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Unstoppable analysis of pesticides residues in black tea using triple quadrupole GC-MS

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Goal: The aim of this application note is to demonstrate the performance of the Thermo Scientific™ TSQ™ 9610 triple quadrupole mass spectrometer coupled to the Thermo Scientific™ TRACE™ 1610 GC for trace level determination of pesticide residues in black tea.

INTRODUCTION

Products of botanical origin, including black tea, have become an increasingly prevalent part of the worldwide health culture with their global market forecast to reach more than $230 billion by 2027. Manufacturers must ensure that these botanicals are safe for consumption, which requires routine/robust trace analysis of pesticide residues. Pesticides are chemicals used for crop protection against a variety of pests such as weeds, fungi, rodents, and insects. Because of their extensive use, pesticides can be found in the air, soil, water, and ultimately in the food chain. Despite their use being highly regulated, misuse of pesticides can lead to unwanted contamination of food and have possible impacts on both human and environmental health.

Laboratories performing the analysis of pesticides in botanical Table 1. GC-MS/MS and autosampler experimental conditions for matrices face numerous challenges. A prominent obstacle to accurate pesticide residue determination is matrix interference. As most botanical ingredients exist in a form of concentrated extracts, smaller sample sizes are needed to overcome heavy matrix interference, in turn requiring highly sensitive instrumentation to detect trace amounts of pesticide residues. Sensitivity is key in this analysis as the regulatory maximum residue levels (MRLs) must be met. The typical global MRL level for pesticides in food is 0.01 mg/kg. High-throughput laboratories must consistently hit the MRL levels in matrix with minimal user intervention on the GC-MS/MS system. Unplanned and prolonged instrument downtime can delay the return of results to the manufacturer of the botanicals. It is therefore essential that the GC-MS/MS produces confident and reliable results over time to ensure consumer safety.

In this study, the suitability of the TSQ 9610 triple quadrupole GC-MS/MS system was assessed for the analysis of more than 200 pesticides in black tea at trace concentrations supplemented with SPE cleanup. The linearity, accuracy, precision, limit of quantitation, and injection reproducibility of 20 selected analytes representative of the different pesticide classes in black tea matrix were demonstrated.

EXPERIMENTAL

Data was acquired with a TSQ 9610 triple quadrupole mass spectrometer equipped with a Thermo Scientific™ NeverVent™ Advanced Electron Ionization (AEI) ion source was coupled to a TRACE 1610 gas chromatograph equipped with a Thermo Scientific™ iConnect™ Split/Splitless (iConnect-SSL) injector and a Thermo Scientific™ AI/AS 1610 liquid autosampler. The NeverVent technology allows for ion source cleaning, filament replacement, and column exchange without breaking instrument vacuum, therefore ensuring minimum downtime to the laboratory workflow. Chromatographic separation was achieved on a Thermo Scientific™ TraceGOLD™ TG-5SILMS analytical column, 30 m × 0.25 mm i.d. × 0.25 μm fused silica capillary, with 10 m SafeGuard, (P/N 26096-1421). The MS/MS method monitored a minimum of three transitions for each compound. Additional method details are in Table 1.

Table 1. GC-MS/MS and autosampler experimental conditions for the analysis of pesticides

Parameter Value	Parameter Value
Injection liner	Thermo Scientific ™ LinerGOLD™ Splitless Liner, Single Taper with Quartz Wool, 4 mm (P/N 453A1925-UI)
GC column	TraceGOLD TG-5SILMS analytical column, 30 m × 0.25 mm i.d. × 0.25 μm fused silica capillary column with 10 m SafeGuard, P/N 26096-1421
Injection volume	1 μL
Injection temperature	260 °C
Splitless time	1 min
Column flow	1.4 mL/min, He
Run time	26.5 min
MS source temperature	320 °C
Emission current	10 μA
Aquistion mode	timed-SRM

Compounds were evaluated using SANTE/12682/2019 (Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed[1]) guidelines:

- Compound recovery should be within a mean recovery of 80–120%. If recovery within this range cannot be achieved, correction for recovery is necessary, or an explanation given for why a correction factor was not applied.
- Ion ratios for detected compounds must be within ±30% of the average of matrix-matched calibration standards.
- Detected compounds must have a signal-to-noise ratio (S/N) of at least 3:1.

