An integrated GC-MS/MS and LC-MS/MS workflow for quantitative analysis of pesticides with cross-confirmation using a single chromatography data system software

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Abstract

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

Methods: Pre-configured "out of the box" pesticide workflow methods have been specifically designed and optimized 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.

Results: The results for all the pesticides in both the wheat and green bean PT test samples, analyzed by LC-MS/MS and GC-MS/MS, were all within the minimum and maximum values with Z-scores mostly below 1. All results were classified as satisfactory, demonstrating the excellent accuracy, precision, and reliability of the integrated pesticides workflow.

Introduction

According to the World Health Organization, more than one thousand different pesticides are used to protect crops from pests to increase yields and to minimize deterioration of agricultural products during storage and transportation. However, inappropriate use of pesticides can result in contamination of the food supply and the environment making it essential to define and monitor pesticide residue targets to protect the environment, consumer health, support trade, and establish food regulatory control. Consequently, laboratories are tasked to develop methods with a broad scope to detect, correctly identify, and quantitate hundreds of different pesticides and their transformation products in diverse sample matrices, and often at levels well below maximum residue levels (MRLs) set by regulatory bodies.

Materials and methods

Sample Preparation

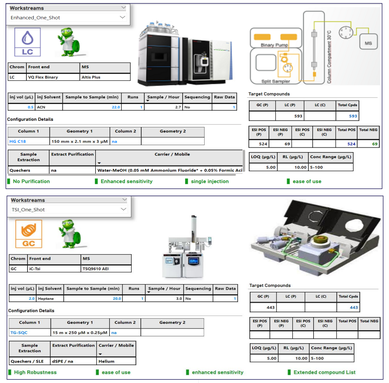
A PT sample of homogenized French green beans and another of wheat, each containing between 10 and 25 unspecified pesticides at concentrations in the range 0.020 to 0.200 mg/kg, from a list of approximately 250 pesticides, were obtained from BIPEA, a European non-profit organization set up to conduct proficiency tests and provide reference materials.

The 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.

Materials and methods (cont.)

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). See Figure 1 for more method details.

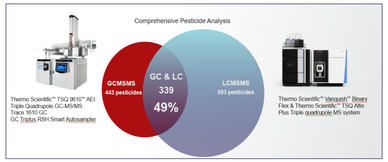
For the GC-MS/MS amenable pesticides, a Thermo Scientific™ TRACE™ 1610 GC equipped with a Thermo Scientific™ iConnect™ Thermospray injector (TSI) and a Thermo Scientific™ TriPlus™ SMART autosampler was connected to a Thermo Scientific™ TSQ™ 9610 triple quadrupole GC-MS/MS system equipped with a Thermo Scientific™ Advanced Electron Ionization (AEI) source. Chromatographic separation was achieved using a Thermo Scientific™ TraceGOLD™ TG-5QC GC-capillary column (15 m × 0.25 mm ID × 0.25 µm film thickness). See Figure 2 for more method details.



Figures 1 and 2. Method details regarding the LC-MS/MS (top) and the GC-MS/MS (bottom) used for analysis of the PT samples.

Results

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 analytical technique is subject to interference. This is especially the case for more difficult samples that contain high amounts of matrix co-extractives. See Figure 3.



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

Results

The dedicated Chromeleon CDS cross-confirmatory user interface allows analysts to confirm identification of the suspected pesticides at-a-glance as shown in Figure 4 for the detected pesticides in the green been PT sample.

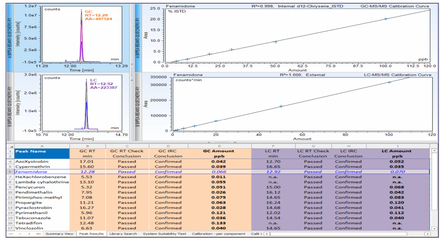


Figure 4. Positive identification and confident quantitation of Fenamidone using Chromeleon CDS cross-confirmatory view while reprocessing the green bean sample.

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.

GREEN BEANS - Program 5		GC Results	LC Results	Target	Value min	Value max	Z-score GC	LC Evaluation	Z-score LC	LC Evaluation
Azoxystrobin	Strobilurine	0.042	0.052	0.048	0.034	0.072	-0.50	Satisfactory	0.33	Satisfactory
Cypermethrin	Pyréthrinoïde	0.039	0.035	0.047	0.023	0.071	-0.67	Satisfactory	-1.00	Satisfactory
Fenamidone	Azole	0.066	0.070	0.064	0.03	0.098	0.12	Satisfactory	0.35	Satisfactory
Pencycuron	Urée	0.051	0.068	0.056	0.028	0.084	-0.36	Satisfactory	0.86	Satisfactory
Pendimethaline	Dinitroaniline	0.026	0.042	0.033	0.016	0.05	-0.82	Satisfactory	1.06	Satisfactory
Pirimiphos-methyl	Organophosphoré	0.075	0.083	0.073	0.039	0.11	0.31	Satisfactory	0.54	Satisfactory
Propargite	Sulfite d'ester	0.063	0.061	0.048	0.024	0.072	1.25	Satisfactory	1.08	Satisfactory
Pyraclostrobin	Strobilurine	0.028	0.041	0.039	0.019	0.059	-1.10	Satisfactory	0.20	Satisfactory
Pyrimethanil	Anilinopyrimidine	0.121	0.112	0.110	0.056	0.164	0.41	Satisfactory	0.07	Satisfactory
Tebuconazole	Azole	0.036	0.040	0.040	0.02	0.06	-0.40	Satisfactory	0.00	Satisfactory
HCB	OrganoChloré	0.011	na	0.019	0.009	0.029	-1.60	Satisfactory		Not tested
Lambda-Cyhalothrin	Pyréthrinoïde	0.055	na	0.045	0.017	0.073	0.71	Satisfactory		Not tested
Tetradifon	OrganoChloré	0.133	na	0.151	0.081	0.221	-0.51	Satisfactory		Not tested
Vinclozolin	Dicarboximide	0.040	na	0.038	0.019	0.057	0.21	Satisfactory		Not tested
Linuron	Urée	0.049	0.052	0.045	0.022	0.068	0.35	Satisfactory	0.61	Satisfactory

