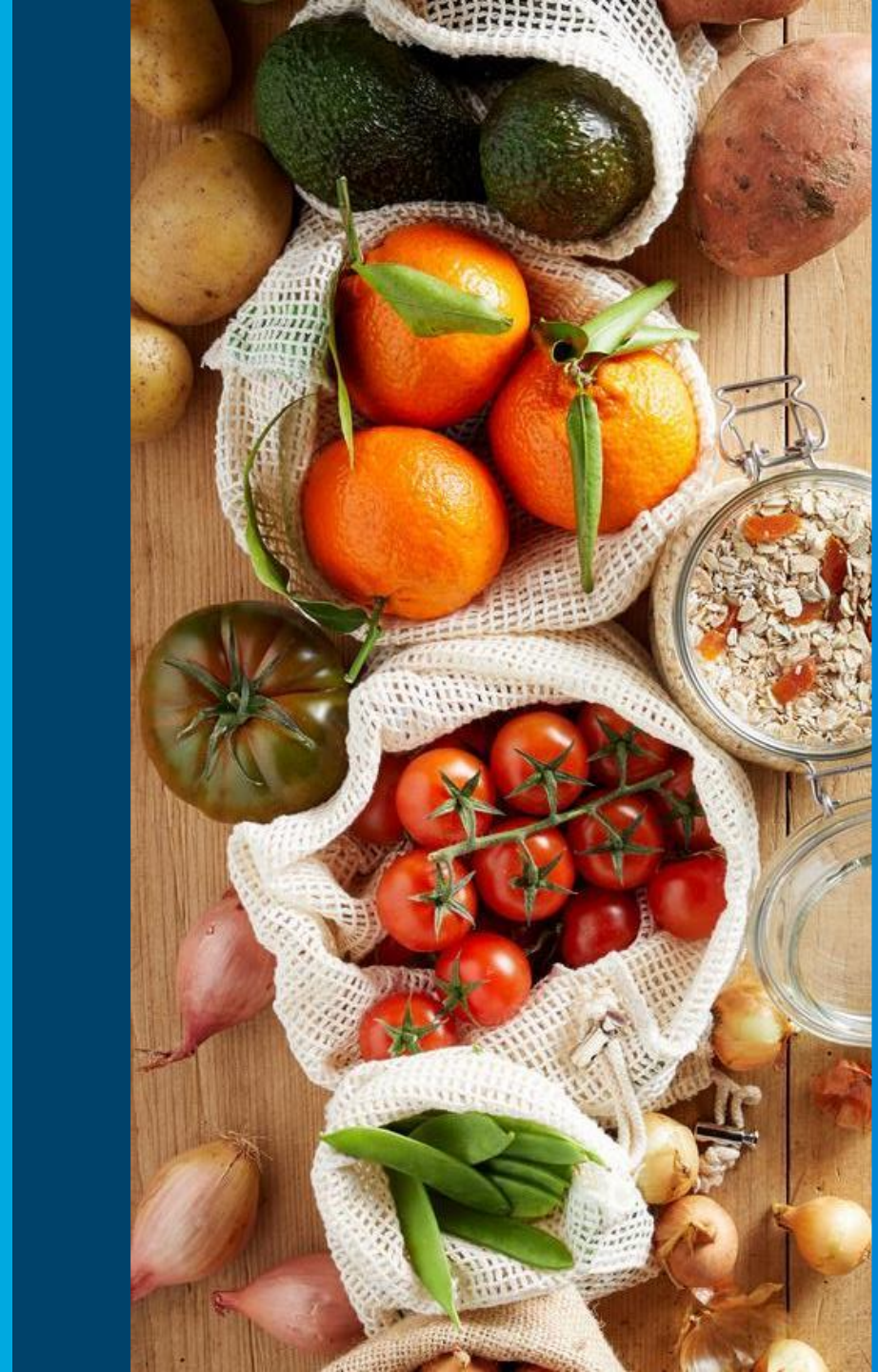
Quantitation 1000 Pesticide Residues in Tomato According to SANTE 11312/2021 Guideline



Laurent Pascaud
GC & GCMS product Specialist

DE97934359

Agenda

- Introduction
- Validation and Method Details
- Results
- Summary

Why Pesticide Analysis in Food Matrices?

- Pesticides are used as crop protection products to prevent, destroy, control harmful organisms or diseases during production, storage and transport
- Pesticide residues in or on food or feed have potential toxicity to humans and other species
- Global regulations require reporting of MRLs for various compounds in different food products

The Challenge of Pesticide Residues Analysis

Pesticide Diversity

Large numbers of analytes from multiple classes are analytically challenging, with more and more new pesticides introduced regularly

Matrix Complexity

Food is a complex matrix with many different commodity groups, adding to the challenge of method development

Low Limits of Detection

Difficult to identify and determine the targets with low concentration in complex matrices

Pesticide Diversity

11 classes of pesticides (based on target organisms)

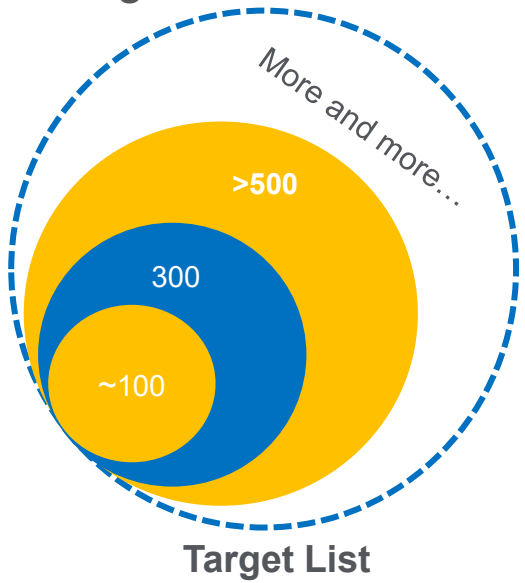
- Insecticide – kills insects
- Nematicide – kills nematode worms
- Miticide – kills mites
- Herbicide – kills weeds
- Fungicide – kills parasitic fungi
- Virucide – kills phages outside the mycobacteria
- Rodenticide – kills rodent
- Avicide – kills birds
- Molluscicide – kills mollusks
- Algaecide – kills or prevents the growth of algae
- Bactericide – kills bacteria

Governing Agency for Pesticides in Europe

European union

The number of new pesticides will keep growing due to the high demand of new pesticides to treat and prevent crops from pests damaging, which results in more and more pesticides are being added to the EU list for monitoring purpose or establishing MRLs.

The coverage of the method is driven by the growing target list!



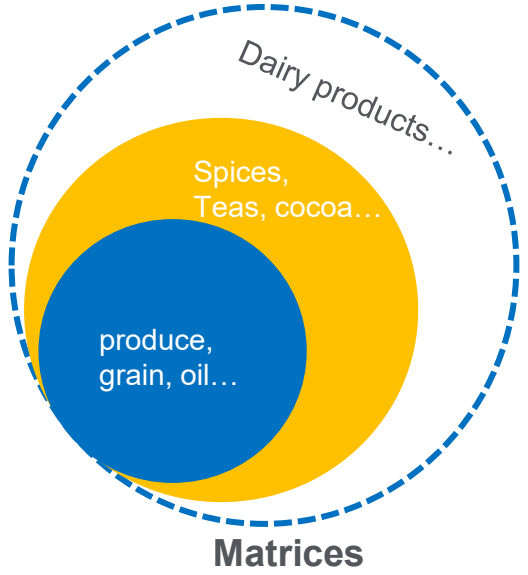
Matrix Complexity

SANTE/11312/2021: Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed

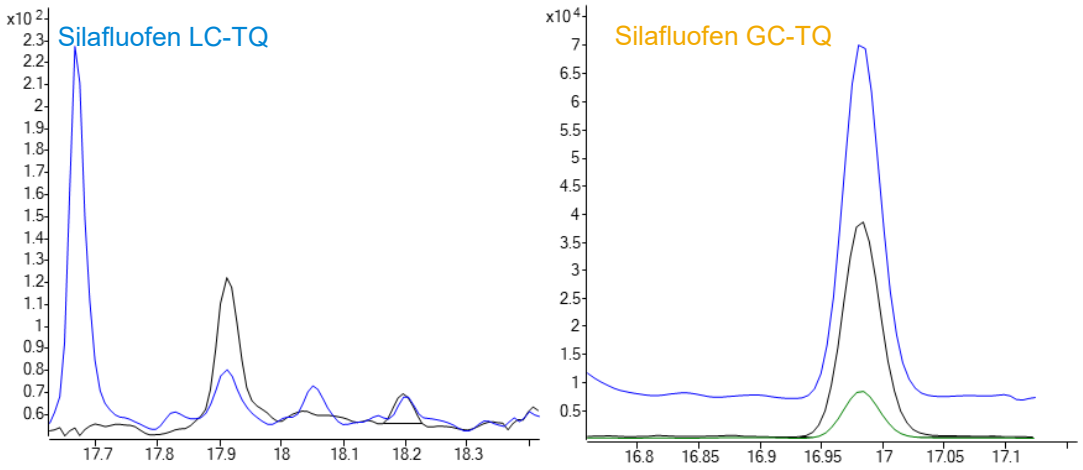
Annex A. Commodity groups and representative commodities[9]
Vegetable and fruits, cereals and food of animal origin

