APPLICATION NOTE 73959

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

## Using dual channel chromatography – triple quadrupole mass spectrometry

Authors: Łukasz Rajski[1], Christopher Elicone[2], Richard J. Fussell[3], Alan Atkins[3], Claudia P.B. Martins[4], Mauro De Pra[5], Amadeo Rodríguez Fernández-Alba[1]

[1]European Union Reference Laboratory for Pesticide Residues in Fruits and Vegetables, University of Almeria; Thermo Fisher Scientific, [2]Franklin, Massachusetts, US; [3]Hemel Hempstead, UK, [4]San Jose, CA, US, [5]Germering, Germany

Keywords: Dual channel chromatography, Vanquish Duo UHPLC, LC-MS/MS, Dual LC-MS, pesticide residues, triple quadrupole mass spectrometry

### 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).

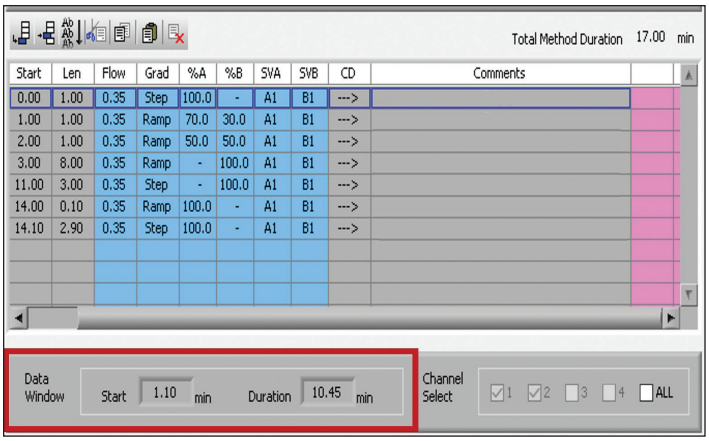


**Figure 1. Aria MX- method editor**

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 |

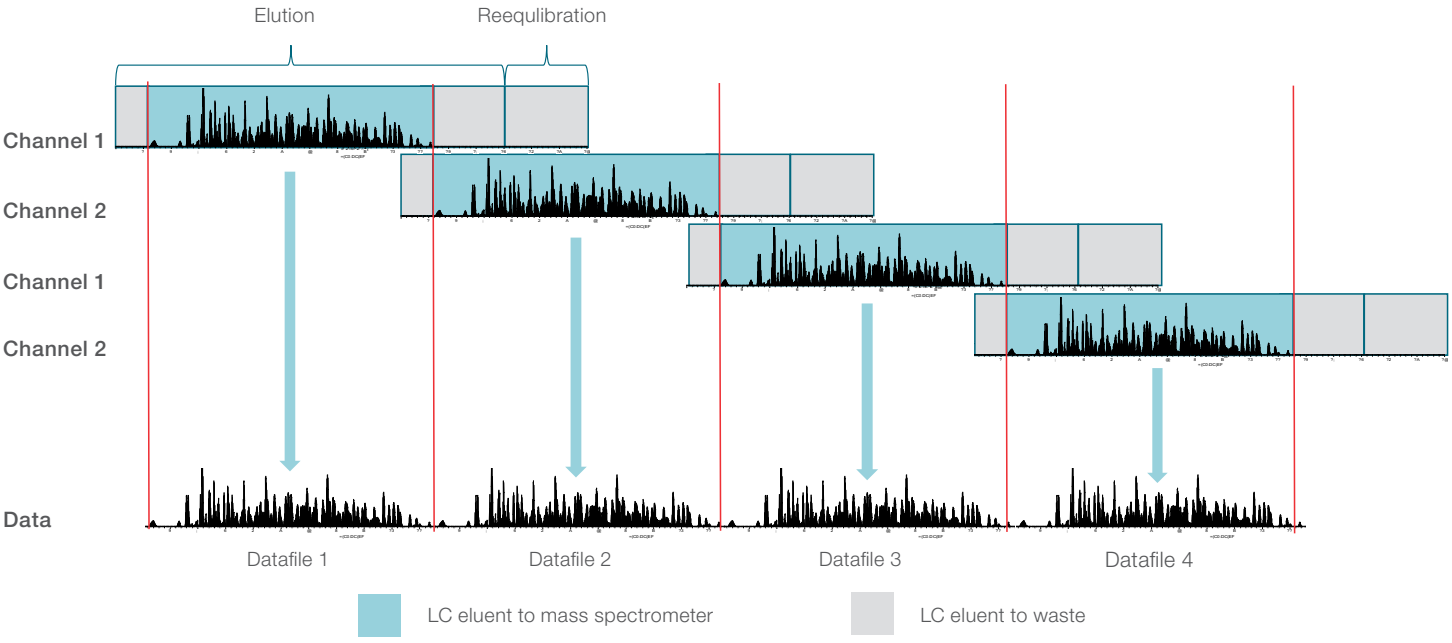


**Figure 2. The principle of the Vanquish Duo UHPLC systems for Dual LC-MS**

## 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).

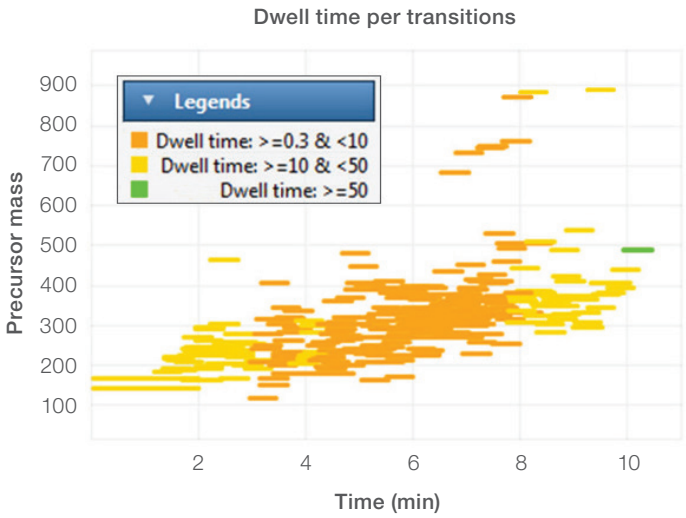


Figure 3. (A) Dwell time per transition

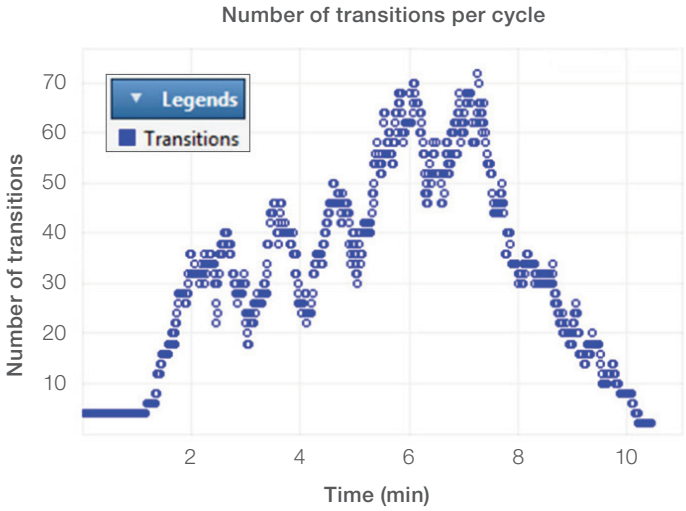


Figure 3. (B) Number of transitions per cycle

## 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.

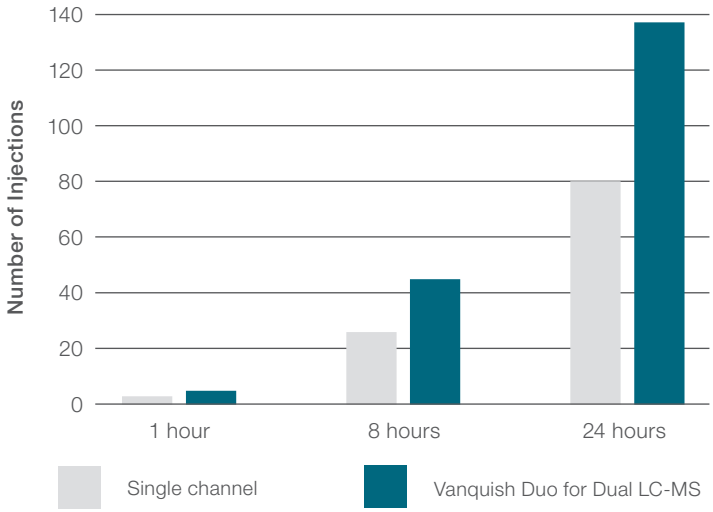


Figure 4. Sample throughput, single-channel vs. dual-channel

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.

