parameters for the application. 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 x 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 mid-column/post run backflushing (Fig. 2).

Parameter	Value
Injection port liner	4-mm Ultra Inert splitless, single taper liner w/wool
Injection mode	Hot-splitless
Inlet temperature	280 °C
Injection volume	1 µL
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
L3 Volume	1 µL
Plunger speed	Slow
Carrier gas	He, constant flow 1.00 mL/min (column 2 = 1.20 mL/min)
Oven program	40 °C/min 60 °C 1 min 10 °C/min 170 °C 0 min 10 °C/min 310 °C 2.25 min
MS transfer line temp.	280 °C



Parameter	Value
Timing	1.5 min duration during post-run
Oven Temperature	310 °C
AUX EPC pressure	~50 psi
Inlet pressure	~2 psi

*Backflush conditions optimized for method.

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
Difficult	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)

Parameter	Value
Electron Energy	70 eV
Tune	atunes.eh.s.tune.xml
EM gain	10*
MS1 & MS2 resolution	dMRM unit
Collision Cell	1.5 mL/min N ₂ & 2.25 mL/min He
Quant/Qual transitions	Matrix Optimized for M7412AA [4]
Dwell times	Optimized by dMRM**
Source temperature	280 °C
Quad temperatures	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

Sandwich Injection

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 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 [5], therefore, flexibility is added to the basic sandwich injection technique, allowing simple and easy specification of the desired aliquots and their order.

There are two ways that sandwich injections can be viewed:

1. The order in which the aliquots are drawn up
2. 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. 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 3).

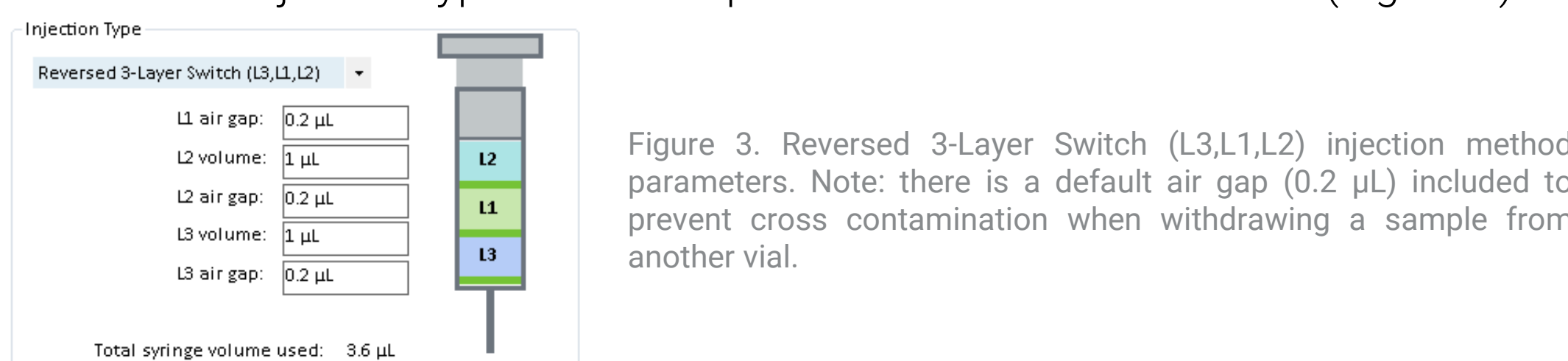


Figure 3. Reversed 3-Layer Switch (L3,L1,L2) injection method parameters. Note: there is a default air gap (0.2 µL) included to prevent cross contamination when withdrawing a sample from another vial.