In this application, difficult-to-analyze compounds, in addition to compounds from different pesticide classes (organophosphates, organochlorides, synthetic pyrethroids, and herbicide methyl esters), are highlighted in the results.

Data acquisition, processing, and reporting

Data were acquired, processed, and reported using the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.3. Integrated instrument control ensures full

automation of the analytical workflow combined with an intuitive user interface for data analysis, processing, customizable reporting, and storage in compliance with the Federal Drug Administration Title 21 Code of Federal Regulations Part 11 (Title 21 CFR Part 11). The advanced reprocessing capability of Chromeleon CDS ensures immediate and easy data analysis, offering the possibility to easily flag and check the compliance of the results with the SANTE/12682/2019 criteria.[1]

Sample preparation

Sample preparation involved the addition of water and acetonitrile to a black tea matrix, followed by QuEChERS extraction. Final clean-up was performed with solid-phase extraction using a cartridge consisting of graphitized carbon black (GCB) and primary−secondary amine sorbent (PSA). The resulting extract solutions were concentrated and re-diluted with toluene for a GC-MS/MS analysis.

RESULTS AND DISCUSSION

Chromatography

Black tea is an extremely complex sample, and it is important that the matrix interferences are removed during sample preparation to produce good chromatography and subsequent quantitation of pesticides at the MRLs defined by the regulations. A timed-selected reaction monitoring (t-SRM) acquisition method allowed for simultaneous acquisition of multiple characteristic ions for each pesticide, maintaining high sensitivity and selectivity to discriminate between the target compounds and the matrix, thus ensuring a confident and selective identification of analytes. Figure 1 shows the t-SRM acquisition of pesticides at 5 µg/kg in black tea.

Linearity

The TSQ 9610 NeverVent AEI is equipped with the Thermo Scientific™ XLXR™ detector, which offers an extended dynamic range and longer lifetime compared with its predecessor. In this experiment, linearity was evaluated for select analytes using matrix-matched calibration curves ranging from 0.01 to 200 ng/mL. The lowest standard to meet SANTE/12682/2019 criteria ranged, for the selected analytes, from 0.01 to 2 ng/mL.

Figure 2 shows an example calibration curve for 4,4'-DDT (0.5 to 200 ng/mL (ppb)) and cypermethrin I (2.0 to 200 ng/mL (ppb)). Table 2 shows the calibration result for the 20 selected pesticides.

To be compliant with the current regulation, it is essential that analytical testing laboratories meet the MRL of 10 ppb (0.01 mg/kg). To demonstrate the reliability of the instrument at such low concentration, the peak area repeatability of a 10 ppb black tea extract was assessed by using n=12 repeated injections. The % RSD of the calculated concentrations for all 20 compounds was <20%, with the majority of compounds giving RSDs <10%. Figure 3 shows a summary of results at the MRL including the %RSDs for n=12 injections and the average percentage recovery for each compound. Appendix 1 shows the chromatograms of the lowest matrix-matched standard conforming to SANTE/12682/2019 guidance for each of the selected pesticides.