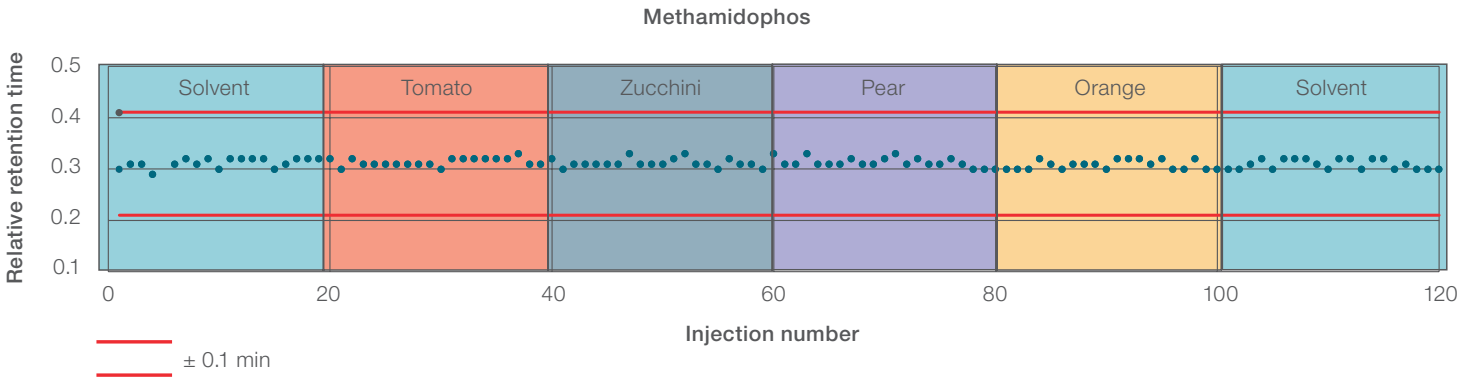


**Figure 5. Retention time stability of methamidophos**

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.

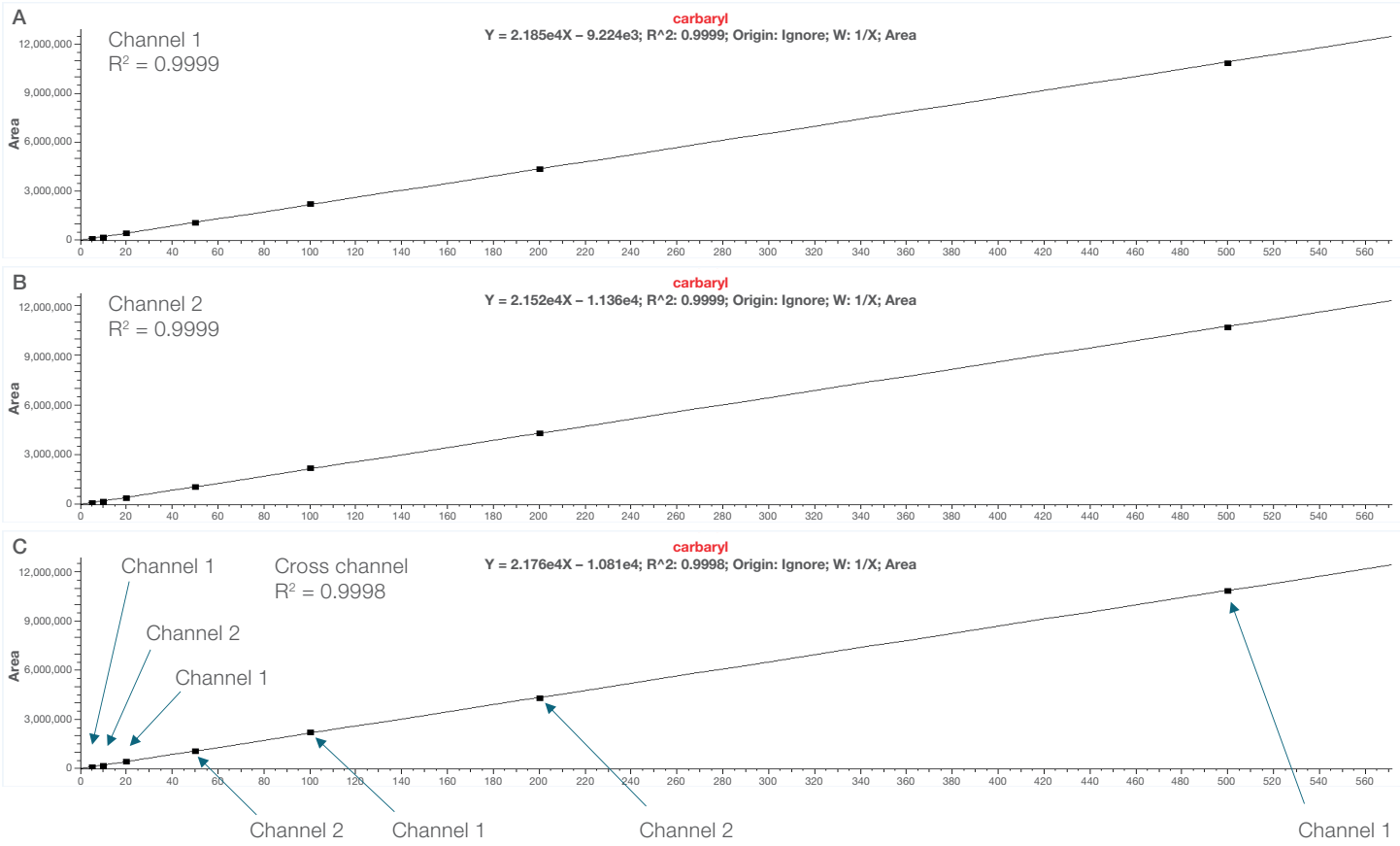


**Figure 6. Comparison of intra-channel calibration (plots A and B) with cross-channel calibration (plot C)**

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%.

