APPLICATION NOTE 73959

Increasing throughput of the LC-MS analysis of pesticide residues in food

Using dual channel chromatography – triple quadrupole mass spectrometry

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Goal

To develop a UHPLC-MS dual channel liquid chromatography workflow for the multiresidue analysis of pesticides in food for increased sample throughput, and without compromise to chromatographic resolution.

Introduction

Comprehensive analysis of pesticide residues requires analysis of samples using gas chromatography- (GC-), ion chromatography (IC-), and liquid chromatography (LC-), each coupled to mass spectrometers. Modern pesticides are quite polar, thermally labile, or not easily vaporized. Thus, they are increasingly more amenable to LC than GC. Today it is typical for laboratories to analyze 250 or more pesticides in a single LC-MS analysis and often in complex matrices. The expectation is that results will comply with regulatory levels/tolerances and method performance criteria, and still be available within short turnaround times, typically 48 hrs. Hence, productivity is a crucial requirement for a pesticide residues laboratory.

With each new generation, mass spectrometers become faster, more sensitive, and more selective. In the quest to improve sample throughput, there have been attempts to eliminate chromatographic separation and to work directly with the mass spectrometer. But chromatography has proved indispensable, providing an orthogonal mode of selectivity, critical for complex samples.

The analysis run time using a single column can be reduced by changing the chromatographic conditions. However, the improvement of sample throughput by modifying the LC gradient or reducing the column length has some drawbacks. Very short run times achieved by the use of a short column, steep gradient, or high flow rate can provide unwanted effects: too narrow peaks (not enough data points), too many co-eluting pesticides (short dwell times or long duty cycle), and increased coelution with matrix co-extractive resulting in higher ion suppression and more chemical interferences. Dual channel chromatography can provide a substantial decrease of the analysis time without compromising the separation and peak width. The Thermo Scientific™ Vanquish™ Duo UHPLC system for Dual LC-MS comprises two independent pumps, a single autosampler with two independent injectors/flow paths, and two columns operated in parallel. Consecutive injections are partially overlapped and synchronized in the way that the first analyte from the second column elutes just after the elution of the last analyte from the first column. In this arrangement, the idle time of the MS is minimized and consequently the sample throughput increased.

Experimental

Chemicals, apparatus, and consumables

- Pesticides standards were obtained from Sigma-Aldrich (Darmstadt, Germany) and LGC (Teddington, UK).
- Ultrapure water was obtained from Fisher Scientific.
- Ammonium formate, formic acid, and QuEChERS salts were obtained from Sigma-Aldrich.
- LC-MS grade methanol and acetonitrile were obtained from Riedel-de Haën (Seeleze, Germany).
- Triple quadrupole system calibration solution was provided by Thermo Fisher Scientific.

Sample preparation

Samples were extracted using the citrate buffered QuEChERS sample preparation procedure. After homogenization, a test portion (10 g) of the sample was weighed in a 50 mL PTFE centrifuge tube. Then, acetonitrile (10 mL) and two surrogate standards (100 µL of each) were added, and the sample then shaken in an automatic axial extractor for 7 min. Afterwards, magnesium sulfate (4 g), sodium chloride (1 g), trisodium citrate dihydrate (1 g), and disodium hydrogen citrate sesquihydrate (0.5 g) were added, and the sample again shaken in the automatic axial extractor for 7 min. The extract was then centrifuged (3,700 rpm) for 5 min.

An aliquot of the supernatant extract (100 µL) was diluted with ultrapure under water (400 µL) containing dimethoate d-6 as an injection standard. Matrix-matched standards were prepared by evaporating an aliquot of the blank matrix extract (100 µL) to dryness under a gentle stream of nitrogen. The dried extract was reconstituted with an acetonitrile solution of 273 target pesticides (100 µL) at the appropriate concentration. Finally, the spiked extract was diluted with of ultrapure water (400 µL) containing dimethoate d-6 (injection standard).

Dual channel UHPLC-MS/MS analysis

An ultra-high-performance liquid chromatography system (Vanquish Duo UHPLC system for Dual LC-MS) was coupled with a tandem mass spectrometer (Thermo Scientific™ TSQ Altis™ mass spectrometer) equipped with a Thermo Scientific™ OptaMax™ NG ion source. The principles and operation of the dual channel chromatography system are described below and the instrument conditions are given in Table 1.

