

Targeted quantitative screening pesticides in food matrices using high resolution DIA spectral library matching.

Alan Barnes¹; Raquel Leonardo²; Emily G Armitage¹; Jonathan McGeehan³; Steve Williams²; <u>Neil J Loftus¹</u> ¹Shimadzu Corporation, Manchester, UK; ²SGS Cambridge Limited, Cambridge, Cambridgeshire, UK; ³Shimadzu UK Limited, Milton Keynes, Buckinghamshire, UK

Overview

- A single, generic untargeted data independent acquisition (DIA) method was developed for the analysis of extended panels of pesticides in food matrices.
- The high-resolution LC-MS/MS method was designed for multi-residue screening and quantitation of pesticides to meet the European Union SANTE 11312/2021 v2 guidelines capable of working with extended target pesticide panels.
- The MS and DIA-MS/MS method was applied to quantify a routine panel of pesticides but also included an additional target panel of fungicides for qualitative screening.

1. Introduction

With increasingly diverse residues applied to commodities, food safety laboratories are looking to high resolution instrumentation to provide both quantitative and screening capability to provide higher specificity compared to nominal mass instruments. Whilst triple quadrupole LC-MS/MS remains the core technology for targeted analysis its scope is limited to the panel of compounds targeted. Expanding this further requires validation and optimization meaning retrospective data review or targeted screening remains limited. An alternative non-biased approach, using high resolution (HR) mass spectrometry by full scan untargeted acquisition, can support quantitative analysis and high reporting confidence using library searching MS/MS data. HR LC-MS/MS was applied to the analysis of a panel of pesticides to meet the needs of the SANTE 11312/2021 v2 guidelines. With an extended list of compounds for screening, retrospective analyses was made possible providing four degrees of confidence in identification including retention time, accurate mass, isotope matching and library identification.

2. Materials and Methods

Unknown samples and calibration samples were prepared using a standard QuEChERS sample preparation method. Spiked matrix calibration curve (olive oil) and deuterated internal standards at a concentration range of 0.1 - 100 µg/kg were prepared. The pesticide target panel previously validated on a routine triple quadrupole LC-MS/MS MRM system.

Reverse phase LC Separation

- Nexera[™] LC system
- Shim-pack Velox[™] Biphenyl (2.1x100mm 2.7µm); 40°C, flow rate 0.4 mL/min
- Binary gradient; water + 2mM ammonium formate + 0.004% formic acid, and methanol + 2mM ammonium formate + 0.004% formic acid
- Injection; $4 \mu L + 40 \mu L$ water co-injection

LC-MS/MS Mass Spectrometry Detection

- High resolution QTOF LC-MS/MS (LCMS-9050, Shimadzu Corporation, Japan)
- TOF MS mass scan m/z 140-925; 100 msecs
- 31 DIA MS/MS scans with a scan time 29 msecs for each mass scan, collision energy spread 5-55V
- m/z 140-540; precursor isolation width of 20 Da
- m/z 540-925; precursor isolation width of 35 Da
- ESI positive ion mode data was acquired using external mass calibration
- Scan cycle time 1 second (31 mass scans in total)

- identification. The cycle time is 1 sec for all mass scans. Target compounds can be simply added to the method without changing any acquisition parameter.

Figure 1. LabSolutions Insight[™] user interface highlighting identified analytes (Flag Result shows 'Report' meeting the SANTE guidelines: mass accuracy and ion ratio tolerance) for a panel of pesticides spiked at 10 µg/kg in olive oil matrix. Flag criteria for QCs were set for mass accuracy (ppm and mDa), ion ratios; for calibration standards further flags were set including Rt difference, accuracy, isotope score, peak symmetry and calibration linearity.

3. Results

3.1 High-resolution quantitation by DIA

Key advantages of a high-resolution LC-MS/MS with DIA-MS/MS in pesticide screening

1 Data **Acquisition**

A single high-resolution LC-MS/MS data acquisition method can be applied to any pesticide panel.

