TECHNICAL BRIEF 74122

Automated data review by compound cross-confirmation

A powerful combination of chromatography data software and LC/GC triple-quadrupole mass spectrometers

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Application benefits

- Enhance productivity by immediate and direct cross-confirmation of quantitative results through a single, combined reprocessing sequence for LC-MS/MS and GC-MS/MS data.
- Increase confidence in the data through an interlaboratory comparison approach with multi-technique, mean-quantitation value.

- Use a single QuEChERS acetonitrile extract for direct injection into LC-MS/MS and GC-MS/MS.
- Obtain part-per-billion (ppb) level sensitivity that is compliant with European Commission directive EC 396/2005.

Goal

The goal of this study is to demonstrate the ability of Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) to reproduce user-specific data review workflows. Key improvements include enhanced productivity, increased confidence in analytical results and compliance with regulatory criteria. By simultaneously processing the same sample that has been analyzed with two orthogonal techniques, we can automatically cross-confirm the presence of a target analyte, minimize the risk of a false positive or negative result and prevent unwanted confirmation re-injections.

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Introduction

Over 70% of potential food contaminants that are routinely monitored by testing laboratories can be detected and quantified at required regulatory concentration levels with LC-MS/MS and GC-MS/MS. However, the selection of the chromatographic front-end technique (either LC or GC) can vary from analyst to analyst and is often based on individual preferences rather than the physical properties of the molecule. With this variation in upstream technologies, analysts face two key challenges: first a complicated, multi-step, manual sample preparation and secondly, time-consuming, disconnected data processing and reporting.

The proven solution for the first challenge is the established Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction method, with the utilization of Chromeleon software enabling users to overcome the second challenge by processing data from different techniques.

Chromeleon CDS can automatically cross-confirm target compounds processed by both LC and GC techniques, with results combined into a single sequence with a single processing method. One consolidated report helps the analyst to quickly validate the analytical results. This report can then be exported to a Laboratory Information Management System (LIMS), such as the Thermo Scientific™ SampleManager™ LIMS.

In this technical brief, we will demonstrate the complete software workflow with a real-life sample of organic honey in accordance to the European Commission directive EC 396/2005, which sets the maximum residue levels for 29 pesticides. The results are compared in a round-robin test.

Strategy

The acetonitrile QuEChERS extract is divided into two samples: one is injected into the Thermo Scientific™ TSQ Altis™ Triple Quadrupole Mass Spectrometer, and the other into the Thermo Scientific™ TSQ 9000™ Triple Quadrupole GC-MS/MS System. All data is acquired, processed and reported using Chromeleon software.

Figure 1. Lab workflow

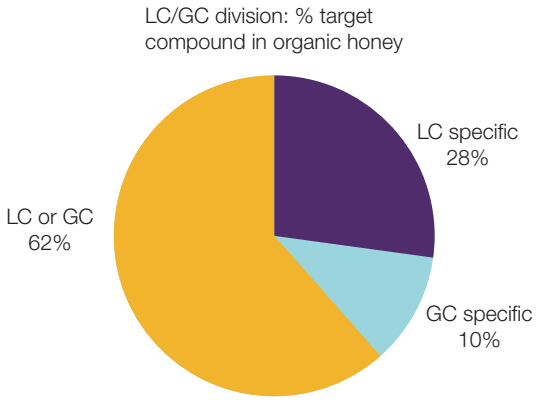


Figure 2. LC/GC division of target pesticides in organic honey

Results and discussion

Separate injection sequences are analyzed on each instrument. Subsequently, the data is combined into a single sequence containing one processing method and report template to provide a holistic analysis.

Figure 3 illustrates the component table of the Chromeleon Processing Method where we can see, for example, that the “Clopyralid” group includes both “Clopyralid_GC” and “Clopyralid_LC” components. Each compound has its corresponding retention time and SRM (Selected Reaction Monitoring) transition quantitation filter.

Component Table

Group Area Drag a column header here to group by that column. Run Component Table Wizard... Show Properties...