Recovery and precision

Analyte recovery was assessed by spiking black tea at three levels (10, 25, and 50 ppb) before extracting with QuEChERS. Six extractions were performed at each level. Calculated recoveries for the spiked compounds were between 80% and 120%, with calculated precision ≤10% with the exception of folpet, which is thermally labile and therefore known to break down in the GC injector. Utilizing the PTV injector can help reduce the breakdown of labile compounds. Table 3 summarizes the recovery and precision results.
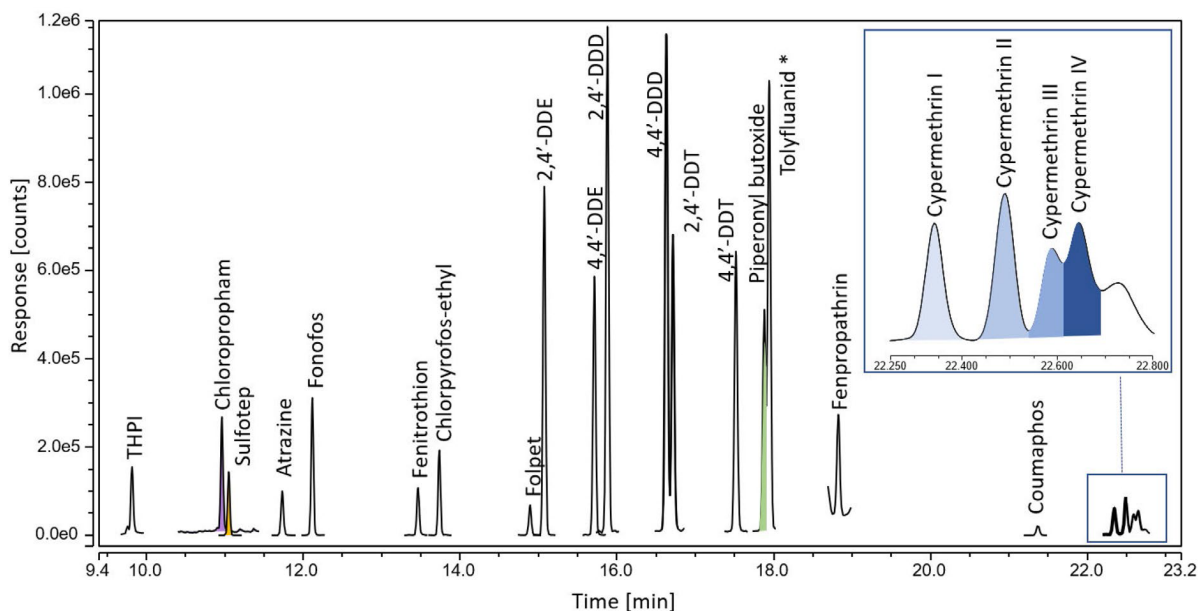
Figure 1. t-SRM acquisition for black tea sample pre-spiked at 5 μg/kg. *A matrix interferent was found to co-elute with tolyfluanid; therefore, the results for this compound were not included in the present work.
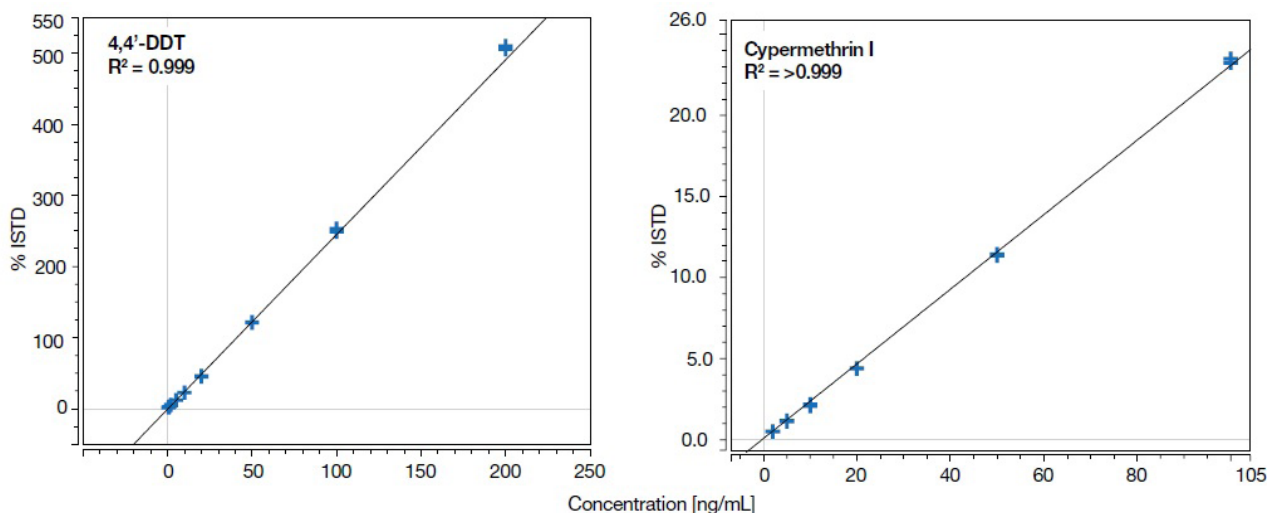
Figure 2. Calibration curve for 4,4'-DDT (range 0.5–200 ng/mL (ppb)) and cypermethrin I (2.0–200 ng/mL (ppb)).