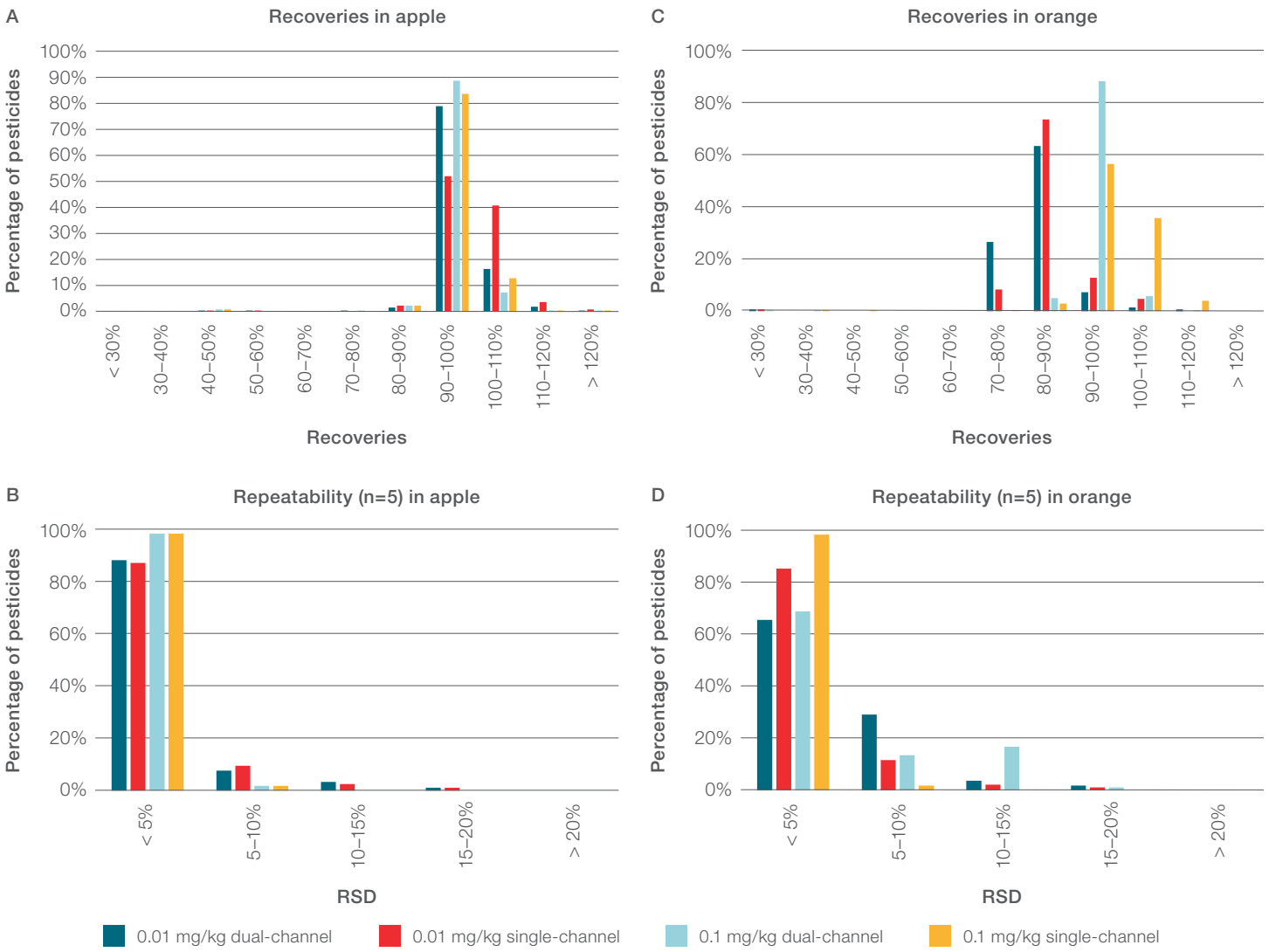


**Figure 7. Summary of validation results in apple and orange matrices**

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 |

thermo scientific

## 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 |

Find out more at **thermofisher.com**



ThermoFisher SCIENTIFIC