Staying ahead of the challenges of pesticides residues analysis using a new triple quadrupole GC-MS

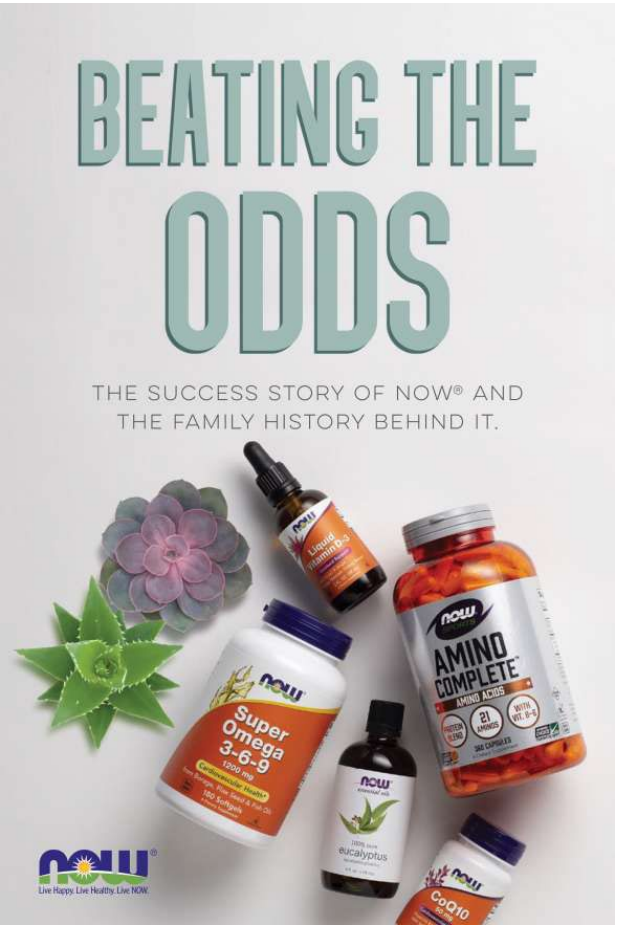
Katie Banaszewski

Sr. Director of Quality

NOW Health Group

NOW Foods

- In 1968 Elwood Richard started the company under the belief that good health was not a luxury available only to the wealthy.
- NOW is still owned by the Richard family and has grown to become one of the natural product industry's most recognizable brands, providing one of the most extensive lines of natural products available.

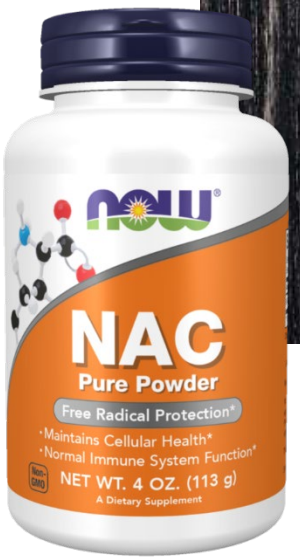


NOW Foods Mission

- NOW was created to empower healthy living. We are a legacy, family-owned brand, absolutely relentless about making the natural products and supplement industry better.
- At NOW empowering people to lead healthier lives is a reward in and of itself, and awards and recognition from others are affirmation that we're fulfilling this mission. These awards are also a testament to the innovation and quality that go into every one of our products.

NOW Foods Regulatory Status

- NOW complies with 21 CFR 111 Current Good Manufacturing Practice in Manufacturing, Packaging, Labeling or Holding Operations for Dietary Supplements (cGMPs) to make sure our products are made according to the best standards in the industry.
- As a leading natural products company for 50 plus years, our dedication to superior quality has always involved sustainable sourcing, transparency at all levels, and exclusive testing methods that go above and beyond.



A Brand Built on Quality

- With our world-class ISO17025 accredited labs and science team we make sure our products are safe from adulteration, contamination, and ingredient substitution by implementing a routine stringent testing program.

Dietary Ingredients Sourcing Challenges

- NOW sources ingredients from all around the world, based on the highest quality and best value available.
- In 2021, as a consequence of the COVID-19 pandemic, global supply chains and shipments slowed, causing worldwide shortages.
- Adulteration and contamination occurrences have spiked due to high demand and low material availability.

What to Watch For?

- Pesticide contamination, which also includes prohibited from use and novel pesticides
- Increase in heavy metal levels
- Microbial contamination
- Illicit drug and steroid residues

Why We Care about Pesticide Residues?

- Pesticides belong to substances which are the most toxic and are persistent
- They do not break down easily, have ability to bio-accumulate, and can be mobile in the environment.
- They can also become mutagenic, carcinogenic, teratogenic, and allergenic.
- Human food and livestock feed should not contain pesticide residues over the maximum residue limits (MRL).

80% of adults, children in US have pesticides in their bodies from food

Certain pesticides are banned from potatoes, grapes, strawberries, green beans, soybeans, almonds and other foods

More than 80% of urine samples drawn from children and adults in a study done by the Centers for Disease Control and Prevention contained a weed-killing chemical linked to cancer.

Pesticide Testing Program at NOW

- Established to ensure safety of our dietary ingredients.
- Response to a high customer demand for clean products.
- Developed and validated in house to create a fit-for-purpose method and workflow.