Same LC methodology widely used for targeted MRM triple quadrupole LC-MS/MS analysis. It is robust, well characterized and has been applied to large target pesticide panels.

The MS method is optimized for precursor detection and quantitation with a TOF MS scan of 100 msecs and DIA-MS/MS mass scans for ion ratio confirmation and library

All data are acquired using an external mass calibration.

- 2 Data **Processing**
- Meeting the reporting guidelines of European Union SANTE 11312/2021 v2 guidelines.
- To meet the reporting criteria of the SANTE quidelines, the TOF MS mass scan event is used to detect the target pesticide quantifier ion (molecular ion) and a product ion in the DIA-MS/MS mass scan event is used for identification as the qualifier ion (reference ion).
- Mass accuracy must be within \leq 5 ppm (or 1 mDa for ions less than m/z 200).
- Ion ratios should be within 30% of the average of calibration standards from the same sequence.
- The method supports the quantitative analysis of a targeted pesticide panel and retrospective analysis with library identification.
- A highly curated MS/MS library of pesticides was used in the library identification. All pesticides were acquired with the same LC conditions and same collision energy spread (5-55 V) using targeted MS/MS

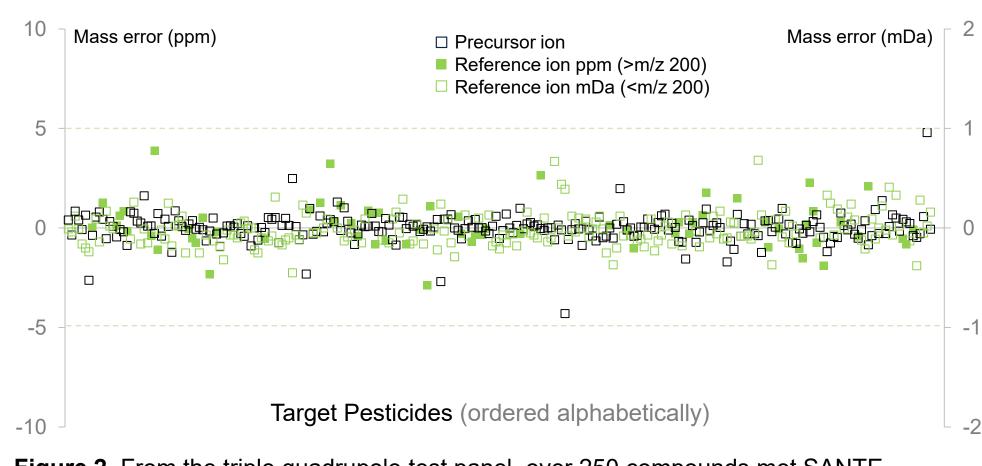


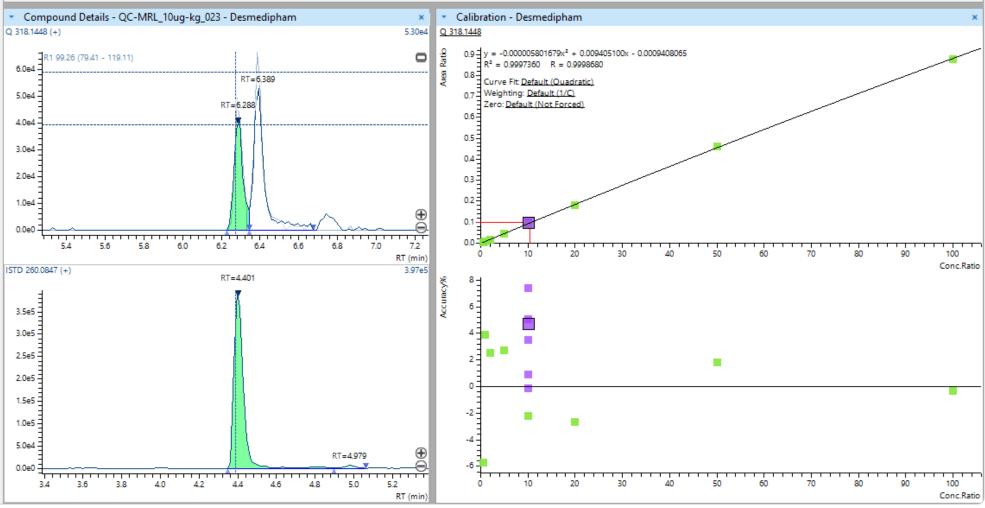
Figure 2. From the triple quadrupole test panel, over 250 compounds met SANTE guidelines at the MRL concentration of 10 µg/kg in olive oil matrix for the MS quantifier ion and MS/MS DIA reference ion. Data were acquired using external mass calibration.

