Application brief | 002783

Food safety

An advanced integrated GC-MS/MS and LC-MS/MS workflow for the comprehensive analysis of pesticide residues in food

Authors

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Keywords

Pesticide residues analysis, comprehensive pesticides workflows, QuEChERS, LC-MS/MS, GC-MS/MS, Chromeleon CDS, cross-confirmation, targeted quantitation, automated data review

Goal

Analysis of proficiency test (PT) samples to demonstrate the benefits of combining GC-MS/MS and LC-MS/MS with the innovative Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) for improving laboratory efficiency in the analysis of multi-class pesticide residues in food.

Introduction

Background

According to the World Health Organization, more than one thousand different pesticides are used to protect crops from pests to increase crop yields and to minimize deterioration of agricultural products during post-harvest storage and transportation. However, inappropriate use of pesticides can result in contamination of the food supply, making it essential to define and monitor pesticide residue targets to protect consumer health, support trade, and establish food regulatory control.[1]

Consequently, laboratories are tasked to develop methods to detect, correctly identify, and quantitate hundreds of different pesticides and their transformation products in hundreds of different sample matrices, in compliance with Maximum Residue Levels (MRLs) set by regulatory bodies.

Data analysis of pesticide residues in food typically requires numerous performance checks including retention time, ion ratios, calibration curves, recovery, and precision. Data review of thousands of data values in a single chromatographic run and checking compliance against quality control criteria such as the EC SANTE guidelines[2] are some of the most time-consuming tasks in pesticides analysis.

Therefore, it is essential that workflows cover a broad pesticides scope, are sensitive and robust, and are as productive and efficient as possible to achieve compliance with regulations while meeting business goals such as return on investment (ROI), sample turnaround times, and cost-per-sample targets.

An innovative approach

In response to this challenge, new pre-configured "out of the box" pesticide workflows based on the latest Thermo Scientific™ GC-MS/MS and LC-MS/MS instruments and Chromeleon CDS[3] have been specifically designed for multi-class pesticides analysis.

These solutions include the hardware, software, built-in instrument acquisition methods, and customizable data processing methods including view settings and report templates, along with details of sample extraction and consumables for fast implementation.

This new approach, which enables the detection, identification, and quantitation of up to 700 pesticides by GC-MS/MS and LC-MS/MS, combines results in a unique software user interface to confirm the identity of residues quickly and accurately, especially those amenable by both techniques.

This automated cross-confirmation increases confidence in the data by minimizing the risk of false positive and false negative results while increasing productivity by reducing the need for repeat sample injections when either the LC-MS/MS or GC-MS/MS analysis is subject to interference. This is especially the case for more difficult samples that contain high amounts of matrix co-extractives.

Proof statement

Laboratories are often required to undertake blind analysis of PT samples containing an unknown number of unspecified pesticides. Assessing PT samples is an independent and more realistic test of the laboratory's methods and procedures.

In this application brief, the analysis of PT samples was undertaken to evaluate the analytical performance of the new integrated GC-MS/MS and LC-MS/MS pesticides workflow and to illustrate the benefits of the new software features.

Experimental

PT samples

A PT sample of homogenized French green beans and another of wheat, each containing between 10 and 25 unspecified pesticides at concentrations in the range 0.020 to 0.200 mg/kg, from a list of approximately 250 pesticides, were obtained from BIPEA, a European non-profit organization set up to conduct proficiency tests and provide reference materials.[4] The PT samples were part of two BIPEA programs[5] related to multi-residue pesticides screening in vegetables and cereals.

Sample extraction

The PT samples were extracted using the Thermo Scientific™ QuEChERS AOAC 2007.01 Method extraction kit (P/N S1-15-AOAC-POT). Acetonitrile (15 mL) was added to 15 g of green beans sample; for wheat, water (15 mL) plus acetonitrile (15 mL) were added to the sample (5 g). No clean-up was applied to extracts for LC-MS/MS before injection. GC-MS/MS extracts were cleaned up using the Thermo Scientific QuEChERS AOAC 2007.01 Method clean-up kit (P/N S2-2-GFV-AOAC-KIT), and an aliquot of the cleaned-up solution was solvent exchanged to heptane before injection.

Instrumentation

For these experiments, two workflows have been selected:

- For the LC-MS/MS amenable pesticides, a Thermo Scientific™ Vanquish™ Flex UHPLC system was connected to a Thermo Scientific™ Altis™ Plus mass spectrometer system. Chromatographic separation was achieved using a Thermo Scientific™ Hypersil GOLD™ C18 column (150 × 2.1 mm, 3 µm, P/N 25003-152130). Method details are shown in Figure 1.