Pesticide Analysis in a QC Lab

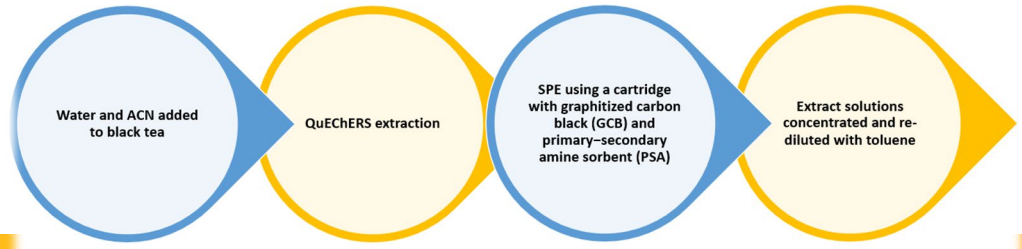
- Laborious and costly
- Requires designated and trained stuff
- Appropriate instrumentation needed for the job
- Methods need to be fit for purpose and validated

Method Choice and Validation

- An extensive list of pesticide analysis methods have been issued by the United States Environmental Protection Agency (USEPA), the European Community (EC), United States Department of Agriculture (USDA), Association of Official Analytical Chemists (AOAC), United States Food & Drug Administration (USFDA), and the World Health Organization (WHO)
- SANTE/11813/2017

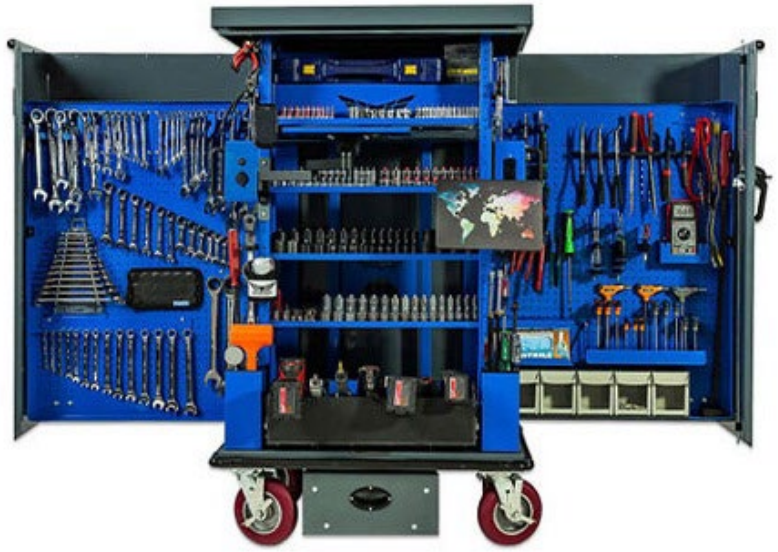
Method Choice and Validation

- Method adapted from “Multiresidue Pesticide Analysis of Botanical Dietary Supplements Using Salt-out Acetonitrile Extraction, Solid-Phase Extraction Cleanup Column, and Gas Chromatography-Triple Quadruple Mass Spectrometry.” D. G. Hayward, J.W. Wong, F. Shi, K. Zhang, N. S. Lee, A. L. DiBenedetto, M. J. Hengel, Anal. Chem. 2013, 85, 4686-4693
- NOW followed European SANTE/11813/2017 Guideline.
- Validation included specificity, linearity, limit of quantification (LOQ), accuracy, precision and measurement uncertainty.



Instrumentation Tool Box

- Large pesticide scope requires orthogonal approach for targeted analysis.
- Considerations for instrument and detectors
- LC-MS/MS
- GC-MS/MS
- Orbitrap MS



When Sensitivity Matters…

- Botanicals present a challenge due to matrix interferences
- Analysis of concentrated, highly pigmented extracts requires smaller sample size
- No globally harmonized tolerances
- 40 CFR 180 does not cover many botanicals
- If no tolerance or exemption, tolerance is zero (action at 0.01 ppm = 10 ppb)
- Chasing “zero”

