Options for Fast, Reliable Pesticide Residues Analysis in Food by Triple Quadrupole GC-MS/MS

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Outline

• The analytical challenge

• User requirements for GC-MS/MS analysis of pesticides

• Collaboration between Fera and Thermo Fisher Scientific; method optimisation, results and learning experiences

• Latest GC-MS/MS developments

• Summary
Analytical Challenges

- Wide range of matrices
  - Food
  - Environment
- Wide analytical scope
- Low limits of detection
- High sample throughput
- Fast turnaround
- Low cost of analysis
Requirements for GC-MS/MS

• Selectivity

• High sensitivity for 2\textsuperscript{nd} transition to enable identification of pesticides at low target concentrations

• Robustness in operation 24/7 and ease of use

• Reproducibility

• Software: Effective and ease of use

• Cost, maintenance and service
1. **Sample Prep:** March 24th
2. **LC-MS Analysis:** April 29th
3. **GC-MS Analysis:** June 17th
4. **Data Processing/Analysis:** July 15th

Register at [www.chromatographyonline.com/LCGCwebseminars](http://www.chromatographyonline.com/LCGCwebseminars)
Pesticide Analysis: Triple Quad GC-MS

Highly Selective Reaction Monitoring (SRM)

✓ Improved detection limits
Selectivity: SIM and SRM

DDE-p,p’, 0.05 mg/kg in green tea, 1.0 uL splitless injection

Ion ratio = 3.5

Ion ratio = 2.7
Analysis of QuEChERS Acetonitrile by GC?

- High expansion coefficient-limited injection volume
- Offline solvent exchange - requires care to avoid losses of volatile or unstable pesticides
- LVI with higher capacity liners or liner packing materials, but potential for adsorption of pesticides?
- Will repeated injection of acetonitrile extracts lead to a decrease in column efficiency?
- How quickly will matrix extracts contaminate the GC-MS system?
Evaluation of high sensitivity GC-MS/MS

Evaluate direct analysis, of QuEChERS acetonitrile extracts

- Thermo Scientific™ TSQ Quantum™ GC-MS/MS system
- Thermo Scientific™ Trace™ Ultra GC Chromatograph
- Thermo Scientific™ TriPlus RSH ™ Autosampler
- Column: TR-Pesticide (II), 0.25 mm i.d., 0.25 µm film
- ‘test matrix’- fruit preserve
- 96 pesticides/metabolites: 192 transitions
SRM chromatograms – 1µL splitless

Dichlorvos

Pirimiphos-methyl

Captan (0.02 mg/kg)

Cyfluthrin (0.02 mg/kg)

Deltamethrin

184.95>92.88
290.09>233.07
148.97>69.98
226.03>206.03
180.99>151.99

219.95>1
305.10>290.09
148.97>104.98
163.02>206.03
252.99>93.00

21.05
24.9
47.03
51.6

Splitless injection (1 µL) of acetonitrile extracts (d-PSA clean-up) of fruit preserve spiked at 0.01 mg/kg (= 10 pg on-column)

Courtesy of Fera, UK
Summary of validation data (1 µL splitless)

- QuEChERS extraction (citrate buffer/d-PSA) of fruit preserve

- Except chlorothalonil, recovery and % RSD data for the other 95 compounds met EU DG SANCO validation criteria

Results courtesy of Fera, UK
Robustness (1 year) of instrument performance

- TSQ Quantum XLS
- Analysed ~ 200 batches of samples
- ~ 6000 matrix injections (PTV-backflush)
- Kidney, liver, fish, meat, crustaceans, milk
- Response does vary but is always sufficient
- Analytical capillary column not replaced
- 2 breakdowns requiring service engineer

Information courtesy of Fera, UK
Thermo Fisher Scientific and Fera GC-MS/MS Collaboration (2)
Thermo Scientific TSQ 8000 GC-MS/MS

The new Trace 1300 Series offers the most versatile GC platform in the market, with unique “instant connect” modularity for ground-breaking ease of use and performance, setting a new era in GC technology.
Repeatability using the baffled liner

- [10ul (1/10 diln with EtAc] speed controlled solvent vent]
- PTV Baffle Liner deactivated (Siltek),

Results courtesy of Fera, UK
Repeatability Using Asymmetric Liners

- QuEChERS extracts of baby food (no IS)
- High number of injector parameters to optimise

Results courtesy of Fera, UK
Validation Results

(0.01 mg/kg, 10 pg on column) (0.001 mg/kg 2.5 pg on column)

• Excellent results obtained
• Cycle time approximately 40 minutes  

Results courtesy of Fera, UK

Dichlorvos
(m/z 185-93, 186.9-93)