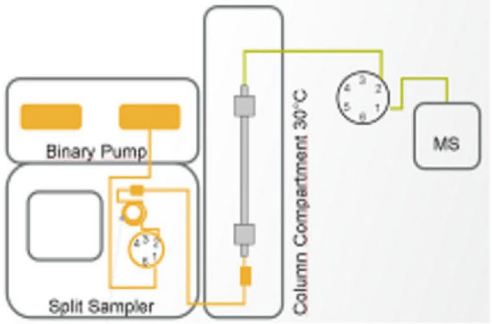
- For the GC-MS/MS amenable pesticides, a Thermo Scientific™ TRACE™ 1610 GC equipped with a Thermo Scientific™ iConnect™ Thermospray injector (TSI) and a Thermo Scientific™ TriPlus™ SMART autosampler was connected to a Thermo Scientific™ TSQ™ 9610 triple quadrupole GC-MS/MS system equipped with a Thermo Scientific™ Advanced Electron Ionization (AEI) source. Chromatographic separation was achieved using a Thermo Scientific™ TraceGOLD™ TG-SQC GC-capillary column (15 m × 0.25 mm ID × 0.25 µm film thickness, P/N 26070-1300). Method details are shown in Figure 2.

The combination of these two workflows enables analysis of up to 697 different pesticides, 443 in a single GC-MS/MS run of 20 minutes and 593 in a single LC-MS/MS run of 22 minutes with a cross correlation rate of 49% (Figures 3 and 4).

Workstreams

Enhanced_One_Shot

Chrom	Front end	MS
LC	VQ Flex Binary	Altis Plus



inj vol (µL)	inj Solvent	Sample to Sample (min)	Runs	Sample / Hour	Sequencing	Raw Data
0.5	ACN	22.0	1	2.7	No	1

Target Compounds

GC (P)	LC (P)	LC (C)	Total Cpds
	593		593

Configuration Details

Column 1	Geometry 1	Column 2	Geometry 2
HG C18	150 mm x 2.1 mm x 3 µM	na	

ESI POS (P)	ESI NEG (P)	ESI POS (C)	ESI NEG (C)	Total POS	Total NEG
524	69			524	69

Sample Extraction	Extract Purification	Carrier / Mobile
Quechers	na	Water-MeOH (0.05 mM Ammonium Fluoride* + 0.05% Formic Acid

LOQ (µg/L)	RL (µg/L)	Conc Range (µg/L)
5.00	10.00	5-100

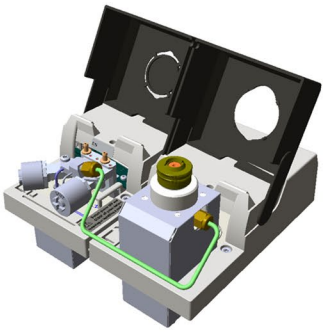
No Purification Enhanced sensitivity single injection ease of use

Figure 1. Method details regarding the LC-MS/MS used for analysis of the PT samples

Workstreams

TSI_One_Shot

Chrom	Front end	MS
GC	iC-Tsi	TSQ9610 AEI



inj vol (µL)	inj Solvent	Sample to Sample (min)	Runs	Sample / Hour	Sequencing	Raw Data
2.0	Heptane	20.0	1	3.0	No	1

Target Compounds

GC (P)	LC (P)	LC (C)	Total Cpds
443			443

Configuration Details

Column 1	Geometry 1	Column 2	Geometry 2
TG-SQC	15 m x 250 µM x 0.25µM	na	

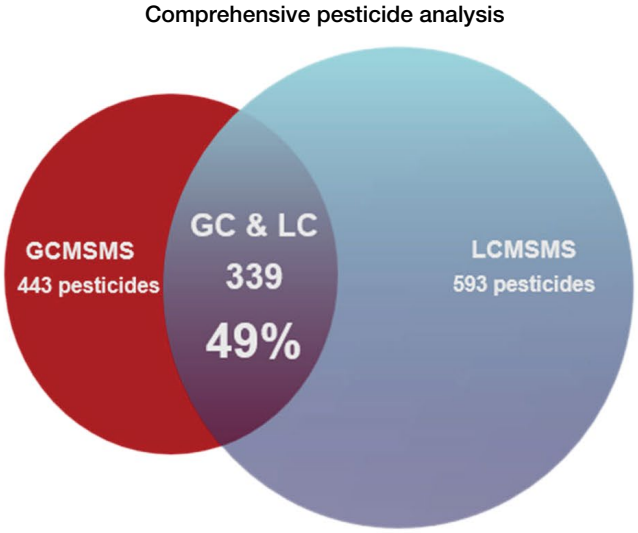
Sample Extraction	Extract Purification	Carrier / Mobile
Quechers / SLE	dSPE / na	Helium

LOQ (µg/L)	RL (µg/L)	Conc Range (µg/L)
5.00	10.00	5-100

High Robustness ease of use enhanced sensitivity Extended compound List

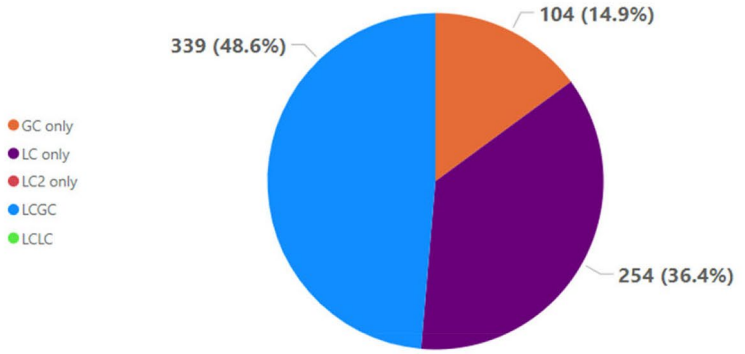
Figure 2. Method details regarding the GC-MS/MS used for analysis of the PT samples

