Determination of pyrethroids and macrocyclic lactone insecticides in spices and tea

Using the SCIEX 7500 system

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Pyrethroids and macrocyclic lactones are groups of commonly used insecticides in the agriculture and horticulture industries. Macrocyclic lactones are naturally occurring, or semisynthetic, compounds produced as fermentation products in soil-dwelling *Streptomyces avermitilis.*[1] Pyrethroids, on the other hand, are synthetic, and were designed based on the naturally occurring family of pyrethrins, which were originally derived from chrysanthemum flowers.[2]

Due to the widespread use of these compounds in the environment, a comprehensive quantitative method is necessary to monitor and control their concentration in final food products destined for human consumption.

Here, a method has been developed using the SCIEX 7500 system for the simultaneous identification and quantification of pyrethroid and macrocyclic lactone insecticides at detection levels below the maximum residue level defined by the European Commission under regulation 2018/1514.[3]

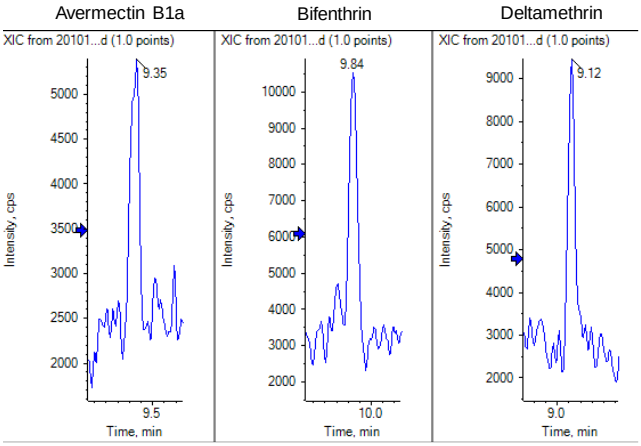

Figure 1. Signals obtained for avermectin B1a, bifenthrin and deltamethrin at their respective LLOQs. A good signal is obtained at a concentration level of 0.02 ng/mL, highlighting the sensitivity that can be achieved when using the SCIEX 7500 system.

Key features of the SCIEX 7500 system for the quantification of pesticides in spices and green tea

- Highly sensitive detection and quantification of avermectin (containing 96% avermectin B1a and 4% avermectin B1b), bifenthrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerat, λ-cyhalothrin, milbemectin A3 and A4 and permethrin from QuEChERS extracts of spices and green tea
- Improved sensitivity over previous assays with lower limits of quantification (LLOQs) down to 0.02 ng/mL in solvent (Figure 1)
- Optimization of new parameter Q0D for milbemectin A3 in spices resulted in greatly reduced background and increased signal to noise
- Increased sensitivity allows for the use of lower sample injection volumes, increasing assay robustness and significantly reducing ion suppression in matrix

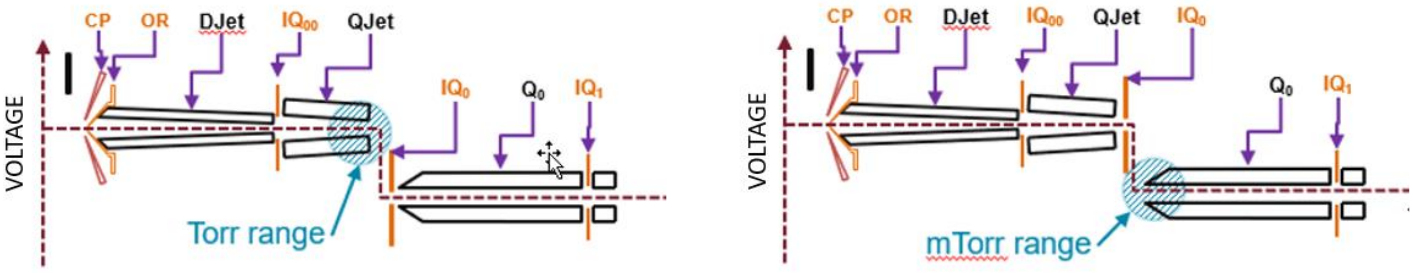

Figure 2. Additional declustering of ions using Q0 dissociation. On the SCIEX 7500 system, additional declustering can be applied during LC-MS analysis using 2 modes: Q0D simple (left) and Q0D enhanced (right). Use of a voltage differential, applied either between the QJet ion guide rods and IQ0 lens or between the IQ0 lens and Q0 on the SCIEX 7500 system, can aid in breaking up clusters, removing interferences and increasing signal to noise.