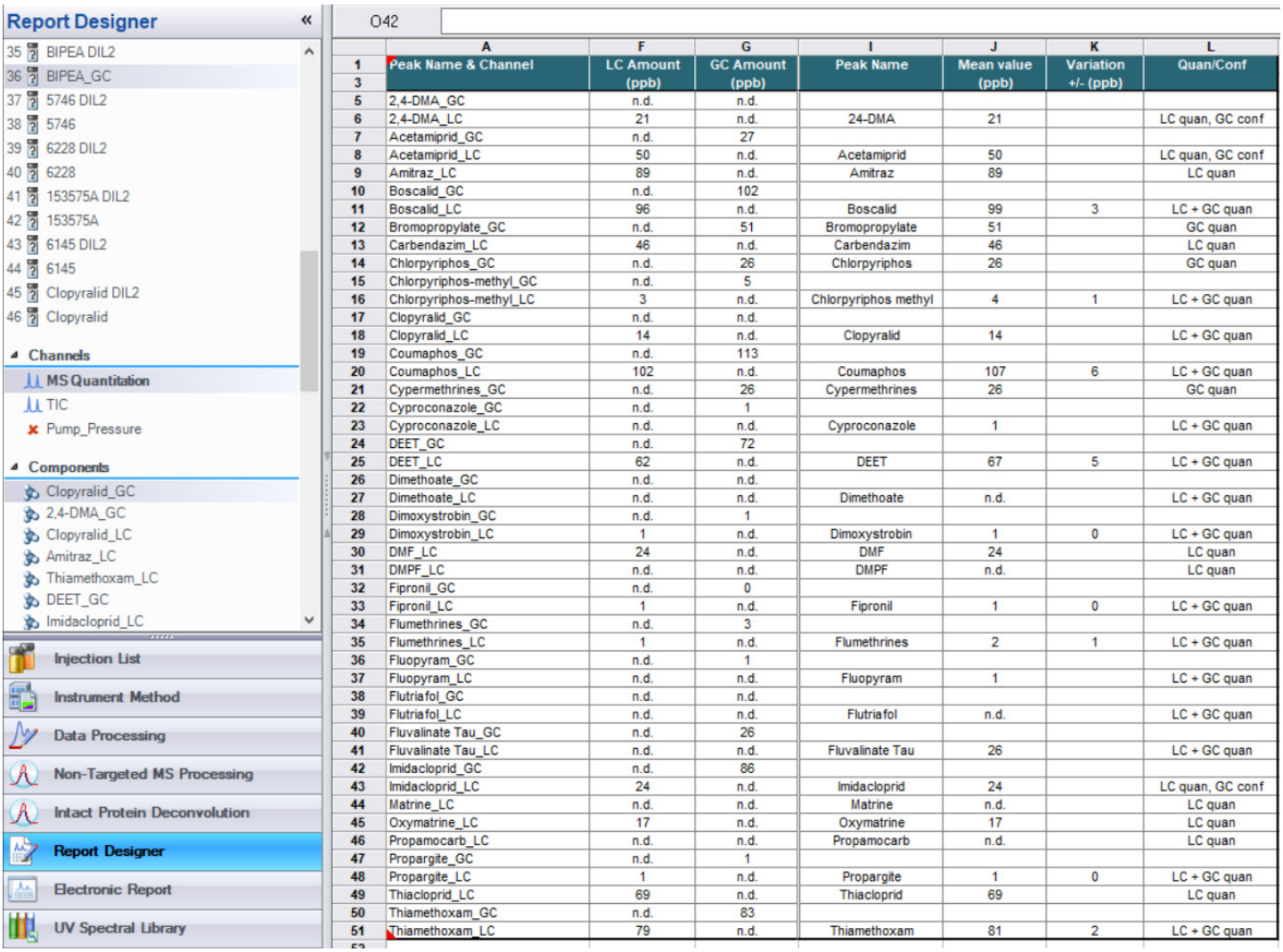
#	Peak Group(s)	Name	Ret.Time	MS Quantitation Peak	MS Confirming Peak 1
15	Chlorpyriphos methyl	Chlorpyriphos-methyl_GC	6.890	285.84 / 93.00	124.90 / 47.00
16	Chlorpyriphos methyl	Chlorpyriphos-methyl_LC	8.200	321.91 / 124.99	321.91 / 289.96
17	Clopyralid	Clopyralid_GC	3.140	146.90 / 76.00	112.00 / 76.00
18	Clopyralid	Clopyralid_LC	4.300	191.90 / 145.90	189.84 / 145.96
19	Coumaphos	Coumaphos_GC	9.680	361.93 / 226.11	226.00 / 163.00
20	Coumaphos	Coumaphos_LC	8.390	363.05 / 227.04	363.05 / 307.04
21	Cypermethrines	Cypermethrines LC_n.a.	10.150		
22	Cypermethrines	Cypermethrines_GC_Quan	10.150	181.00 / 152.00	181.00 / 151.10
23	Cyproconazole	Cyproconazole_GC	7.920	224.00 / 126.20	222.00 / 124.20
24	Cyproconazole	Cyproconazole_LC	7.700	292.10 / 125.04	292.10 / 70.07
25	DEET	DEET_GC	5.770	190.10 / 115.00	190.10 / 145.00
26	DEET	DEET_LC	7.280	192.11 / 65.04	192.11 / 100.07
27	Dimethoate	Dimethoate_GC	6.330	124.90 / 46.30	93.00 / 63.00
28	Dimethoate	Dimethoate_LC	5.960	230.05 / 199.04	230.05 / 124.93
29	Dimoxystrobin	Dimoxystrobin_GC	8.670	205.10 / 58.00	205.10 / 116.00
30	Dimoxystrobin	Dimoxystrobin_LC	8.090	327.10 / 205.15	327.10 / 116.06