3.3 Extended capabilities of a DIA-MS/MS method

-										
	🖁 LabSol	utior	ns Insight	(Admin) ·						
	 Sam 	ple L	.ist							
	#									
		23	QC-MRL	10ug-kg						
		✓ 'QC-MRL' ✓ 23 QC-MRL_10ug-kg ✓ 24 QC-MRL_10ug-kg ✓ 25 QC-MRL_10ug-kg ✓ 37 QC-MRL_10ug-kg ✓ 38 QC-MRL_10ug-kg ✓ 39 QC-MRL_10ug-kg Compound Details - QC-MI 2181448 (+) 5.0e4								
		37	OC-MRI	10ua-ka						
		38	OC-MRI	10ug-kg						
	Q 518.1448 (+)									
	1 1	R1-11	9.48 (79.41 -	119.11)						
	5.0e4 -									
	4.0e4 -									
	-									
	2014									
	5.064 -									
	2.0e4 -									
	1.0e4 -									
>										
	-									
	0.0e0 -									
			5.4	5.6						
	 Library Hits - Desmedinham 									
	±			-						
				compo						
		1		Desmod						
		2								
		2	07	Phenme						
			~							
	<u> </u>									
			\rightarrow							
		~		N						

10 µg/kg in olive oil matrix.

LabSolutions Insight (Admin) - (Quant-DIA-ref-ions.iproc - DIA.Icm)											_		×
Compound Results - QC-MRL_10ug-kg_023													
#	Flag Result	Name	Conc.	RT	m/z	Mass Error (ppm)	Ref 1 m/z	Ref 1 Mass Error (ppm)	Ref 1 Mass Error	Ref 1 Result	Ref 1 Actual Ratio	Ref 1 Rang	e
	'Report'. 🔻	T	Ŧ	Ŧ	Ŧ	Ŧ	Ŧ	Ŧ	Ŧ	T	•		Ŧ
67 🔽	Report 📒	Cycluron	9.6691	5.885	199.1805	0.502	72.0444	-1.804	-0.13	Pass	17.26	16.76 - 27.9	33
68 🔽	Report 📒	Cyenopyrafen	9.3613	10.141	394.2489	-0.304	310.1914	-0.193	-0.06	Pass	40.09	29.26 - 48.7	77
69	Report 📒	Cyflumetofen	9.7687	9.875	465.1996	-0.924	173.0209	-1.098	-0.19	Pass	103.23	84.29 - 126	.43
71 🗹	Report 📒	Cyromazine	9.8478	1.823	167.1040	-0.299	68.0243	-4.704	-0.32	Pass	4.52	2.70 - 8.11	
75 🗹	Report 📒	Demeton-S-methyl sul	9.6174	3.639	247.0222	0.040	109.0049	-0.642	-0.07	Pass	44.19	41.03 - 61.5	54
🛛 🔽 77	Report 🛛	Desmedipham	10.4713	6.273	318.1448	-0.283	136.0393	0.368	0.05	Pass	99.26	79.41 - 119	.11
79	Report 📒	Dicrotophos	9.5463	3.909	238.0839	-0.420	127.0155	0.551	0.07	Pass	17.59	15.72 - 26.2	20
🖂 80	Report 📒	Diethofencarb	9.4600	6.574	268.1543	0.112	124.0393	-0.081	-0.01	Pass	106.21	82.64 - 123	.95
2 81	Report 📒	Difenacoum	9.9892	10.025	445.1798	-0.899	179.0855	0.000	0.00	Pass	125.73	89.35 - 134	.03
2 82	Report 📒	Difenoxuron	10.4917	6.609	287.1390	0.000	123.0441	-1.788	-0.22	Pass	20.06	16.76 - 27.9	94
2 83	Report 📒	Diflubenzuron	9.8379	7.604	311.0393	-0.611	141.0147	-1.773	-0.25	Pass	89.26	72.24 - 108	.36