Table 1. Vanquish Duo for Dual LC-MS method, UHPLC column, and mobile phases

Columns	Thermo Scientific™ Accucore™ C18 100 mm × 2.1 mm, 2.6 µm		
Column temperature	30 °C		
Mobile phase A	98% water and 2% methanol, 5 mM of ammonium formate and 0.1% formic acid		
Mobile phase B	98% methanol and 2% water, 5 mM of ammonium formate and 0.1% formic acid		
Flow rate	0.35 mL/min (for each column)		
Injection volume	2.5 µL		
Data window start	1.1 min		
Data window duration	10.45 min		
Mobile phase gradient	Time	A%	%B
	0	100	0
	1	100	0
	2	70	30
	3	50	50
	11	0	100
	14	0	100
	14.1	100	0
	17	100	0

The dual-channel method was created using Thermo Scientific™ Aria™ MX software (Figure 1).

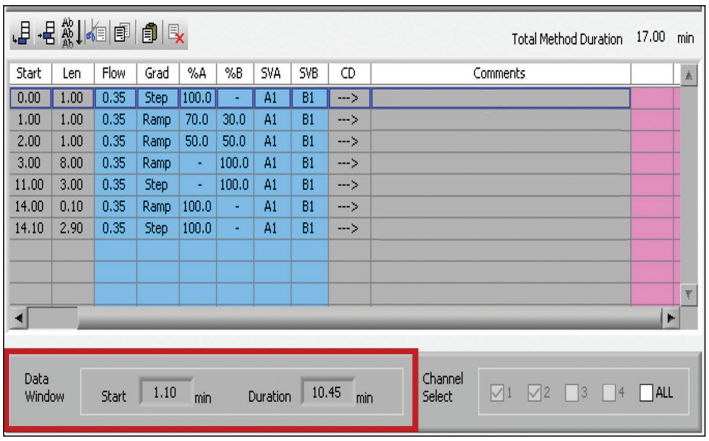

Figure 1. Aria MX- method editor

To convert a single-channel gradient into a dual-channel method the "Data Window", which corresponds to the portion of the chromatogram that contains the analytes, was established. This simply required two parameters to be specified: the "Start" (retention time when the "Data Window" begins), and the "Duration" (the length of the "Data Window"), effectively the length of time the mass spectrometer is acquiring data. The Aria MX software uses these two parameters to correctly synchronize the two channels. To obtain the highest throughput, the "Data Window" should start just before the elution of the first analyte and it should end just after the elution of the last analyte.

The result of synchronizing the two columns, operated in parallel, is illustrated in Figure 2. The highlighted blue segment is the "Data Window" and it covers all the SRM transitions. Only this portion of the chromatogram is directed to the MS. The grey parts where no analytes are present are directed to waste.

The optimized settings for the TSQ Altis mass spectrometer are given in Table 2.

Table 2. Parameters used for the TSQ Altis triple quadrupole mass spectrometer

Parameter	Value
Positive ion spray voltage	3,500 V
Negative ion spray voltage	2,500 V
Sheath gas	50 (arbitrary units)
Aux gas	10 (arbitrary units)
Sweep gas	1 (arbitrary units)
Ion transfer tube temperature	325 °C
Vaporizer temperature	350 °C
Q1 resolution	0.7 FWHM
Q3 resolution	1.2 FWHM
CID gas	1.5 mTorr