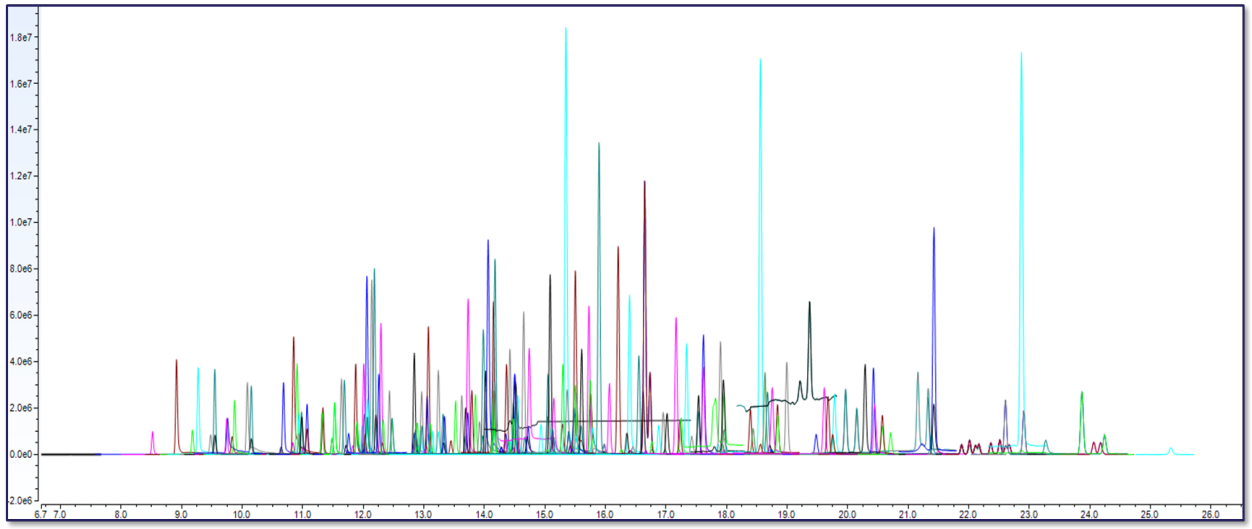
Exciting Upgrade

- TSQ 9610 GC-MS/MS with TRACE™ 1610 GC with NeverVent Technology
- Chromeleon™ Chromatography Data System 7.3 Software

TSQ 9610 GC-MS/MS

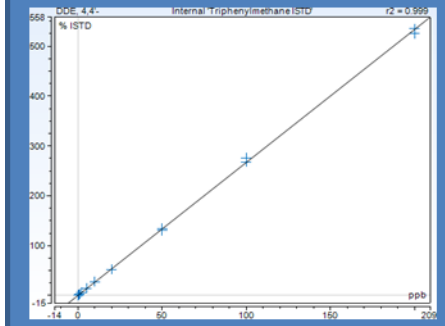
A Case Study: Analysis of 200+ Pesticides in Black Tea

- Chromatography performed using Thermo Scientific™ TRACE™ 1610 GC with Thermo TSQ 9610 MS/MS.
- The analytes were detected in Auto SRM mode.
- Data acquisition and processing performed using Chromeleon™ 7.3 Software.

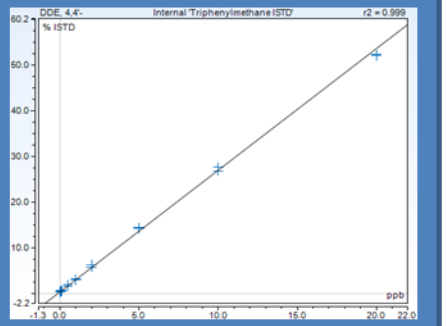


Parameter	Value
Injection liner	Thermo Scientific™ LinerGOLD™ Splitless Liner, Single Taper with Quartz Wool, 4 mm (P/N 453A1925-UI)
GC column	Thermo Scientific™ TG-5SILMS analytical column, 30 m x 0.25 mm i.d. x 0.25 µm fused silica capillary column with 10 m Safe Guard, P/N 26096-1421
Injection volume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

