# **SHIMADZU**

# Using HRAM LC/QTOF for Target and Suspect Screening in Multi-Residue Pesticide Analysis

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### **Overview**

- Applying high resolution accurate mass (HRAM) QTOF analysis for routine quantitative pesticide monitoring programs to meet EU SANTE/12682/2019 validation guidance.
- The analytical method was based upon a validated triple quadrupole LC-MS/MS analysis (LCMS-8060) and transferred to a QTOF (LCMS-9030)
- A HRAM QTOF method was applied to the analysis of a panel of over 200 pesticides quantified using a TOF MS mass scan and DIA-MS/MS ion ratio confirmation with 31 DIA-MS/MS mass scan events. The cycle time was less than 0.875 seconds to acquire 32 mass scans for MS and DIA-MS/MS.

### **1. Introduction**

The EU SANTE/12682/2019 guidelines for the Analytical Quality Control and Method Validation Procedures for Pesticides Residues Analysis in Food and Feed identifies the following criteria;

- The measurement of 2 ions with a mass accuracy  $\leq$  5 ppm (for masses below m/z 200 the tolerance is  $\leq$  1 mDa) preferably including the molecular ion (or de-protonated molecule or adduct ion) and at least one characteristic product ion.
- Precision (expressed as repeatability RSDr) ≤ 20% for each spike level.
- Rt variance ± 0.1 minute

### 2. Methods

### **Sample Preparation**

Pesticide spiked samples, extracted using established QuEChERS based methods, were provided by Concept Life Sciences, UK. Matrices included apple, tomato and orange. Final extracts were prepared in acetonitrile without dilution and directly injected into the LC-MS/MS. A water co-injection method, performed automatically in the auto-sampler, was used to improve early eluting peak shapes.

#### LC separation

The panel of pesticides were separated using a Restek Raptor Biphenyl (100 x 2.1mm 2.7um) column using a binary gradient of Solvent A (formic acid (0.004%) in 2mM ammonium formate solution) and Solvent B (formic acid (0.004%) and 2mM ammonium formate solution in methanol). Flow rate 0.4 mL/min.

#### **Mass Spectrometry**

The LCMS-9030 HRAM QTOF (Shimadzu Corporation, Japan) system with Electrospray Ionization (ESI) was used in positive ion mode.

TOF-MS mass scan 140-900 Da; 100 msec.

31 dependent MS/MS mass scans 65-900 Da; each DIA-MS/MS mass scan was acquired for 25 msec; isolation width of 20 Da up to m/z 540, above m/z 540 variable isolation windows were used; CE spread of 5-55 V. The total cycle time for all 32 mass scans was 0.875 seconds.

All data were acquired using external mass calibration only (a TOF mass calibration was typically performed after 3-4 days of continuous analysis). LabSolutions Insight software was used for data review.

