Quadrupole-Resolved All Ions MS/MS for Reliable Quantitation of Pesticides in Complex Matrices Using Untargeted Acquisition

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pesticides screening using LC/MS High Resolution Mass Spectrometry has attracted considerable attention in recent years.

Untargeted data collection produces full spectrum data which allows laboratories to continuously evolve/extend/widen the scope of targets without the need to change a base method. These spectra are delivered with high resolution and accurate mass, therefore highly selective EIC’s can be produced, enabling required sensitivity in this application for both quantitating and qualifying ions.

Measurement of fragment ions can sometimes suffer from interferences, effecting the reliability of ion ratio and ultimately the ability to detect much reduced fragments. To overcome this, precursors are resolved into smaller bands of ions on a prototype QTOF which has the ability to swiftly cycle between different bands of ions.

![Image](image.png)

**Figure 1: Quadrupole scan in 2 amu steps and EIC of calibrant masses show 90 amu wide windows.**

Figure 1 shows a quadrupole full scan acquired with a step size of 2 amu, with an effective window width of 90 amu. Once the calibrant signal is outside the range of the selected window, no detectable remaining signal is observed, demonstrating the selectivity over this broad quadrupole isolation range.

<table>
<thead>
<tr>
<th>Window</th>
<th>amu</th>
<th>m/z range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium 1</td>
<td>127</td>
<td>120-172</td>
</tr>
<tr>
<td>Medium 2</td>
<td>180</td>
<td>172-221</td>
</tr>
<tr>
<td>Medium 3</td>
<td>229</td>
<td>221-268</td>
</tr>
<tr>
<td>Medium 4</td>
<td>276</td>
<td>268-314</td>
</tr>
<tr>
<td>Medium 5</td>
<td>322</td>
<td>314-359</td>
</tr>
<tr>
<td>Wide</td>
<td>379</td>
<td>359-478</td>
</tr>
</tbody>
</table>

The quadrupole isolation windows are adjustable by changing the DAC values ranging from 0 to 255, affecting the applied RF values of the quadrupole. Tuning solution was introduced via calibrant delivery system. By using calibrant and background ions, the width and rectangularity of the isolation window could be determined. Multiple acquisition methods were generated, allowing to divide the mass range into sections between 10 amu and up to several hundred amu.

For proof of concept evaluation, QuEChERS extracts of avocado, orange and black tea were spiked to 2 and 10 ppb using a pesticide standard mixture. UHPLC separation was carried out with an Agilent 1290 Infinity 2 UHPLC system. An Agilent Zorbax Eclipse Plus C18 2.1x100 mm 1.8 µm column was used at a flow rate of 0.4mL/min. Column temperature was maintained at 40°C. Mobile phase A consist of 20% methanol in water, mobile phase B of 100% methanol, with both phases containing 5mM NH4-formate and 0.1% FA as modifier. A linear gradient from 0%B to 95% (0.5min to 11min) was used to separate the spiked extracts.

At For All Ions, a 6 Hz acquisition rate with two collision energies of 20 eV and 40eV yielded 12-15 data points across the chromatographic peak.

For the Quadrupole resolved All Ions experiments, two collision energies of 15 and 40eV were used with an Acquisition rate of 10Hz in MS and 15 Hz in MS/MS.

With a fixed DAC value of 22, the following window sizes were empirically determined, with the amu in the table the equivalent of a precursor in a classic targeted MS/MS spectrum:

The AJS source conditions were as follow: 180°C for the Dry gas at 6 l/min, and 375°C for the Sheath gas at 11 l/min, with a nebulizer pressure of 50psi.

Mass Spectrometric analysis was performed on a new Q-TOF prototype allowing wide quadrupole window selection. To preserve fragile analytes and enhance sensitivity, the Q-TOF prototype used SWARM autotune selection for 250 m/z fragile tune.
Basic characterization

The rectangularity of the quadrupole window width needed to be determined, because the shape of the isolated window determines the necessary overlap when moving from one window to the next. After generating empirically the DAC values for a 25 and 50 amu wide window, and operating the quadrupole with a step size of 0.1Da, less than 1 amu was needed to reach FWHM on both sides of the isolation window. Calibrant mass 322 was used to establish beginning, end and half maximum of the window.

However, in the case of Imazalil the AI spectra showed a matrix interference of one of its fragment ions, leading to a mass shift as well as an EIC height consisting of both pesticide and matrix interference (Fig. 4). The effect of matrix contribution went up with lower concentration of the pesticide. By using 50amu isolation window width, the matrix interference was completely removed, allowing the reliable quantitation of Imazalil in both avocado and black tea matrices (Fig. 5) with identical ion ratio compared to the standard in neat solvent matrix.

Pesticide Analysis:

Comparing the analyses of All Ions (AI) MS/MS with quadrupole resolved All Ions MS/MS using the spiked pesticide samples, we have observed that the fragmentation spectra looked substantially cleaner in the quadrupole resolved AI MS/MS spectra. Nevertheless, for multiple cases the ion-ratio values of the fragment ions remained the same. Figure 3 shows as an example the pesticide Prometron, with the EIC's of precursor (highest abundance) and two fragment ions. Apex spectra are shown below.

Figure 2: Quadrupole scan in 0.1 amu steps of EIC of calibrant masses 322 show for a 25 and a 50 amu wide window.

Figure 3: 2 ppb Prometron in black tea matrix.

Figure 4: 10 ppm (left) and 2 ppm (right) of Imazalil in black tea. Both top spectra were acquired by All Ions, whereas the bottom one by Q-resolved All Ions.

Figure 5: 2 ppm of Imazalil in black tea (left) and avocado (right). Top spectra were acquired by All Ions, the bottom one by Q-resolved All Ions.
Quantitative Analysis

The vast majority of quantitation on Q-TOF instrumentation is done on the MS level. In order to ensure that a) the correct compound is identified, and b) the compound is free from interferences, ion-ratios are an effective analytical tool to ensure that the measurement and quantitative results are valid. Using our prototype based experimental setup, it was possible to shift the bias to MS by using different acquisition rates in the MS mode compared to the quadrupole resolved All Ions spectra. Using this approach, a better ion-statistic (and as a result a better dynamic range) can be achieved.

Here, we present data utilizing the MS/MS domain for quantitation, and the MS domain as an additional qualifier. This workflow offers apart from high-resolution and accurate mass the additional advantage of untargeted acquisition. This allows at a later point in time the retro perspective analysis of the data with previously unknown targets.

Figure 6 shows the concentration curve of the pesticide Prochloraz, ranging from 1 ppb to 50 ppb in orange matrix. Data was acquired in All Ions mode at 6Hz.

Using the MS level for quantitation, at lower concentration levels the background starts to interfere with the compounds, leading to higher response values than theoretically calculated from the calibration curve (Fig. 6 inset).

Figure 7 shows the same pesticide, Prochloraz, as in figure 6, but instead using the MS domain for identification, the EIC peak area of the fragment 308 in the MS/MS domain was used. The inset shoes the fragment spectrum at 308 m/z, which is baseline separated from neighboring peaks and interferences.

Conclusions

Quadrupole resolved All Ions offers:

- Cleaner fragmentation spectra
- Less interference of co-eluting compounds with similar fragments
- As a consequence, fragments show better mass accuracy and better ion ratios
- Reliable Quantitation possible in MS and MS/MS

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