The separation and detection of over 100 pesticides in complex matrices in less than 4 minutes using UPLC-MS/MS

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Abstract

Ultra Performance Liquid Chromatography (UPLC) with electrospray tandem mass spectrometry (ESI-MS/MS) has become an established front-line technique used by the Scottish Agricultural Science Agency (SASA) for the quantitative determination of over 100 pesticide residues in a variety of complex matrices. The method is applied routinely to support the annual UK and EU pesticide residue in food monitoring programs.

Analytical methodologies employed in the determination of pesticide residues in foodstuffs must be capable not only of quantifying very low levels of incurred residues but also of confirming the identity and magnitude of these residues. The requirements to provide unambiguous evidence become increasingly challenging as reporting limits and maximum residue levels are decreased and the number of target pesticides and metabolites is increased (from 125 in 2006 to over 200 in the 2015 UK surveillance programme).

A group of 50 compounds from the suite of 103 pesticides routinely sought using LC methods has been chosen to illustrate the power of fast analytical techniques using UPLC-ESI-MS/MS. These representative compounds cover a wide range of chemical functionality and pesticide class.

Performance data for various commodities and results from vegetable samples screened as part of the 2007 surveillance program are presented.

Methodology

Sample Extraction Procedure

Samples of fruit and vegetables are frozen and cryo-milled on receipt prior to extraction by homogenisation with ethyl acetate. An aliquot of this crude extract is solvent-exchanged into methanol, filtered and preserved for LC-MS/MS analysis (105 analysis units).

The remainder is passed through a clean-up stage using gel permeation chromatography prior to analysis by GC-MS/MS (97 analysis units).

UPLC Performance LC™ (UPLC)

Small-stationary phase technology (1.7µm) and the ability to operate at high back pressures (15,000psi) allow this system to achieve higher sensitivity and peak capacity at optimum flow rates.

UPLC Experimental Parameters

Instrument: Waters ACQUITY UPLC system
Column: ACQUITY UPLC BEH C18 1.7µm
ACQUITY LC Pump Initial Conditions
A: H2O/MeOH 95/5 v/v, 5mM ammonium acetate solution
B: MeOH, 5mM ammonium acetate solution
Solvents (gradient elution), Flow 0.48 ml/min

<table>
<thead>
<tr>
<th>Min</th>
<th>A%</th>
<th>B%</th>
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<tbody>
<tr>
<td>0</td>
<td>100</td>
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<td>30</td>
</tr>
<tr>
<td>15.9</td>
<td>70</td>
<td>30</td>
</tr>
</tbody>
</table>

Step Time (min) | 6.5
Min Pressure (psi) | 15000
Oven Temperature (°C) | 35.0
Injection Volume (µl) | 4.9

Mass Spectrometry Experimental Parameters

- To utilise chromatographic performance common in UPLC (narrow peaks 1-3s FWHM) the mass spectrometer must be capable of acquiring MRM data at a sufficiently high rate (short dwell times <10ms).
- The present method comprises 7 consecutive acquisition functions covering the time from 0.1 - 4.0 minutes. Each function contains MRM acquisition parameters for between 6 to 27 compounds. This single experimental method acquires data for 103 pesticides plus internal standards in less than 4 minutes.

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Summary

It is highly likely that the spread trend in pesticide numbers sought in all commodities will continue. This will place greater demands on the capabilities of analytical instrumentation and methods. We believe that the development and validation of fast, efficient methods such as that described will be a cornerstone in meeting this challenge.

Advantages of Rapid Analysis by UPLC-ESI-MS/MS

- Reduces instrument time and increases efficiency
- Sample batches run times of ca. 2.5 hours are typical with no observed adverse effects on system stability or chromatographic integrity during or between batches analysis. Sample batches run times of ca. 2.5 hours are common in conventional UPLC. A typical batch comprises ca. 25 samples and includes sample extracts, matrix matched standards, AQC, blanks and controls.
- Allows a greater throughput of samples
- Reduces solvent usage
- Allows rapid confirmation of identity and quantity as required by the Pesticide Residues Committee
- Allows faster response to identified pesticides
- Minimises possible analyst bias in sample extracts over time

Performance Data for Selected Pesticides

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Unit</th>
<th>Conc.</th>
<th>REC</th>
<th>Mean</th>
<th>RSD</th>
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<tr>
<td>Azoxystrobin</td>
<td>ng/g</td>
<td>1.40</td>
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<td>Flusilazole</td>
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<td>Boscalid</td>
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<td>Fenpropimorph</td>
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<tr>
<td>Fenhexamid</td>
<td>ng/g</td>
<td>4.90</td>
<td>15.7</td>
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<tr>
<td>Fenbuconazole</td>
<td>ng/g</td>
<td>13.1</td>
<td>15.5</td>
<td>15.5</td>
<td></td>
</tr>
</tbody>
</table>

- The supplier is notified and asked to investigate the cause.
- A risk assessment is undertaken to determine whether consumers would be at risk from consuming the product.
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- If residues found are a health concern, other matrices are informed (ES: major crops).

- Alert monitoring may be initiated.

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