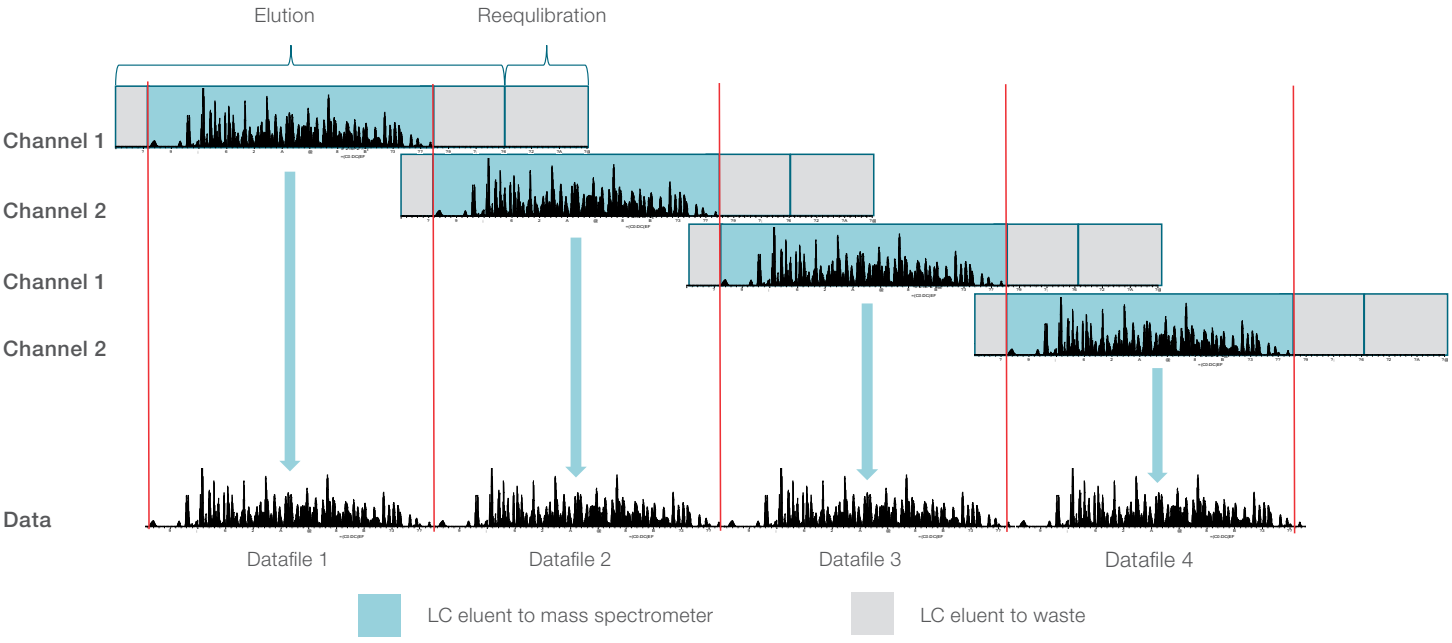

Figure 2. The principle of the Vanquish Duo UHPLC systems for Dual LC-MS

Data acquisition and processing

Data acquisition was performed in selected reaction monitoring mode (SRM). The product ions were individually tuned for each target analyte using TSQ Altis 3.1 Tune software by infusing the corresponding standard solution (1 mg/L). Data processing was carried out using Thermo Scientific™ TraceFinder™ software. The minimum dwell time was set at 5 ms. The number and distribution of SRM transitions throughout the run are visualized in Figures 3(A) and 3(B).

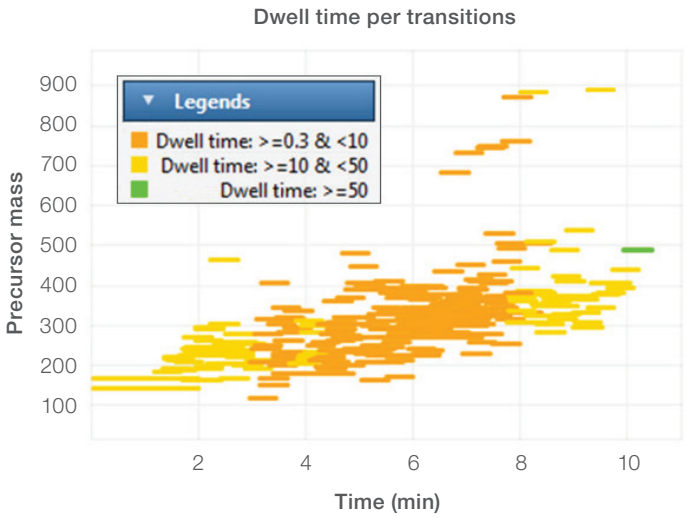

Figure 3. (A) Dwell time per transition

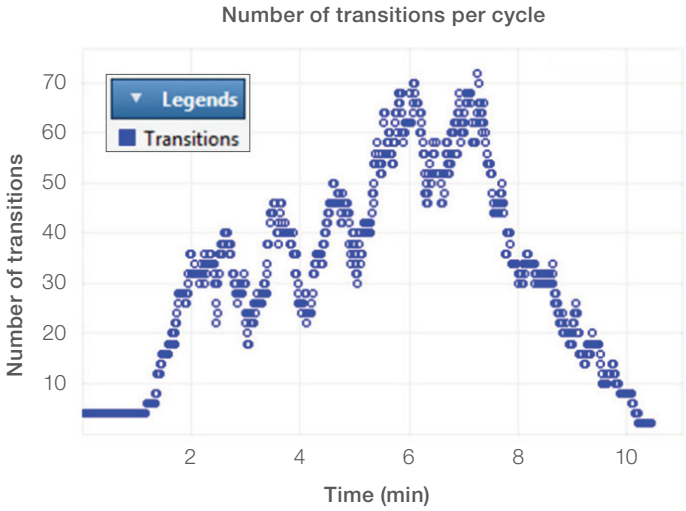

Figure 3. (B) Number of transitions per cycle

Results and discussion

With the Vanquish Duo UHPLC system, the sample throughput was increased by 70% when compared to a single channel system (80 vs. 137 injections in 24 hours), as shown in Figure 4. The time necessary for the analysis of one sample is reduced from 18 minutes (14 min of gradient elution + 3 min of column equilibration + 1 min of needle wash, sample aspiration, sample injection) to 10.45 minutes. Although a throughput increase of 100% was possible, the LC gradient was optimized to minimize potential matrix effects and co-elutions to achieve the highest data quality.