3.2 Mass accuracy in a food commodity

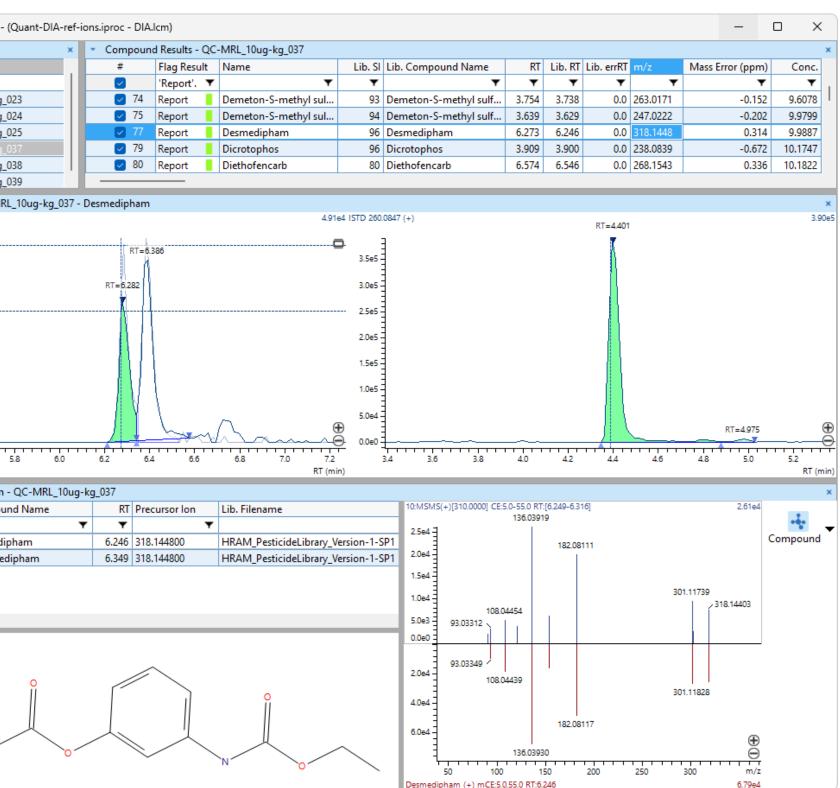


Figure 3. A single LC-MS with DIA-MS/MS acquisition method not only has the capability of supporting quantitation and ion ratio confirmation but also supports MS/MS library identification. The library contains over 500 compounds measured from authentic standards, each spectra structurally mass assigned, noise ions removed, and retention time defined. In this example, desmedipham has been highlighted as the library identification at the MRL of

