Use of HRAM with LC, GC and IC for the analysis of pesticide residues in food

Choosing the best tools for the job at hand

Jim Garvey
Summary

Introduction
Multi residue method – extraction
GC Method
Validation data
LC- Method
MS² options
Data

IC Methods
PRM
Data
Screening
Introduction

- HRAM methods offer a number of advantages over current QQQ methods
- They allow for unlimited expansion of analysis scope
- No loss of data
- Multiple MS2 options depending on what we want to do
- Comparable sensitivity to other techniques
Sample preparation
Extraction

15g of homogenised sample is taken

Acetone (30ml) is added and the sample is homogenised

DCM (30ml), Petroleum Ether 40-60°C (30ml) and Sodium Sulphate (30g) is added and the sample homogenised again

The extract is centrifuged, 60ml of the extract is reduced and reconstituted in Ethyl Acetate to a final volume of 10ml

The neat extract is injected on the GC-MS and a 1/20 dilution with methanol is injected onto the LCMS
Orbitrap – basic components

1. Ion source – EI / HESI
2. Focussing lenses
3. Quadrupole - Parallel Reaction Monitoring
4. C-Trap
5. HCD Cell – Secondary ionisation
   • All ions fragmentation – AlF
   • Data dependent analysis – ddMS2
   • Variable data independent analysis – vDIA
6. Orbitrap mass analyser
GC Method

- Ion source – EI
- Quadrupole left open
- Ions are allowed accumulate in the C-trap before being sent to the orbitrap
- Full scan data
- No MS\(^2\) options used
- The simplest way to use this system
**GC Method**

- **Column** = TG-SilMS with 5m safeguard – 5% diphenyl, 95% dimethyl polysiloxane
- **0.25mm x 0.25 \( \mu \)m**
- **Max Temp 320/350°C**
Raw data – α-HCH
Raw data - Chlorpropham
Linearity

7 point calibration curve between 5 and 250 µg/l

Spikes bracketed between calibration standards
93.2% of the compounds in the method met the reproducibility criteria.
Overall reproducibility

Reproducibility Mean - overall

60 – 140%

RSD - R

0 – 25%
Mass Accuracy by matrix

0 – ±3ppm

0 – ±3ppm

0 – ±3ppm
Mass Accuracy – confirming ions

An Roinn Talmhaíochta, Bia agus Mara │ Department of Agriculture, Food and the Marine
Ion Ratio data

- HRAM: Ion ratio 30% Tolerance
- Analyte peaks for Target Ion and Confirming Ion must fully overlap.
  - We Measured a total of 515 Ion Ratio’s For Each Matrix!
  - No easy way to summarise as the ion ratio tolerance is slightly different from batch to batch
  - The mean ratio at each level is compared with the mean tolerance across all levels
  - 93.2% of the mean ratio’s meet the tolerance
## Problematic compounds

<table>
<thead>
<tr>
<th>Problem</th>
<th>Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor</td>
<td>Captafol, Phorate, folpet,</td>
</tr>
<tr>
<td>Inconsistent recoveries</td>
<td>Azinphos-methyl, pp-ddt, omethoate</td>
</tr>
<tr>
<td>Poor extraction from lemon</td>
<td>Pirimicarb, Pirimicarb-desmethyl, Prochloraz &amp; Triflumizole</td>
</tr>
<tr>
<td>High recovery, except in broccoli</td>
<td>Acephate</td>
</tr>
<tr>
<td>Enhancement in broccoli</td>
<td>Dichlofluanid (chlorthalonil)</td>
</tr>
<tr>
<td>Suppression in lemon</td>
<td>Metribuzin</td>
</tr>
<tr>
<td>Enhancement in cucumber</td>
<td>Phosmet</td>
</tr>
<tr>
<td>Enhancement, except in lemon</td>
<td>Resmethyl</td>
</tr>
<tr>
<td>Some poor recoveries in lemon</td>
<td>Trifluralin</td>
</tr>
</tbody>
</table>
Overall conclusion