Linearity

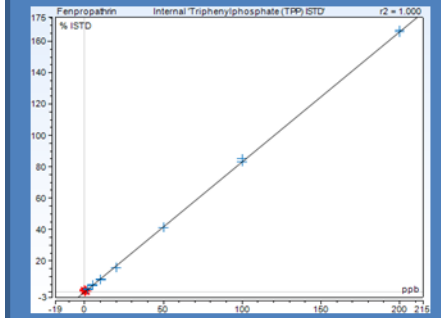


Full Range: 0.01-200 ng/mL

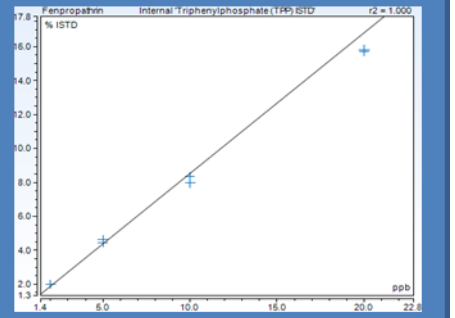


Zoomed in: 0.01-20 ng/mL

DDE, 4,4'-

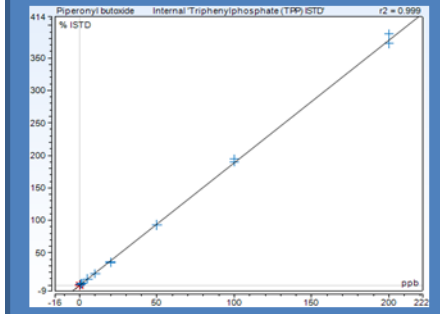


Full Range: 2-200 ng/mL

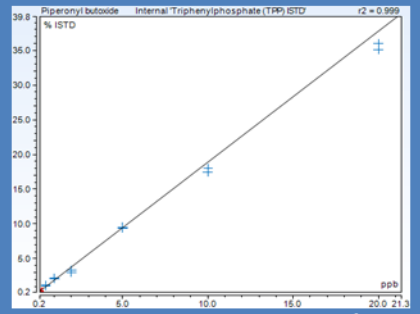


Zoomed in: 2-20 ng/mL

Fenpropathrin

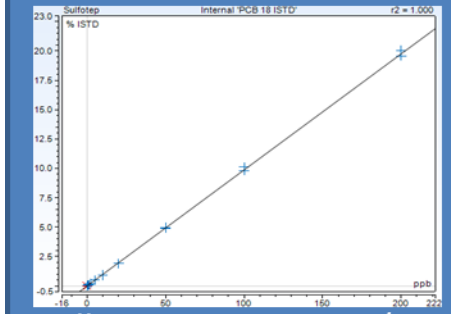


Full Range: 0.5-200 ng/mL

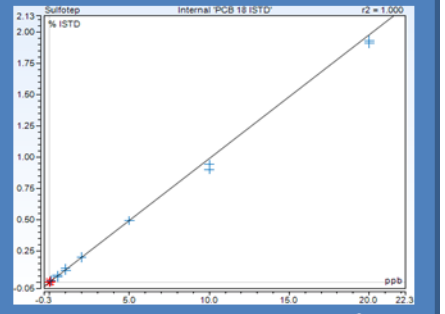


Zoomed in: 0.5-20 ng/mL

Piperonyl Butoxide



Full Range: 0.1-200 ng/mL



Zoomed in: 0.1-20 ng/mL

Sulfotep

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Recovery and Precision

	Concentration %RSD			Average %Recovery		
	10 ppb Spike	25 ppb Spike	50 ppb Spike	10 ppb Spike	25 ppb Spike	50 ppb Spike
	n=6	n=6	n=6	n=6	n=6	n=6
Atrazine	3.363	4.145	5.49	83.26	84.54	85.5
Chlorpropham	7.802	7.041	2.547	77.72	78.67	74.32
Chlorpyrifos-ethyl	4.683	5.973	3.451	83.95	78.92	81.54
Coumaphos	6.1	6.533	5.508	91.34	91.97	91.09
Cypermethrin I	8.015	3.366	4.121	92.94	91.46	91.28
Cypermethrin II	4.966	3.409	3.696	88.3	89.55	89.17
Cypermethrin III	9.684	4.115	3.621	101.17	91.61	90.68
Cypermethrin IV	7.712	5.931	6.164	88.92	87.87	90.06
DDD, 2,4'-	3.439	5.048	2.218	93.29	91.81	91.25
DDD, 4,4'-	3.074	5.708	2.559	96.95	95.28	93.58
DDE, 2,4'-	3.807	4.747	2.238	86.12	83.38	81.59
DDE, 4,4'-	5.824	4.213	3.527	94.49	88.35	85.56
DDT, 2,4'-	3.808	4.428	3.768	89.63	90.23	87.62
DDT, 4,4'-	3.622	2.982	4.191	96.94	92.71	89.31
Fenitrothion	9.8	7.723	3.788	83.22	93	90.91
Fenpropathrin	5.979	7.456	5.021	99.1	92.15	92.56
Folpet	20.606	10.297	6.635	107.61	86.22	61.09
Fonofos	4.429	3.81	6.125	76.02	73.24	72.58
Piperonyl butoxide	4.808	3.582	4.261	98.53	95.68	95.02

- Chromatography analysis was performed using Thermo Scientific™ TRACE™ 1610 GC equipped with Thermo Scientific TSQ 9610 Triple Quadrupole Mass Spectrometer.
- The analytes were detected in Auto SRM mode.
- Data acquisition and processing was performed using Thermo Scientific™ Chromeleon™ Chromatography Data System 7.3 Software.