Methods

Sample preparation: Avermectin (containing 96% avermectin B1a and 4% avermectin B1b), bifenthrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerat, λ-cyhalothrin, milbemectin A3 and A4 and permethrin were diluted into mobile phase A for analysis. Calibration curves were constructed in mobile phase A by spiking in compounds at concentrations of 0.02–20 ng/mL.

Organic green tea (1 g) was milled to a fine powder, homogenized and added to 10 mL of water and 10 mL of acetonitrile. For the spices (organic paprika powder), a starting mass of 2 g was used. The sample was shaken for 1 min and added to Macherey-Nagel QuEChERS Mix I (ref 730970). The sample was shaken for another 3 min and centrifuged for 5 min. Next, 8 mL of the organic (upper) phase was added to Macherey-Nagel QuEChERS Mix III (ref 730648). The sample was shaken for 3 min, centrifuged for 5 min and 7 mL was then taken and acidified with 5% formic acid in acetonitrile (10 µL per 1 mL of sample taken). Compounds were spiked into the tea extract at 1 ng/mL and 10 ng/mL, corresponding to 10 ppb and 100 ppb in the original tea and 5 ppb and 50 ppb in the original spice sample. Samples were analyzed on the SCIEX 7500 system without further dilution.

Chromatography: Chromatographic separation was performed using the ExionLC AD system and a Phenomenex Synergi Fusion-RP (4 µm, 50 x 2.1 mm) column at a flow rate of 0.25 mL/min. Mobile phase A was 5 mM ammonium formate in 20% methanol and 80% water (v/v); mobile phase B was 5 mM ammonium formate in 90% methanol and 10% water (v/v). A gradient of 0–100% mobile phase B over 8 min was used. The total analysis time was 17 min, using either a 2 µL or 5 µL injection volume. The column temperature was 40°C.

Mass spectrometry: Multiple reaction monitoring (MRM) analysis was performed using a SCIEX 7500 system. The system was operated in positive electrospray ionization (ESI) mode using the OptiFlow Pro ion source. Data was acquired using SCIEX OS software. Source conditions, which were optimized using the guided optimization tool in SCIEX OS software, were ISV 3500 V, GS1 40, GS2 70, TEM 250ºC and CUR 50.

Data processing: Data was processed using SCIEX OS software.

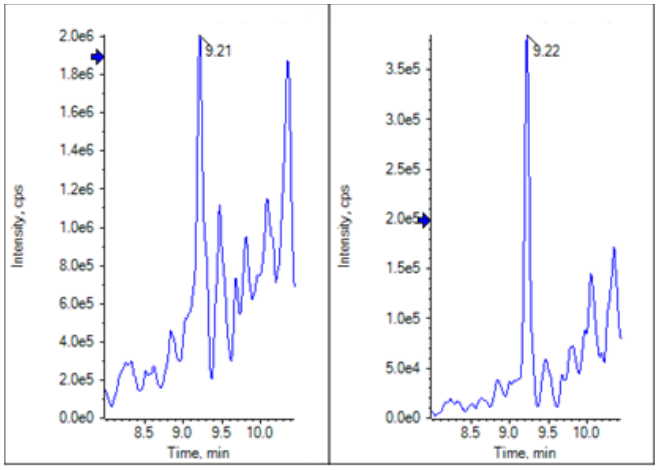

Figure 3. Sensitivity increase resulting from optimization of parameters for Q0D enhanced for milbemectin A3 in spices. XIC for milbemectin A3 using default Q0D value of -10 V (left); XIC for milbemectin A3 using optimized Q0D enhanced mode value of 25 V (right). Milbemectin was analyzed at a concentration of 50 ppb (2 µL injection volume). A signal to noise increase of 3.5-fold was observed after optimization of Q0D.