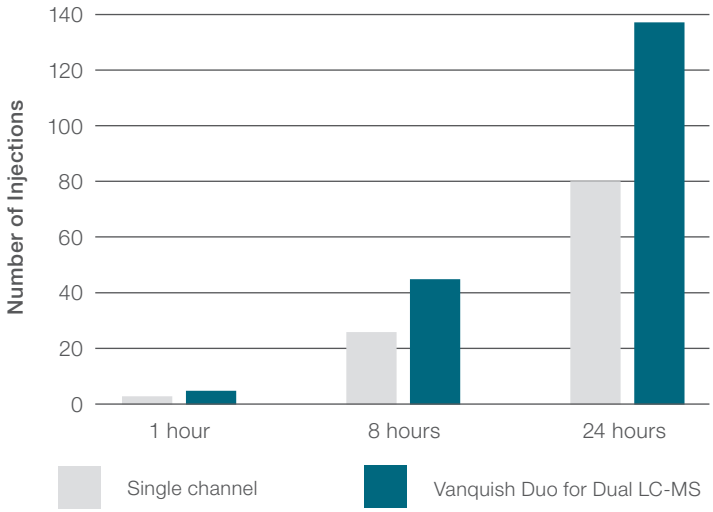

Figure 4. Sample throughput, single-channel vs. dual-channel

Although the samples were injected alternating on two independent columns, the retention times showed excellent stability. The deviation was well within a 0.1 min deviation, as permitted by the DG SANTE guidelines.[1] A typical retention time variation observed is depicted in Figure 5. The chart shows a sequence of 120 injections of methamidophos in solvent and in four matrices. The red horizontal lines mark the tolerance of ± 0.1 minute.

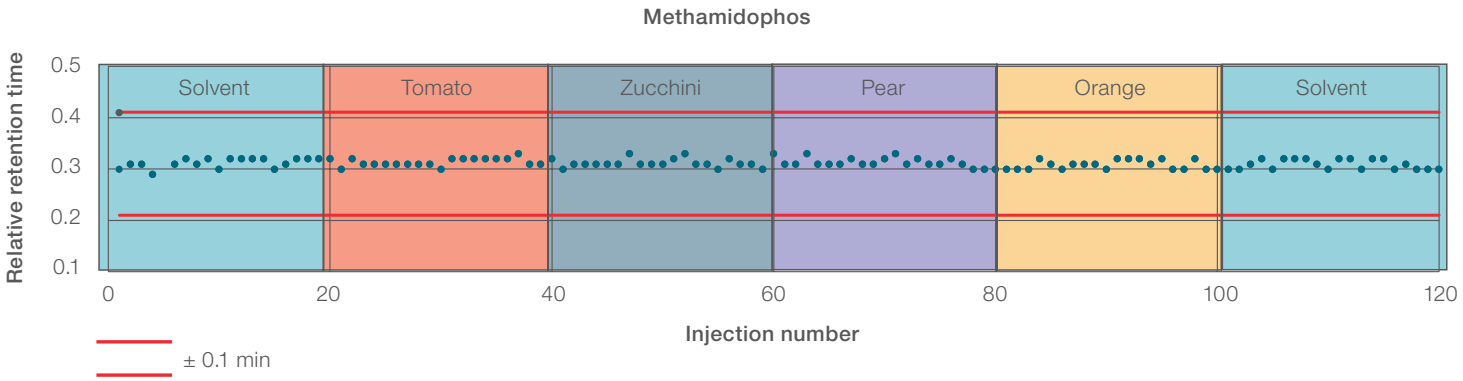

Figure 5. Retention time stability of methamidophos

The excellent stability of the system supported the application of the cross-channel calibration. Figure 6 shows the calibration curve of carbaryl injected on channel 1 (Figure 6A), on channel 2 (Figure 6B) and cross-channel (Figure 6C). As can be seen in Figure 6, there is no significant difference between the intra-channel and the cross-channel calibration. Utilizing cross-channel calibration saves time since the entire calibration curve does not need to be injected on both channels.