## 3. Results

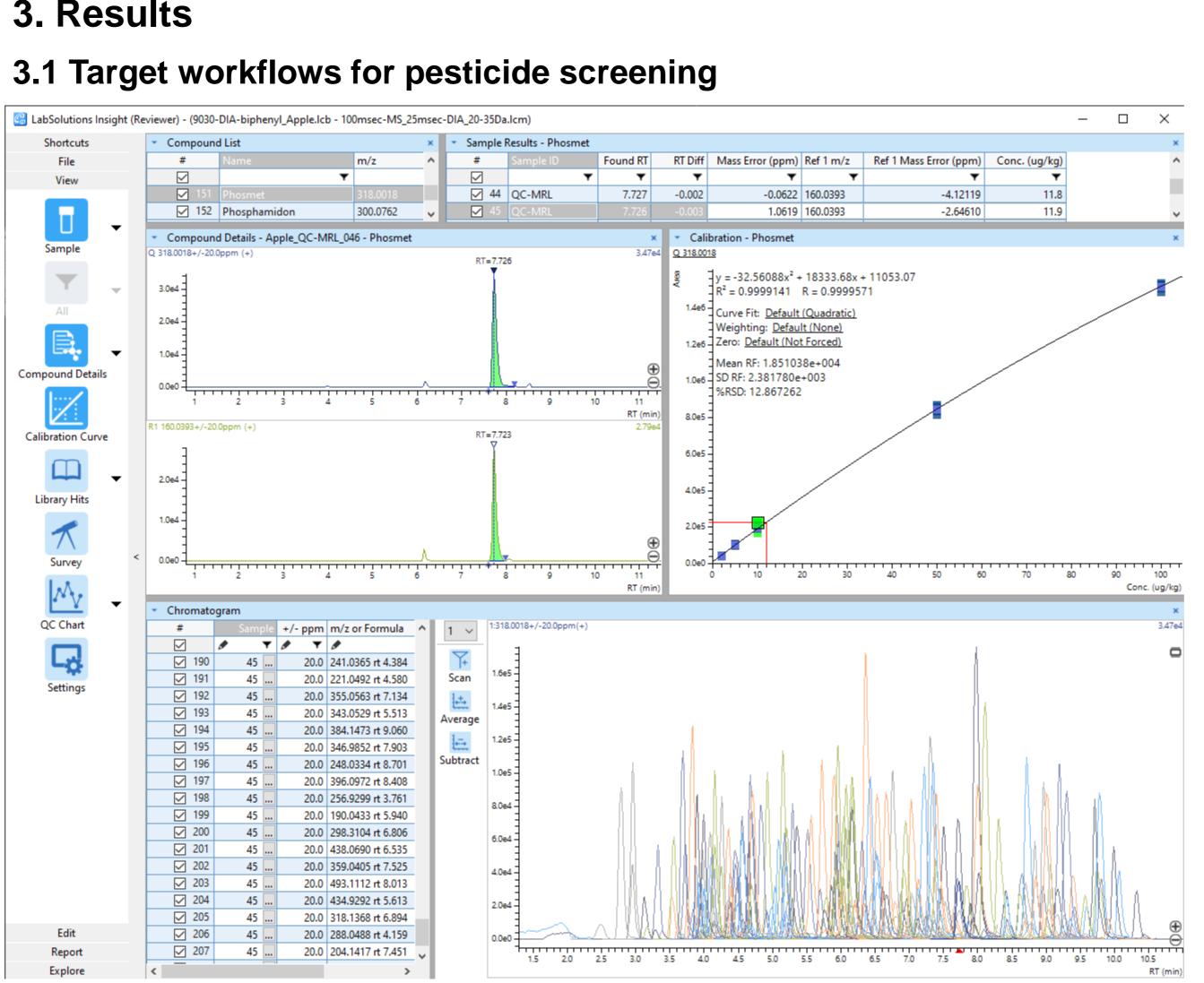


Figure 1. To meet the needs of EU SANTE/12682/2019 validation guidance, LabSolutions Insight software was set up to highlight mass accuracy error of the quantitative precursor (TOF Survey scan) and product ion (DIA-MS/MS mass scan), precursor and product ion mass chromatograms and Rt variance.

- (RSD) less than 20%.

Mass chromatograms for a panel of over 200 pesticides at 0.010 mg/kg (± 5 ppm). All compounds were detected at the default MRL of 0.010 mg/kg in the TOF Survey mass scan. As the cycle time was 0.875 seconds for all MS and DIA-MS/MS mass scans it was possible to acquire to acquire between 10-20 data points across a target peak generating reproducible peak integration and robust quantitation.

Precision (expressed as repeatability-RSDr); n=6. The SANTE/12682 /2019 guidance states that the variability of (at least 5) replicate injections (expressed as repeatability-RSDr) should be taken into account. In the batch analysis of 92 samples, calibration standards spiked into an apple matrix over a concentration range of 0.002-0.1 mg/kg were repeatedly injected (n=6). At the lowest calibration standard of 0.002 mg/kg, 183 target compounds resulted in a variance

Precision (expressed as repeatability-RSDr); n=50 at the MRL level. To assess the system robustness the default MRL sample at a concentration of 0.010 mg/kg sample was repeatedly injected (n=50) and automatically processed using default peak integration parameters (iPeakFinder algorithm). 194 target compounds resulted in with a peak area variance (RSD) less than 10%; 203 target compounds were found with a variance less than 20%.

### 3.2 Suspect screening workflows for pesticide screening.

A key advantage of acquiring TOF Survey scan and DIA-MS/MS mass scans is the capability to analyze the data retrospectively as every data point has precursor and product ion information.