Table 2. Calibration results for 20 selected pesticides in black tea (each point was injected in duplicate)

Peak name	Internal standard	Calibration type	R²	Linear range (ppb)
Atrazine	Internal PCB 18 ISTD	Lin, WithOffset, 1/A	>0.999	0.5–200
Chlorpropham	Internal PCB 18 ISTD	Lin, WithOffset, 1/A	>0.999	0.5–200
Chlorpyrifos-ethyl	Internal PCB 52 ISTD	Lin, WithOffset, 1/A	0.999	0.5–200
Coumaphos	Internal Triphenylphosphate (TPP) ISTD	Lin, WithOffset, 1/A	0.999	1.0–200

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Table 2. Calibration results for 20 selected pesticides in black tea (each point was injected in duplicate) [continuation]

Peak name	Internal standard	Calibration type	R^2	Linear range (ppb)
Cypermethrin I	Internal Triphenylphosphate (TPP) ISTD	Lin, WithOffset, 1/A	>0.999	2.0–200
Cypermethrin II	Internal Triphenylphosphate (TPP) ISTD	Lin, WithOffset, 1/A	>0.999	2.0–200
Cypermethrin III	Internal Triphenylphosphate (TPP) ISTD	Lin, WithOffset, 1/A	0.998	2.0–200
Cypermethrin IV	Internal Triphenylphosphate (TPP) ISTD	Lin, WithOffset, 1/A	0.999	2.0–200
DDD, 2,4'-	Internal Triphenylmethane ISTD	Lin, WithOffset, 1/A	0.999	0.1–200