Method development and optimization

An MRM method was first developed and optimized on the SCIEX Triple Quad 5500 system and targeted more than 500 pesticides and their metabolites. Eleven of the most challenging compounds from the multi-component method were selected for evaluation on the SCIEX 7500 system, and the method was adapted. Using the guided optimization tool in SCIEX OS software, collision energies were re-optimized for the SCIEX 7500 system. Source conditions were also optimized using the guided optimization tool in SCIEX OS software.

Additional declustering of ions can be achieved on the SCIEX 7500 system by using a voltage differential applied either between the QJet ion guide and IQ0 lens (Q0 dissociation, Q0D simple) or between the IQ0 lens and Q0 rods (Q0D enhanced), illustrated in Figure 2. Q0D is useful for breaking up clusters, decreasing interferences and increasing signal to noise in some cases. Q0D was optimized for a subset of the analytes that were the most difficult to detect in green tea and spices. MRM extracted ion chromatograms (XICs) for milbemectin A3 in spices that were acquired using both default settings and optimized Q0D enhanced settings are shown in Figure 3. In this case, a signal to noise increase of 3.5-fold was observed after optimization of Q0D.

The final MRM assay comprised 1 MRM transition per compound. Separation of target compounds is shown in Figure 4.

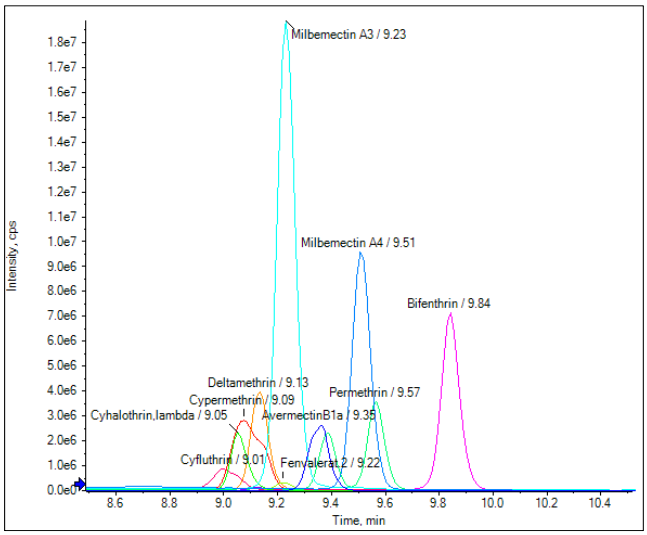

Figure 4. Chromatographic separation of 10 compounds. Avermectin (containing 96% avermectin B1a and 4% avermectin B1b), bifenthrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerat, λ-cyhalothrin, milbemectin A3 and A4 and permethrin at a concentration of 10 ng/mL (5 µL injection volume) in mobile phase A.

Dilution series in solvent

The optimized MRM assay was applied to the analysis of a dilution series of target compounds in mobile phase A. Standards were prepared in the range 0.01–20 ng/mL; 5 µL sample was injected per analysis. Each sample was analyzed 3 times. Examples of calibration curves and MRM XICs from the low end of the dilution series for 3 compounds are shown in Figure 5. LLOQs and associated statistics for all compounds are shown in Table 1.

Table 1. LLOQs and associated statistics for a dilution series of 10 compounds in solvent. Avermectin contains 96% avermectin B1a and 4% avermectin B1b. For each compound, linearity was observed from the LLOQ to the 20 ng/mL top standard. A linear regression with 1/x weighting was applied to the data.