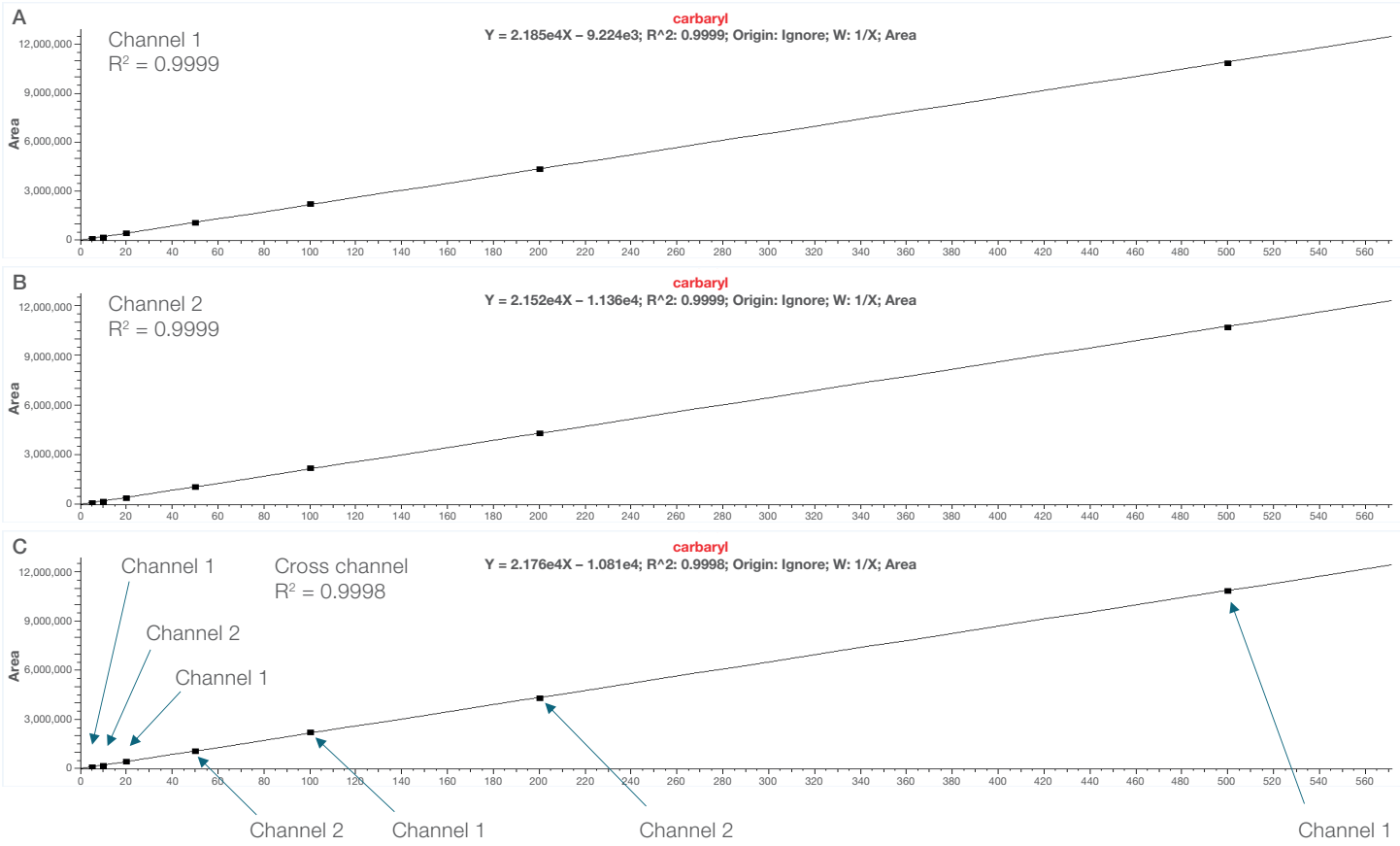

Figure 6. Comparison of intra-channel calibration (plots A and B) with cross-channel calibration (plot C)

To verify the utility of the Vanquish Duo UHPLC system for Dual LC-MS for pesticide residue analysis, a recovery study was carried out. Three matrices of various degree of complexity (apple, bell pepper, orange) were spiked with 273 pesticide compounds (see Appendix) at two concentration levels (0.01 mg/kg and 0.10 mg/kg) and extracted according to the QuEChERS protocol with no clean-up applied. The samples were injected first in the dual LC-MS mode and then in the single-channel LC-MS mode.