DDD, 4,4'-	Internal Triphenylmethane ISTD	Lin, WithOffset, 1/A	>0.999	0.5–200
DDE, 2,4'-	Internal Triphenylmethane ISTD	Lin, WithOffset, 1/A	0.999	0.05–200
DDE, 4,4'-	Internal Triphenylmethane ISTD	Lin, WithOffset, 1/A	0.999	0.01–200
DDT, 2,4'-	Internal Triphenylmethane ISTD	Lin, WithOffset, 1/A	0.999	0.5–200
DDT, 4,4'-	Internal Triphenylphosphate (TPP) ISTD	Lin, WithOffset, 1/A	0.999	0.5–200
Fenitrothion	Internal PCB 52 ISTD	Lin, WithOffset, 1/A	0.996	0.5–200
Fenpropathrin	Internal Triphenylphosphate (TPP) ISTD	Lin, WithOffset, 1/A	>0.999	2.0–200
Folpet	Internal PCB 52 ISTD	Lin, WithOffset, 1/A	0.997	2.0–200
Fonofos	Internal PCB 18 ISTD	Lin, WithOffset, 1/A	>0.999	0.5–200
Piperonyl butoxide	Internal Triphenylphosphate (TPP) ISTD	Lin, WithOffset, 1/A	0.999	0.5–200
Sulfotep	Internal PCB 18 ISTD	Lin, WithOffset, 1/A	>0.999	0.1–200
Tetrahydrophthalimide (THPI)	Internal PCB 18 ISTD	Lin, WithOffset, 1/A	0.998	1.0–200
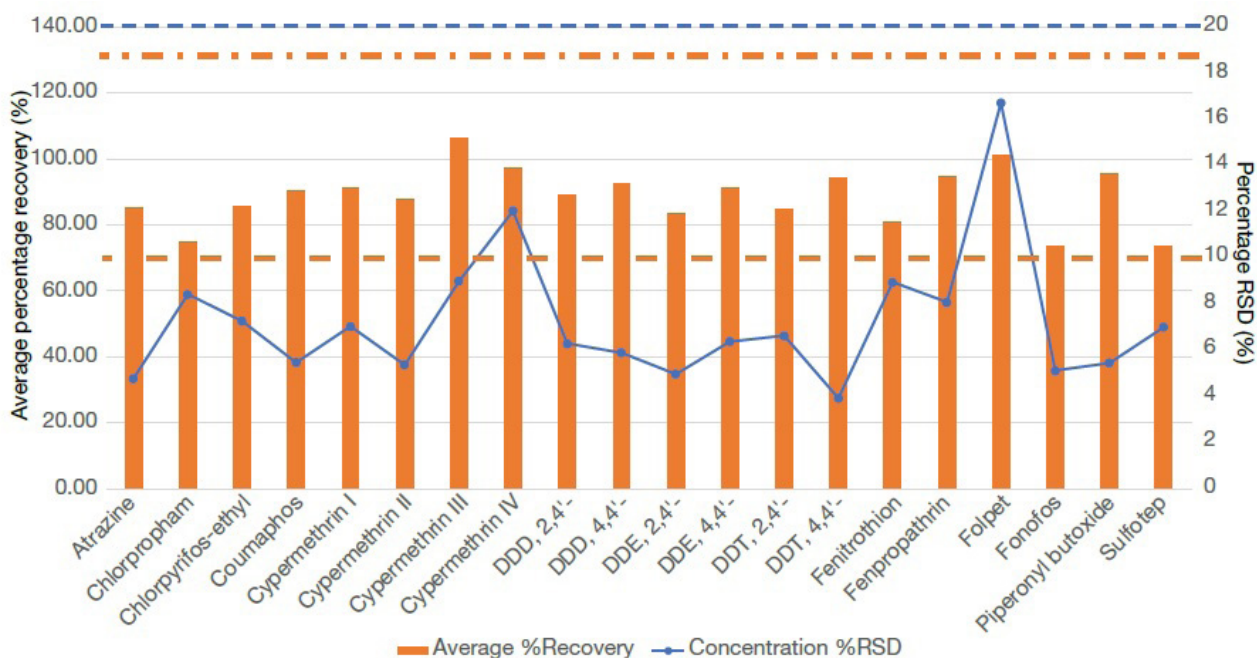
Figure 3. MRL results with %RSDs for n=12 injections and the average percentage recovery for each pesticide.

Table 3. Precision and spike recovery at 10, 25, and 50 ppb (ng/mL) (n=6 extracts at each level)