Compound	Q1 (*m/z*)	Q3 (*m/z*)	Retention time (min)	LLOQ (ng/mL)	# Values at LLOQ	% Accuracy at LLOQ	% CV at LLOQ	Linearity (r value)
AvermectinB1a	*890.5*	*305.1*	*9.35*	*0.02*	*3 of 3*	*142*	*7.4*	*0.9964*
AvermectinB1b	*876.5*	*291.1*	*9.12*	*0.50*	*3 of 3*	*99.9*	*6.6*	*0.9958*
Bifenthrin	*440.2*	*181.1*	*9.84*	*0.02*	*3 of 3*	*154*	*4.1*	*0.9937*
Cyfluthrin	*450.9*	*434.0*	*8.99*	*0.50*	*3 of 3*	*96.9*	*5.1*	*0.9890*
Cypermethrin	*433.1*	*191.0*	*9.09*	*0.02*	*3 of 3*	*106*	*10.5*	*0.9918*
Deltamethrin	*522.9*	*280.7*	*9.13*	*0.02*	*3 of 3*	*97.4*	*3.4*	*0.9924*
Fenvalerat	*437.0*	*125.0*	*9.22*	*0.20*	*3 of 3*	*93.4*	*5.6*	*0.9915*
λ-cyhalothrin	*467.0*	*225.0*	*9.06*	*0.05*	*3 of 3*	*110*	*12.6*	*0.9897*
Permethrin	*408.2*	*355.1*	*9.57*	*0.05*	*3 of 3*	*107*	*12.6*	*0.9914*
Milbemectin A3	*546.1*	*511.2*	*9.24*	*0.05*	*3 of 3*	*99.3*	*6.6*	*0.9987*
Milbemectin A4	*560.0*	*525.2*	*9.52*	*0.05*	*3 of 3*	*99.5*	*10.4*	*0.9965*