The results of recoveries and repeatability for all pesticides spiked at 0.01 mg/kg and 0.1 mg/kg in a relatively simple matrix (apple) and in a complex matrix (orange) are presented in Figure 7A-D. Recoveries and repeatability in bell pepper (intermediate complexity), showed similar results. The DG SANTE guidance document for the initial validation recommends recoveries within 70–120% and RSD <20%.[1] In both approaches (single- and dual LC-MS), the results were essentially the same. The only exception was for emamectin B1b in apple at 0.01 mg/kg. In the single-channel analysis, the recovery was slightly above 120%, whereas in the dual-channel analysis, it was in the 70–120% range. For all pesticide-matrix combinations, the repeatability % RSDs were below 20 for 0.01 and 0.1 mg/kg spiked levels measured using single and dual channel chromatography calibration. In apple and bell pepper, RSDs were <5% for more than 87% of the pesticides at 0.01 mg/kg, and in oranges, the more complex matrix, more than 65%.

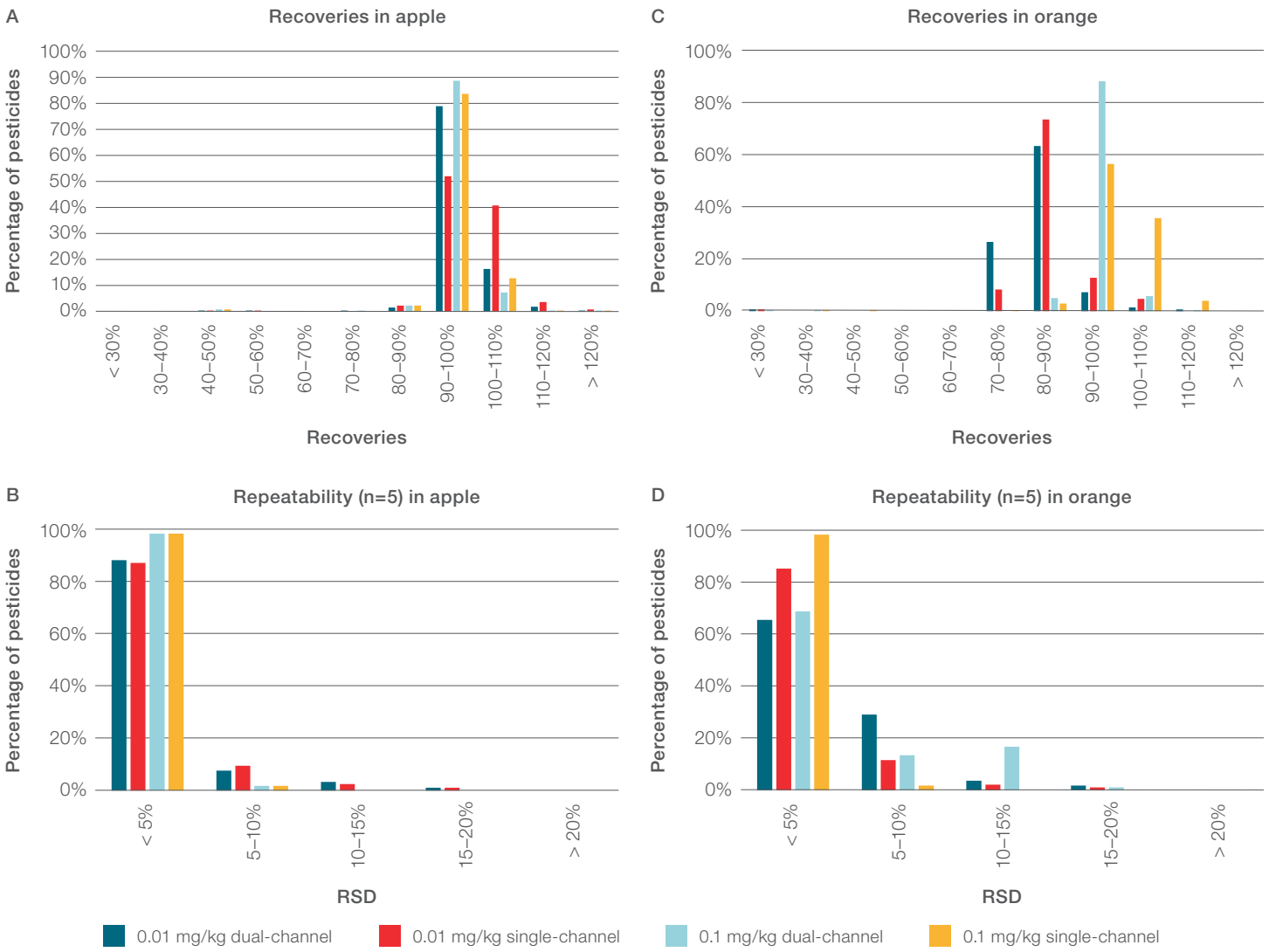

Figure 7. Summary of validation results in apple and orange matrices

Following validation of the method, six proficiency test samples were analyzed to test the applicability of the dual-channel system for the analysis of real-life samples. The test samples (FAPAS FCPM2-VEG76; EUPT-FV 13; EUPT-FV 14; EUPT-FV 16; EUPT-FV 17; EUPT-FV 18) were injected and quantified using the intra-channel and cross-channel calibration, and the z-scores were calculated for each analyte for each calibration set.