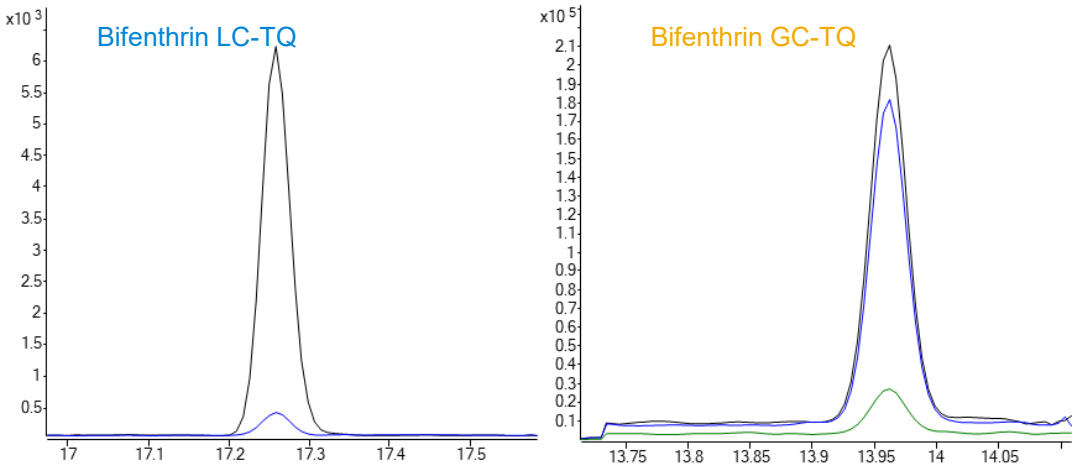
Commodity groups	*Typical commodity categories wthin the group*	*Typical representative commodities within the category*
1. High water content	Pome fruit	Apples, pears
	Stone fruit	Apricots, cherries, peaches,
	Other fruit	Bananas
	Alliums	Onions, leeks
	Fruiting vegetables/cucurbits	Tomatoes, peppers, cucumbers, melons
	Brassica vegetables	Cauliflowers, Brussels-sprouts, cabbages, broccoli
	Leafy vegetables and fresh herbs	Lettuce, spinach, basil
	Stem and stalk vegetables	Celery, asparagus
	Fresh legume vegetables	Fresh peas with pods, peas, mange tout, broad beans, runner beans, French beans
	Fresh Fungi	Champignons, chanterelles
	Root and tuber vegetables	Sugar beet, carrots, potatoes, sweet potatoes
2. High acid content and high water content[10]	Citrus fruit	Lemons, mandarins, tangerines, oranges
	Small fruit and berries	Strawberries, blueberries, raspberries, black currants, red currants, white currants, grapes
3. High sugar and low water content[11]	Honey, dried fruit	Honey, raisins, dried apricots, dried plums, fruit jams
4a. High oil content and very low water content	Tree nuts	Walnuts, hazelnuts, chestnuts
	Oil seeds	Oilseed rape, sunflower, cotton-seed, soybeans, peanuts, sesame etc.
	Pastes of tree nuts and oil seeds	Peanut butter, tahina, hazelnut paste
4b. High oil content and intermediate water content	Oily fruits and products	Olives, avocados and pastes thereof
5. High starch and/or protein content and low water and fat content	Dry legume vegetables/pulses	Field beans, dried broad beans, dried haricot beans (yellow, white/navy, brown, speckled), lentils
	Cereal grain and products thereof	Wheat, rye, barley and oat grains; maize, rice wholemeal bread, white bread, crackers, breakfast cereals, pasta, flour.
6. "Difficult or unique commodities"[12]		Hops Cocoa beans and products thereof, coffee, tea Spices
7. Meat (muscle) and Seafood	Red muscle	Beef, pork, lamb, game, horse
	White muscle	Chicken, duck, turkey
	Offal	Liver, kidney
	Fish	Cod, haddock, salmon, trout
8. Milk and milk products	Milk	Cow, goat and buffalo milk
	Cheese	Cow and goat cheese
	Dairy products	Yogurt, cream
9. Eggs	Eggs	Chicken, duck, quail and goose eggs
10. Fat from food of animal origin	Fat from meat	Kidney fat, lard
	Milk fat[13]	Butter

The difficulties in pesticide analysis (such as matrix effect and extraction efficacy) are driven by a growing number of complex matrices!



Advantages of using GC- and LC-TQ Detection





Silafluofen 10 µg/kg

- Bad sensitivity and results with LC-TQ
- Good sensitivity and results with GC-TQ

→ Using GC for analysis and quantification

Bifenthrin 10 µg/kg

- Good sensitivity and results with LC-TQ
- Good sensitivity and results with GC-TQ

→ Using LC for quantification and GC for verification or vice versa

What Would the Ideal Analysis Solution Look Like?

Comprehensive Method

An analytical method that covers all GC and LC amenable pesticides residues in a single method

Robust Workflow

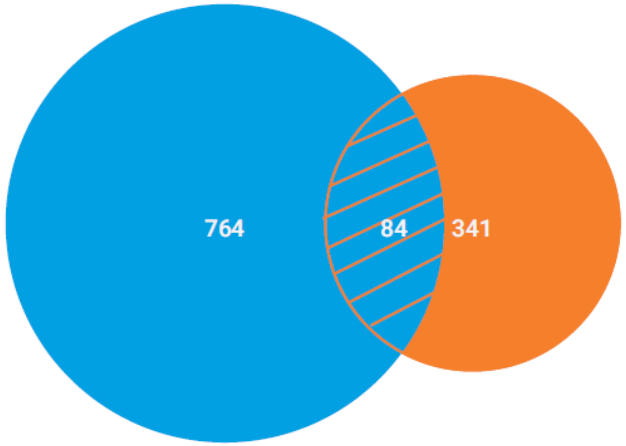
A simple to use workflow that produces reliable results, regardless of matrices or target compounds

Powerful Instrument

High selectivity and sensitivity to meet MRLs requirement that is also robust and reproducible

GC/MS(MS)
Small non-polar compounds

LC/MS(MS)
Larger, polar & thermally labile compounds



Validation and Method Details








Agilent

Verification Parameters and Criteria

SANTE/11312/2021: Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed

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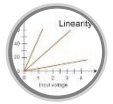
	Cereal grain and products thereof	Wheat, rye, barley and oat grains; maize, rice wholemeal bread, white bread, crackers, breakfast cereals, pasta, flour.

Verification Parameters and Criteria

SANTE 11312/2021: Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in the Food and Feed

Parameter	What/how	Criterion	Cross reference to AQC document
Sensitivity/linearity	Linearity check from five levels	Deviation of back-calculated concentration from true concentration ≤± 20 %	C14-C19
Matrix effect	Difference of response from standard in matrix extract and standard in solvent	*	C21-C29 Glossary
LOQ	Lowest spike level meeting the identification and method performance criteria for recovery and precision	≤ MRL	G6
Specificity	Response in reagent blank and blank control samples	≤ 30 % of RL	C41
Recovery	Average recovery for each spike level tested	70-120 %	G3,G6
Precision (RSD_r)	Repeatability RSD_r for each spike level tested	≤ 20 %	G3, G6
Precision (RSD_{wR})	Within-laboratory reproducibility, derived from on-going method validation / verification	≤ 20 %	G3, G6
Robustness	Average recovery and RSD_{wR}, derived from on-going method validation / verification	See above	G6, C39-C44
Ion ratio	Check compliance with identification requirements for MS techniques	Table 3	Section D
Retention time		± 0.1 min.	D2

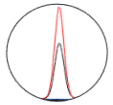


Linearity using matrix-matched calibration levels

Sensitivity (LOD & LOQ) using matrix-matched calibration levels and prespiked QCs

Mean Recovery using one level of prespiked QC (10 µg/kg)

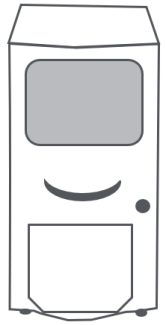
Method Precision using Intra batch recovery analysis



Qualifier Ratio 2 Product Ions, Ratio ± 30 %

Sample Preparation

Agilent QuEChERS EN Extraction Kits



Mechanical shaker

Centrifuge

Agilent QuEChERS dSPE Kits

Centrifuge

Extract with 10 mL Acetonitrile

Add Extraction Kit

- 4 g $MgSO_4$
- 1 g NaCl
- 1 g Na_3Citrate
- 0.5 g Na_2HCitrate

dSPE clean-up of Raw Extract

- 150 mg $MgSO_4$
- 50 mg C18
- 7.5 mg Carbon S (GCB)
- 50 mg PSA

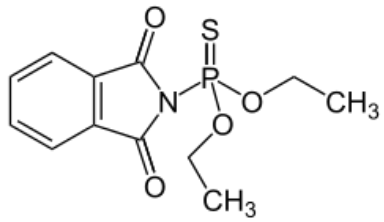
→Universal dSPE kit, recommended for general pigmented fresh products