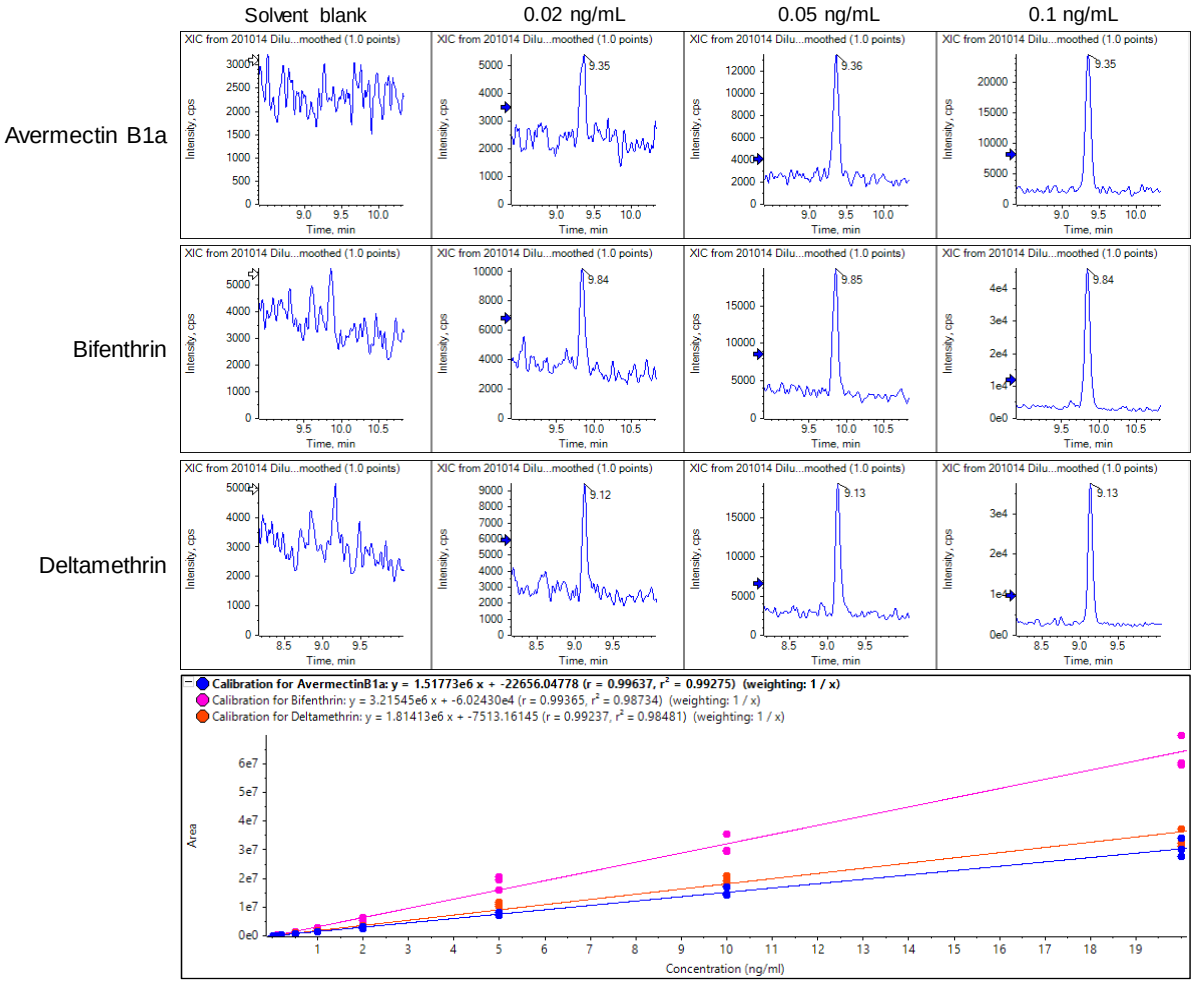

Figure 5. MRM XICs from the low end of the calibration curves for avermectin B1a, bifenthrin and deltamethrin. An LLOQ of 0.02 ng/mL was obtained for each of these 3 compounds in solvent with linearity maintained across the entire dilution series (all r values ≥ 0.9924).

Compounds spiked into spices and green tea

Compounds were next spiked into QuEChERS extracts of spices and green tea at 1 ng/mL and 10 ng/mL, corresponding to 5 ppb and 50 ppb in spices and 10 ppb and 100 ppb in tea. Quantification of target compounds in matrix against the dilution series in solvent was performed using both 2 µL and 5 µL injection volumes to assess the effect of lowering the injection volume on matrix ion suppression. It was found that the reduction in ion suppression observed for a 2 µL injection compared with a 5 µL injection mostly compensated for the reduction in injection volume to the extent that the sensitivity of the assay was not significantly impacted (Table 2 and Figure 6). Figure 7 demonstrates the background signal and ion suppression observed with a 2 µL injection volume; however, the level of ion suppression was much less than the ion suppression observed with higher volume injections (Figure 6).

It is noted that for bifenthrin, an unexpected increase in signal was observed in the QuEChERS spiked samples compared with the dilution series in solvent. The source of this anomalous result is unknown and requires further investigation. See Table 3 and Table 4 for a summary of each compound's ion suppression results and concentrations of detection (mean calculated concentration) in the matrices analyzed.

Compound	Q1	Q3	Spices, 2 µL Injection			Tea, 2 µL Injection		
			Blank	5 ppb	50 ppb	Blank	10 ppb	100 ppb
AvermectinB1a	890.5	305.1	×	✓	✓	×	✓	✓
AvermectinB1b	876.5	291.1	×	×	×	×	×	×
Bifenthrin	440.2	181.1	✓	✓	✓	×	✓	✓
Cyfluthrin	450.9	434.0	×	×	✓	×	×	✓
Cypermethrin	433.1	191.0	×	×	✓	×	✓	✓
Deltamethrin	522.9	280.7	×	✓	✓	×	✓	✓
Fenvalerat	437.0	125.0	×	×	✓	×	✓	✓
Cyhalothrin,lambda	467.0	225.0	×	×	✓	×	✓	✓
Permethrin	408.2	355.1	×	×	✓	×	✓	✓
Milbemectin A3	546.1	511.2	×	×	✓	×	×	✓
Milbemectin A4	560.0	525.2	×	×	✓	×	×	✓