• Method was successfully validated for more than 93% of the 167 pesticides & PCBs.
• The method has been used successfully in parallel with existing methods.
• PT results excellent
• The screening capability of the technology has been successfully tested
• Next steps - Validation of other matrices, cereals, IF, Milk, eggs, honey.
1. Ion source – HESI
2. Focussing lenses
3. Quadrupole - Parallel Reaction Monitoring
4. C-Trap
5. HCD Cell – Secondary ionisation
   - All ions fragmentation – AIF
   - Data dependent analysis – ddMS2
   - Variable data independent analysis vDIA
6. Orbitrap mass analyser
LC Method

- Column = Accucore Vanquish C18+
- 100 x 2.1mm
- Particle size = 1.5µm
- Mobile phase – 5mmol Ammonium Formate : Methanol
- Injection volume = 1.0µl
Quantitation

In all cases the initial experiment is a full scan experiment across the full mass range covered by the method.

From this experiment a target ion is chosen and this is used to construct a calibration curve.

This target ion is also used to calculate the theoretical isotope ratio’s, when this function is enabled.
Confirmation options – MS2

1. All ions fragmentation – AIF
2. Data dependent MS$^2$ analysis – ddMS$^2$
3. Variable data independent analysis – vDIA
4. ddMS$^2$ / AIF
Data dependent analysis – ddMS2

1. Requires an inclusion list

2. Once a compound from the inclusion list is found this is fragmented in a secondary experiment – one scan

3. MS² fragments are used for confirmation of presence

4. Efficient use of cycle time

5. Secondary fragmentation doesn’t always fire on the apex of the peak and sometimes doesn’t fire at all
**Data dependent analysis – ddMS2**

- The total cycle time or scan rate will now be dependent on the two experiments carried out.
- Imazalil elutes at 8.74 minutes and therefore gives the average number of points across the peak for this method.
- In this case approximately 30 points are being collected across the peak which gives a cycle time of ~**0.35 secs**.
Data dependent analysis – ddMS2

- When a mass from the inclusion list is found one MS² scan is carried out.
- Because only one scan is carried out across the peak centroid data is collected.
Data dependent analysis – ddMS2
Data independent analysis - vDIA

1. The mass window is broken up into a number of mass ranges

2. Secondary fragmentation is carried out on each range independently

3. MS² fragments used for confirmation

4. Reduces the risk of common fragments causing interference

5. Heavy on cycle time – 0.8sec
Methamidaphos elutes at a retention time of 1.2min.

The peak width is approximately 9secs.

The number of points collected across the peak is approximately 15.

This is the worst case scenario as the peak width will increase with increasing retention time.
SRM 14 – Bovine liver – Ad hoc method

Bixafen-desmethyl in Liver – 56 ppb

Boscalid-5-hydroxy in Liver – 90 ppb
Fluopyram benzamide in Liver – 83.5 ppb

<table>
<thead>
<tr>
<th>SRM 14</th>
<th>Reported</th>
<th>Assigned value</th>
<th>Z-score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bixafen desmethyl</td>
<td>56.0</td>
<td>50</td>
<td>0.5</td>
</tr>
<tr>
<td>Boscalid-5-ydroxy</td>
<td>90.2</td>
<td>81</td>
<td>0.5</td>
</tr>
<tr>
<td>Fluopyram benzamide</td>
<td>83.5</td>
<td>101</td>
<td>-1.0</td>
</tr>
</tbody>
</table>
Linearity 0.5 – 250 ppb
Average recovery for fruit and veg matrices

In most cases the recoveries are within the AQC criteria

% RSD’s are less than 25% in most cases
Screening PT EUPT SM11

Designed to test the laboratory’s screening capability

Sample has to be analysed and results reported within 72 hours of receipt of sample

No target list – anything could be present !!

Semi-quantitative

Sample analysed using a targeted method on GC and LC-Orbitrap
Targeted screening
Isopyrazam
Spinetoram I and II
Conclusion

Compound found, not yet on PCL scope

Fenpyrazamine
Penthiopyrad
Spinetoram I and II
Metrafenone
Isopyrazam
Bifenazate

To find that needle in the haystack you just need to use the right tools