voedsel en waren autoriteit - VWA

Food and Consumer Product Safety Authority

Chemistry Laboratory, R&D - Pesticide Analysis Group
Amsterdam, The Netherlands

André de Kok, ***Peter Rensen and Maurice Hiemstra***

"Comparison of HPLC- with UPLC-MS/MS Multiresidue Methods for the Determination of Pesticide Residues: Extension of Scope vs. Shorter Run Times"

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Florida Pesticide Residue Workshop (FPRW2008)
– St. Pete Beach, FL, USA , July 20-23, 2008

OUTLINE

- Status of Multiresidue Methods in Pesticide Analysis
- LC-MS/MS Applications
  - Fruits and Vegetables
  - Soy bean samples
- Expansion of Scope: *Quattro Ultima → Premier XE*
- Faster Run Times: *HPLC-MS/MS → UPLC-MS/MS*
- Conclusion

VWA - Food and Consumer Product Safety Authority

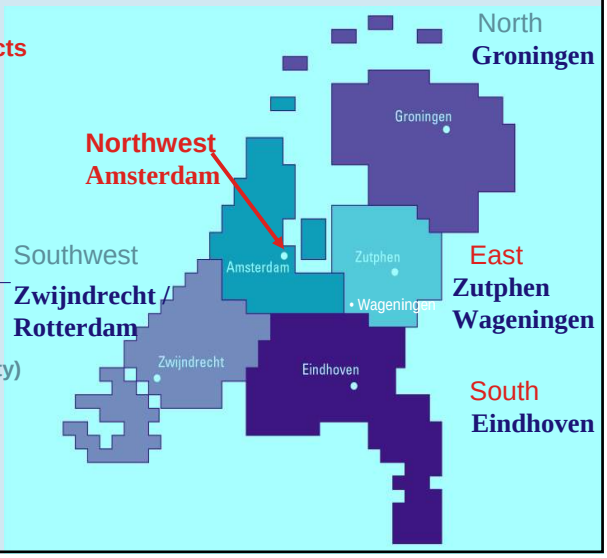
Northwest
- Primary Agricultural Products

East
- Veterinary Products

South
- Composite Food Products

North
- Non-Food Products
  (chemical/microbiological safety)

Southwest
- Non-Food Products
  (mechanical/electrical safety)