| Compound	Concentration %RSD			Average %Recovery		
	10 ppb spike (n=6)	25 ppb spike (n=6)	50 ppb spike (n=6)	10 ppb spike (n=6)	25 ppb spike (n=6)	50 ppb spike (n=6)
Atrazine	3.4	4.1	5.5	83.3	84.5	85.5
Chlorpropham	7.8	7.0	2.5	77.7	78.7	74.3
Chlorpyrifos-ethyl	4.7	6.0	3.5	84.0	78.9	81.5
Coumaphos	6.1	6.5	5.5	91.3	92.0	91.1
Cypermethrin I	8.0	3.4	4.1	92.9	91.5	91.3
Cypermethrin II	5.0	3.4	3.7	88.3	89.6	89.2
Cypermethrin III	9.7	4.1	3.6	101.2	91.6	90.7
Cypermethrin IV	7.7	5.9	6.2	88.9	87.9	90.1
DDD, 2,4'-	3.4	5.0	2.2	93.3	91.8	91.3
DDD, 4,4'-	3.1	5.7	2.6	97.0	95.3	93.6
DDE, 2,4'-	3.8	4.7	2.2	86.1	83.4	81.6
DDE, 4,4'-	5.8	4.2	3.5	94.5	88.4	85.6
DDT, 2,4'-	3.8	4.4	3.8	89.6	90.2	87.6
DDT, 4,4'-	3.6	3.0	4.2	96.9	92.7	89.3
Fenitrothion	9.8	7.7	3.8	83.2	93.0	90.9

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Table 3. Precision and spike recovery at 10, 25, and 50 ppb (ng/mL) (n=6 extracts at each level) [continuation]

Compound	Concentration %RSD			Average %Recovery		
	10 ppb spike (n=6)	25 ppb spike (n=6)	50 ppb spike (n=6)	10 ppb spike (n=6)	25 ppb spike (n=6)	50 ppb spike (n=6)
Fenpropathrin	6.0	7.5	5.0	99.1	92.2	92.6
Folpet	20.6	10.3	6.6	107.6	86.2	61.1
Fonofos	4.4	3.8	6.1	76.0	73.2	72.6
Piperonyl butoxide	4.8	3.6	4.3	98.5	95.7	95.0

Robustness

It is important that the results produced over time are consistent and minimal user intervention is required to operate the system. Mass calibration and resolution tuning are two of the most important aspects ensuring system performance. The Thermo Scientific™ SmartTune™ tool allows the user to check the tune status of the system with a few mouse clicks in an easy and quick fashion. To demonstrate instrument robustness, black tea extracts at a concentration of 50 µg/kg were injected continuously over 124 injections. Neither inlet nor ion source maintenance was performed on the system during this extended sequence, which was equivalent to over 2.5 days of continuous analysis. Peak area %RSDs were <10% for all representative analytes (Figure 4 and Table 4) with the exception of folpet, which produces inconsistent results due to breakdown in the hot GC inlet.
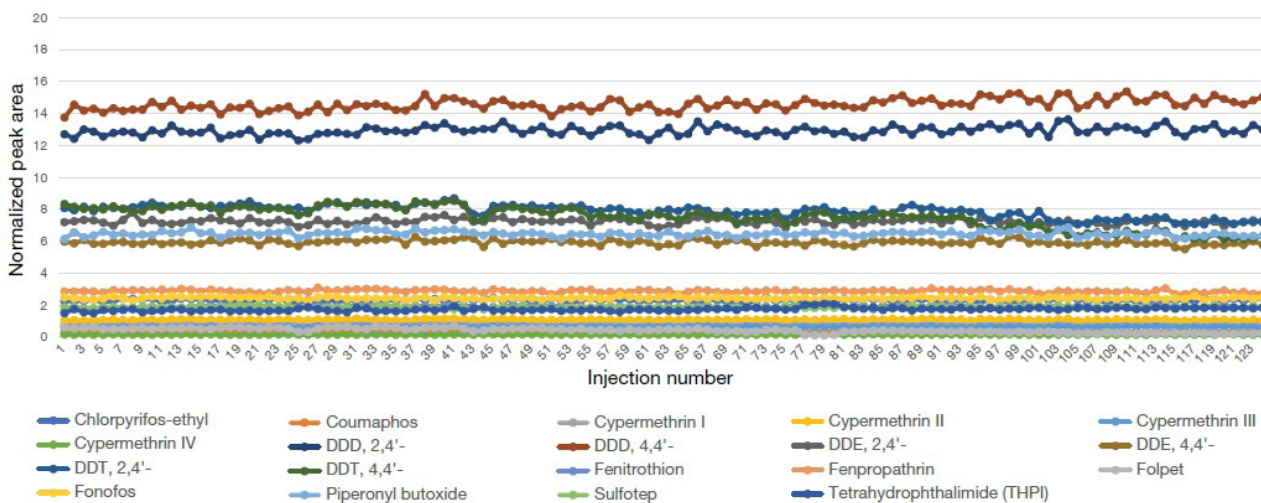
Figure 4. Normalized peak area response (analyte peak area / ISTD peak area) obtained for n=124 consecutive injections of matrix samples spiked at 50 µg/kg.