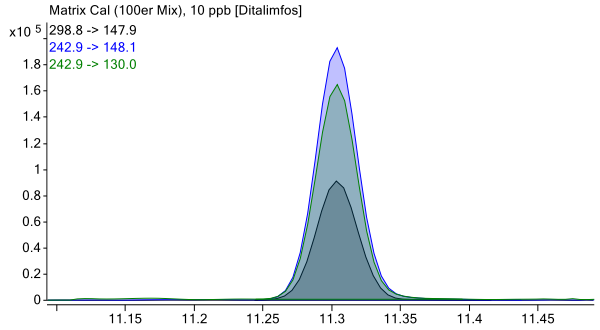
Examples for Clean-up Recovery

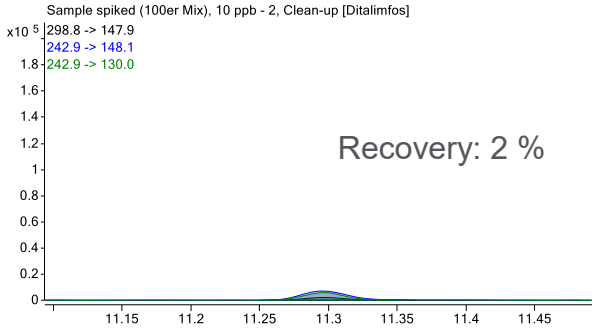
Matrix Cal 10 ppb

Tomato Sample 10 ppb with clean-up

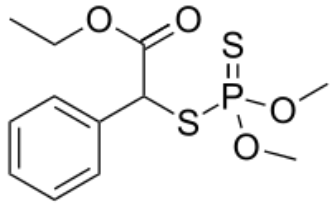
Ditalimfos

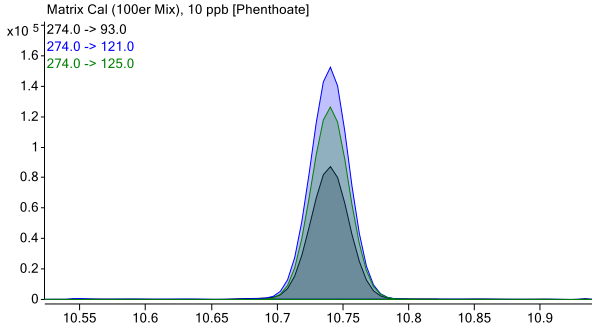


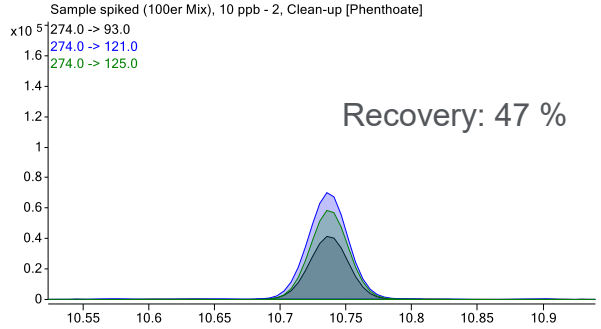




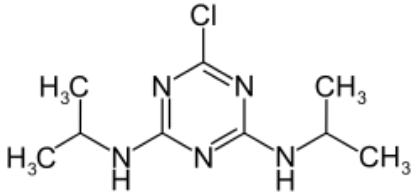
Phenthoate

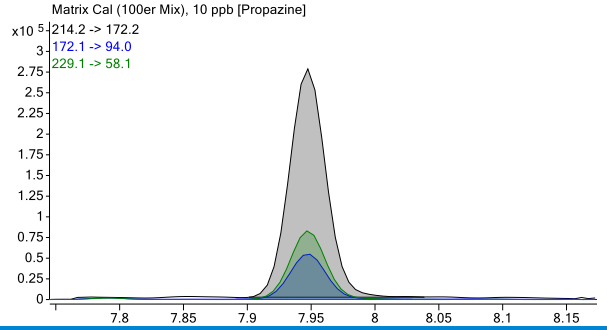


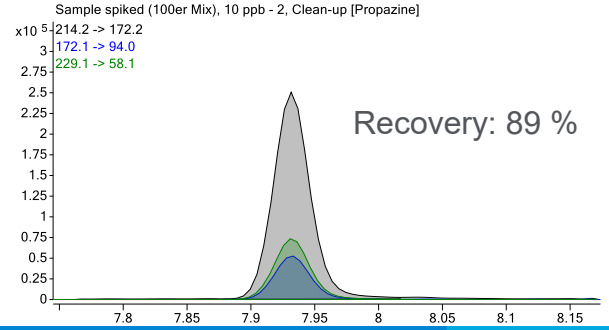




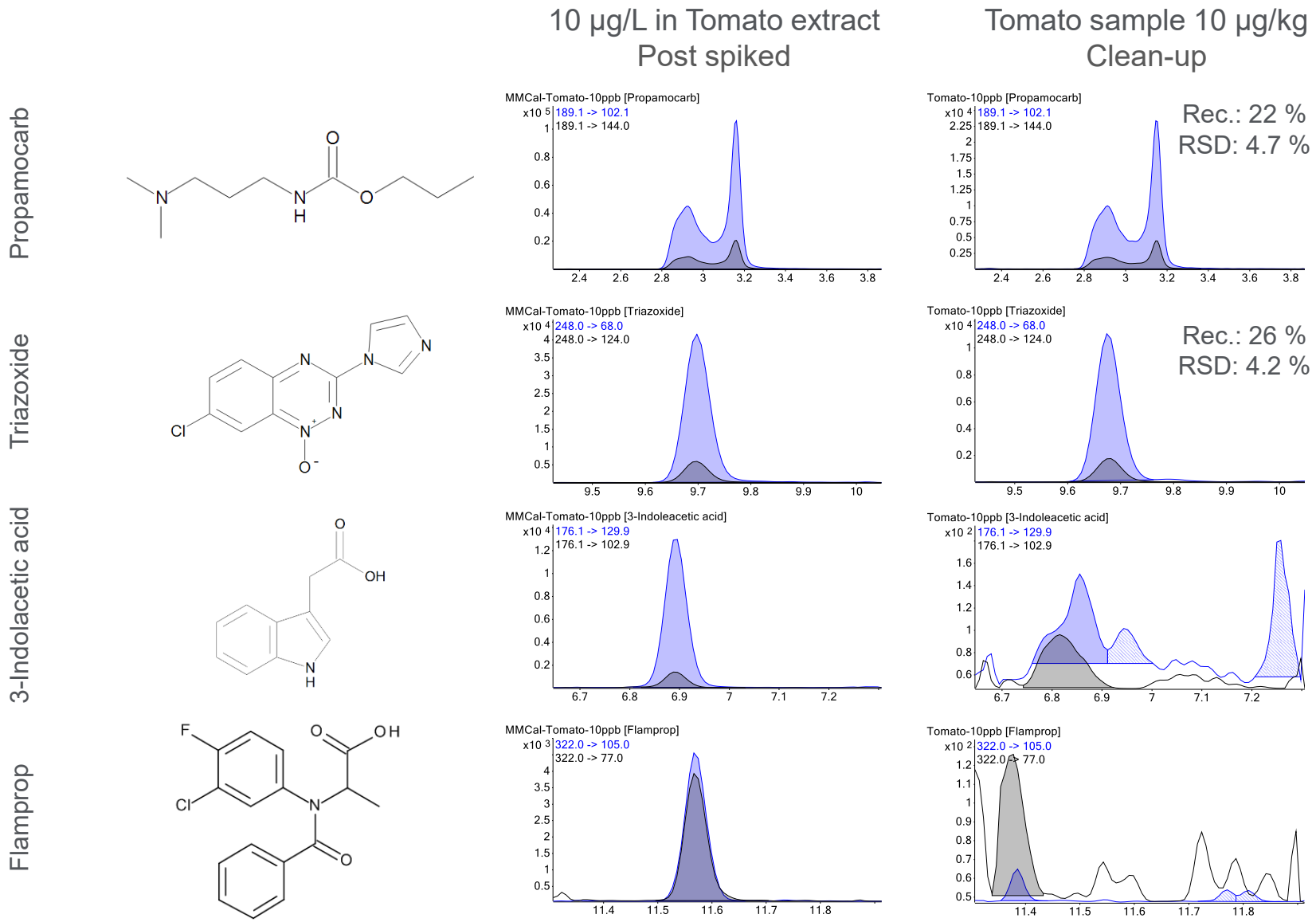
Propazine



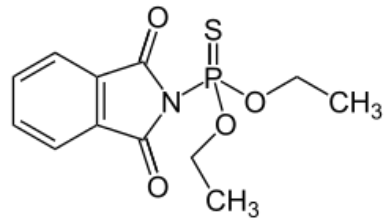
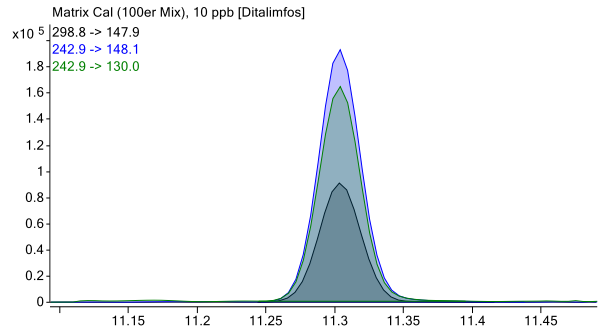
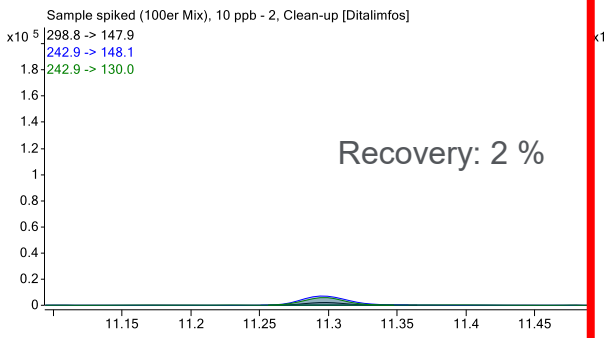
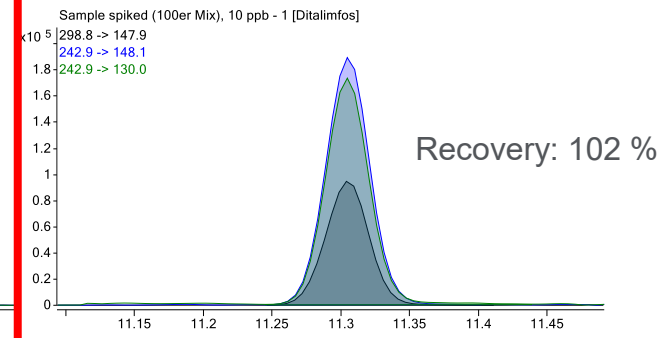
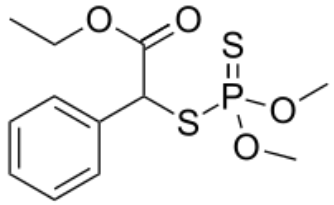
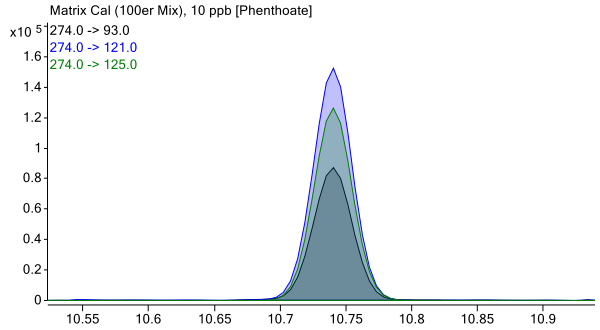
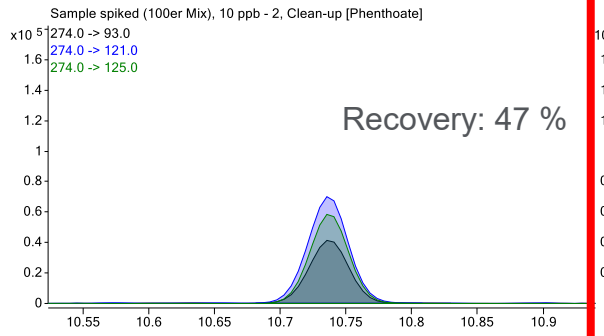
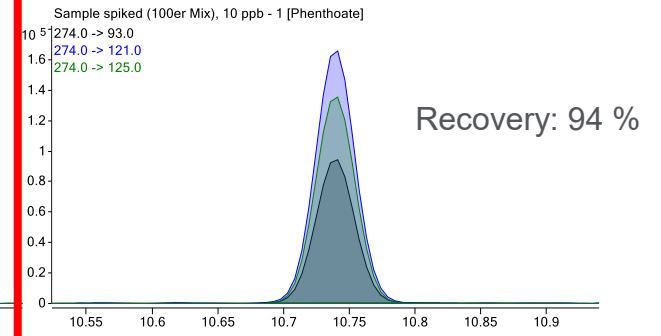
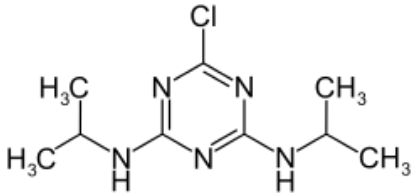
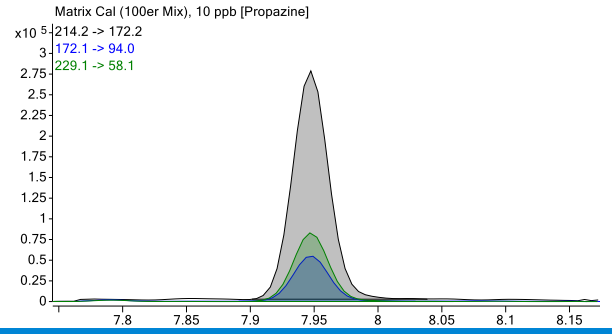
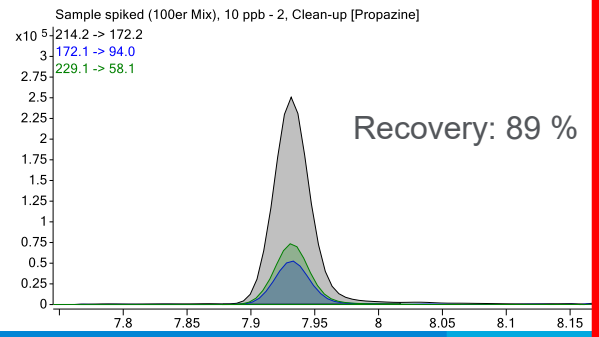
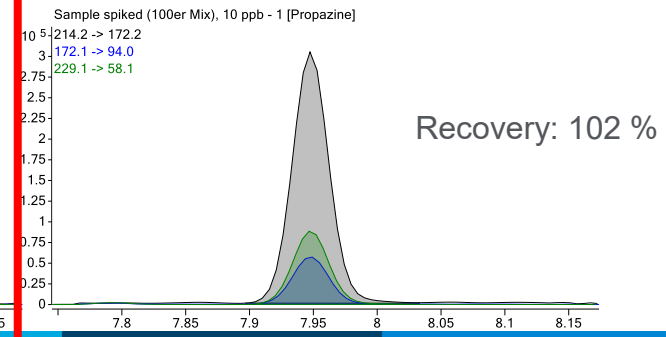