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

Figure 5 shows cross confirmatory results for the wheat PT sample between GC-MS/MS and LC-MS/MS for the detected pesticides.

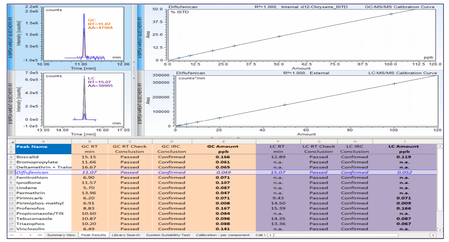


Figure 5. Positive identification and quantitation of Diflufenican using Chromeleon CDS cross-confirmatory view while reprocessing wheat sample.

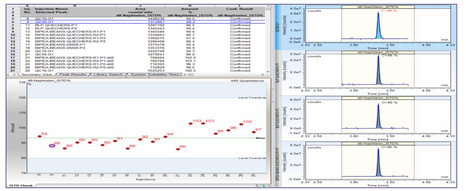
Results (Cont.)

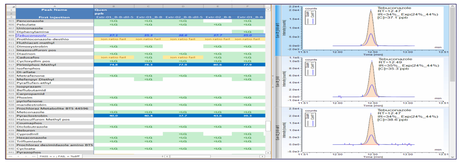
The results for all the pesticides in the wheat PT test sample were within the minimum and maximum values with Z-scores mostly below 1. All results were classified as satisfactory as shown in Table 2.

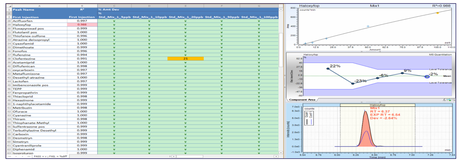
WHEAT - Program 2		GC Results	LC Results	Target	Value min	Value max	Z-score GC	GC Evaluation	Z-score LC	LC Evaluation
Boscalid	Carboxamide	0.166	0.119	0.147	0.078	0.216	0.55	Satisfactory	-0.81	Satisfactory
Bromopropylate	Carbinol	0.061	na	0.070	0.035	0.105	-0.51	Satisfactory		Satisfactory
Deltamethrin	Pyréthrinoïde	0.061	na	0.099	0.049	0.149	-1.36	Satisfactory		Satisfactory
Diflufenican	Carboxamide	0.049	0.052	0.07	0.035	0.105	-1.20	Satisfactory	-1.20	Satisfactory
Fenitrothion	Organophosphoré	0.071	na	0.049	0.024	0.074	1.76	Satisfactory		Not tested
Gamma-HCH	OrganoChloré	0.087	na	0.089	0.042	0.136	-0.09	Satisfactory		Not tested
Iprodione	Urée	0.107	na	0.080	0.038	0.122	1.29	Satisfactory		Not tested
Permethrin	Pyréthrinoïde	0.047	na	0.073	0.036	0.11	-1.41	Satisfactory		Not tested
Pirimicarb	Carbamate	0.071	0.071	0.075	0.037	0.133	-0.21	Satisfactory	-0.21	Satisfactory
Pirimiphos-methyl	Organophosphoré	0.088	0.069	0.051	0.005	0.057	1.00	Satisfactory	-0.67	Satisfactory
Prothioconazole	Organophosphoré	0.167	0.166	0.135	0.071	0.199	1.00	Satisfactory	0.97	Satisfactory
Propiconazole	Azole	0.084	na	0.092	0.046	0.138	-0.35	Satisfactory		Not tested
Tebuconazole	Azole	0.096	0.087	0.107	0.054	0.16	-0.42	Satisfactory	-0.75	Satisfactory
Triazophos	Organophosphoré	0.088	0.067	0.070	0.033	0.107	0.97	Satisfactory	-0.16	Satisfactory
Vinclozolin	Dicarboximide	0.141	na	0.137	0.072	0.202	0.12	Satisfactory		Not tested

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

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, 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 SOPs***. Preconfigured view settings have been designed for pesticide analysis through GC-MS/MS and LC-MS/MS (examples displayed in Figures 6, 7, and 8).







Figures 6, 7, and 8. Top: Easily check calculated amounts of ISTDs if they are in the expected range prior to sample analysis; Middle: Ion Ratio check and calculated amount for samples. Bottom: Peak calibration curve checking.

Conclusions

- 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 unique feature for cross confirmation of identity, 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 negative results.
- The 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.
- The Chromeleon CDS version 7.3.2, built specifically for pesticides, provides fast data processing of large datasets comprising of 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.

References

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    1. PTS 66b : Multi-residue screening of pesticides – Cereals
    2. PTS 66c : Multi-residue screening of pesticides – Vegetables

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