2. Select the correct keyword in the sequence (Figure 4).

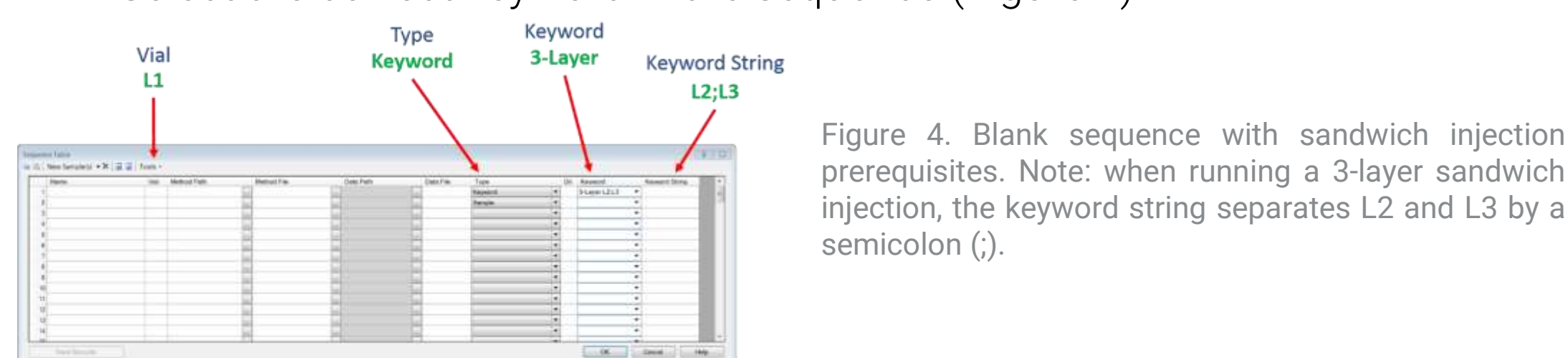


Figure 4. Blank sequence with sandwich injection prerequisites. Note: when running a 3-layer sandwich injection, the keyword string separates L2 and L3 by a semicolon (;).

3. Specify the vial locations in the keyword string of the sequence (Figure 5).



Figure 5. Selection of organic honey matrix-matched calibration curve. Note that when a vial change is made, a new keyword line is required.

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 [5]. 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 6) and as the bottom layer (drawn up by the syringe last; after the I and S; Figure 7).

Area counts were compared at a mid calibration level (~12–25 µg/µL, compound dependent). Figures 8–10 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; green bars), and when the matrix was injected as the top layer (drawn up first by the syringe; orange bars).

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.

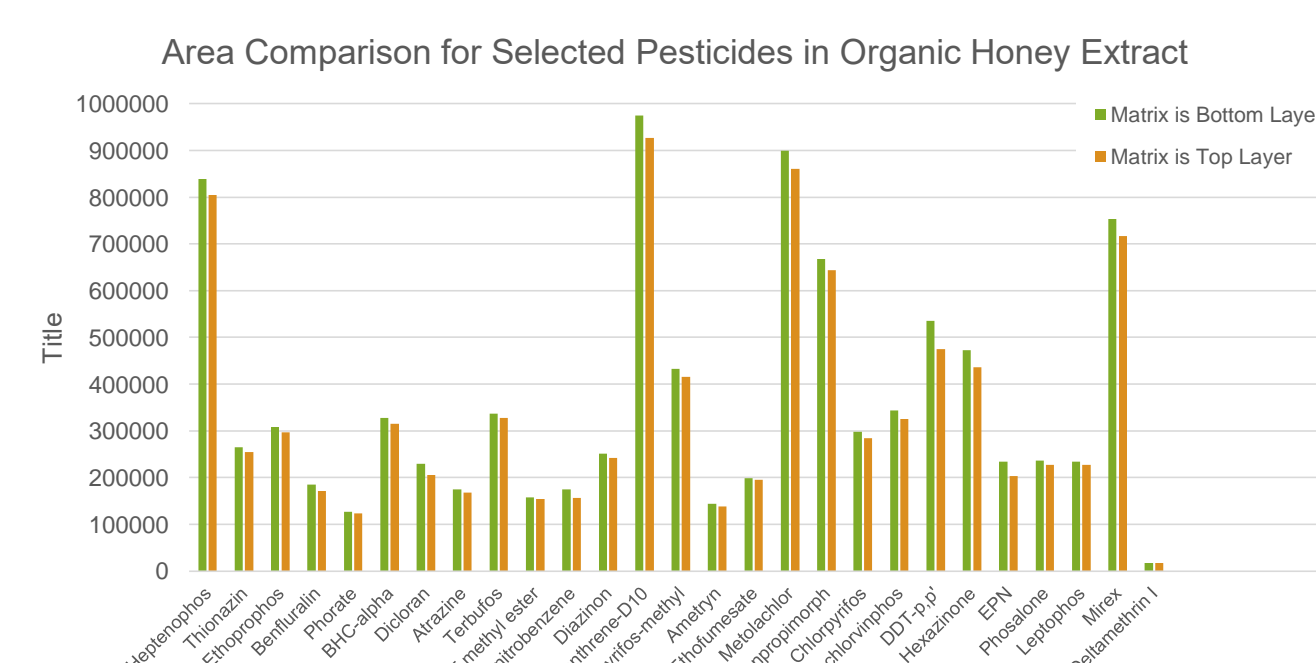


Figure 8. Area Comparison for Selected Pesticides in Organic Honey Extract.

