The rapid analysis of fungicide residues in crude extracts of fruit and vegetables using UPLC-ESI-MS/MS detection

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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)w. 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 analysis. The number of pesticides to be analysed is also increasing (eg from 125 in 2006 to 200 in 2007 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 screened as part of the PRC 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 presented for LC-MSMS analysis (103 analytes sought).
The remainder is passed through a clean-up stage using gel permeation chromatography prior to analysis by GC-MSMS (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 this system to achieve higher sensitivity and peak capacity at optimum flow rates.

UPLC Experimental Parameters

UPLC ***Gradient method***

Instrument: Waters Acquity UPLC system
Column: Acquity UPLC BEH C18 **1.7µm** (2.1mm i.d. x 50mm)

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)

A%	70
B%	30

Flow (ml/min) 0.48

Initial	A%	B%
	70	30
mins		
0.52	70	30
0.66	40	60
1.05	40	60
3.31	15	85
4.90	15	85
4.91	0	100
5.5	0	100
5.51	70	30
6.5	70	30

Stop Time (mins)	**6.5**
Min Pressure (psi)	0
Max Pressure (psi)	15000
Oven Temperature (°C)	35.0
Injection Volume(µl)	**3.0µl**

HPLC Experimental Parameters

HPLC ***Gradient method***

Instrument: Agilent 1100 HPLC system
Column: Thermo HPLC BDS Hypersil C18 **3.0µm** (4.6mm i.d. x 100mm)

Agilent LC Pump Initial Conditions
A: H2O/MeOH 95/5 v/v , 5mM ammonium acetate solution
B: MeOH, 5mM ammonium acetate solution

Solvents (GRADIENT ELUTION)

A%	70
B%	30

Flow (ml/min) 0.5

Initial	A%	B%
	70	30
mins		
1.00	70	30
2.00	40	60
5.00	40	60
22.00	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**
Min Pressure (psi)	0
Max Pressure (psi)	5800
Oven Temperature (°C)	35.0
Injection Volume(µl)	**10.0µl**

Typical Mass Spectrometry Experimental Parameters

To utilize chromatographic performance (narrow peaks 1-3s FWHM) common in UPLC the mass spectrometer must be capable of acquiring data at a sufficiently high rate (short dwell times ≤20ms).

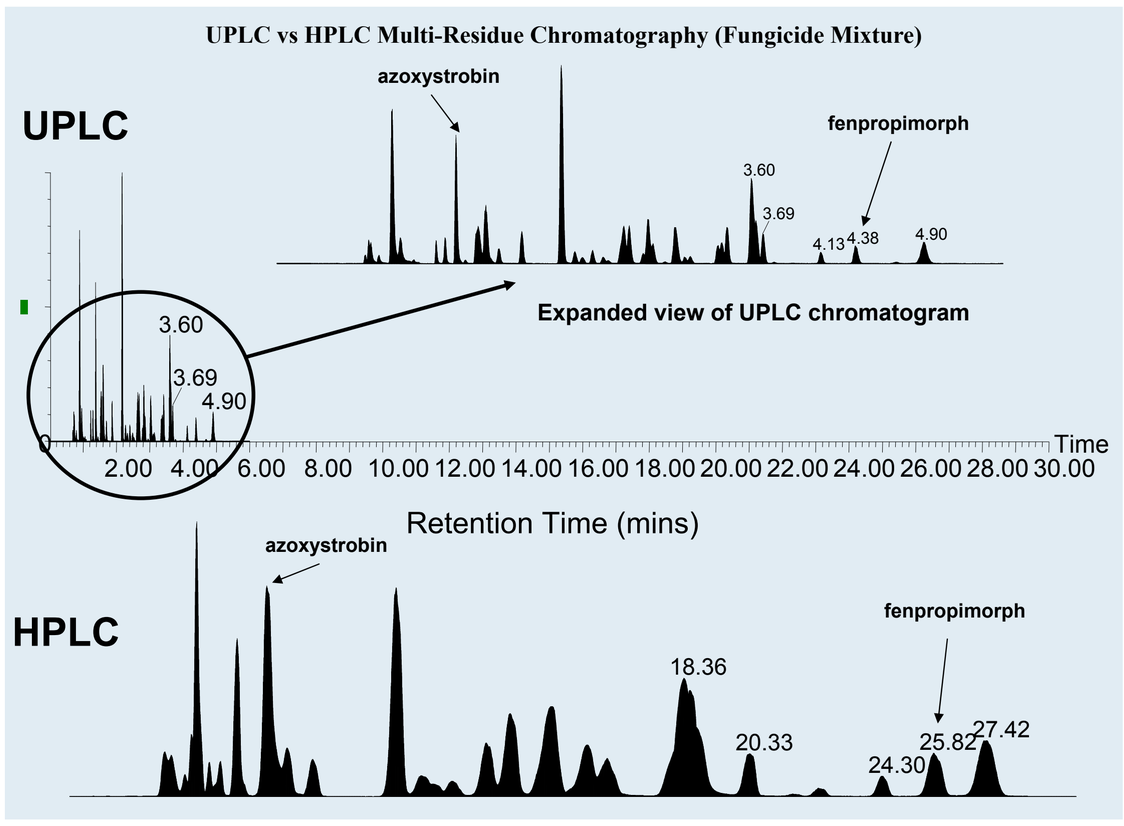
Instrument ***Waters-Micromass Quattro Premier-XE***