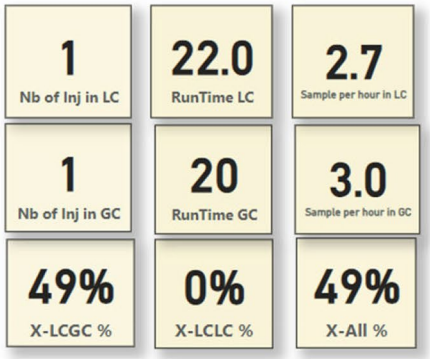
- TSQ 9610 triple quadrupole GC-MS/MS with AEI source
- TRACE 1610 GC
- TriPLUS RSH SMART autosampler

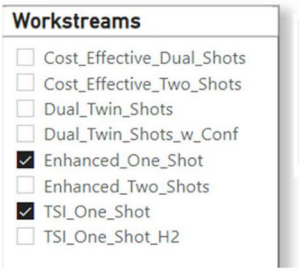


- Vanquish Flex Binary UHPLC
- TSQ Altis Plus triple quadrupole MS system

Figure 3. Analysis of up to 697 pesticides, 443 in a single GC-MS/MS analysis and 593 in a single LC-MS/MS analysis with a cross correlation rate of 49%







Workstreams	Chrom	Front end	MS	inj vol (µL)	Sample to Sample (min)	Sequencing	Runs	Sample / Hour
Enhanced_One_Shot	LC	VQ Flex Binary	Altis Plus	1	22	No	1	2.70
TSI_One_Shot	GC	iC-Tsi	TSQ9610 AEI	1	20	No	1	3.00

Figure 4. Summary of the overall outcome of using the GC-MS/MS and LC-MS/MS workflows combination

Data review

Chromeleon CDS version 7.3.2 was used for instrument control, data acquisition, data processing, and reporting of results for both GC-MS/MS and LC-MS/MS.

Automatic filtering and flagging options within the software enable laboratories to carry out exception-focused review (review by exclusion). Results falling outside laboratory-defined specifications are highlighted, allowing analysts to focus attention on problematic data to facilitate error-free reporting.

The View Settings in Chromeleon CDS can be customized to display the information required at each step of data review (Retention Time check, Calibration Curve check, QC sample check, Internal Standard (ISTD) check, and Ion Ratio check). This customizability guides operators through the review process, and reduces the time needed to train analysts and help ensure adherence to laboratory Standard Operating Procedures (SOPs).

Pre-configured view settings have been designed for pesticide analysis through GC-MS/MS and LC-MS/MS (examples displayed in Figures 5, 6, and 7).