TSQ 9610 GC-MS/MS

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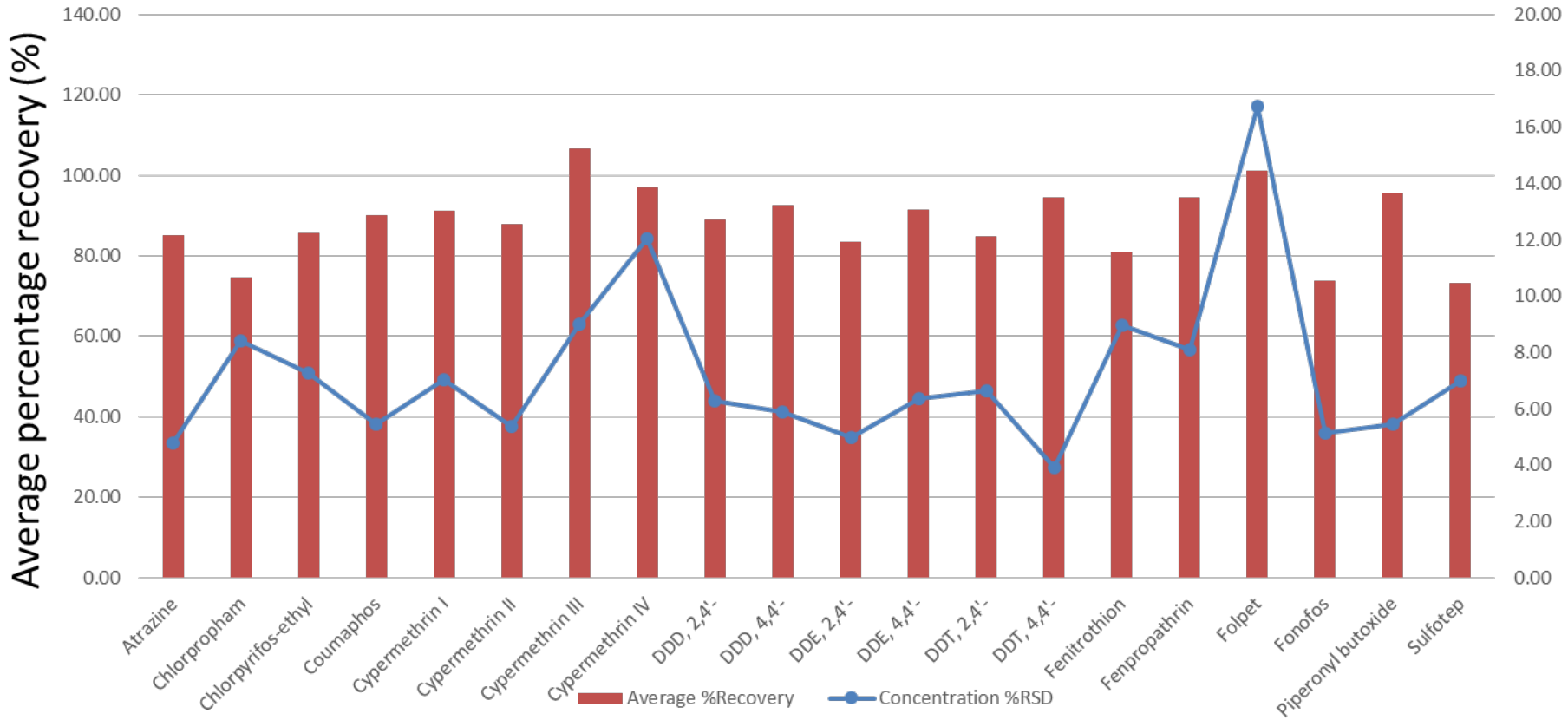
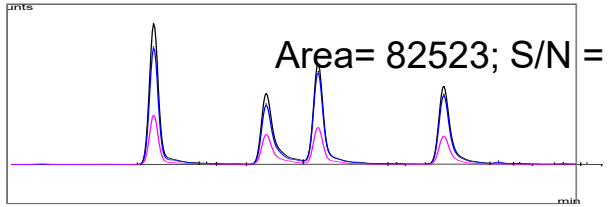
Pushing the Sensitivity Limits



MRL results with %RSDs for n=12 injections and the average percentage recovery for each pesticide

Improvements

AEI TSQ 9000 MS/MS
50ppb BHC Isomers Sensitivity At 50µA Emission Current



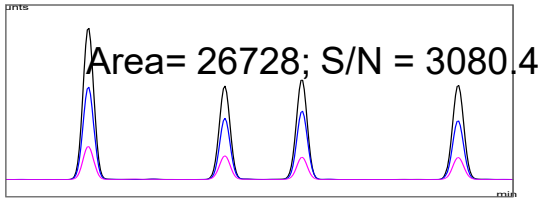
Area= 65413; S/N = 683.4

Area= 55684; S/N = 481.1

Area= 56612; S/N = 511.4

Improvements

AEI TSQ 9610 MS/MS
50ppb BHC Isomers Sensitivity At 10µA Emission Current

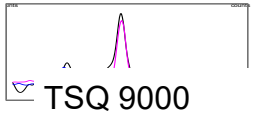


Area= 17755; S/N = 2047.0

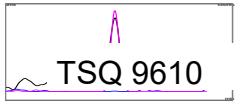
Area= 16407; S/N = 1922.5

Area= 17460; S/N= 1727.9

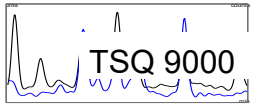
Improved Sensitivity in Matrix



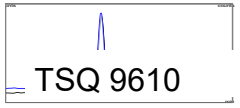
Coumaphos
Area = 5699: S/N= 12.4



Coumaphos
Area = 1290; S/N = 51.1

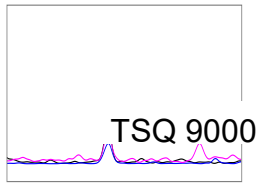


Fonofos
Area = 6628; S/N= 8.2

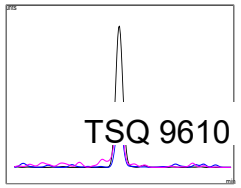


Fonofos
Area = 1642; S/N = 142.6

Not Only Sensitivity



Fenitrothion
Area = 1961; S/N = 0.6



Fenitrothion
Area = 1639; S/N = 12243

What We Saw?

- Chromatography analysis was performed using Thermo Scientific™ TRACE™ 1610 GC equipped with Thermo Scientific TSQ 9610 Triple Quadrupole Mass Spectrometer.
- The analytes were detected in Auto SRM mode.
- Data acquisition and processing was performed using Thermo Scientific™ Chromeleon™ Chromatography Data System 7.3 Software.

Robustness Study

- Chromatography analysis was performed using Thermo Scientific™ TRACE™ 1610 GC equipped with Thermo Scientific TSQ 9610 Triple Quadrupole Mass Spectrometer.
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What Have We Learned?