Compound	Q1	Q3	Spices, 5 µL Injection			Tea, 5 µL Injection		
			Blank	5 ppb	50 ppb	Blank	10 ppb	100 ppb
AvermectinB1a	890.5	305.1	×	✓	✓	×	✓	✓
AvermectinB1b	876.5	291.1	×	×	×	×	×	×
Bifenthrin	440.2	181.1	✓	✓	✓	×	✓	✓
Cyfluthrin	450.9	434.0	×	×	✓	×	×	✓
Cypermethrin	433.1	191.0	×	×	✓	×	✓	✓
Deltamethrin	522.9	280.7	×	✓	✓	×	✓	✓
Fenvalerat	437.0	125.0	×	×	✓	×	✓	✓
Cyhalothrin,lambda	467.0	225.0	×	×	✓	×	✓	✓
Permethrin	408.2	355.1	×	×	✓	×	✓	✓
Milbemectin A3	546.1	511.2	×	×	✓	×	×	✓
Milbemectin A4	560.0	525.2	×	×	✓	×	×	✓

Table 2. Detection of spices and green tea. Top: 2 µL sample injected per analysis. Bottom: 5 µL sample injected per analysis. No difference in sensitivity was observed when using a 2 µL injection compared to a 5 µL injection volume. This may be due to the reduction in ion suppression observed for a lower volume injection (see Figure 6).

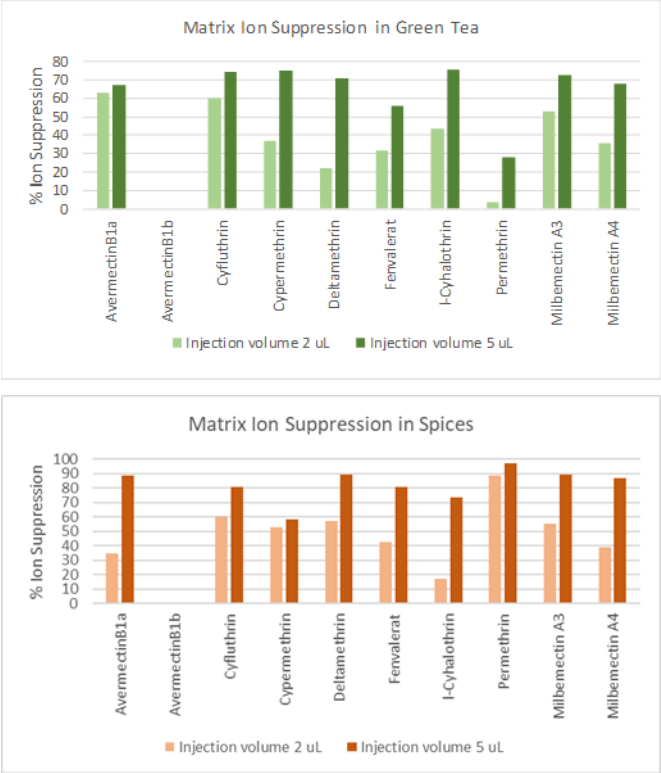

Figure 6. Ion suppression in green tea at 100 ppb (top) and spices at 50 ppb (bottom) versus solvent for injection volumes of 2 µL and 5 µL. Each sample was analyzed 3 times at each injection volume, and concentrations were calculated against a dilution in solvent. The % ion suppression values were calculated based on expected concentrations. See Table 3 and Table 4 for more detail.