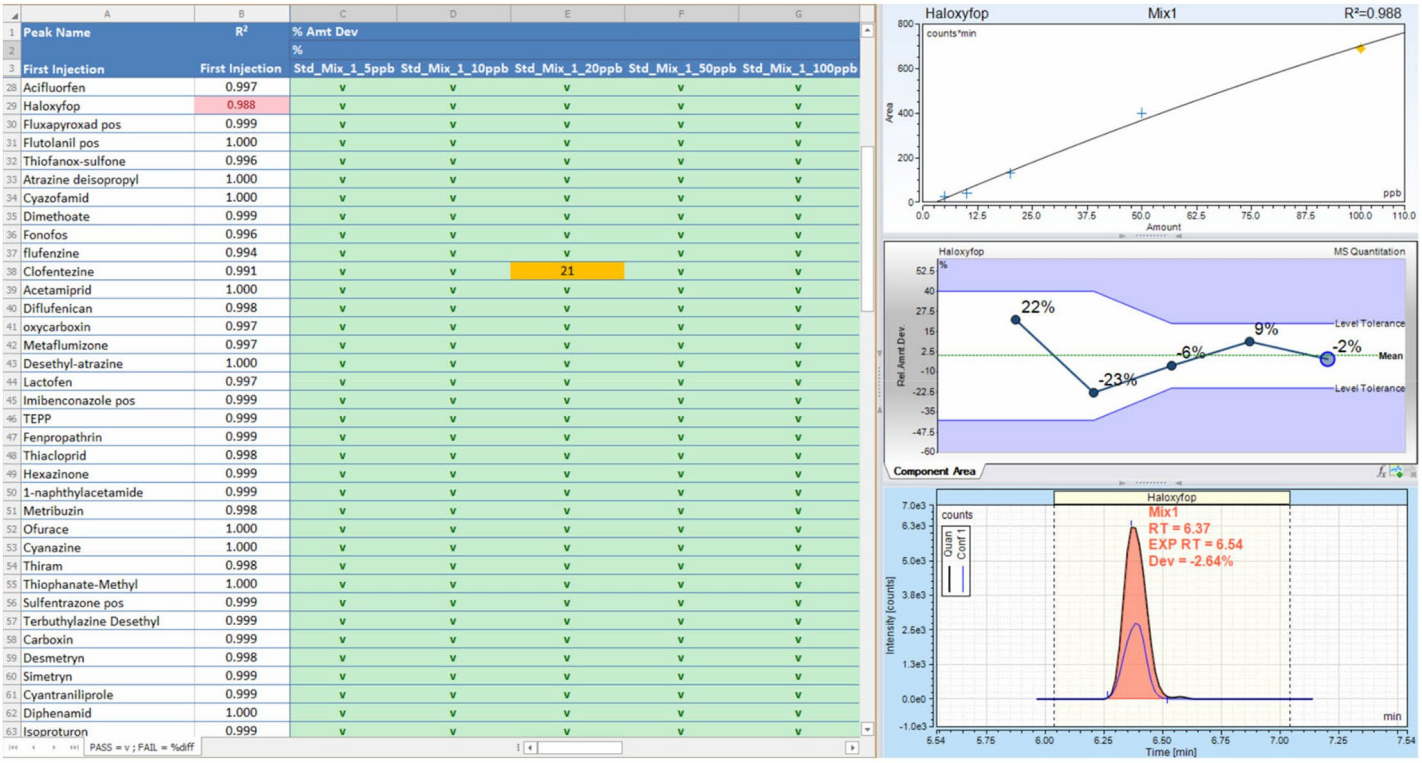


Figure 5. Data review: peak calibration curve checking. For each compound, the user can quickly check all criteria related to calibration curve linearity, accuracy, and peak shape.

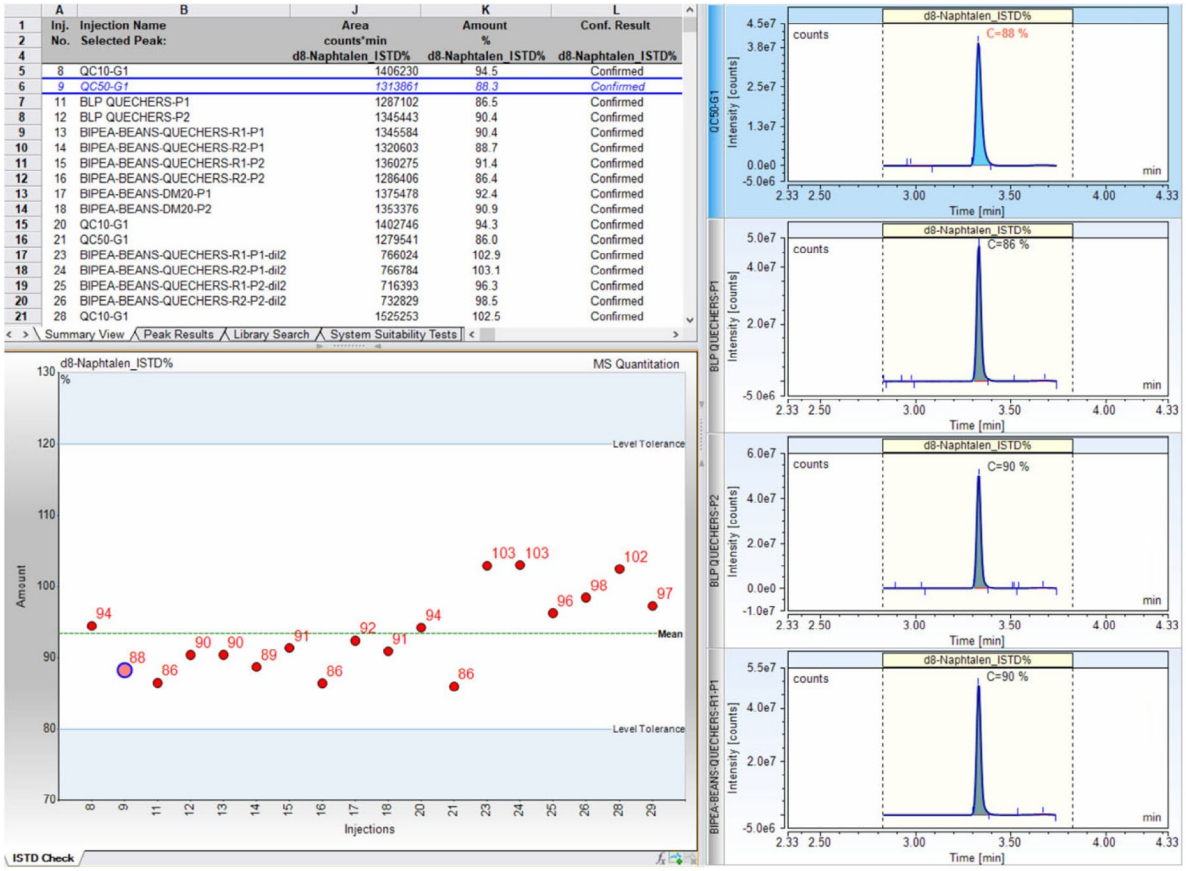


Figure 6. Data review: ISTD checking. This view allows quick confirmation that all calculated amounts of ISTDs are in the expected range prior to sample analysis.