🔐 LabSolutions Insight (Reviewer) - (9030-DIA-biphenyl\_Apple.lcb - 100msec-MS\_25msec-DIA\_20-35Da.lcm) ing Mode: Screening-Target-List.xlsx [m/z Hits:205 - RT Hits:205 File Target Formula larget m∕z∣m/z View **T** Available MS Event: MS/MS Edit 385 Omethoate C5H12NO4PS 214.02974 214.02947 -1.262 2.960 2,958 341.04542 341.04556 5179 Oxadiargyl C15H14Cl2N2O 0.411 8.145 8.150 Report 941 Oxycarboxin C12H13NO4S 268.06381 268.06348 -1.231 4.460 4.460 Explore Spectral Intensity: 5528 Pencycuron C19H21CIN2C 329.14152 329.14166 318.19762 318.19760 147 Penflufen C18H24FN3O -0.063 7.262 360.13519 360.13521 0.056 3814 Penthiopyrad C16H20F3N3O 6.802 6.750 User Defined Response: C16H16N2O4 318.14483 318.14474 -0.283 43 Phenmedipham 6.009 6.006 Chromatogram C7H17O4PS3 293.00994 293.00986 -0.273 265 Phorate-sulfone 6.271 6.196 C7H17O3PS3 277.01502 277.01484 3098 Phorate-sulfoxide -0.650 6.019 5.948 Chromatogra C11H12NO4PS2 318.00181 318.00208 0.849 7.805 7.713 Spectrum 300.07621 300.07597 354 Phosphamidon C10H19CINO5P -0.800 4.924 4.927 C12H15N2O3PS 299.06138 299.06136 0.067 8.482 Phoxim 8.41 Advanced 377.09077 377.09090 365 Picolinafen C19H12F4N2O 0.345 8.833 8.835 01 Pinoxaden C23H32N2O4 401.24348 401.24358 0.249 7.969 Calculate Mr 162 Pirimicarb-desmethyl C10H16N4O2 225.13460 225.13457 -0.133 4.713 4.665 376.03809 376.03789 010 Prochlora C15H16CI3N3O -0.532 8.688 208.13321 208.13305 -0.769 232 Promecarb C12H17NO2 6.203 6.152 C9H20N2O2 189.15975 189.15956 -1.004 2.851 287 Propamocarb Calculate Formula 877 Propaguizafor C22H22CIN3O 444.13207 444.13223 0.360 9.707 9.710 266 Propargite C19H26O49 368.18901 368.1889 0.190 9.429 9.375 C11H15NO3 210.11247 210.11248 336 Propoxur 0.048 4.952 4.913 373.04075 373.04425 C14H17IN2C 9.382 9.543 9.535 Analyze 7568 Proquinazio 312.06649 312.0663 -0.577 7.191 7.129 4085 Prothioconazole-desthio C14H15Cl2N3 Т<sup>р</sup> Chromatogram Assign # +/- ppm m/z or Formula 'E ▼ 🖉 5.0 368.1889 rt 9.375 Scan 5.0 186.0909 rt 4.694 Precursor 5.0 194,1172 rt 5,219 5.0 226.1086 rt 4.548 Average 5.0 184.0188 rt 2.492 5 0 210 9989 rt 7 46 Subtract 5.0 240.1012 rt 3.323 8 5.0 207.0796 rt 3.163 9 5.0 276.2183 rt 7.348 8.0e4 -10 5.0 228.1275 rt 6.021 5.0 209.1282 rt 4.081 6.0e4 -12 5.0 248.0702 rt 3.279 13 5.0 895.4828 rt 9.915 14 5.0 743.2531 rt 6.269 5.0 404.1243 rt 8.106 5.0 304.2999 rt 7.290 5.0 224.0918 rt 4.985 ✓ 18 5.0 411.1947 rt 9.229 45 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10.0 10.5 19 45 ... 5.0 398.0677 rt 7.931

Figure 2. In suspect screening workflows Insight Analyze algorithms are used to detect components and match to a list of suspected target compounds. Using the same data file and same compound search list as shown in figure 1, the resultant matches from the suspect screening workflow agree with the targeted data presented in figure 1. Key steps;

- Component detection. The first step is to locate components in the raw data file. The algorithm locates ions that behave as a recognized chromatographic feature (ion intensities rise and fall in abundance in a covariant manner) and applies several grouping and filtering steps to give a single component for grouped ions.
- Suspect search list. A search list of target compounds (a spreadsheet with compound name, target m/z, target formula and target retention time together with a mass tolerance and retention time window) is then used to match detected components with the search list. If the match is within the expected mass tolerance and retention time tolerance the target compound is reported. In figure 2, the resultant target compound matches are reported as mass chromatograms which agree with the targeted workflow for the same data file.

### 4. Conclusion

A HRAM DIA-MS/MS method was applied to the quantitation of over 200 pesticides using high data acquisition speeds (in agreement with the SANTE/12682/2019 guidelines). This approach results in a robust quantitative method which can be used for targeted and untargeted data processing.