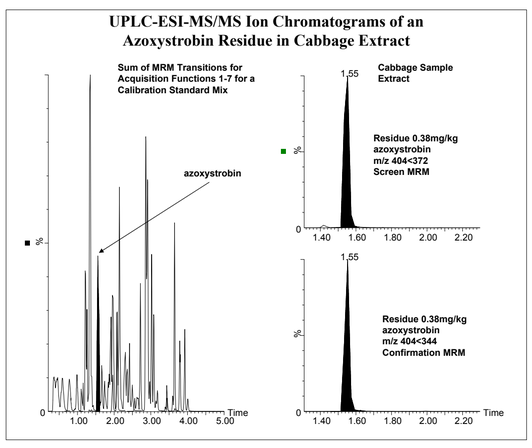
Data System	MassLynx 4.1
Acqusition	Electrospray ± ionisation; Multiple Reaction Monitoring (MRM)
Dwell Times	10-20ms
Collision Gas	Argon

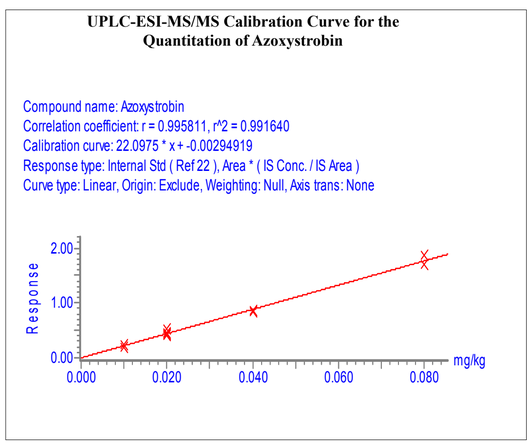
MRM Transitions for Selected Fungicides

ANALYTE	Ret Times mins	Transitions (MRM)	Cone (v)	Collision (ev)	ANALYTE	Ret Times mins	Transitions (MRM)	Cone (v)	Collision (ev)
cymoxanil	0.92	199.1>128	15	10	kresoxim-methyl	2.18	314>222	25	10
thiabendazole	1.12	202>175	30	20	cyprodinil	2.24	226>108	25	25
azoxystrobin	1.52	404>372	21	16	penconazole	2.24	284>159	30	30
diethofencarb	1.55	268.2>226.1	15	10	tebuconazole	2.27	308>70	30	24
pyrimethanil	1.64	200>107	40	28	imazalil	2.27	297>159	32	25
boscalid	1.66	343>307	25	25	zoxamide	2.32	336.2>186.9	25	20
dimethomorph	1.74	388>301	25	15	propiconazole	2.32	342>159	25	30
cyproconazole	1.78	292.3>125	25	30	famoxadone	2.38	392.2>331	15	10
myclobutanil	1.81	289>70	25	20	pyraclostrobin	2.38	388.1>194	25	10
mepanipyrim	1.86	224>106	25	30	phorate	2.41	261>75	15	25
iprovalicarb	1.89	321>119	25	20	hexaconazole	2.41	314.1>158.9	25	25
fenhexamid	1.89	302>97	36	26	prochloraz	2.44	376>308	25	10
pyrifenox	1.93	295.1>93	30	25	bitertanol	2.47	338>269	25	8
fenarimol	1.95	331>268	25	25	pencycuron	2.53	329>218	30	15
epoxiconazole	1.95	330.2>121	20	25	difenoconazole	2.61	406.2>250.9	30	25
tetraconazole	1.96	372>159	25	15	trifloxystrobin	2.70	409>186	20	25
fenbuconazole	2.04	337>125	25	25	spiroxamine	2.81	298.2>143.9	30	22
bupirimate	2.10	317>272	25	25	quinoxyfen	3.10	308>197	20	30
flusilazole	2.10	316>165	20	25	fenpropimorph	3.89	304>147	25	30
picoxystrobin	2.13	368>145	25	20	fludioxonil	1.65	247>180 -ve	40	30
dimoxystrobin	2.16	349>260	25	18					

** coloured segments represent individual MS acquisition functions*







Results for Rapid Analysis of Fungicides in Cabbage

The reporting limit for the majority of pesticides is 0.02mg/kg. A limit of quantitation of at least 0.01mg/kg must be achieved.

SASA Sample No.	Residue	Conc. mg/kg	Reporting Limit (RL) mg/kg	Maximum Residue Level (MRL) mg/kg
CAB 001	boscalid	0.32	0.02	1
CAB 001	pyraclostrobin	0.02	0.02	0.2
CAB 001	difenoconazole	0.03	0.02	no MRL set
CAB 002	tebuconazole	0.07	0.02	0.8
CAB 007	tebuconazole	0.04	0.02	0.8
CAB 017	boscalid	0.06	0.02	1
CAB 017	tebuconazole	0.05	0.02	0.8
*CAB 047	azoxystrobin	0.38	0.02	0.3
CAB 047	difenoconazole	0.14	0.02	no MRL set

* The residue level of azoxystrobin identified in cabbage sample CAB 047 exceeded the MRL.
Under these circumstances the sample is re-extracted and the residue re-quantified before informing the Pesticide Residue Committee.

- A risk assessment is undertaken to determine whether consumers would be at risk from residues present
- The supplier is told and asked to investigate the cause
- If residues found are a health concern other member states are informed (EU rapid alerts)
- Enforcement monitoring possible

Summary

It is highly likely that the upward 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 techniques 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-batch run times of ca. 2.5 hours for UPLC are typical compared to ca. 20 hours for conventional HPLC.
  No adverse effects on either system stability or chromatographic integrity during or between batch analyses are observed.
- Reduces solvent usage
- Allows rapid confirmation of identity and quantity as required by PRC
- Allows faster response to identified pesticides
- Allows a greater throughput of samples.