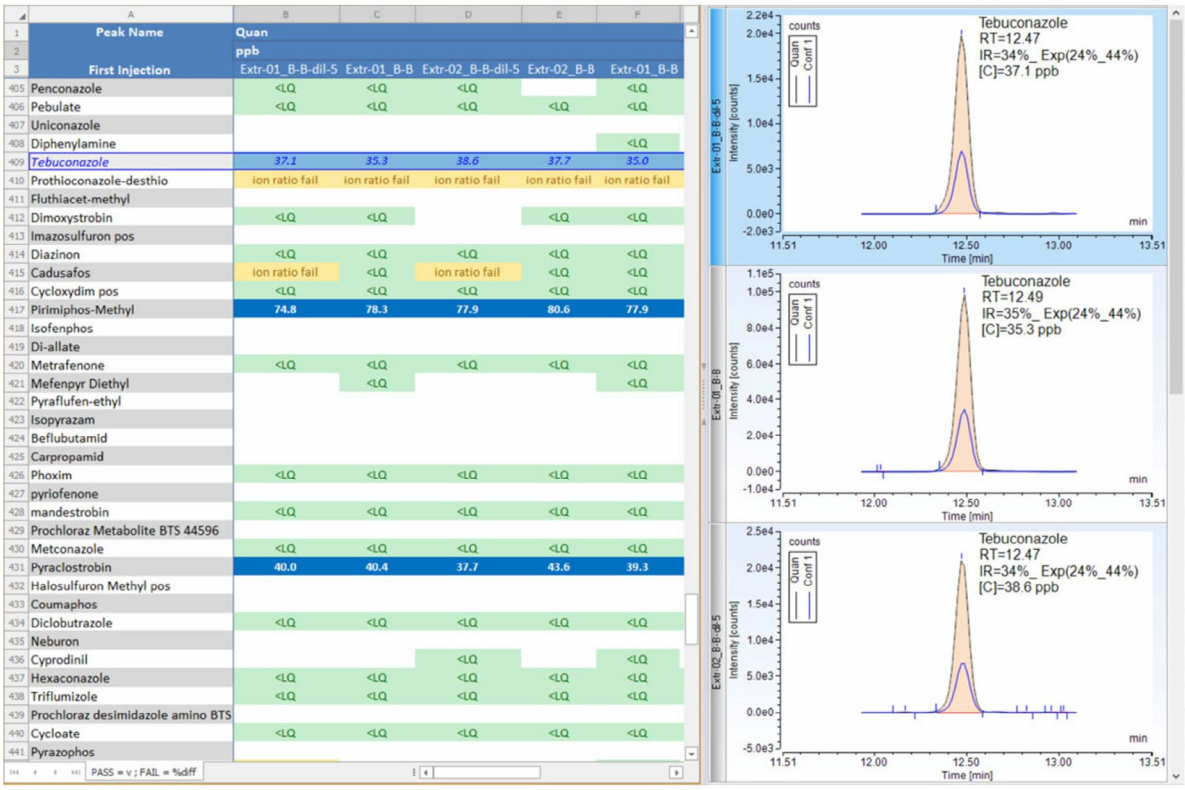


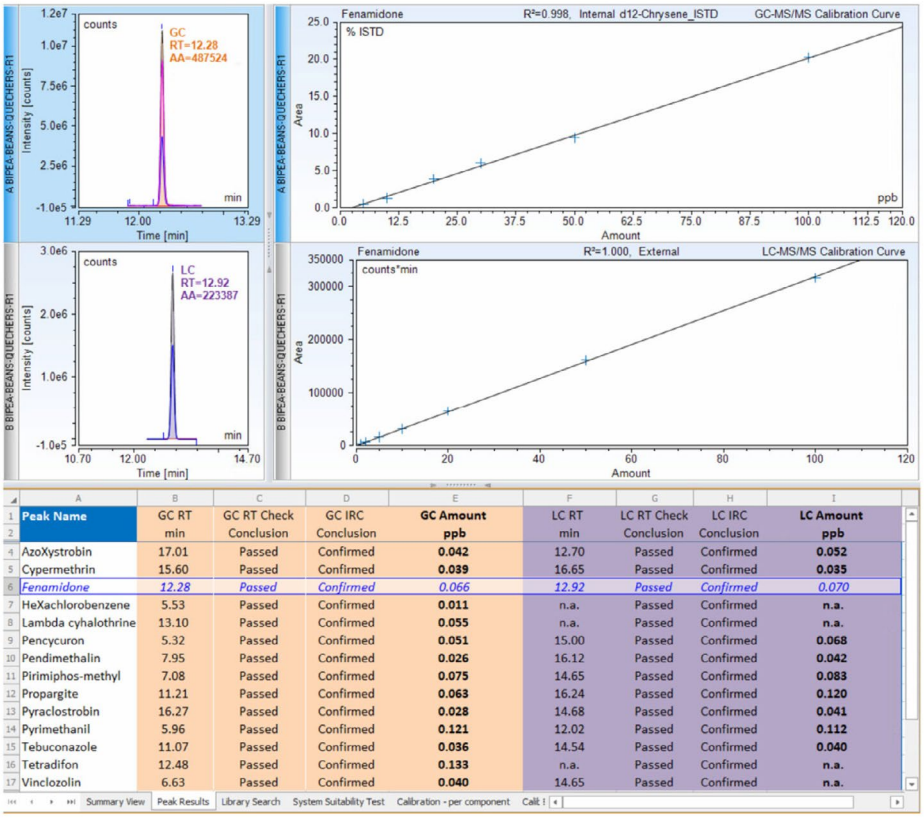
Figure 7. Data review: Ion Ratio check and calculated amount for samples. This view allows quick confirmation that ion ratios are in the expected range and provides sample results.