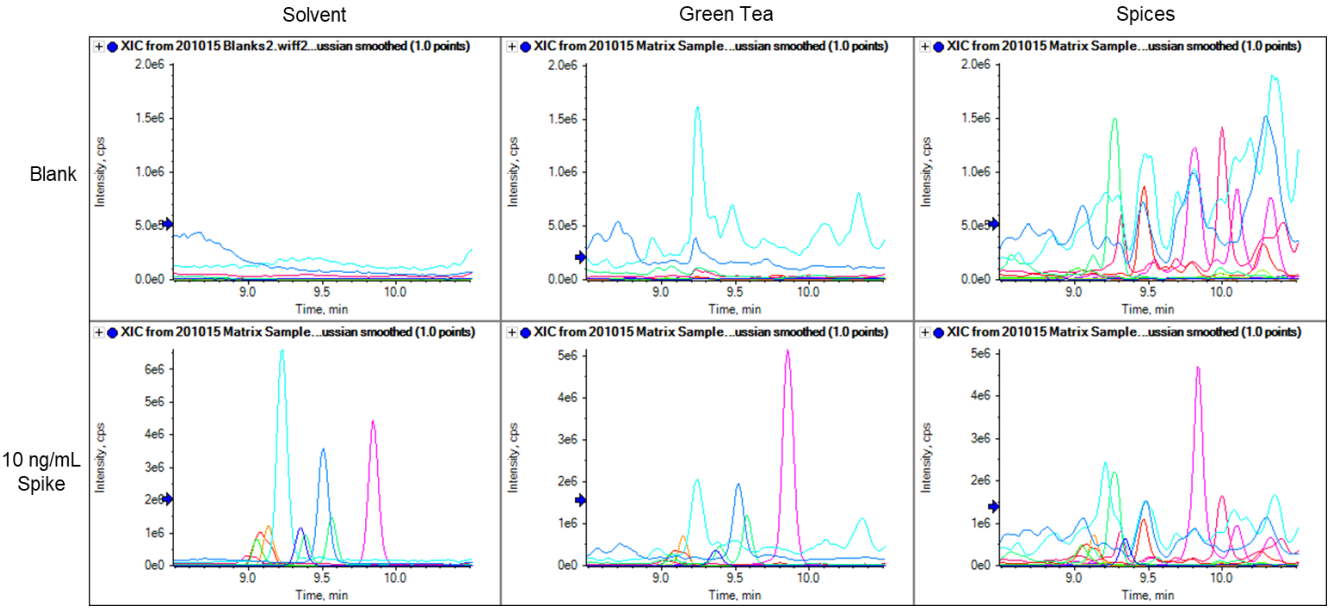

Figure 7. Analysis of 10 compounds in different matrices. MRM XICs for 10 compounds spiked into solvent (left), and into QuEChERS extracts of green tea (middle) and spices (right) using a 2 µL injection volume. Data is from blank samples, shown at the top, and samples with a concentration of 10 ng/mL (100 ppb in green tea and 50 ppb in spices), shown at the bottom. High levels of background signals are present in the green tea and spice QuEChERS extracts compared with analysis of compounds spiked into solvent alone.

Table 3. Quantification of 10 compounds in green tea against a dilution series in solvent. A QuEChERS extract of green tea was spiked at 1 ng/mL and 10 ng/mL (10 ppb and 100 ppb) with each of 10 compounds and analyzed using the SCIEX 7500 system. Two injection volumes (2 µL and 5 µL) were used. Each sample was analyzed 3 times at each injection volume. Calculated concentrations against solvent curves were adjusted for injection volume.

Compound	Solvent RT (min)	Green tea, 10 ppb, 2 µL injection			Green tea, 100 ppb, 2 µL injection			Green tea, 10 ppb, 5 µL injection			Green tea, 100 ppb, 5 µL injection		
		Matrix RT (min)	Mean calc. conc. (ppb)	Matrix % ion suppression	Matrix RT (min)	Mean calc. conc. (ppb)	Matrix % ion suppression	Matrix RT (min)	Mean calc. conc. (ppb)	Matrix % ion suppression	Matrix RT (min)	Mean calc. conc. (ppb)	Matrix % ion suppression
AvermectinB1a	9.35	9.35	4.1	59.5	9.36	36.4	63.3	9.35	2.7	73.4	9.33	32.7	67.3
AvermectinB1b	9.12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bifenthrin	9.84	9.84	23.9	-138.8	9.85	264.0	-163.9	9.84	14.3	-42.5	9.83	164.0	-63.8
Cyfluthrin	8.99	ND	ND	ND	9.02	39.8	60.3	ND	ND	ND	9.00	25.4	74.6
Cypermethrin	9.09	9.10	3.5	64.8	9.10	63.0	37.1	9.10	1.9	80.7	9.10	24.9	75.1
Deltamethrin	9.13	9.13	4.7	53.3	9.13	77.9	22.1	9.13	3.0	70.2	9.13	29.5	70.5
Fenvalerat	9.22	9.21	6.9	31.5	9.22	68.1	31.9	9.21	3.8	62.2	9.21	44.2	55.8
λ-cyhalothrin	9.06	9.05	3.5	65.5	9.07	56.3	43.7	9.05	2.5	74.9	9.05	24.6	75.4
Permethrin	9.57	9.56	7.4	26.0	9.57	96.5	3.5	9.56	9.8	1.9	9.56	72.1	27.9
Milbemectin A3	9.24	ND	ND	ND	9.23	47.2	52.9	ND	ND	ND	9.21	27.5	72.5
Milbemectin A4	9.52	ND	ND	ND	9.51	64.3	35.8	ND	ND	ND	9.45	32.2	67.9