Figure 3. Component table with single processing method as shown in Chromeleon CDS

The interactive and consolidated results for the combined analysis by LC-MS/MS and GC-MS/MS of an organic honey sample in a round-robin test are described in Figure 4.

- LC and GC results are reported respectively in the LC amount/GC amount columns.
- Final results are shown in the 'Mean value' column as either the mean value of LC + GC amount, with the corresponding variation in ppb, or as the required single result.
- The last column describes the requirement for the mean value, i.e. LC only, GC only, or LC+GC.

In this example, the 18 components of the 29 target compounds that are compatible with analysis by GC and/or LC have all been automatically cross-confirmed.



	A	F	G	I	J	K	L
1	Peak Name & Channel	LC Amount (ppb)	GC Amount (ppb)	Peak Name	Mean value (ppb)	Variation +/- (ppb)	Quan/Conf
3							
5	2,4-DMA_GC	n.d.	n.d.				
6	2,4-DMA_LC	21	n.d.	24-DMA	21		LC quan, GC conf
7	Acetamiprid_GC	n.d.	27				
8	Acetamiprid_LC	50	n.d.	Acetamiprid	50		LC quan, GC conf
9	Amitraz_LC	89	n.d.	Amitraz	89		LC quan
10	Boscalid_GC	n.d.	102				
11	Boscalid_LC	96	n.d.	Boscalid	99	3	LC + GC quan
12	Bromopropylate_GC	n.d.	51	Bromopropylate	51		GC quan
13	Carbendazim_LC	46	n.d.	Carbendazim	46		LC quan
14	Chlorpyriphos_GC	n.d.	26	Chlorpyriphos	26		GC quan
15	Chlorpyriphos-methyl_GC	n.d.	5				
16	Chlorpyriphos-methyl_LC	3	n.d.	Chlorpyriphos methyl	4	1	LC + GC quan
17	Clopyralid_GC	n.d.	n.d.				
18	Clopyralid_LC	14	n.d.	Clopyralid	14		LC + GC quan
19	Coumaphos_GC	n.d.	113				
20	Coumaphos_LC	102	n.d.	Coumaphos	107	6	LC + GC quan
21	Cypermethrines_GC	n.d.	26	Cypermethrines	26		GC quan
22	Cyproconazole_GC	n.d.	1				
23	Cyproconazole_LC	n.d.	n.d.	Cyproconazole	1		LC + GC quan
24	DEET_GC	n.d.	72				
25	DEET_LC	62	n.d.	DEET	67	5	LC + GC quan
26	Dimethoate_GC	n.d.	n.d.				
27	Dimethoate_LC	n.d.	n.d.	Dimethoate	n.d.		LC + GC quan
28	Dimoxystrobin_GC	n.d.	1				
29	Dimoxystrobin_LC	1	n.d.	Dimoxystrobin	1	0	LC + GC quan
30	DMF_LC	24	n.d.	DMF	24		LC quan
31	DMPF_LC	n.d.	n.d.	DMPF	n.d.		LC quan
32	Fipronil_GC	n.d.	0				
33	Fipronil_LC	1	n.d.	Fipronil	1	0	LC + GC quan
34	Flumethrines_GC	n.d.	3				
35	Flumethrines_LC	1	n.d.	Flumethrines	2	1	LC + GC quan
36	Fluopyram_GC	n.d.	1				
37	Fluopyram_LC	n.d.	n.d.	Fluopyram	1		LC + GC quan
38	Flutriafol_GC	n.d.	n.d.				
39	Flutriafol_LC	n.d.	n.d.	Flutriafol	n.d.		LC + GC quan
40	Fluvalinate Tau_GC	n.d.	26				
41	Fluvalinate Tau_LC	n.d.	n.d.	Fluvalinate Tau	26		LC + GC quan
42	Imidacloprid_GC	n.d.	86				
43	Imidacloprid_LC	24	n.d.	Imidacloprid	24		LC quan, GC conf
44	Matrine_LC	n.d.	n.d.	Matrine	n.d.		LC quan
45	Oxymatrine_LC	17	n.d.	Oxymatrine	17		LC quan
46	Propamocarb_LC	n.d.	n.d.	Propamocarb	n.d.		LC quan
47	Propargite_GC	n.d.	1				
48	Propargite_LC	1	n.d.	Propargite	1	0	LC + GC quan
49	Thiacloprid_LC	69	n.d.	Thiacloprid	69		LC quan
50	Thiamethoxam_GC	n.d.	83				
51	Thiamethoxam_LC	79	n.d.	Thiamethoxam	81	2	LC + GC quan