Results

PT sample: Green beans

The dedicated Chromeleon CDS cross-confirmatory user interface allows analysts to confirm identification of the suspected pesticides at a glance as shown in Figure 8.

The results for all the pesticides in the PT test, analyzed by LC-MS/MS and GC-MS/MS, were all within the minimum and maximum values with Z-scores mostly below 1. Thus, all results were classified as satisfactory as shown in Table 1, demonstrating the excellent accuracy, precision, and reliability of the integrated pesticides workflow.



Peak Name	GC RT min	GC RT Check Conclusion	GC IRC Conclusion	GC Amount ppb	LC RT min	LC RT Check Conclusion	LC IRC Conclusion	LC Amount ppb
AzoXystrobin	17.01	Passed	Confirmed	**0.042**	12.70	Passed	Confirmed	**0.052**
Cypermethrin	15.60	Passed	Confirmed	**0.039**	16.65	Passed	Confirmed	**0.035**
Fenamidone	*12.28*	*Passed*	*Confirmed*	*0.066*	*12.92*	*Passed*	*Confirmed*	*0.070*
HeXachlorobenzene	5.53	Passed	Confirmed	**0.011**	n.a.	Passed	Confirmed	**n.a.**
Lambda cyhalothrine	13.10	Passed	Confirmed	**0.055**	n.a.	Passed	Confirmed	**n.a.**
Pencycuron	5.32	Passed	Confirmed	**0.051**	15.00	Passed	Confirmed	**0.068**
Pendimethalin	7.95	Passed	Confirmed	**0.026**	16.12	Passed	Confirmed	**0.042**
Pirimiphos-methyl	7.08	Passed	Confirmed	**0.075**	14.65	Passed	Confirmed	**0.083**
Propargite	11.21	Passed	Confirmed	**0.063**	16.24	Passed	Confirmed	**0.120**
Pyraclostrobin	16.27	Passed	Confirmed	**0.028**	14.68	Passed	Confirmed	**0.041**
Pyrimethanil	5.96	Passed	Confirmed	**0.121**	12.02	Passed	Confirmed	**0.112**
Tebuconazole	11.07	Passed	Confirmed	**0.036**	14.54	Passed	Confirmed	**0.040**
Tetradifon	12.48	Passed	Confirmed	**0.133**	n.a.	Passed	Confirmed	**n.a.**
Vinclozolin	6.63	Passed	Confirmed	**0.040**	14.65	Passed	Confirmed	**n.a.**

Figure 8. Positive identification and confident quantitation of *Fenamidone* using Chromeleon CDS cross-confirmatory view while reprocessing the green bean sample

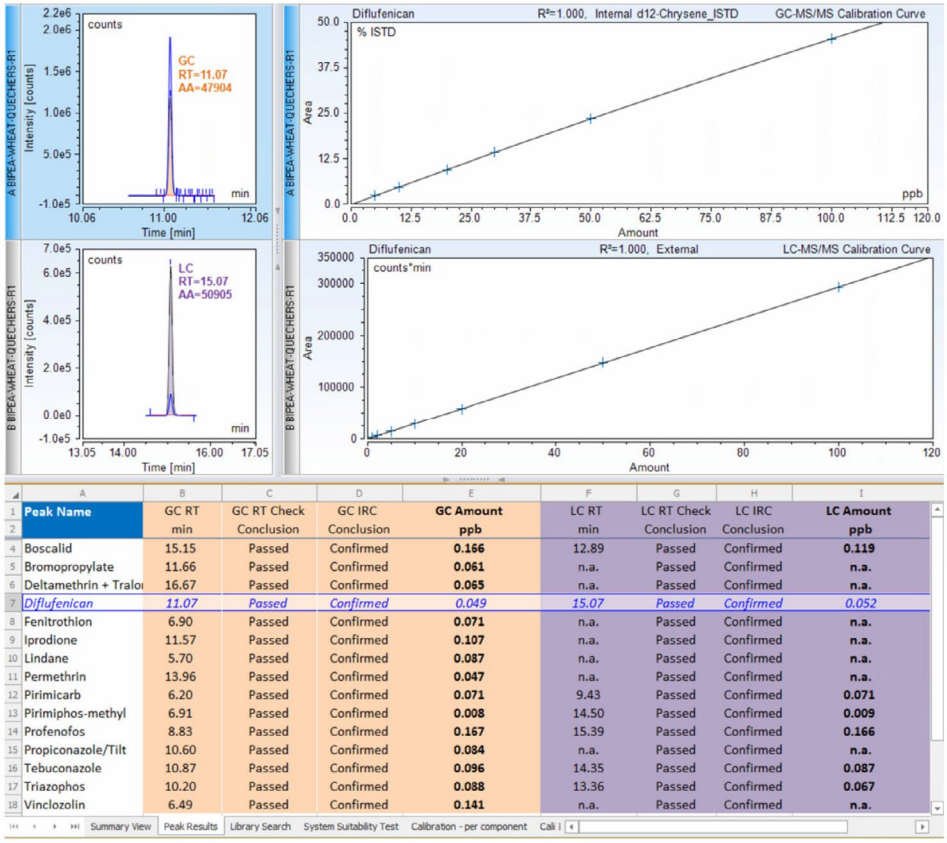
Table 1. Summary of calculated amount (mg/kg) and Z-score obtained based on GC-MS/MS and LC-MS/MS analysis performed on proficiency test sample of green beans