Cyfluthrin
(m/z 226.0-206.1, 163.0-127)
## Preliminary Data

- According to SANCO/12571/2013 criteria
- Recovery 70-120 %, RSDr (<20%)

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Recovery (%)</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Nº out-with</td>
</tr>
<tr>
<td></td>
<td>Nº out-with</td>
<td>criteria</td>
</tr>
<tr>
<td>Apple</td>
<td>95</td>
<td>3</td>
</tr>
<tr>
<td>Grapes</td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>lettuce</td>
<td>104</td>
<td>10</td>
</tr>
<tr>
<td>Oats</td>
<td>96</td>
<td>16</td>
</tr>
</tbody>
</table>

- Splitless (1 µL) with cyclo-liner

Results courtesy of Fera, UK
Increasing Laboratory Productivity

• Decrease analysis time by shortening the GC run times.
  • More samples in less time.

• Increase the number of pesticides in a run.
  • More SRMs to accommodate within an analytical run.

• Improve selectivity for various matrices.
  • Increased number of SRMs per compound.

• See beyond the targets.
  • Full Scan and SRM data acquisition in the same experiment.

Expect More Performance
Fast GC-MS Pesticide Residue Analysis

**Challenges:**
- Complexity of elution when using fast GC
- Large number of compounds (SRMs) in short time
- Many SRM transitions can result in sensitivity loss

**Solution:**
- High speed analyzer
- Fast collision cell
- Short SRM dwell times with very short inter-scan delays
TSQ 8000 Evo GC-MS/MS

**Analytical instrumentation:**

- TSQ 8000 Evo GC-MS/MS Pesticide Analyzer
- TRACE 1310 GC Gas Chromatograph
- TriPlus RSH Autosampler (liquid injection set-up)

**EvoCell**

- Rapid, innovative collision cell technology
- Increased method capacity
- More compounds
- More SRM transitions
- Up to 4x more transitions whilst maintaining method sensitivity low analyte concentrations

Expect **More Capacity**
Pesticide MRM Database

The problem:

• Growing list of target compounds require continuous adjustment to an existing SRM database.

• Some SRM transitions are not suitable for all matrices. Addition of new SRM transitions can be time consuming.

The solution:

• Automated SRM development with AutoSRM.
AutoSRM: Fast, Simple Route to Optimized SRM

1) Precursor ion selection

2) Product ion selection

3) Collision energy optimization

AutoSRM automates the development of SRM methodology
Highlights of AutoSRM

- Automates the following:
  - Creation of full scan, product ion scan, and SRM methods
  - Creation of sample sequences
  - Creation of data layouts for analyzing results
  - Selection of precursor, product, and collision energies

End result showing optimized transition
Timed-SRM: Using Dwell Times Efficiently

Classical segmented SRM:
- Complex to set up
- Wasted dwell time
- Reduced sensitivity
- Reduced tolerance to RT shifts

TSQ 8000 Evo timed-SRM:
- Automated set-up
- Full optimized dwell time
- Optimal sensitivity
- Increased resistance to RT shifts
Increasing Laboratory Productivity

- Decrease the analysis time by shortening the GC run times.
  - More samples in less time.

  - More SRMs Increase the number of pesticides in a run.
  - Increase the number of SRMs per compound.

- Improved selectivity for various matrices

  - Increase the number of SRMs per compound.

- Seeing beyond the targets

  - Full Scan and SRM data acquisition in the same experiment.
Decrease the Analysis Time

Full scan
144 pesticides in baby food @ 0.2 mg/kg
TG-5 SILMS, 30m x 0.25 mm x 0.25 µm
GC run time: ~37 min
Fast GC: 144 Pesticides in Baby Food

As more compounds are added to a single run, more complexity arises with the management of acquisition windows and compound co-elution.

**SRM acquisition** of 144 pesticides in baby food @ 0.2 mg/kg
GC column: TG-5 SILMS, 20m x 0.18 mm x 0.18 µm, faster temperature ramp
GC run time: <11 min
Increase Laboratory Productivity

• Decrease the analysis time by shortening the GC run times.
  • More samples in less time.

• Increase the number of pesticides in a run.
  • More SRMs to accommodate within an analytical run.

• Improved selectivity for various matrices
  • Increase the number of SRMs per compound.