Figure 4. Customized report table from Chromeleon CDS

Below are 2 examples of cross-confirmed peaks for Thiamethoxam and Coumaphos.

LC_TSQ Altis

GC_TSQ 9000

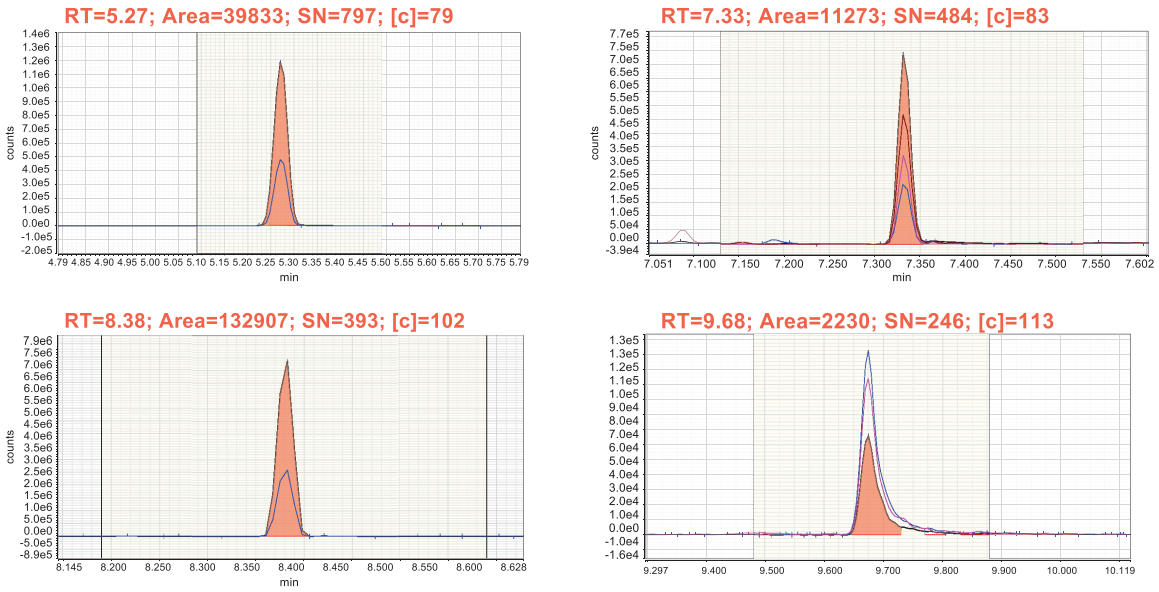


Figure 5. Cross-confirmation of thiamethoxam and coumaphos. TOP: chromatogram of Thiamethoxam (left LC, right GC) and BOTTOM: chromatogram of Coumaphos (left LC, right GC)