ND = not detected

Table 4. Quantification of 10 compounds in spices against a dilution series in solvent. A QuEChERS extract of spices was spiked at 1 ng/mL and 10 ng/mL (5 ppb and 50 ppb) with each of 10 compounds and analyzed using the SCIEX 7500 system. Two injection volumes (2 µL and 5 µL) were used. Each sample was analyzed 3 times at each injection volume. Calculated concentrations against solvent curves were adjusted for injection volume.

Compound	Solvent RT (min)	Spices, 5 ppb, 2 µL injection			Spices, 50 ppb, 2 µL injection			Spices, 5 ppb, 5 µL injection			Spices, 50 ppb, 5 µL injection		
		Matrix RT (min)	Mean calc. conc. (ppb)	Matrix % ion suppression	Matrix RT (min)	Mean calc. conc. (ppb)	Matrix % ion suppression	Matrix RT (min)	Mean calc. conc. (ppb)	Matrix % ion suppression	Matrix RT (min)	Mean calc. conc. (ppb)	Matrix % ion suppression
AvermectinB1a	9.35	9.33	2.6	47.8	9.34	32.6	34.8	9.29	0.7	86.8	9.29	5.8	88.4
AvermectinB1b	9.12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bifenthrin	9.84	9.82	8.8	-75.3	9.84	69.0	-38.0	9.81	1.5	70.6	9.82	15.4	69.2
Cyfluthrin	8.99	ND	ND	ND	9.02	20.0	60.2	ND	ND	ND	9.00	9.6	80.8
Cypermethrin	9.09	ND	ND	ND	9.09	23.6	52.9	ND	ND	ND	9.07	21.0	58.1
Deltamethrin	9.13	9.13	1.7	66.8	9.13	21.6	56.9	9.13	0.6	88.3	9.12	5.6	88.9
Fenvalerat	9.22	ND	ND	ND	9.21	28.9	42.3	ND	ND	ND	9.19	9.7	80.6
λ-cyhalothrin	9.06	ND	ND	ND	9.04	41.6	16.8	ND	ND	ND	9.02	13.3	73.4
Permethrin	9.57	ND	ND	ND	9.56	5.8	88.4	ND	ND	ND	9.54	1.4	97.2
Milbemectin A3	9.24	ND	ND	ND	9.21	22.3	55.5	ND	ND	ND	9.17	5.4	89.2
Milbemectin A4	9.52	ND	ND	ND	9.48	30.6	38.8	ND	ND	ND	9.49	6.7	86.7

ND = not detected

Conclusions

To summarize, the SCIEX 7500 system provides impressive levels of sensitivity, robustness and accuracy for trace quantification of insecticides in food matrices. In this study, excellent sensitivity has been demonstrated with LLOQ values down to 0.02 ng/mL. Quantification of pyrethroids and macrocyclic lactones in both green tea and spices has also been shown. By using a higher sensitivity LC-MS platform, injection volumes could be reduced, lowering ion suppression without impacting the overall assay sensitivity. The reduced amount of matrix injected for large studies can help to improve total system uptime.

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