- Chromatography analysis was performed using Thermo Scientific™ TRACE™ 1610 GC equipped with Thermo Scientific TSQ 9610 Triple Quadrupole Mass Spectrometer.
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TSQ 9610 GC-MS/MS

What Did the Team Say?

- Chromatography analysis was performed using Thermo Scientific™ TRACE™ 1610 GC equipped with Thermo Scientific TSQ 9610 Triple Quadrupole Mass Spectrometer.
- The analytes were detected in Auto SRM mode.
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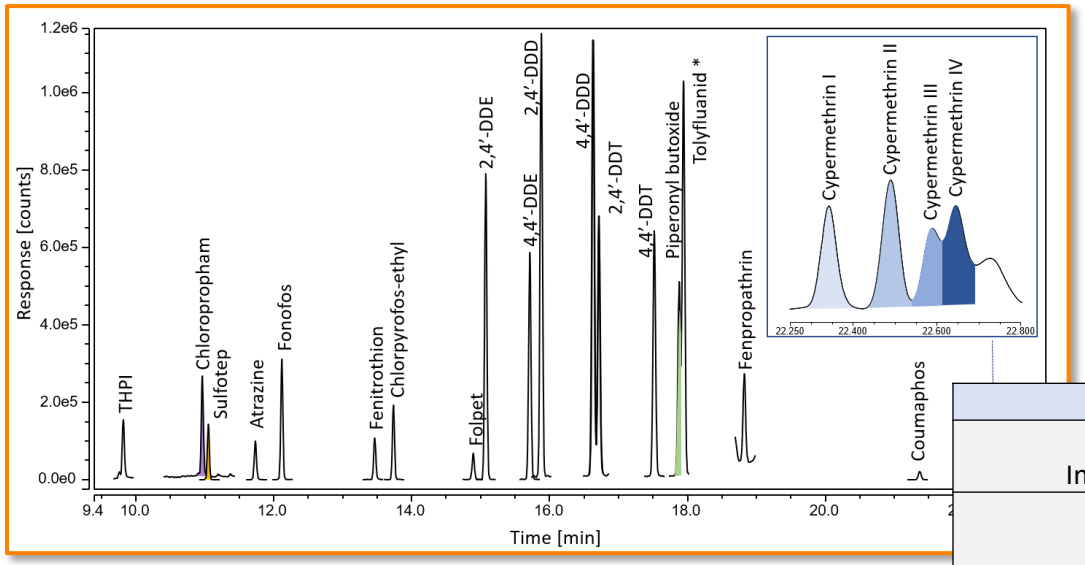
TSQ 9610 GC-MS/MS