Comparison Raw Extract and Extract after Clean-up

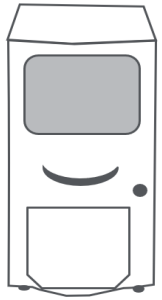


Examples for Clean-up Recovery

	Matrix Cal 10 ppb	Tomato Sample 10 ppb w/ clean-up	Tomato Sample 10 ppb raw extract (w/o clean-up)
Ditalimfos				
Phenthoate				
Propazine				

Sample Preparation

Agilent
QuEChERS EN
Extraction Kits



Mechanical shaker + Centrifuge

Agilent 8890 GC + 7010C
(1:5 dilution)

Agilent 1290
Infinity II
LC System

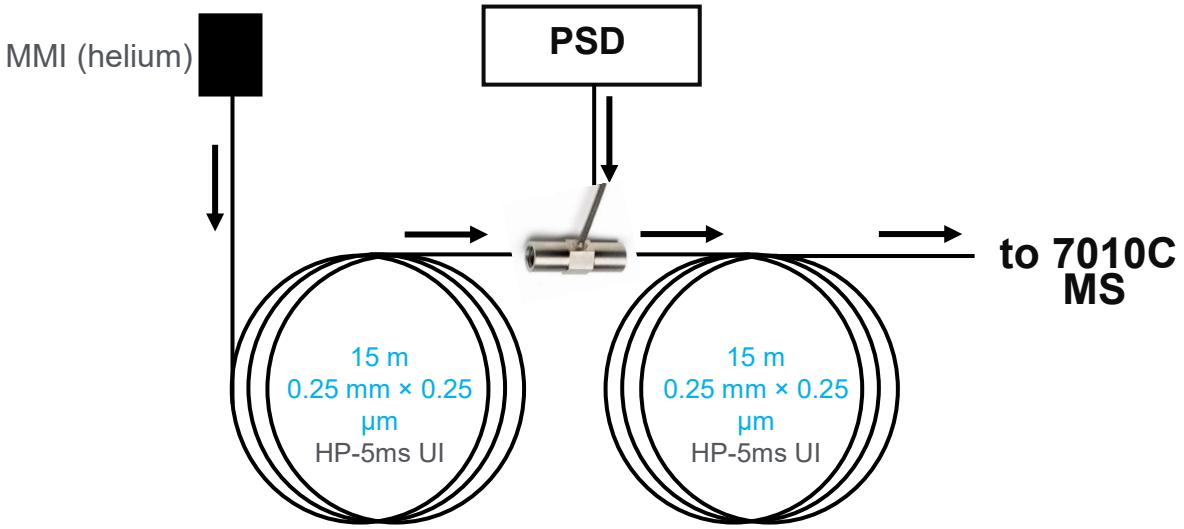
Agilent 6470 LC/TQ

Extract with 10 mL Acetonitrile

Add Extraction Kit

- 4 g $MgSO_4$
- 1 g NaCl
- 1 g Na_3Citrate
- 0.5 g Na_2HCitrate

Agilent 8890 GC – Method Details



Midcolumn backflush configuration

PUU = Purge Ultimate union

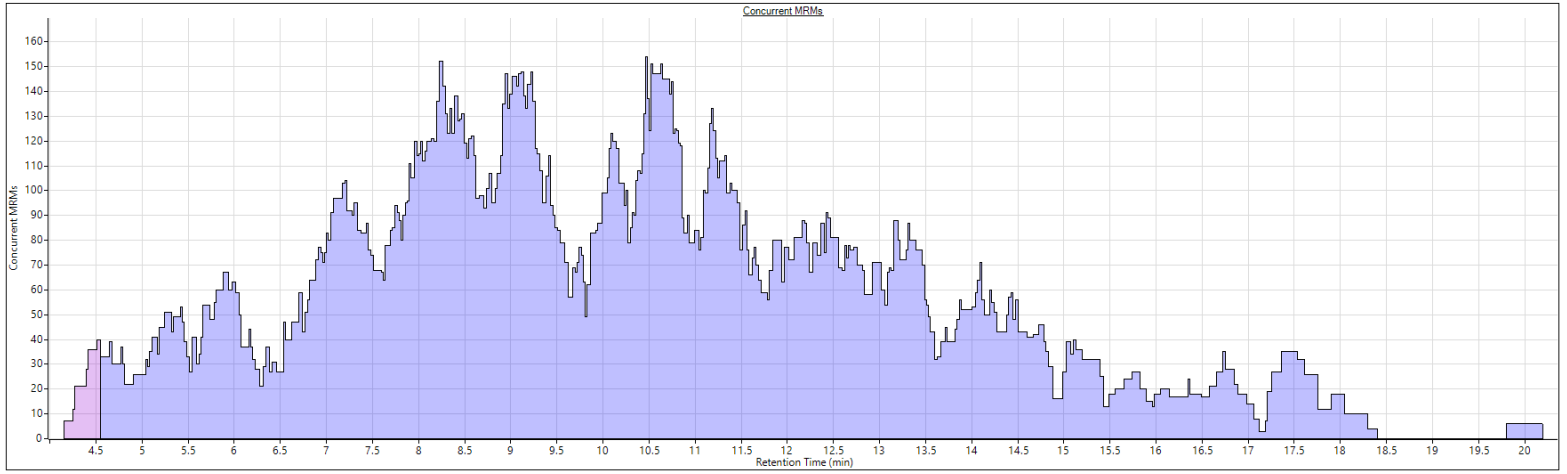
Parameter	
Column	HP-5ms Ultra Inert (15 m × 0.25 mm × 0.25 µm), two columns coupled with PUU and backflush function
Injection Volume	1 µL
Injection Mode	Solvent Vent on MultiMode Inlet (MMI), dimpled liner
Inlet Temp. Program	60 °C (0.06 min), 720 °C/min to 280 °C
Column Flow	0.94 mL/min (column 1) and 1.14 mL/min (column 2)
Oven Gradient	60 °C (1 min), 40 °C/min to 170 °C, 10 °C/min to 310 °C (3 min)
Carrier Gas	Helium
Post Run	Backflush for 1.5 minutes

7010C TQ-MS Method Details

Parameter	
Ion Source	High Efficiency Source (HES)
Quad Temperature	150 °C
Source Temperature	280 °C
Mode	dMRM
Gain Factor	10
Resolution	Wide/Wide
Total MRMs	2.093
min/max Dwell Time	1.2 ms / 100.2 ms

Agilent 8890 GC with 7693A Automatic Liquid Sampler and 7010C Triple Quad

Overview of monitored MRMs over retention time:



6470B Triple Quadrupole LC/MS: A deeper look

- 6470 (A or B) Triple Quadrupole LC/MS
- 1290 Infinity II LC System

MCT

Fast chromatography and day-to-day reproducibility

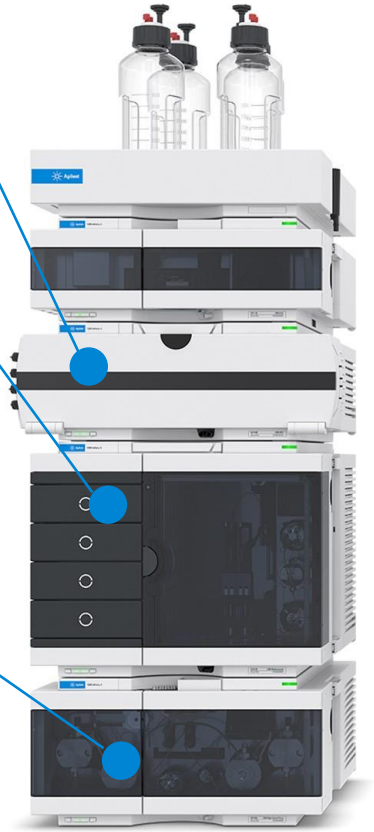
Multisampler

High sample capacity, precise injection, reduced carryover, best for complex matrices