Green beans - PT sample		GC results	LC results	Target	Std deviation	Min value	Max value	Z-score GC	GC evaluation	Z-score LC	LC evaluation
Compound	Class										
Azoxystrobin	**Strobilurin**	**0.042**	**0.052**	0.048	0.012	0.024	0.072	-0.50	Satisfactory	0.33	Satisfactory
Cypermethrin	**Pyrethroid**	**0.039**	**0.035**	0.047	0.012	0.023	0.071	-0.67	Satisfactory	-1.00	Satisfactory
Fenamidone	**Triazole**	**0.066**	**0.070**	0.064	0.017	0.03	0.098	0.12	Satisfactory	0.35	Satisfactory
Pencycuron	**Phenylurea**	**0.051**	**0.068**	0.056	0.014	0.028	0.084	-0.36	Satisfactory	0.86	Satisfactory
Pendimethaline	**DinitroAniline**	**0.026**	**0.042**	0.033	0.009	0.016	0.05	-0.82	Satisfactory	1.06	Satisfactory
Pirimiphos-methyl	**Organophosphorus**	**0.075**	**0.083**	0.073	0.019	0.039	0.11	0.11	Satisfactory	0.54	Satisfactory
Propargite	**Sulfite ester**	**0.063**	**0.061**	0.048	0.012	0.024	0.072	1.25	Satisfactory	1.08	Satisfactory
Pyraclostrobin	**Strobilurin**	**0.028**	**0.041**	0.039	0.010	0.019	0.059	-1.10	Satisfactory	0.20	Satisfactory
Pyrimethanil	**Anilinopyrimidine**	**0.121**	**0.112**	0.110	0.027	0.056	0.164	0.41	Satisfactory	0.07	Satisfactory
Tebuconazole	**Triazole**	**0.036**	**0.040**	0.040	0.01	0.02	0.06	-0.40	Satisfactory	0.00	Satisfactory
HCB	**OrganoChloride**	**0.011**	***na***	0.019	0.005	0.009	0.029	-1.60	Satisfactory		*Not tested*
Lambda-Cyhalothrin	**Pyrethroid**	**0.055**	***na***	0.045	0.014	0.017	0.073	0.71	Satisfactory		*Not tested*
Tetradifon	**OrganoChloride**	**0.133**	***na***	0.151	0.035	0.081	0.221	-0.51	Satisfactory		*Not tested*
Vinclozolin	**Dicarboximide**	**0.040**	***na***	0.038	0.01	0.019	0.057	0.21	Satisfactory		*Not tested*
Linuron	**Phenylurea**	**0.049**	**0.052**	0.045	0.012	0.022	0.068	0.35	Satisfactory	0.61	Satisfactory

PT sample: Wheat

Figure 9 shows cross-confirmatory results between GC-MS/MS and LC-MS/MS.

The results for all the pesticides in the PT test were within the minimum and maximum values with Z-scores mostly below 1. All results were classified as satisfactory as shown in Table 2.



Peak Name	GC RT	GC RT Check	GC IRC	GC Amount	LC RT	LC RT Check	LC IRC	LC Amount
	min	Conclusion	Conclusion	ppb	min	Conclusion	Conclusion	ppb
Boscalid	15.15	Passed	Confirmed	**0.166**	12.89	Passed	Confirmed	**0.119**
Bromopropylate	11.66	Passed	Confirmed	**0.061**	n.a.	Passed	Confirmed	**n.a.**
Deltamethrin + Tralo	16.67	Passed	Confirmed	**0.065**	n.a.	Passed	Confirmed	**n.a.**
Diflufenican	*11.07*	*Passed*	*Confirmed*	*0.049*	*15.07*	*Passed*	*Confirmed*	*0.052*
Fenitrothion	6.90	Passed	Confirmed	**0.071**	n.a.	Passed	Confirmed	**n.a.**
Iprodione	11.57	Passed	Confirmed	**0.107**	n.a.	Passed	Confirmed	**n.a.**
Lindane	5.70	Passed	Confirmed	**0.087**	n.a.	Passed	Confirmed	**n.a.**
Permethrin	13.96	Passed	Confirmed	**0.047**	n.a.	Passed	Confirmed	**n.a.**
Pirimicarb	6.20	Passed	Confirmed	**0.071**	9.43	Passed	Confirmed	**0.071**
Pirimiphos-methyl	6.91	Passed	Confirmed	**0.008**	14.50	Passed	Confirmed	**0.009**
Profenofos	8.83	Passed	Confirmed	**0.167**	15.39	Passed	Confirmed	**0.166**
Propiconazole/Tilt	10.60	Passed	Confirmed	**0.084**	n.a.	Passed	Confirmed	**n.a.**
Tebuconazole	10.87	Passed	Confirmed	**0.096**	14.35	Passed	Confirmed	**0.087**
Triazophos	10.20	Passed	Confirmed	**0.088**	13.36	Passed	Confirmed	**0.067**
Vinclozolin	6.49	Passed	Confirmed	**0.141**	n.a.	Passed	Confirmed	**n.a.**