No false positives or false negatives were detected. To consider a quantitative result as "acceptable", the z-score should be equal or lower than 2. All the results obtained during the study fulfilled that condition and the majority of them with a z-score below 1.

Conclusions

The Vanquish Duo UHPLC system for Dual LC-MS is a technique that facilitates increased throughput in a pesticide residues laboratory.[2] It helps to significantly reduce the time of analysis in comparison to a standard single-channel UHPLC system. The improvement is achieved without compromising the separation or LC-MS data quality. As it was demonstrated, the results obtained with the dual-channel system are the same as in the single-channel system. The results of the proficiency tests demonstrated that the dual-channel chromatography can be used for the quantitative analysis of pesticide residues in fruit and vegetables.

References

1. DG SANTE, *Analytical quality control and method validation procedures for pesticide residues analysis in food and feed.* SANTE/12682/2019, 2019.
2. L Rajski et al., Dual-channel chromatography a smart way to improve the analysis efficiency in liquid chromatography coupled to mass spectrometry. *J Chromatogr A,* **2020**, 1633, 461614 (2020). DOI: 10.1016/j.chroma.2020.461614

Appendix

List of pesticide parent compounds, isomers and metabolites sought in method

No.	Pesticide compound	No.	Pesticide compound	No.	Pesticide compound
1	2,4-D	16	Azinphos-methyl	31	Bupirimate
2	3,4-Dichloroaniline	17	Azoxystrobin	32	Buprofezin
3	3-hydroxycarbofuran	18	BAC 10	33	Butoxycarboxim
4	Acephate	19	BAC 8	34	Carbaryl
5	Acetamiprid	20	Benalaxyl	35	Carbendazim
6	Alachlor	21	Bendiocarb	36	Carbofuran
7	Albendazole	22	Bifenazate	37	Chlorantraniliprole
8	Aldicarb	23	Bifenthrin	38	Chlorbromuron
9	Aldicarb-sulfone	24	Bitertanol	39	Chlorfenvinphos
10	Aldicarb-sulfoxide	25	Boscalid	40	Chlorfluazuron
11	Ametoctradin	26	Bromacil	41	Chloridazon
12	Anilofos	27	Bromuconazole	42	Chlorotoluron
13	Atrazine	28	BTS_44595	43	Chloroxuron
14	Avermectin B1a	29	BTS_44596	44	Chlorpropham
15	Azinphos-ethyl	30	BTS-40348	45	Chlorpyrifos

Appendix (continued)