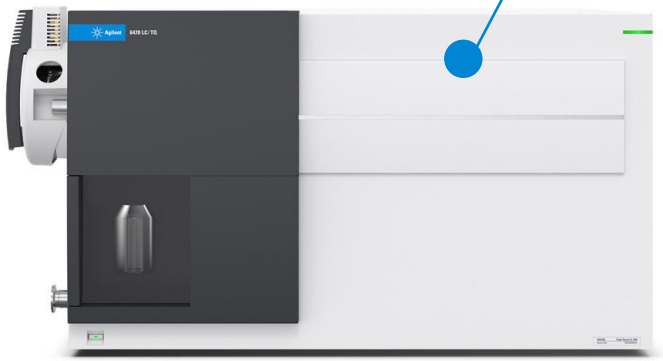
High speed Binary Pump

Ideal UHPLC front-end for LC/MS applications

1290 Infinity II



6470B LC/TQ



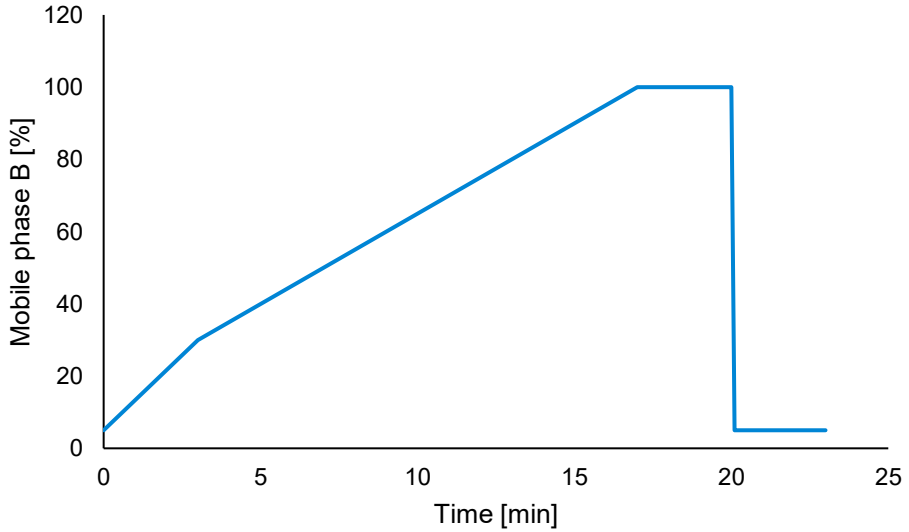
Robust, dMRM acquisition for reliable target detection at low levels in complex matrices, offers uninterrupted lab productivity

Agilent 1290 Infinity II LC- Method Details

Agilent 1290 Infinity II UHPLC

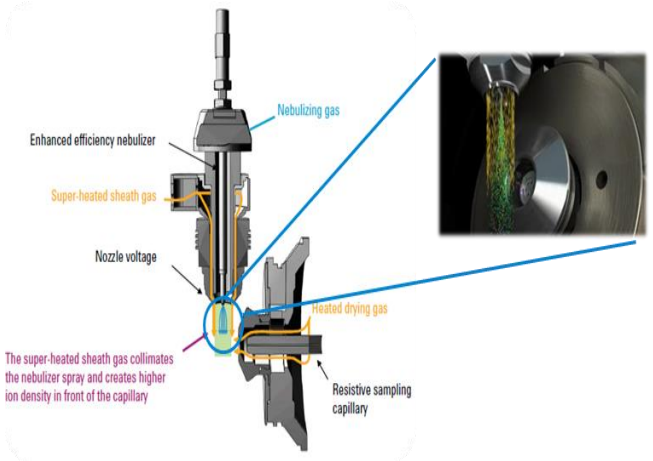
Parameter	
Column	Zorbax Eclipse Plus C18, 2.1 x 150 mm; 1.8 µm
Temperature	40 °C
Injection volume	2 µL
Flow rate	0.4 mL/min
Mobile phase A	5 mM Ammonium Formate in Water with 0.1 % Formic acid
Mobile phase B	5 mM Ammonium Formate in Methanol with 0.1 % Formic acid

Time [min]	Mobile Phase B [%]
0	5
3	30
17	100
20	100
Post Time	3 min



6470B TQ-MS Method Details

Parameter	
Ionization mode	Positive / Negative ESI with AJS
Scan type	dMRM
Gas temperature	200 °C
Gas flow	9 L/min
Nebulizer	35 psi
Sheath gas temperature	400 °C
Sheath gas flow	12 L/min
Capillary voltage	2500 V (+) / 3000 V (-)
Nozzle voltage	0 V
MS1 / MS2 resolution	Unit / Wide



Dynamic MRM Mode – Improved Data Quality in complex Analysis

Scan segments

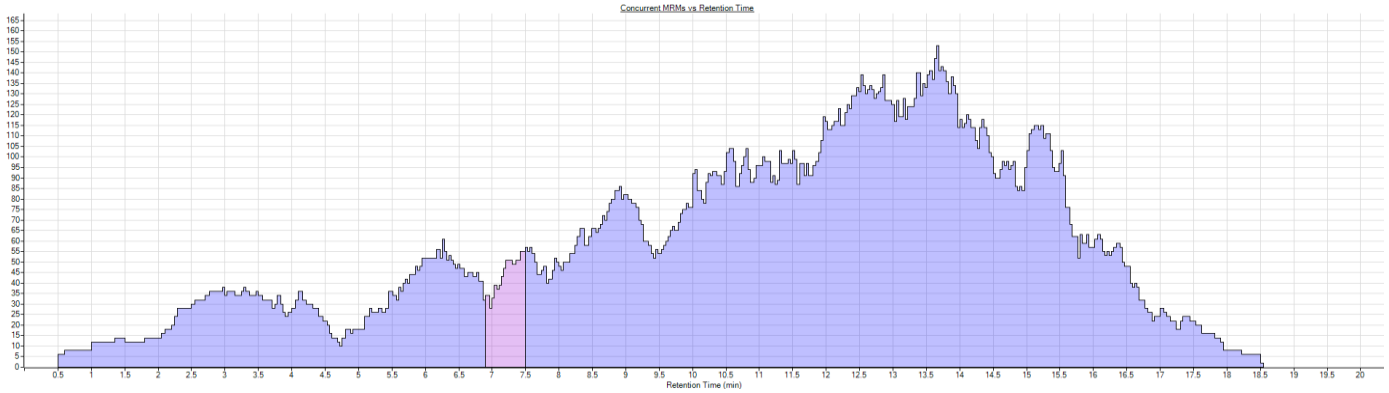
Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Ret Time (min)	Delta Ret Time	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
AccuSTD S-96086-C	1-(4-Chlorophenyl)ur	☐	171	Unit	128.1	Wide	7.21	0.6	105	15	2	Positive
AccuSTD S-96086-C	1-(4-Chlorophenyl)ur	☐	171	Unit	93.2	Wide	7.21	0.6	105	24	2	Positive
LGC 1	1,2,4-dichloropheny	☐	257	Unit	136	Wide	6.15	0.6	120	39	2	Positive
LGC 1	1,2,4-dichloropheny	☐	257	Unit	125	Wide	6.15	0.6	120	35	2	Positive
AccuSTD S-96086-C	1,2-Benzisothiazol-3	☐	152	Unit	134	Wide	6.1	1.4	140	20	3	Positive
AccuSTD S-96086-C	1,2-Benzisothiazol-3	☐	152	Unit	109	Wide	6.1	1.4	140	20	3	Positive
CUS-635	1-Naphthylacetamid	☐	186.09	Unit	141	Wide	8.21	0.6	120	17	3	Positive
CUS-635	1-Naphthylacetamid	☐	186.09	Unit	115	Wide	8.21	0.6	120	45	4	Positive
Agilent-Mix #6	2,3,5-Trimethacarb	☐	194.1	Unit	137	Wide	10.63	0.6	90	9	4	Positive
Agilent-Mix #6	2,3,5-Trimethacarb	☐	194.1	Unit	122.1	Wide	10.63	0.6	90	28	4	Positive
CUS-638	2,4,5-TP	☐	266.9	Wide	194.9	Wide	12.69	0.6	150	16	1	Negative
CUS-638	2,4,5-TP	☐	266.9	Wide	158.9	Wide	12.69	0.6	150	32	1	Negative
AccuSTD S-96086-C	2,4-Dimethylaniline	☐	122	Unit	107	Wide	4.6	1	120	18	3	Positive
AccuSTD S-96086-C	2,4-Dimethylaniline	☐	122	Unit	77	Wide	4.6	1	120	34	3	Positive
LGC 1	2-Hydroxypropoxyca	☐	415.1	Unit	174.2	Wide	6.02	0.6	120	17	4	Positive

Dynamic MRM Parameters

Cycle Time 490 ms Total MRMs = 1590 Max Concurrent MRMs = 151 Min/Max Dwell = 0.52 ms/242.30 ms

Triggered MRM

☐ Triggered Repeats 3



The number of MRM transitions is adjusted dynamically during LC run resulting in shorter MS scan cycle times and excellent quantification of very narrow peaks

Total 1590 MRM

Benefits include:

- Manage many MRMs efficiently and maximize MRM collection time at relevant elution times
- Flexible to add more MRMs with uncompromised quality of data
- Enable to perform sensitive detection of hundreds of targets simultaneously