Table 4. %RSD of pesticides at 50 µg/kg over n = 124 injections

Compound	%RSD (n = 120)
Atrazine	4.3
Chlorpropham	3.6
Chlorpyrifos-ethyl	3.6

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Table 4. %RSD of pesticides at 50 µg/kg over n = 124 injections [continuation]

Compound	%RSD (n = 120)
Coumaphos	4.4
Cypermethrin I	2.8
Cypermethrin II	2.9
Cypermethrin III	3.6
Cypermethrin IV	6.0
DDD, 2,4'	2.1
DDD, 4,4'	2.4
DDE, 2,4'	2.3
DDE, 4,4'	2.6
DDT, 2,4'	5.0
DDT, 4,4'	9.0
Fenitrothion	4.1
Fenpropathrin	2.9
Folpet	34.1
Fonofos	2.8
Piperonyl butoxide	2.4
Sulfotep	2.7
Tetrahydrophthalimide (THPI)	5.9

CONCLUSIONS

The results obtained in these experiments demonstrate that the TSQ 9610 mass spectrometer equipped with the NeverVent AEI ion source in combination with the TRACE 1610 GC and the AI/AS 1610 liquid autosampler delivers excellent analytical performance for multi-residue analysis of pesticides in black tea:
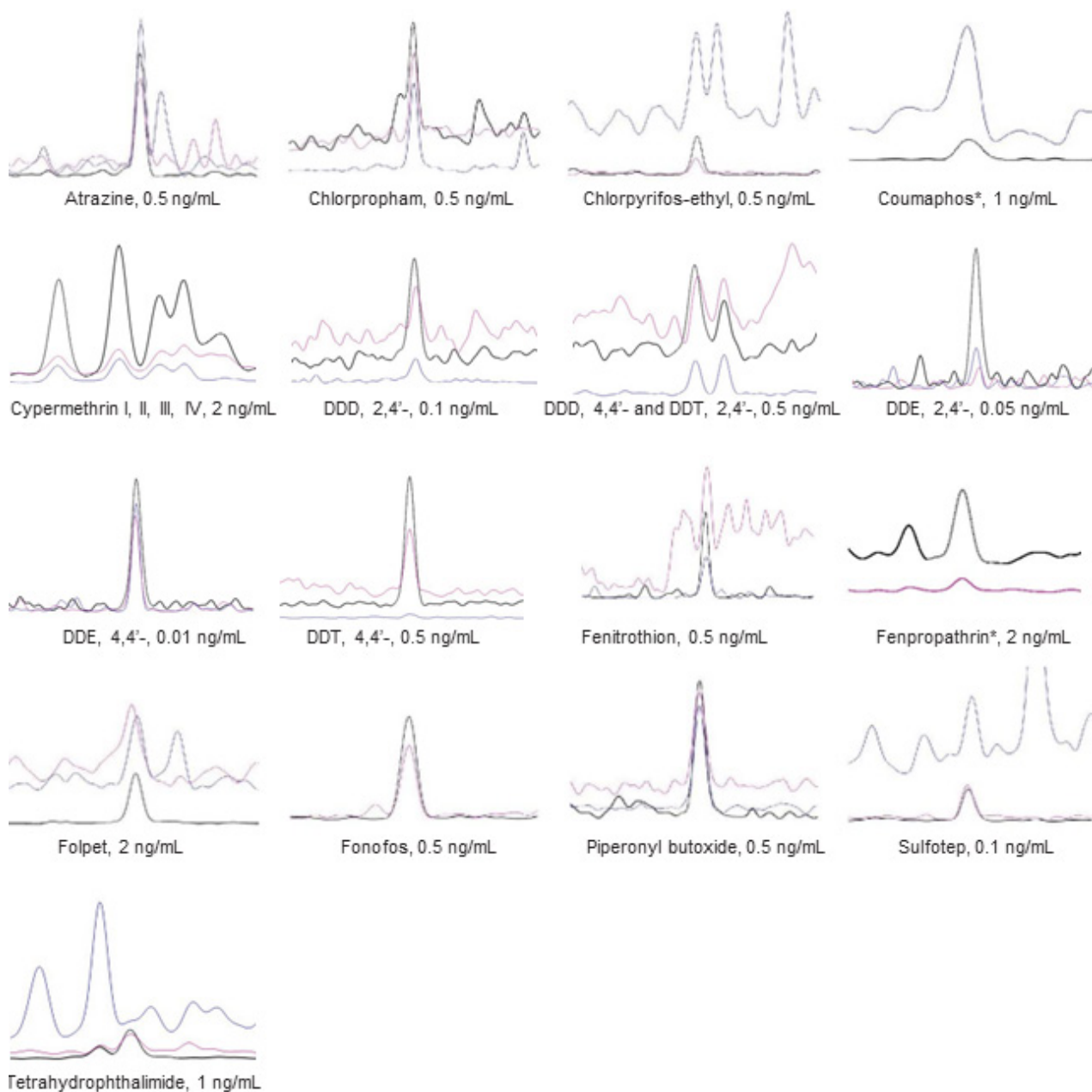
- The excellent sensitivity and good linearity between 0.01 ng/mL and 200 ng/mL allowed detection and accurate quantitation of numerous pesticides in the challenging black tea matrix.