Figure 9. Positive identification and confident quantitation of *Diflufenican* using Chromeleon CDS cross-confirmatory view while reprocessing wheat sample

Table 2. Summary of calculated amount (mg/kg) and Z-score obtained based on GC-MS/MS and LC-MS/MS analysis performed on proficiency test sample of wheat

Wheat- PT sample		GC results	LC results	Target	Std deviation	Min value	Max value	Z-score GC	GC evaluation	Z-score LC	LC evaluation
Compound	Class										
Boscalid	**Carboximide**	**0.166**	**0.119**	0.147	0.035	0.078	0.216	0.55	Satisfactory	-0.81	Satisfactory
Bromopropylate	**Carbinol**	**0.061**	***na***	0.070	0.018	0.035	0.105	-0.51	Satisfactory		Satisfactory
Deltamethrin	**Pyrethroid**	**0.065**	***na***	0.099	0.025	0.049	0.149	-1.36	Satisfactory		Satisfactory
Diflufenican	**Carboxamide**	**0.049**	**0.052**	0.07	0.018	0.035	0.105	-1.20	Satisfactory	-1.20	Satisfactory
Fenitrothion	**Organophosphorus**	**0.071**	***na***	0.049	0.013	0.024	0.074	1.76	Satisfactory		*Not tested*
Gamma-HCH	**OrganoChloride**	**0.087**	***na***	0.089	0.024	0.042	0.136	-0.09	Satisfactory		*Not tested*
Iprodione	**Phenylurea**	**0.107**	***na***	0.080	0.021	0.038	0.122	1.29	Satisfactory		*Not tested*
Permethrin	**Pyrethroid**	**0.047**	***na***	0.073	0.019	0.036	0.11	-1.41	Satisfactory		*Not tested*
Pirimicarb	**Carbamate**	**0.071**	**0.071**	0.075	0.019	0.037	0.133	-0.21	Satisfactory	-0.21	Satisfactory
Pirimiphos-methyl	**Organophosphorus**	**0.008**	**0.009**	0.011	0.003	0.005	0.017	-1.00	Satisfactory	-0.67	Satisfactory
Profenofos	**Organophosphorus**	**0.167**	**0.166**	0.135	0.032	0.071	0.199	1.00	Satisfactory	0.97	Satisfactory
Propiconazole	**Triazole**	**0.084**	***na***	0.092	0.023	0.046	0.138	-0.35	Satisfactory		Not tested
Tebuconazole	**Triazole**	**0.096**	**0.087**	0.107	0.027	0.054	0.16	-0.42	Satisfactory	-0.75	Satisfactory
Triazophos	**Organophosphorus**	**0.088**	**0.067**	0.070	0.019	0.033	0.107	0.97	Satisfactory	-0.16	Satisfactory
Vinclozolin	**Dicarboximide**	**0.141**	***na***	0.137	0.033	0.072	0.202	0.12	Satisfactory		*Not tested*

Conclusion

- The pre-configured Thermo Scientific GC-MS/MS and LC-MS/MS pesticide workflows for analysis of approximately 700 pesticides are available as out-of-the-box solutions which include the hardware, software, built-in instrument and data processing methods, details of sample extraction, and consumables for fast implementation into any laboratory.
- The Chromeleon CDS version 7.3.2, built specifically for pesticides, provides fast data processing of large datasets comprising hundreds of pesticides, in any configuration, from a workstation on a single computer to a complete Chromeleon Enterprise environment connecting multiple MS instruments and computers.
- The multi-instrument and intuitive Chromeleon CDS user interface, with pre-configured customizable templates and view settings, requires laboratory analysts to learn only a single software platform. This saves time on training and enables more effective deployment of staff resources whatever analytical technique is involved.
- The unique feature for cross-confirmation of identity, by combining GC-MS/MS and LC-MS/MS data, provides increased confidence in results and reduces the need for re-analysis while minimizing the possibility of false positive and false negative results.
- The satisfactory results obtained from analysis of the PT samples demonstrate how the integrated workflows can quantitate GC-MS/MS and LC-MS/MS amenable pesticides with the required accuracy and precision in a blind test, mimicking a real-world lab situation.

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