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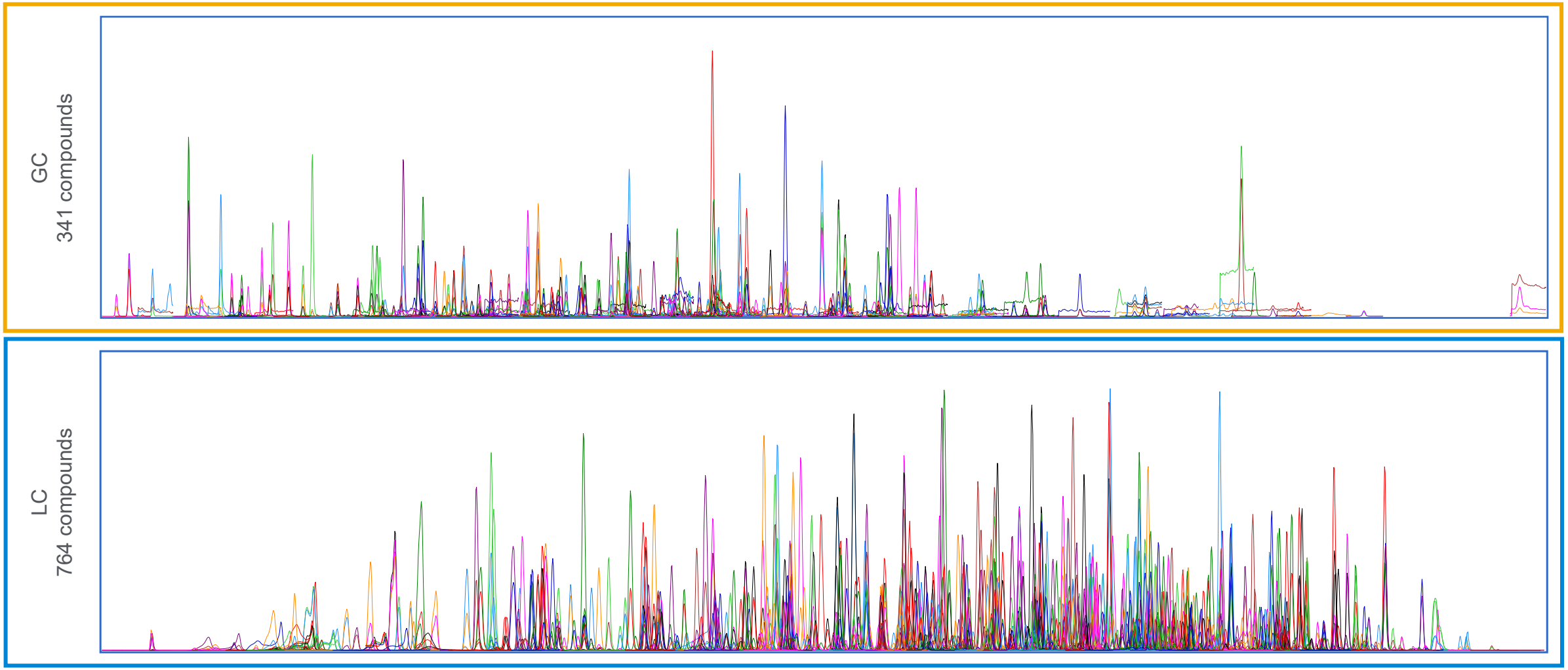
Optimized Methodology Leads to Higher Throughput



Sharp, symmetric peaks demonstrate the efficient chromatographic separation within 20 minutes runtimes

Validation and Method Details





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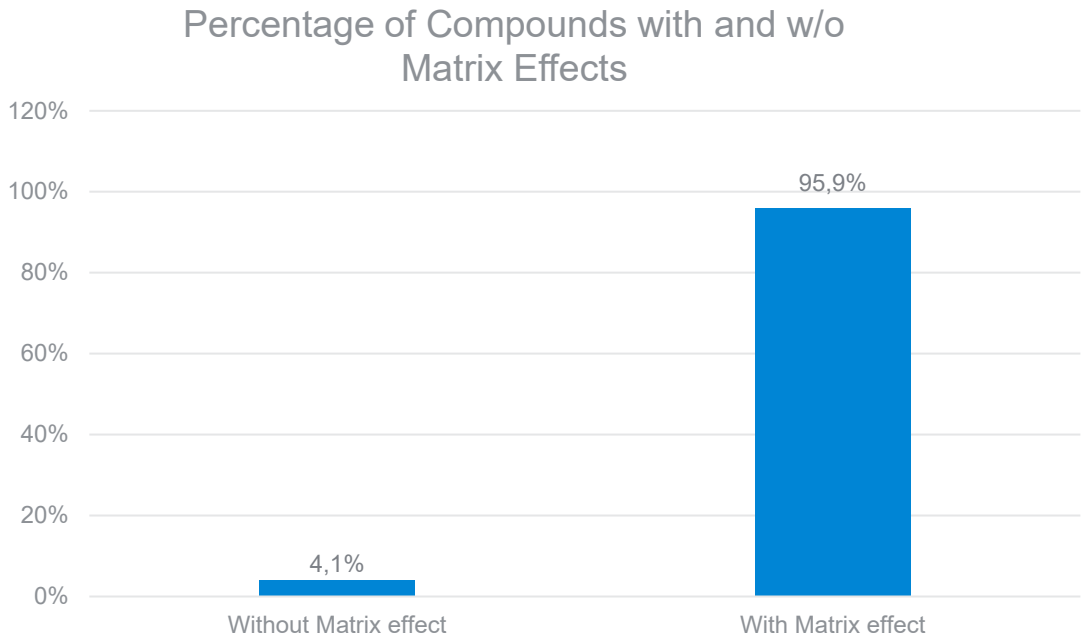
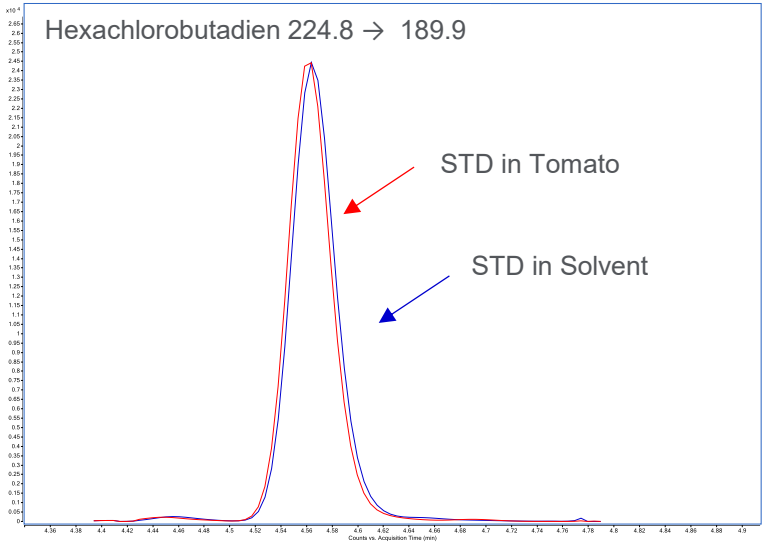
Matrix Effect (ME) Evaluation

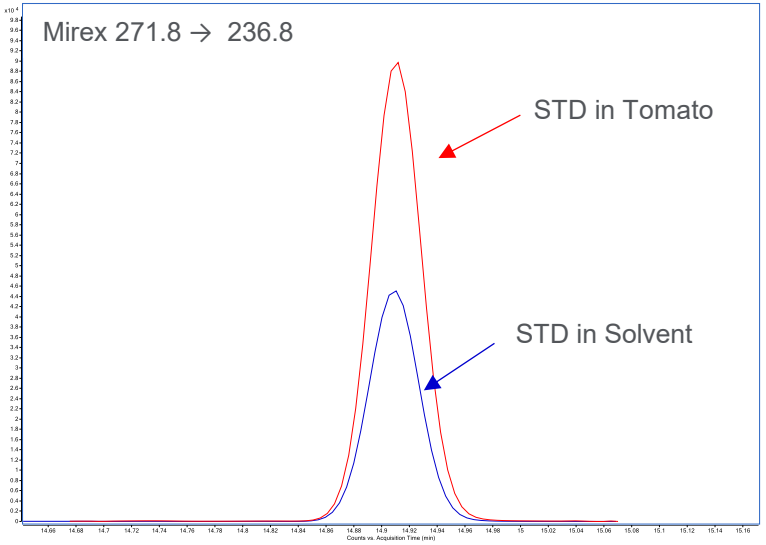
$$ME = \frac{Target\ Response\ in\ matrix}{Target\ Response\ in\ Solvent} x100\%$$

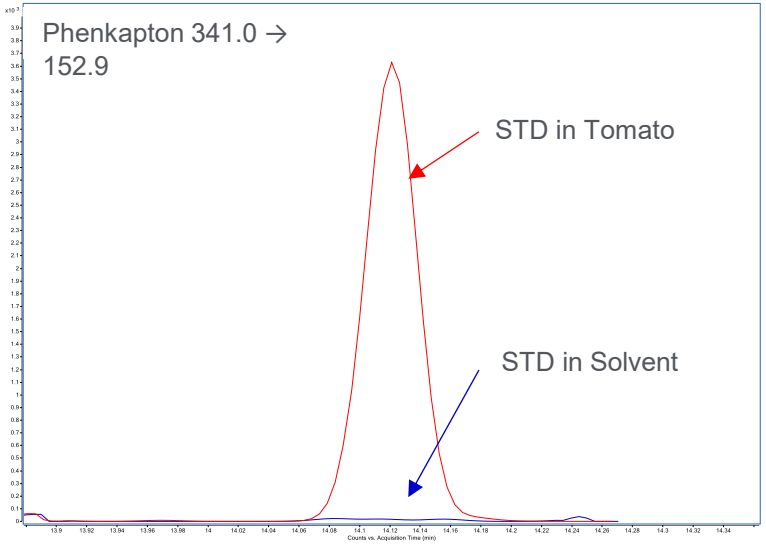
ME % Range*	Ion suppression
80 - 120%	No significant ME
<80%	Significant ion-suppression
>120%	Significant enhancement