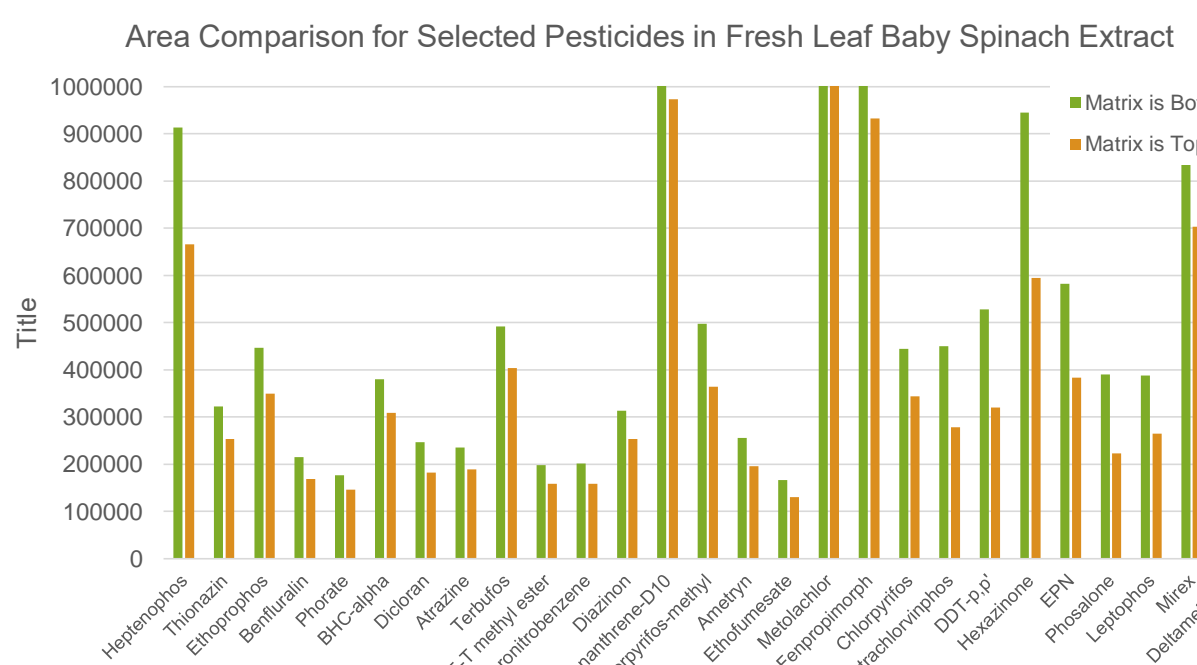


Figure 9. Area Comparison for Selected Pesticides in Fresh Leaf Baby Spinach Extract.