- Peak area %RSDs of calculated concentrations at 10 ppb (0.01 mg/kg) were <10%, which are in compliance with SANTE/12682/2019 (Analytical guidelines).
- Calculated recoveries at three different spiking levels (10, 25, 50 ppb (ng/mL)) were within the 80–120% limits established in the SANTE guidelines.
- The TSQ 9610 mass spectrometer uptime is improved due to the NeverVent technology, allowing for laboratory productivity to be maximized with an uninterrupted workflow.

REFERENCE
1. SANTE/12682/2019, Analytical quality control and method validation procedures for pesticide residues analysis in food and feed. https://ec.europa.eu/food/system/files/2020-01/pesticides_mrl_guidelines_ wrkdoc_2019-12682.pdf

Appendix 1. Chromatograms of lowest matrix-matched standard conforming to SANTE guidance

Atrazine, 0.5 ng/mL

Chlorpropham, 0.5 ng/mL

Chlorpyrifos-ethyl, 0.5 ng/mL

Coumaphos*, 1 ng/mL

Cypermethrin I, II, III, IV, 2 ng/mL

DDD, 2,4'-, 0.1 ng/mL

DDD, 4,4'- and DDT, 2,4'-, 0.5 ng/mL

DDE, 2,4'-, 0.05 ng/mL

DDE, 4,4'-, 0.01 ng/mL

DDT, 4,4'-, 0.5 ng/mL

Fenitrothion, 0.5 ng/mL

Fenpropathrin*, 2 ng/mL

Folpet, 2 ng/mL

Fonofos, 0.5 ng/mL

Piperonyl butoxide, 0.5 ng/mL

Sulfotep, 0.1 ng/mL

Tetrahydrophthalimide, 1 ng/mL

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