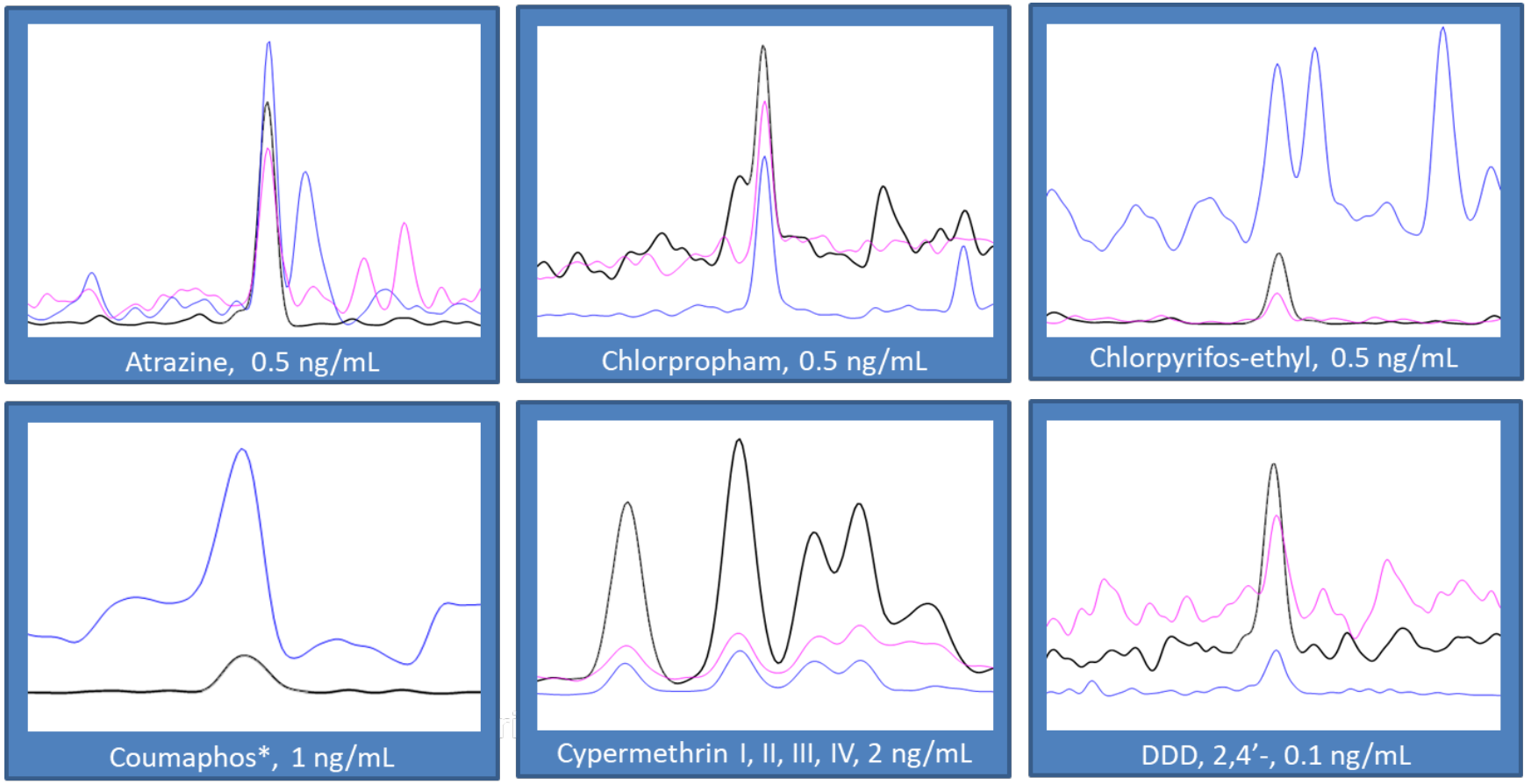
Chromatography



t-SRM acquisition for black tea sample pre-spiked at 5 µg/kg for selected compounds

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

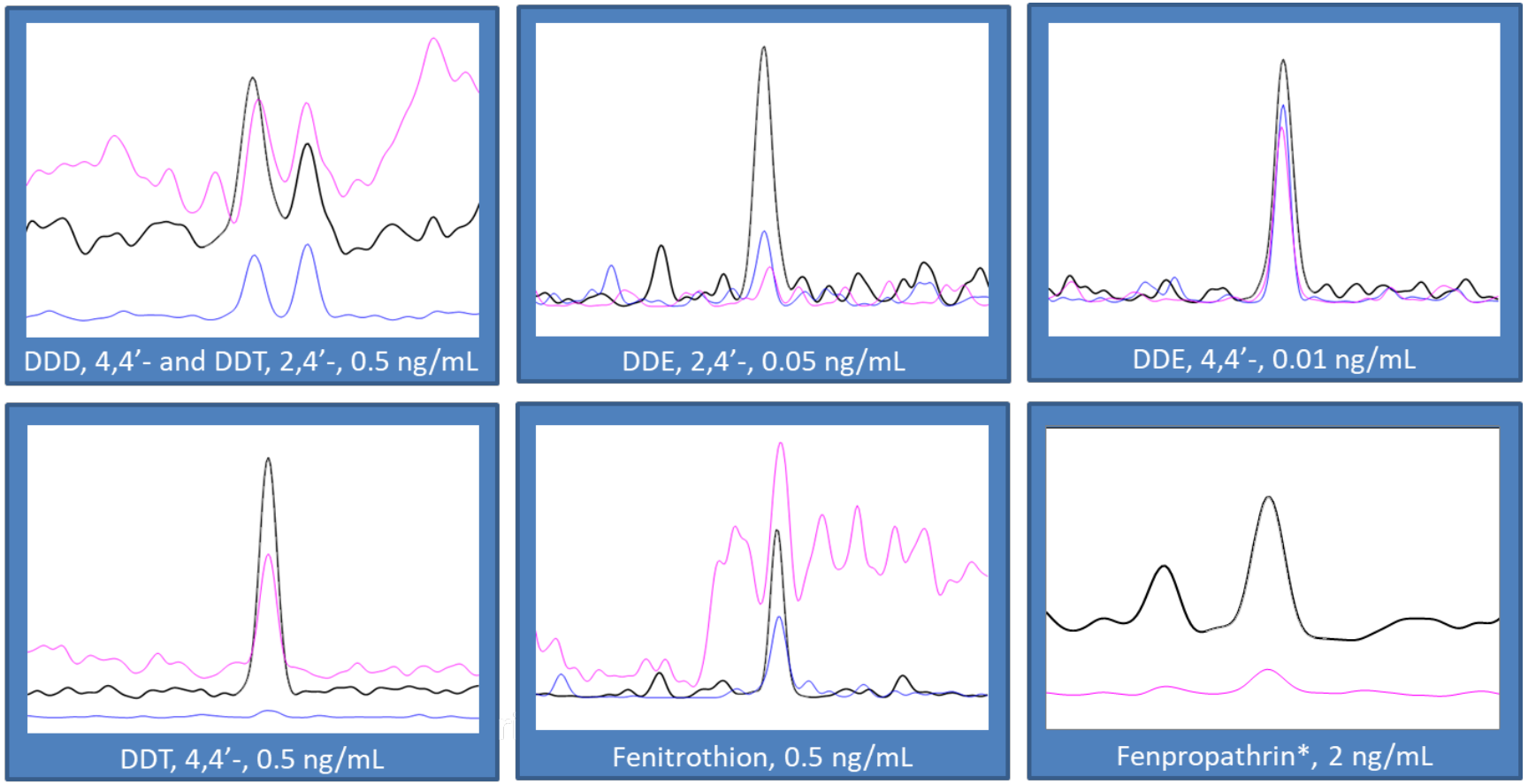
Sensitivity



Chromatograms of Lowest Matrix-Matched Standard Conforming to SANTE Guidance

*No suitable secondary qualifier ion was available at this level.