* SANTE 11312/2021









Matrix Effect Evaluation

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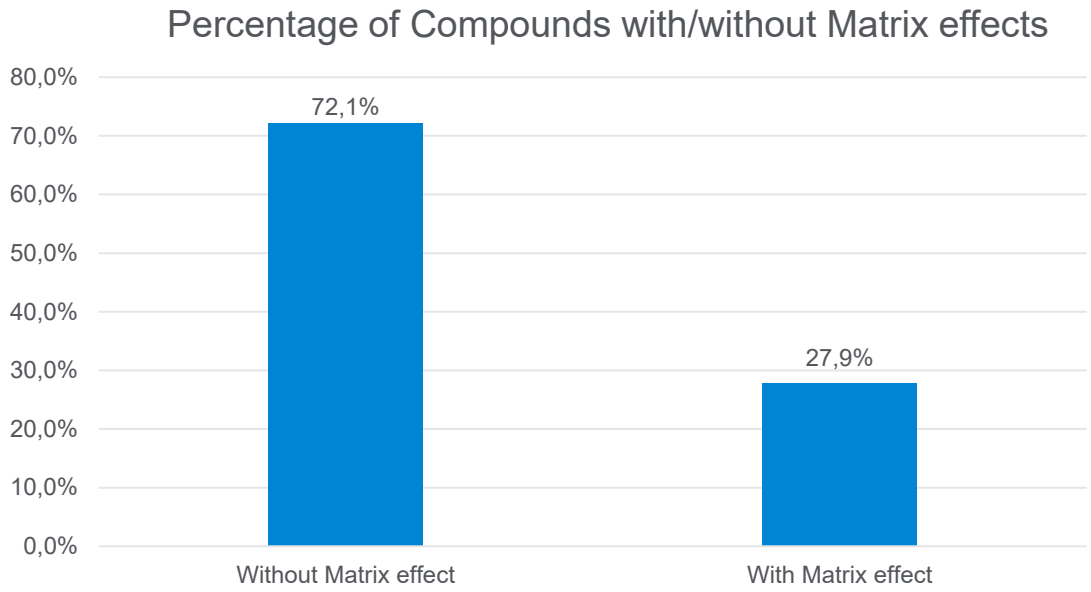
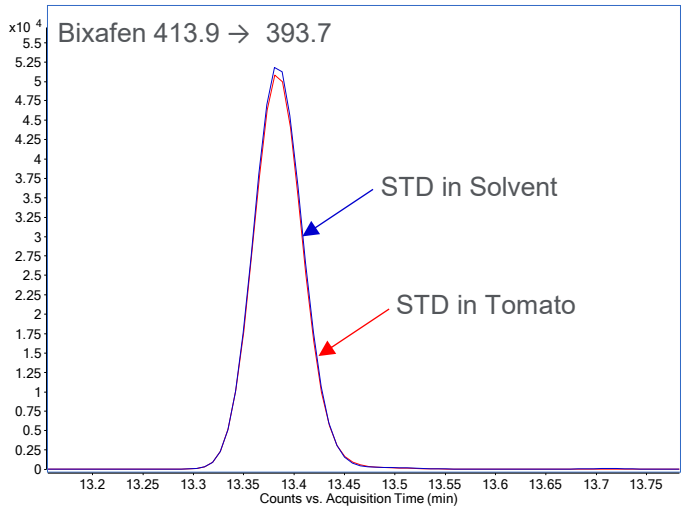
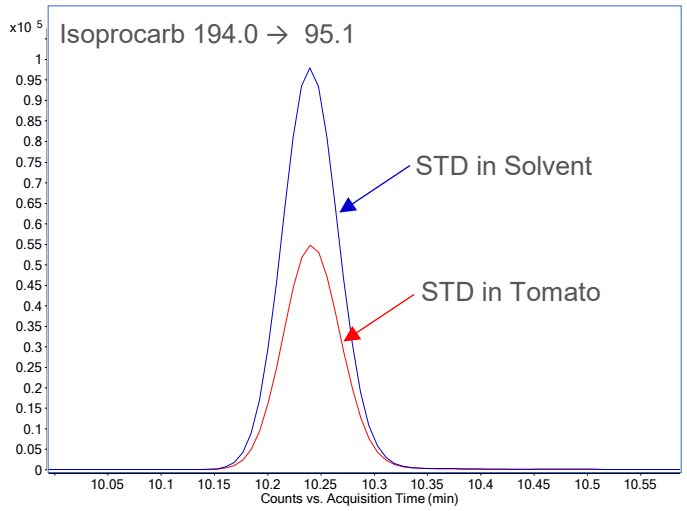
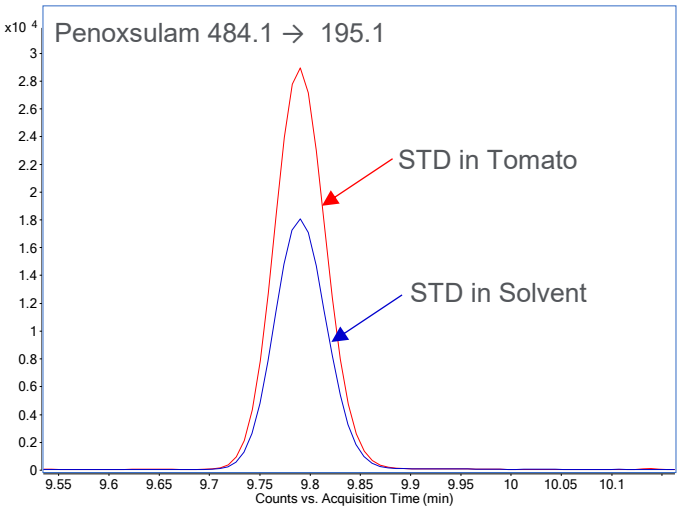