List of pesticide parent compounds, isomers and metabolites sought in method

No.	Pesticide compound	No.	Pesticide compound	No.	Pesticide compound
46	Chlorpyrifos-methyl	92	Fenamiphos	138	Imidacloprid
47	Chromafenozide	93	Fenamiphos-sulfone	139	Indoxacarb
48	Cinerin I	94	Fenamiphos-sulfoxide	140	Ioxynil
49	Cinerin II	95	Fenarimol	141	Iprovalicarb
50	Clofentezine	96	Fenazaquin	142	Isocarbophos
51	Clomazone	97	Fenbuconazole	143	Isofenphos-methyl
52	Clothianidin	98	Fenhexamide	144	Isoprocarb
53	Coumaphos	99	Fenitrothion	145	Isoprothiolane
54	Cyazofamid	100	Fenobucarb	146	Isoproturon
55	Cyflufenamid	101	Fenoxycarb	147	Isoxaflutole
56	Cyazofamid	102	Fenpropathrin	148	Jasmolin I
57	Cyhalofop-butyl	103	Fenpropidin	149	Jasmolin II
58	Cymoxanil	104	Fenpropimorph	150	Kresoxim-methyl
59	Cyproconazole	105	Fenpyrazamine	151	Lenacil
60	Cyprodinil	106	Fenpyroximate	152	Linuron
61	Cyromazine	107	Fenthion	153	Lufenuron
62	Deet	108	Fenthion-oxon	154	Malaoxon
63	Demeton-S-methyl	109	Fenthion-oxon-sulfone	155	Malathion
64	Demeton-S-methyl sulfone	110	Fenthion-sulfone	156	Mandipropamid
65	Demeton-S-methyl sulfoxide	111	Fenthion-sulfoxide	157	Mepanipyrim
66	Diazinon	112	Fenuron	158	Metaflumizone
67	Dichlorvos	113	Fipronil	159	Metalaxyl
68	Dicrotophos	114	Fipronil-sulfone	160	Metamitron
69	Diethofencarb	115	Flazasulfuron	161	Metconazole
70	Difenoconazole	116	Flonicamid	162	Methamidophos
71	Difenoxuron	117	Fluacypirim	163	Methidathion
72	Diflubenzuron	118	Fluazifop	164	Methiocarb
73	Dimethoate	119	Flubendiamide	165	Methiocarb-sulfone
74	Dimethomorph	120	Fludioxonil	166	Methiocarb-sulfoxide
75	Dimethylvinphos	121	Flufenacet	167	Methomyl
76	Diniconazole	122	Flufenoxuron	168	Methoxyfenozide
77	Diuron	123	Fluometuron	169	Metobromuron
78	Edifenphos	124	Fluopicolide	170	Metolachlor
79	Emamectin B1a	125	Fluopyram	171	Metolcarb
80	Emamectin B1b	126	Fluquinconazole	172	Metrafenone
81	EPN	127	Flusilazole	173	Monocrotophos
82	Epoxiconazole	128	Flutriafol	174	Monolinuron
83	Ethiofencarb	129	Fluxapyroxad	175	Monuron
84	Ethion	130	Formetanate-hydrochloride	176	Myclobutanil
85	Ethiprole	131	Fosthiazate	177	Neburon
86	Ethirimol	132	Furathiocarb	178	Nitenpyram
87	Ethoprop (ethoprofos)	133	Haloxyfop	179	Novaluron
88	Etofenprox [M+NH_4]	134	Hexaconazole	180	Omethoate
89	Etoxazol	135	Hexaflumuron	181	Oxadiargyl
90	Famoxadone	136	Hexythiazox	182	Oxadixyl
91	Fenamidone	137	Imazalil	183	Oxamyl

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Appendix (continued)

List of pesticide parent compounds, isomers and metabolites sought in method

No.	Pesticide compound	No.	Pesticide compound	No.	Pesticide compound
184	Oxasulfuron	214	Pymetrozine	244	Sulfoxaflor
185	Oxfendazole	215	Pyraclostrobin	245	Tebuconazole
186	Paclobutrazol	216	Pyrethrins I	246	Tebufenozide
187	Paraoxonmethyl	217	Pyrethrins II	247	Tebufenpyrad
188	Penconazole	218	Pyridaben	248	Teflubenzuron
189	Pencycuron	219	Pyridalyl	249	Terbuthylazine
190	Pendimethalin	220	Pyridaphenthion	250	Terbuthylazine-desethyl
191	Penflufen	221	Pyridate	251	Terbutryn
192	Penthiopyrad	222	Pyrifoenone	252	Tetraconazole
193	Permethrin	223	Pyrimethanil	253	Thiabendazole
194	Phenthoate	224	Pyriproxyfen	254	Thiacloprid
195	Phosalone	225	Quinalphos	255	Thiamethoxam
196	Phosmet	226	Quinoclamine	256	Thiobencarb
197	Phoxim	227	Quinoxyphen	257	Thiophanate-methyl
198	Pirimicarb	228	Quizalofop	258	Tolclofos-methyl
199	Pirimicarb-desmethyl	229	Quizalofop-P-ethyl	259	Tolfenpyrad
200	Pirimiphos-methyl	230	Rotenone	260	Triadimefon
201	Prochloraz	231	Simazine	261	Triadimenol
202	Profenophos	232	Spinetoram (XDE-175J)	262	Triallate
203	Promecarb	233	Spinetoram (XDE-175L)	263	Triazophos
204	Prometryn	234	Spinosyn A	264	Trichlorfon
205	Propamocarb	235	Spinosyn D	265	Triclocarban
206	Propaquizafop	236	Spirodiclofen	266	Tricyclazole
207	Propargite	237	Spiromesifen	267	Trifloxystrobin
208	Propazine	238	Spirotetramat	268	Triflumizole
209	Propiconazole	239	Spirotetramat-enol	269	Triflumuron
210	Propoxur	240	Spirotetramat-enol-glucoside	270	Triticonazole
211	Propyzamide	241	Spirotetramat-ketohydroxy	271	Tritosulfuron
212	Proquinazid	242	Spirotetramat-monohydroxy	272	XMC
213	Prosulfocarb	243	Spiroxamine	273	Zoxamide

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