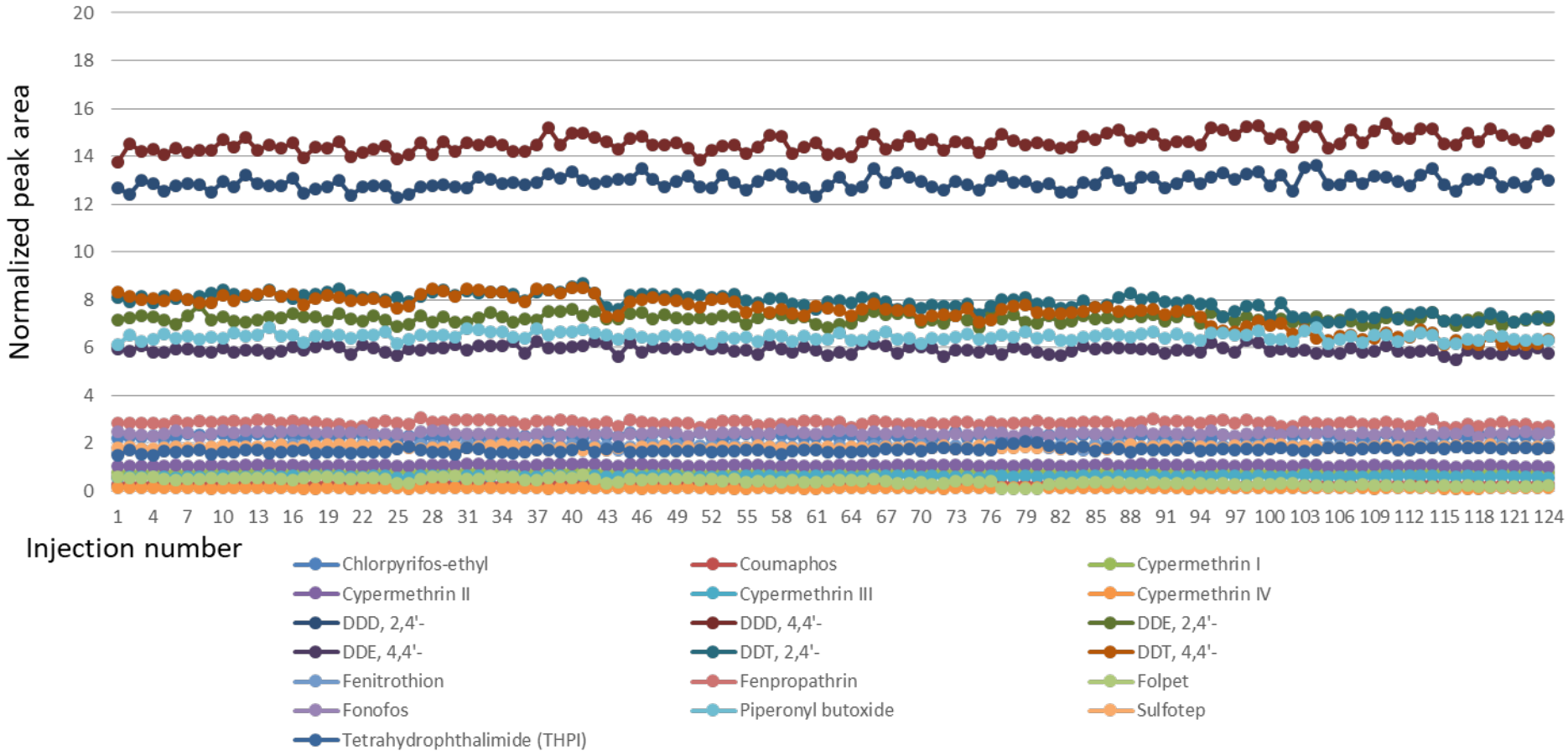
Sensitivity



Chromatograms of Lowest Matrix-Matched Standard Conforming to SANTE Guidance

*No suitable secondary qualifier ion was available at this level.

Reproducibility



Normalized peak area response (analyte peak area / ISTD peak area) obtained for n=124 consecutive injections of matrix samples spiked at 50 µg/kg

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TSQ 9610 Advantages

NeverVent Technology

Thermo Scientific™ NeverVent™ technology allows analytical laboratories to perform maintenance without interrupting their workflow

Available on the TSQ 9610 with the Thermo Scientific™ ExtractaBrite™ and Advanced Electron Ionization (AEI) source

Conclusions

- Excellent sensitivity and good linearity allowed detection and accurate quantitation of numerus pesticides in the challenging black tea matrix.
- Calculated recoveries at three different spiking levels (10, 25, and 50 ppb) are within the 80-120% limits established in the SANTE guidelines.
- Peak area %RSDs of calculated concentrations at 10ppb are <10% which are in compliance with SANTE guideline
- The TSQ 9610 uptime has improved due to the NeverVent technology, allowing for laboratory productivity to be maximized with an uninterrupted workflow.

Find Out More About NOW Foods

- **NOW Foods** www.nowfoods.com
- k.banaszewski@nowfoods.com

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Ada Powers, Thermo Scientific
Mark Belmont, Thermo Scientific

In partnership with