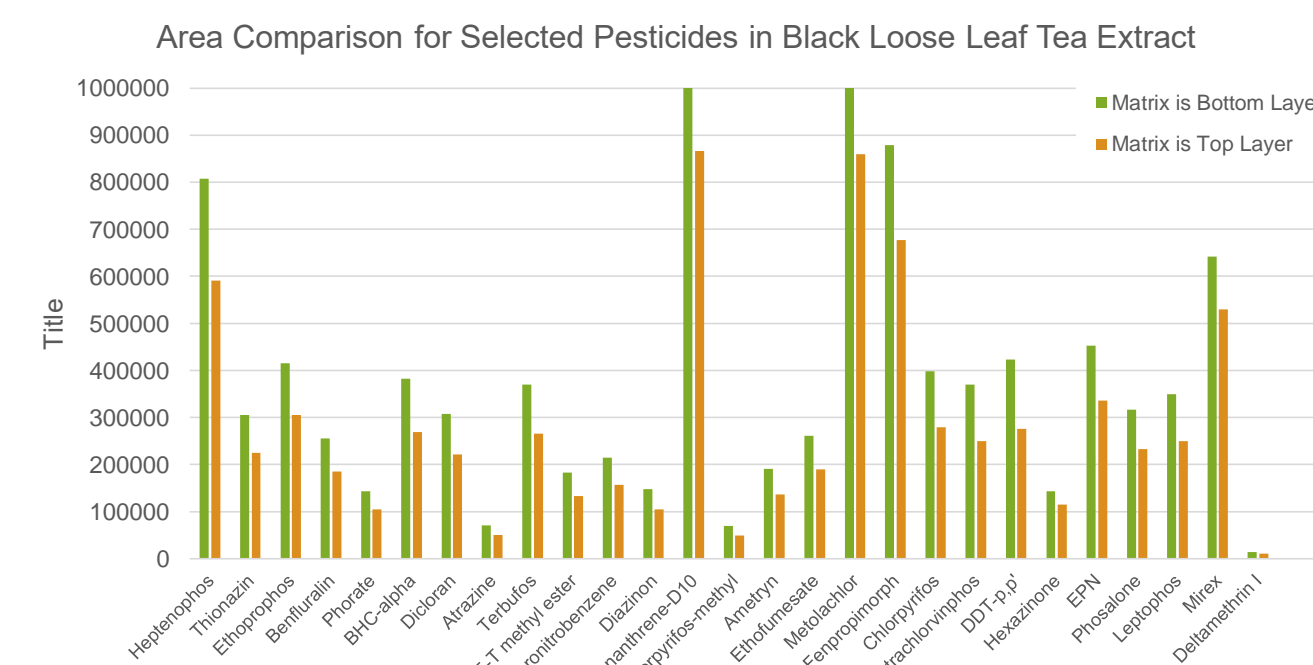


Figure 10. Area Comparison for Selected Pesticides in Black Loose Leaf Tea Extract.

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
Dicloran	4.78	5.91	2.73	9.08
Atrazine	2.73	3.24	2.19	11.63
Terbufos	1.90	3.29	4.18	15.89
2,4,5-T methyl ester	0.91	6.06	1.12	13.74
Pentachloronitrobenzene	4.59	6.49	3.25	11.27
Diazinon	1.90	9.02	2.89	10.16
Phenanthrene-D10	2.01	5.64	1.79	10.59
Chlorpyrifos-methyl	4.62	7.47	3.44	11.68
Ametryn	1.56	5.84	0.92	11.01
Ethofumesate	4.90	5.88	0.53	12.25
Metolachlor	1.13	2.58	1.74	13.73
Fenpropimorph	1.34	4.91	2.02	11.55
Chlorpyrifos	5.95	7.12	0.62	11.20
Tetrachlorvinphos	2.76	3.16	0.97	13.30
DDT-p,p'	3.25	9.02	10.35	26.08
Hexazinone	1.59	6.63	1.79	2.28
EPN	3.01	13.14	1.79	2.28
Phosalone	1.45	9.25	3.23	14.00
Laptophos	2.43	10.09	3.96	13.83
Mirex	2.11	7.02	1.89	14.08
Deltamethrin I	1.44	2.21	0.71	13.80
Deltamethrin I	0.56	9.74	5.00	8.81

Results

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 $R^2 \geq 0.991$ (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 $\leq 30\%$, and 85 % of the analyzed pesticides were found to have a Limit of Quantitation (LOQ) ≤ 0.1 ppb.

Compound	Organic Honey		Black Tea	
	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	3.40	0.79	2.91
Benfluralin	0.65	2.38	0.38	1.41
Phorate	3.18	11.70	0.11	0.41
BHC-alpha	0.92	3.37	0.48	1.77
Dicloran	0.99	3.66	0.50	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	0.70	2.59	0.20	0.73
Pentachloronitrobenzene	0.50	1.84	0.22	0.81
Diazinon	1.27	4.67	0.20	0.72
Phenanthrene-D10	3.10	11.40	1.70	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	1.40	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	0.50	1.84	0.29	1.05
Laptophos	0.32	1.19	0.28	1.03
Mirex	3.12	11.47	0.19	0.70
Deltamethrin I	26.98	85.83	0.03	0.10

Conclusions

The Agilent 7693A Automatic Liquid Sampler (ALS) Reversed 3-Layer Switch sandwich injection allowed for: 1) the placement of the calibration standards or samples in order starting with Tray 1 Vial 1, 2) drawing up the ISTD first to avoid cross contamination, and 3) 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&E M7412AA method delivers an optimal analysis for trace pesticide residues.

References

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