• Seeing beyond the targets
  • Full Scan and SRM data acquisition in the same experiment.
More SRM Transitions/Compound for More Confidence

Tecnazene in baby food at 0.01 mg/kg level
More Speed Maintaining the Sensitivity

DDE-p,p’ in green tea, 44 SRMs
Inj. 0.01 mg/kg on column

DDE-p,p’ in green tea, 1917 SRMs
Inj. 0.01 mg/kg on column

RT: 14.84 - 16.62

RT: 15.79
AA: 3837440
NL: 2.35E6
TIC F: + c EISRM
ms2
246.000@cid28.00
[176.095-176.105]
MS Genesis
19May2014_03

NL: 2.60E6
TIC F: + c EISRM
ms2
246.000@cid28.00
[176.095-176.105]
MS Genesis
19May2014_03

27 ms

RT: 14.95 - 16.71

RT: 15.79
AA: 4093580
NL: 1.21E6
TIC F: + c EISRM
ms2
317.800@cid20.00
[245.995-246.005]
MS Genesis
19May2014_03

NL: 1.03E6
TIC F: + c EISRM
ms2
317.800@cid20.00
[245.995-246.005]
MS Genesis
19May2014_03

0.7 ms
Linearity: Dichlorvos

- Dichlorvos peak area response over 0.5–10 ppb (mg/kg), matrix-matched standard (baby food).
- Chromatograms (quantification and confirmation ions) at 10 ppb level.

![Graph showing linearity with R² values]

2 SRMs/compound

![Graph showing linearity with R² values]

8 SRMs/compound
Examples of Peak Area Repeatability: Fast GC method

%RSD pesticides (0.001 mg/kg on column) baby food matrix

~144 compounds in <10 minutes

<table>
<thead>
<tr>
<th>Compound</th>
<th>% RSD (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHC, Alpha</td>
<td>7.0</td>
</tr>
<tr>
<td>BHC, Beta</td>
<td>8.8</td>
</tr>
<tr>
<td>BHC, gamma</td>
<td>9.2</td>
</tr>
<tr>
<td>Chlorobenzilate</td>
<td>12.5</td>
</tr>
<tr>
<td>Chlorothalonil</td>
<td>12.6</td>
</tr>
<tr>
<td>Clomazone</td>
<td>6.3</td>
</tr>
<tr>
<td>Cyfluthrin peaks 1-4</td>
<td>9.3</td>
</tr>
<tr>
<td>DDE p, p</td>
<td>8.2</td>
</tr>
<tr>
<td>Dichlobenil</td>
<td>5.3</td>
</tr>
<tr>
<td>Dichlorvos</td>
<td>8.1</td>
</tr>
<tr>
<td>EPTC</td>
<td>5.3</td>
</tr>
<tr>
<td>Etridiazole (Terrazole)</td>
<td>4.6</td>
</tr>
<tr>
<td>Hexachlorobenzene</td>
<td>8.9</td>
</tr>
<tr>
<td>Methacrifos</td>
<td>7.6</td>
</tr>
<tr>
<td>Propachlor</td>
<td>11.0</td>
</tr>
<tr>
<td>Propham</td>
<td>11.5</td>
</tr>
<tr>
<td>Simazine</td>
<td>9.1</td>
</tr>
<tr>
<td>Tecnazene</td>
<td>5.6</td>
</tr>
<tr>
<td>Tefluthrin</td>
<td>7.0</td>
</tr>
<tr>
<td>Triallate</td>
<td>11.0</td>
</tr>
</tbody>
</table>

Routine results

- GC Performance Maintenance
- MS Performance Maintenance
- Acquisition Method Maintenance
- Data analysis method maintenance
Comprehensive Detection of Pesticides in a Sample

• Targeted screening and quantification of a number of pesticides is important.

• Increased interest in screening the samples for additional compounds beyond a target list.

• Less common or new pesticides or unknown metabolic/transformation products could be detected in addition to targeted compounds.

• This requires fast analytical instrumentation able to acquire both full scan and SRM/SIM data simultaneously.
Summing up

Fast, easy, and robust pesticide analysis can be achieved using:

- Compound database management:
  - Auto-SRM automates SRM transitions development.

- Using the available dwell time wisely:
  - Timed-SRM ensures minimal loss of time spent to acquire data.

- Enhancing the number of compounds and increasing the number of SRM transitions per compound:
  - EvoCell fast collision cell technology.

- Comprehensive detection of target pesticides and of additional contaminants potentially hazardous to human health:
  - Simultaneous full scan and SRM/SIM data acquisition.

- A full validation of this fast method is ongoing and will be available soon.
Thermo Scientific Food and Beverage Community

- View application notes, on-demand webinars, product information, and many more resources on our Pesticides and Food Communities Libraries:
  - www.thermoscientific.com/pesticides
  - www.thermoscientific.com/foodandbeverage
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