The rapid analysis of fungicide residues in crude extracts of fruit and vegetables using UPLC-ESI-MS/MS detection
Kirsty Reid, George Keenan & Michael J Taylor.
Scottish Agricultural Science Agency, Roddinglaw Road, Edinburgh, EH12 9FJ

Abstract
The Scottish Agricultural Science Agency (SASA) carries out annual surveillance monitoring of pesticides in fruit and vegetables as part of a UK programme organised by the Pesticide Residues Committee (PRC)’s. Monitoring is essential to support enforcement of legislation, to ensure trading compliance and to carry out surveillance programmes on regional and national dietary components. Analytical methodologies employed in the determination of pesticide residues in foodstuffs must be capable of quantifying very low levels of incurred residues and confirming the identity and magnitude of these residues.

This requirement to provide unambiguous evidence of residues is becoming increasingly challenging as reporting limits and maximum residue levels are decreased whenever new legislation is introduced e.g. infant food feed. The number of pesticides to be analysed is also increasing (eg from 125 in 2006 to 208 for the UK surveillance programme).

A group of 41 fungicides 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.

Results from cabbage samples obtained as part of the PRC surveillance programme 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 eluate of this crude extract is solvent exchanged into methanol, filtered and presented for LC-MS/MS analysis (103 analytes sought).

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

Comparison of ACQUITY Ultra Performance LC™ (UPLC) and HPLC

Small stationary phase particle technology (1.7µm) and the ability to operate at high back pressures (15,000psi) allow the use of a maximum sample injection volume of 3µl.

UPLC Experimental Parameters

Instrument: Waters Acquity UPLC system
Column: Acquity UPLC BEH C18 1.7µm, 2.1mm x 50mm
Acquity LC Pump Initial Conditions
A: H2O/MeOH 95/5 v/v, 5mM ammonium acetate solution
B: MeOH, 5mM ammonium acetate solution
Samples (GRADIENT ELUTION)
A% B% Flow (µl/min)
Initial 70 30 0.48
1.00 70 30
2.00 40 60
3.11 15 85
4.90 15 85
4.91 0 100
5.51 0 100
6.5 70 30
Stop Time (mins) 6.5
Max Pressure (psi) 3500
Oven Temperature (°C) 55.0
Injection Volume(µl) 0.15

HPLC Experimental Parameters

Instrument: Agilent 1100 HPLC system
Column: Thermo Hypersil Gold C18 3µm (1.14 mm x 100mm)
Agilent LC Pump Initial Conditions
A: H2O/MeOH 95/5 v/v, 5mM ammonium acetate solution
B: MeOH, 5mM ammonium acetate solution
Samples (GRADIENT ELUTION)
A% B% Flow (µl/min)
Initial 70 30 0.5
1.50 70 30
2.00 40 60
3.00 40 60
22.50 15 85
34.00 15 85
35.00 0 100
36.00 0 100
37.00 70 30
40.00 70 30
Stop Time (mins) 40.00
Max Pressure (psi) 15000
Oven Temperature (°C) 55.0
Injection Volume(µl) 10

Sample-batch run times of ca. 2.5 hours for UPLC are typical compared to ca. 20 hours for conventional HPLC.

● Reduces instrument time and increases efficiency
● Reduction in solvent usage

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

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● Reduction in solvent usage

Summary

It is highly likely that the spread/trad of 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 techniques such as that described will be a cornerstone in meeting this challenge.

Acknowledgements

This study was funded by the Scottish Government. We thank Graham Mitchell of SASA for his assistance with sample processing and Pesticide Residues Committee for access to the sample batches and their valuable comments.

Results for Rapid Analysis of Fungicides in Cabbage

The reporting limit for the majority of pesticides is 0.2mg/kg. A limit of quantification of at least 0.1mg/kg must be achieved.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>azoxystrobin</td>
<td>0.21</td>
<td>0.18</td>
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<tr>
<td>fenbuconazole</td>
<td>0.15</td>
<td>0.14</td>
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<td>iprovalicarb</td>
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<td>myclobutanil</td>
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<tr>
<td>cyproconazole</td>
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<td>0.21</td>
</tr>
<tr>
<td>pyrimethanil</td>
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<td>0.19</td>
</tr>
<tr>
<td>azoxystrobin</td>
<td>0.20</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Results shown are the mean of two replicates, each spiked at 200% of the MRL.

References


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