3.4 Advanced flag capability for extended screening

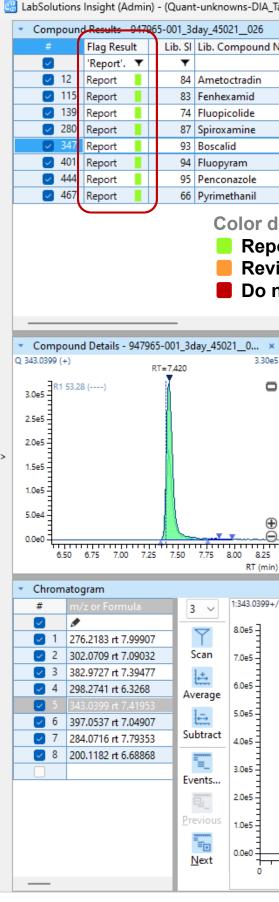


Figure 4. Using an extended target list of pesticides the fungicides, ametoctradin and fenhexamid were detected in a grape sample. In another example pyrimethanil was detected in a nectarine sample which is not routinely quantified in the tripe quadrupole method Compound results were filtered by Flag Result to show 'Report' (meeting the reporting criteria).

Flag Criteria set for **Report**

All criteria must be met to **Report**

- Lib. Compound Name: match • Min. Library Similarity Index > 20
- Iso Score > 20.
- RT Diff (absolute) 0.5 min
- Mass Error (ppm) \leq 5 ppm for ions > m/z 200
- Mass Error (mDa) \leq 1 mDa for ions \leq m/z 200

4. Conclusions

- library identification were supported.
- concentration (10 μ g/kg).

Disclaimer: The products and applications in this presentation are intended for Research Use Only (RUO). Not for use in diagnostic procedures. The authors declare no competing financial interest.

_Targeted	-Screening.iproc - DIA.lcm)								_	
						1				×
l Name	Name	RT		Lib. errRT		Mass Error (ppm)	Conc.	Area		Ref 1 m/z
•		Y	•	T	T	•	T	T	T	
	Ametoctradin	7.984	7.975		276.2183		N.I.(Hig	5059750	524504	
	Fenhexamid	7.082	7.054		302.0709		N.I.(Hig	5035367		97.1012
	Fluopicolide	7.387	7.358		382.9727	-1.828	0.5235	19947		172.9555
	Spiroxamine	6.274	6.317		298.2741	-0.402	0.3659	19087		144.1383
	Boscalid	7.388	7.388		343.0399	-0.233		1278603		307.0633
	Fluopyram	7.015	7.015		397.0537	0.403		4514724		208.0135
	Penconazole During ath a mil	7.763	7.763 6.645		284.0716	-0.176		633254		158.9763
	Pyrimethanil	6.645	0.045	0.0	200.1182	0.899		1019047	200000	107.0604
oort Passes all set flag criteria /iew Failed to meet some flag criteria not report No flag criteria are met										
× × L	ibrary Hits - Boscalid - 9479	65-001 3	day 450	21 026						×
e5	Lib. SI Compou				MS(+)[350.000	00] CE:5.0-55.0 RT:[7.382-	7.449]		1.12e5	
	T Composition			-	_			30	7.06316	-÷-
	/ 1 93 Boscalid			1.0e5	5-			271.08652	0	Compound
				- 1	-					
)))		5.0e4 0.0e6 2.5e4 7.5e4 1.0e5		39.98989		3.09135 273.09674 3.09167 272.0944 271.08659 307.0632 250 30	1 1 17 343.03994 💬	
in)				Bosca	lid (+) mCE:5.0	0,55.0 RT:7.388			9.33e4	
										×
By extending the search space, 4 additional pesticides were identified in a grape extract outside the scope of the initial target panel. Boscalid, fluopyram, penconazole and pyrimethanil were detected in the extended search space (the compounds were not present in the quantitative pesticide compound panel).										3.30∈
	1 2 3		4	5		7	8	9	10 11	12 RT (mir

Flag Criteria set for **Review** If mass error is out of tolerance it is identified as a **Review**

Flag Criteria set for **Do not report** If any remaining flags fail it is categorized as **Do not report**

Using a single generic LC-MS and DIA-MS/MS method targeted quantitation using ion ratio confirmation (to meet the EU SANTE guidelines) and extended search capabilities with

The method results in the quantitation of >250 pesticides at or below the default MRL