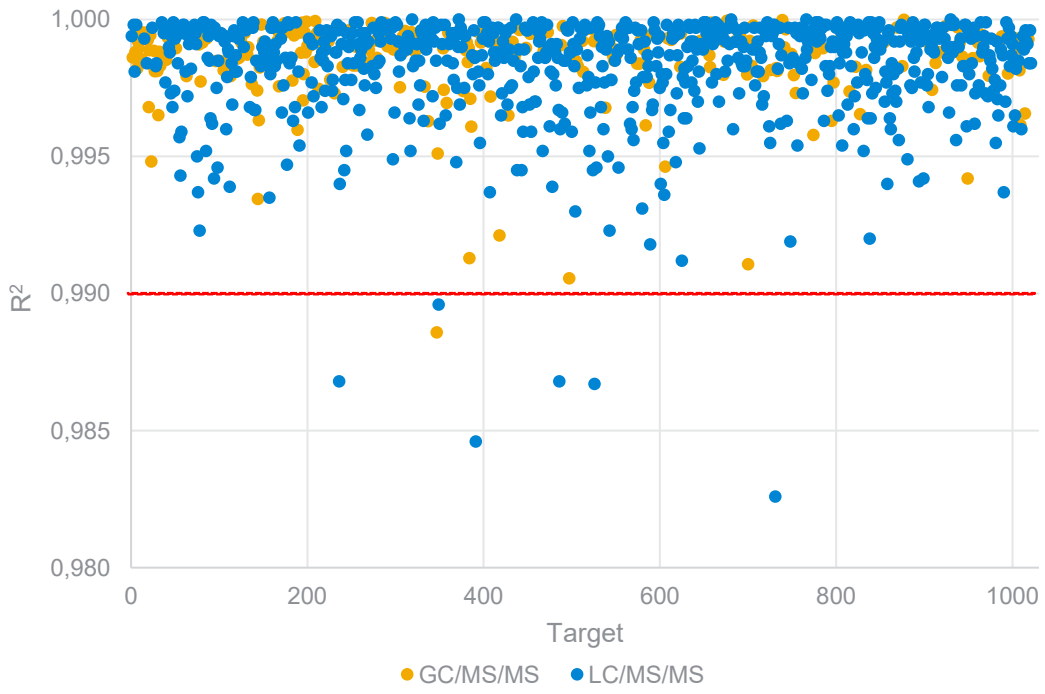




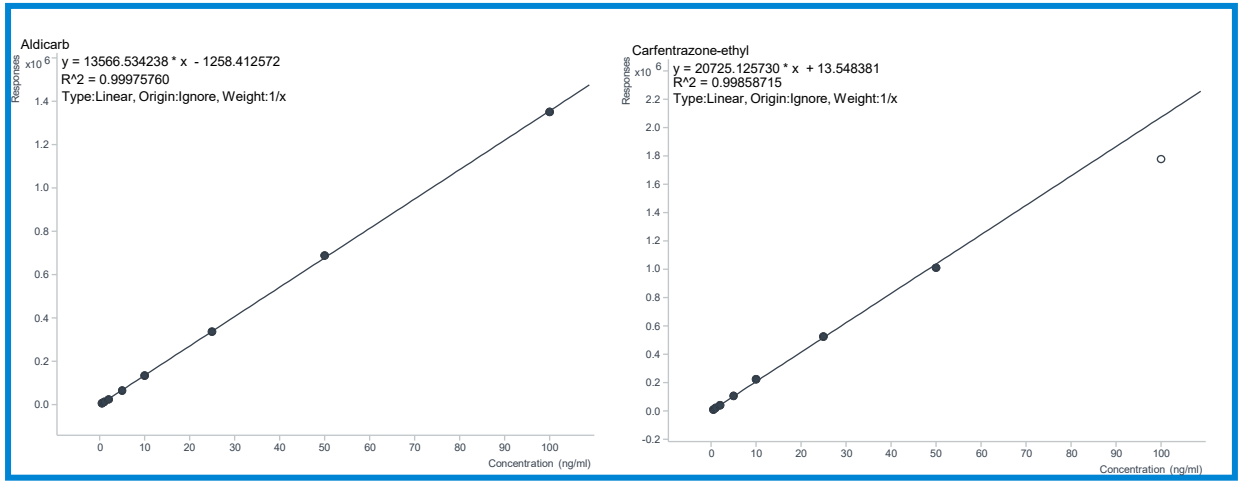


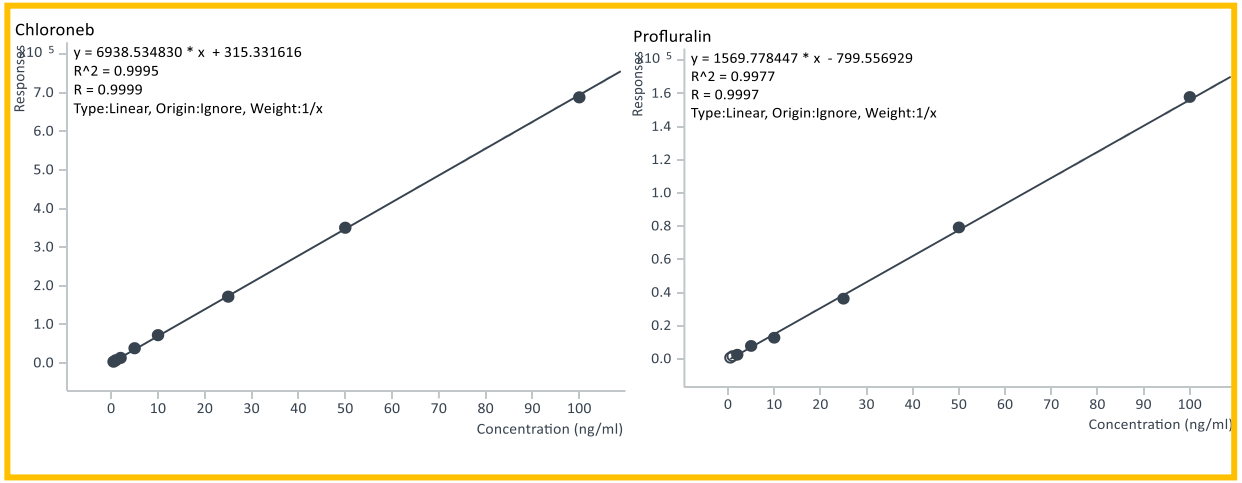
Linearity

Calibration range: 0.5 – 100 µg/L with 8 calibration levels

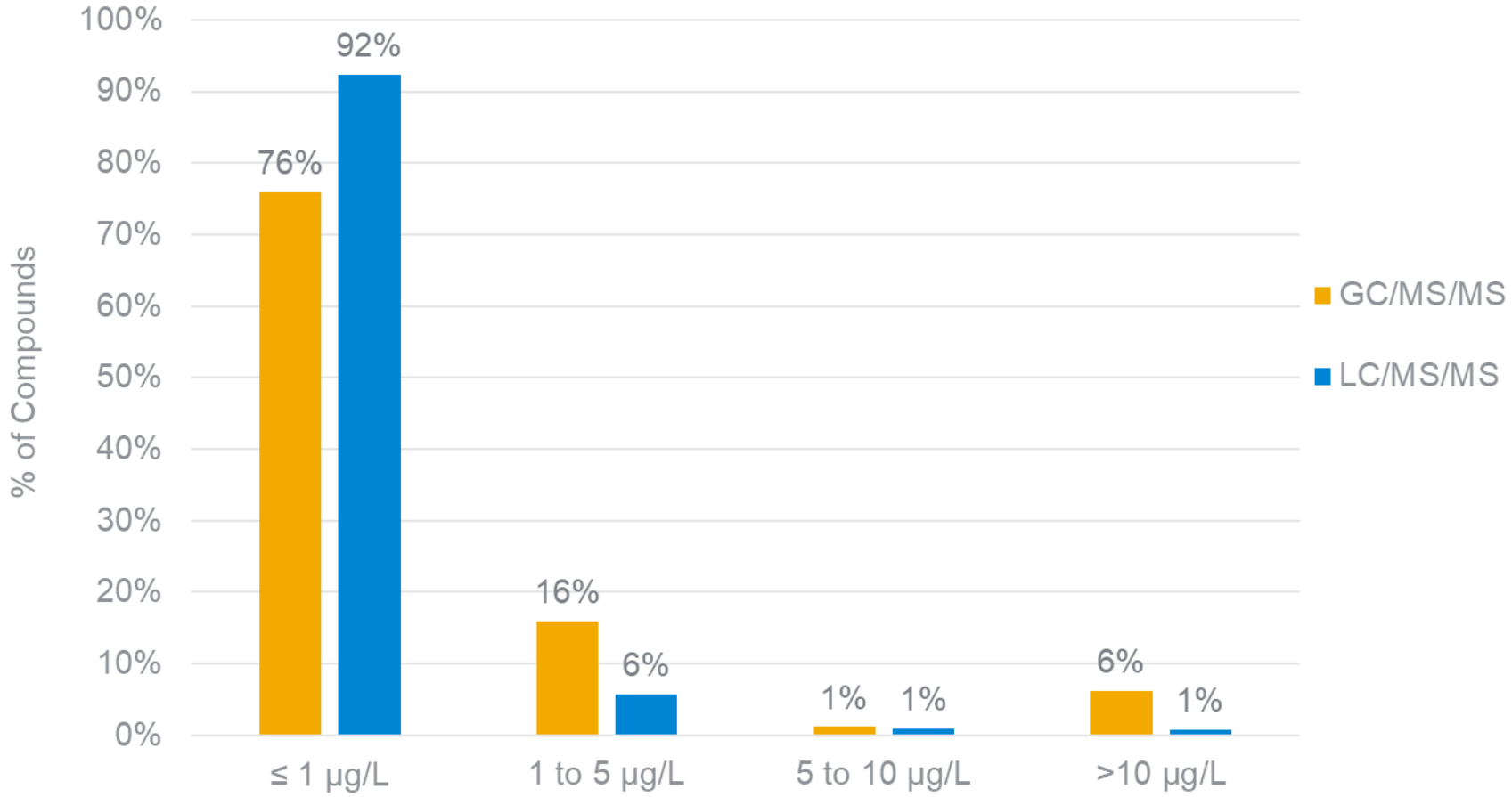


99 % of compounds demonstrated $R^2 \geq 0.99$

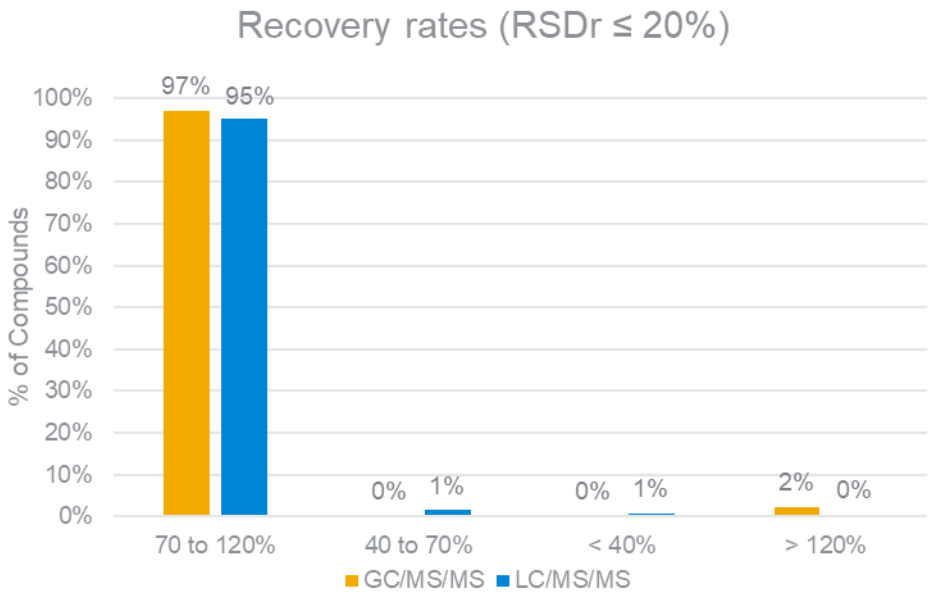




Sensitivity



Method Limits of Quantification – Meeting Regulatory Requirements



Matrix	Criteria for Limits of Quantification	Number of compounds meeting Criteria
Tomato	• S/N ≥ 10 • Qualifier ratios variation ≤ 30% • R^2 ≥ 0.99 • Recovery within 70% - 120% • RSD ≤ 20%	10 µg/kg: 959 (94 %)

Conclusions





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Summary

Application Note
Food and Beverage Testing

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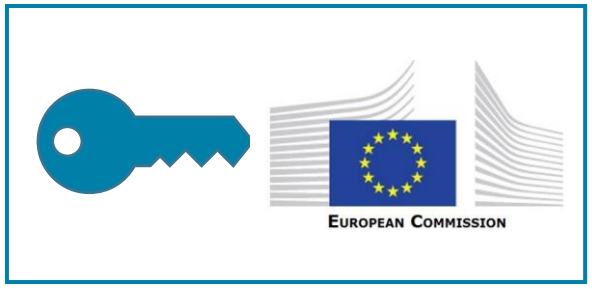
Trusted Answers

Wide Coverage

- 1021 targets in dMRM
- Only sample prep
- 20 minutes run time

Full Verification



- Verified based SANTE
- Linearity, Matrix effects, RSD, Recover

Excellent Results

- Sensitivity: 97 % of compounds with LoD ≤ 10 µg/kg
- Linearity: More than 98 % of compounds with R2 ≥ 0.99
- Quality: ≥ 94 % of compounds fulfilled SANTE Quality criteria

Quantitation of Over 1,000 Pesticide Residues in Tomato According to SANTE 11312/2021 Guideline

Using LC/MS/MS and GC/MS/MS detection

Authors

Peter Kornas and Teresa Klink
Agilent Technologies, Inc.

Abstract

A comprehensive multiresidue workflow was developed and validated for the simultaneous quantitation of over 1,000 pesticide residues in tomato to accelerate and simplify routine laboratory food testing. The workflow analyzes a wide range of pesticide residues simultaneously in 20 minutes and uses a single sample preparation method for both LC/MS/MS and GC/MS/MS analyses, leading to increased turnaround time, simplified analysis, and lower laboratory costs.

The workflow includes sample preparation, chromatographic separation, mass spectrometric (MS) detection, data analysis, and data interpretation using Agilent LC/MS/MS and GC/MS/MS systems. For sample preparation, the Agilent QuEChERS extraction kit was used without further cleanup. Compound transitions and associated optimized parameters were developed based on the Agilent pesticide MRM databases for both LC/MS and GC/MS workflows.

Workflow performance was evaluated and verified according to the SANTE 11312/2021 guideline based on instrument limit of detection (LOD), calibration curve linearity, recovery, and precision using matrix-matched calibration standards from 0.5 to 100 µg/L. Over 98% of analytes demonstrated linearity with $R^2 \geq 0.99$. Method precision was assessed using recovery repeatability (RSD_r). At the 10 µg/kg level, RSD_r values of 98% of compounds were within the limit of 20%. The mean recoveries of the six technical replicates were within the limits of 40 to 120% for 98% of target analytes.

5994-6895EN

Thank you!