Table 1. Interpretation of results and recommended action

Result	Interpretation of detected peak	Example (Figure 4)	Recommended action for analyst
Positive	Reciprocally confirmed by GC and LC	Boscalid at 99 ± 3 ppb, Coumaphos at 107 ± 6 ppb, and DEET 67 ± 5 ppb	No need to visually check the chromatograms.
Confirmed	Not considered as positive because concentration is below regulated limit of 5ppb for organic honey	Chlorpyriphos-methyl at 4 ± 1 ppb and Dimoxystrobin at 1 ± 0 ppb	No need to visually check the chromatograms.
Positive, but	Reciprocally confirmed by GC and LC but standard deviation is out of tolerance: target is preferentially quantified by LC	Acetamiprid at 50ppb, Imidacloprid at 24ppb	No need to visually check the chromatograms, the LC calculated amount will be the only reported value.
	Reciprocally confirmed by GC and LC but standard deviation is out of tolerance: target should be both LC and GC amenable	None in this example	Check peak integration (double-click on quantitation value's cell – this will automatically show the corresponding chromatogram). If a manual integration occurs, the new result is automatically implemented into the result table.

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Table 2. Interlaboratory comparison results

Target in Honey	TSQ ALTIS µg/kg	TSQ 9000 µg/kg	Mean value LC/GC µg/kg	Assigned value µg/kg	Automatically Cross confirmed
AMITRAZ (including the metabolites containing the 2,4 -dimethylaniline moiety expressed as amitraz). CAS 33089-61-1	89 + 21 = 110	N/A	110	121 ± 58	✓ LC only
BOSCALID. CAS 188425-85-6	96	102	99 ± 6	115 ± 56	✓
BROMOPROPYLATE. CAS 18181-80-1	N/A	51	51	65 ± 33	✓ GC only
COUMAPHOS. CAS 56-72-4	102	113	107 ± 11	112 ± 55	✓
CYPERMETHRIN. CAS 52315-07-8	N/A	26	26	36 ± 18	✓ GC only
DIETHYLTOLUAMIDE. CAS 134-62-3	62	72	67 ± 5	76 ± 38	✓
THIAMETHOXAM. CAS 153719-23-4	79	83	81 ± 4	121 ± 58	✓

Round-robin, interlaboratory comparisons approach

BIPEA (Bureau Interprofessionnel d'Etudes Analytiques) is a European non-profit organization located in France. Serving nearly 2500 laboratories worldwide in 120 countries, it offers more than 150 regular proficiency testing programs. BIPEA is ISO 9001 certified by the Lloyd's Register Quality Assurance (LRQA) and ISO/IEC 17043 accredited for the organization of interlaboratory comparisons. BIPEA creates and organizes proficiency testing plans with an annual series of one or more tests.

The cross-confirmation is defined in Table 2, which shows the analysis results in a BIPEA sample of organic honey (April 2020). All the values conform with BIPEA target values. This table also presents LC, GC and mean value (second, third and fourth column respectively), the BIPEA assigned target value and tolerance value (fifth column) according to their procedure. Target values and tolerance are generally assigned using a combination of the original formulation value of the spike matrix and participating laboratories values, encompassing different technologies such as LC or GC, MS or MS/MS, or a specific detector.

Conclusion

A single processing sequence that combines and automates cross-confirmation of LC-MS/MS and GC-MS/MS data with Chromeleon CDS is a clear enhancement to laboratory productivity. This approach drastically reduces the number of time-consuming tasks such as visualizing each sample chromatogram or checking all chromatographic peak integrations. Overall, the reviewing process was at least three times faster than normal, considering that only one sequence has to be checked, and more than 60% of target compounds were automatically validated. This method also improves confidence in analytical results, allowing data to be consolidated and validated immediately after injection, even before exporting into LIMS.

Find out more at **thermofisher.com/chromeleon**

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