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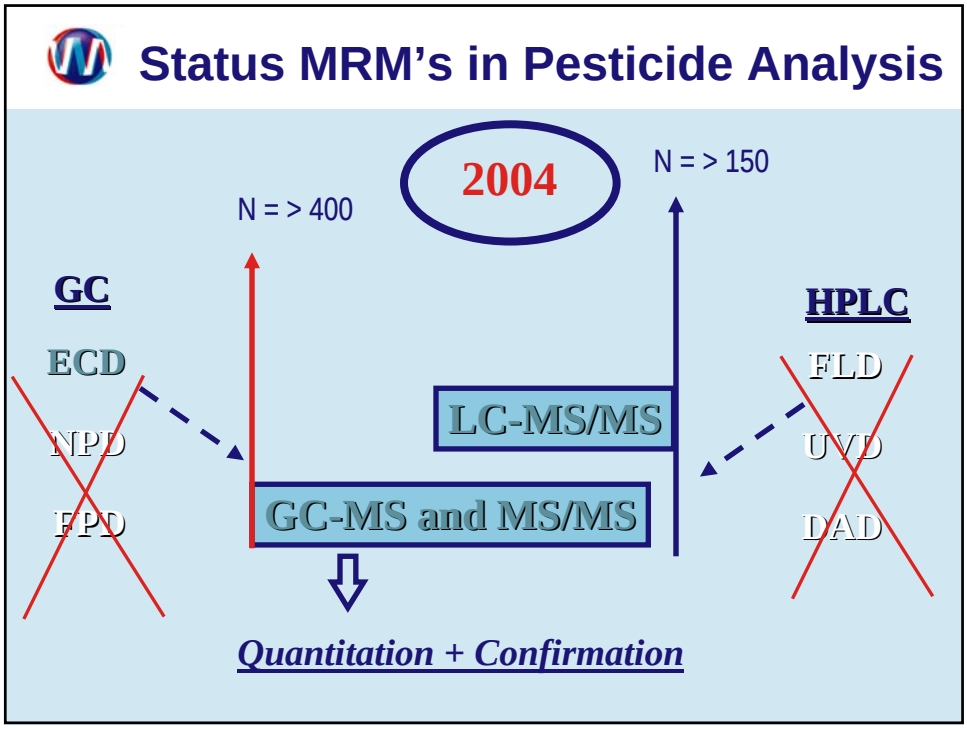
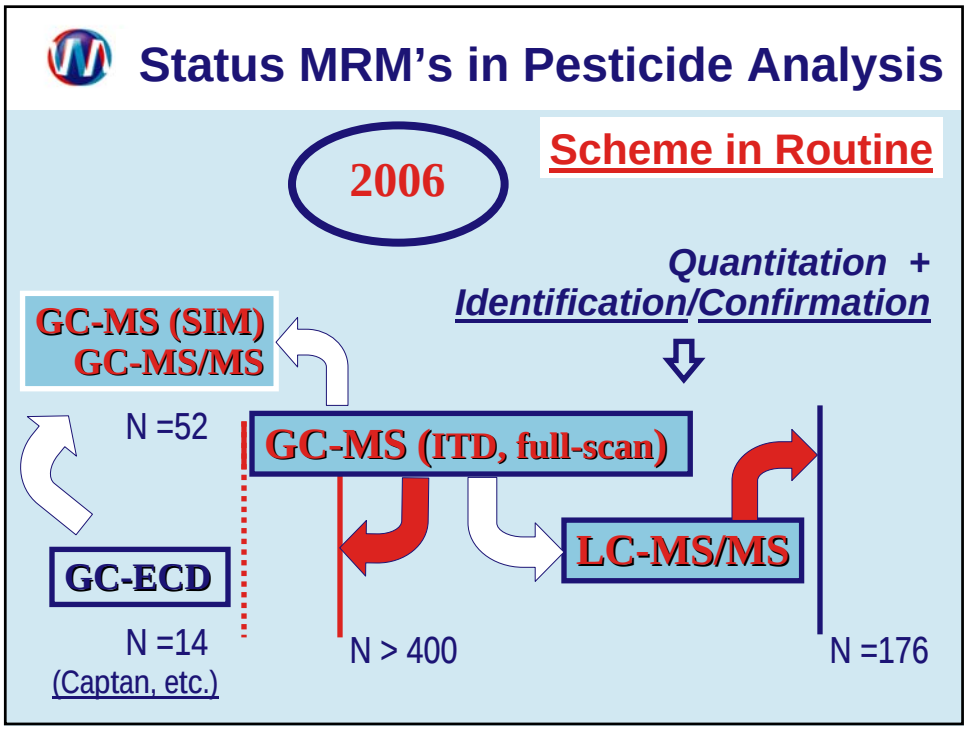
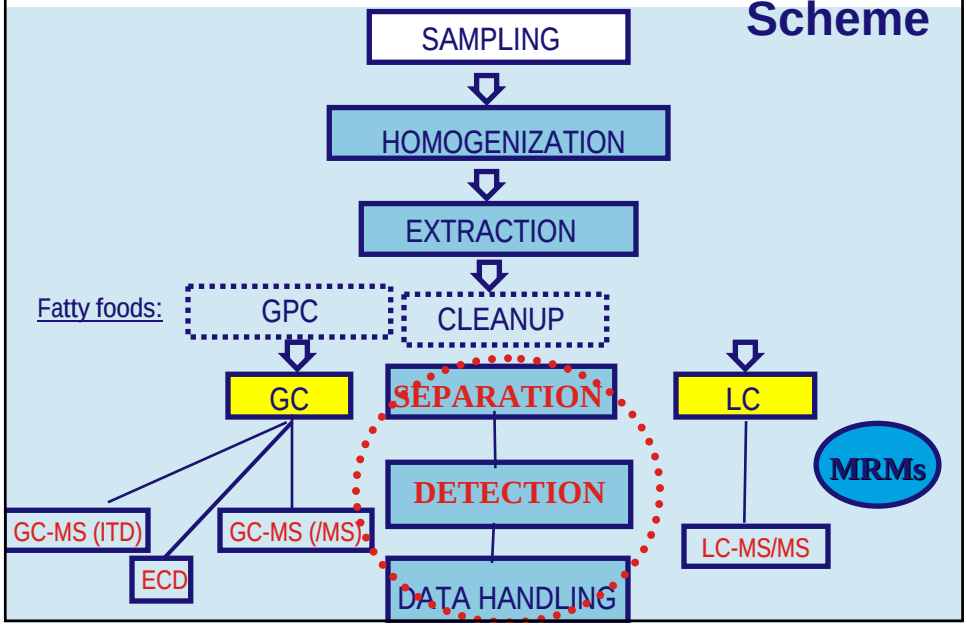
Official Food Control – Pesticides Monitoring/Enforcement Requirements:

- Accredited lab / methods (ISO 17025)
- Quality Assurance / Quality Control (QA/QC)
  - ✓ (Calibration) Standards stability check
  - ✓ Calibration curve check
  - ✓ Recovery check
  - ✓ Detector Response check (RL, drift)
  - ✓ Proficiency Tests
- Up to 1000 pesticides to measure – wide scope: priorities
- Low MRL's → Low Reporting Limits (0.01 mg/Kg)
- High Sample Throughput (24-72 hr throughput time)
- Low-cost, robust methods


Status MRM's in Pesticide Analysis
2004
N = > 400
N = > 150
GC
ECD
NPD
FPD
HPLC
FLD
UVD
DAD
LC-MS/MS
GC-MS and MS/MS
Quantitation + Confirmation

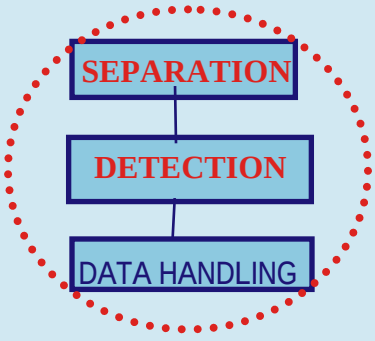

Status MRM's in Pesticide Analysis
2006
Scheme in Routine
Quantitation + Identification/Confirmation
GC-MS (SIM)
GC-MS/MS
N =52
GC-MS (ITD, full-scan)
LC-MS/MS
GC-ECD
N =14
(Captan, etc.)
N > 400
N =176

VWA-Amsterdam MRM Scheme



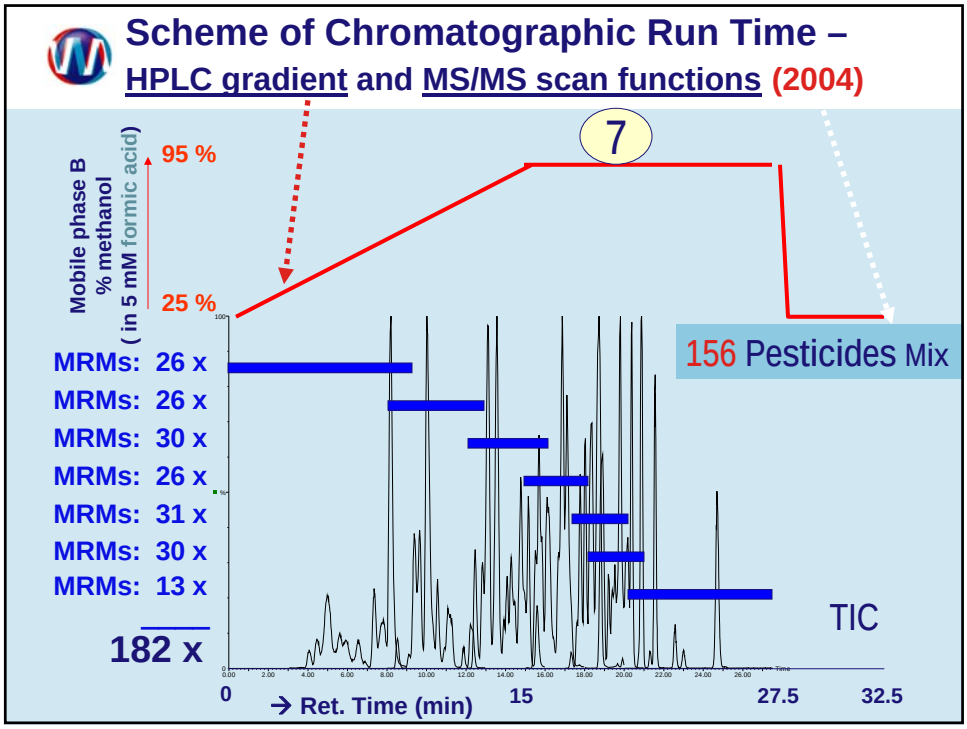
Status MRM's in Pesticide Analysis

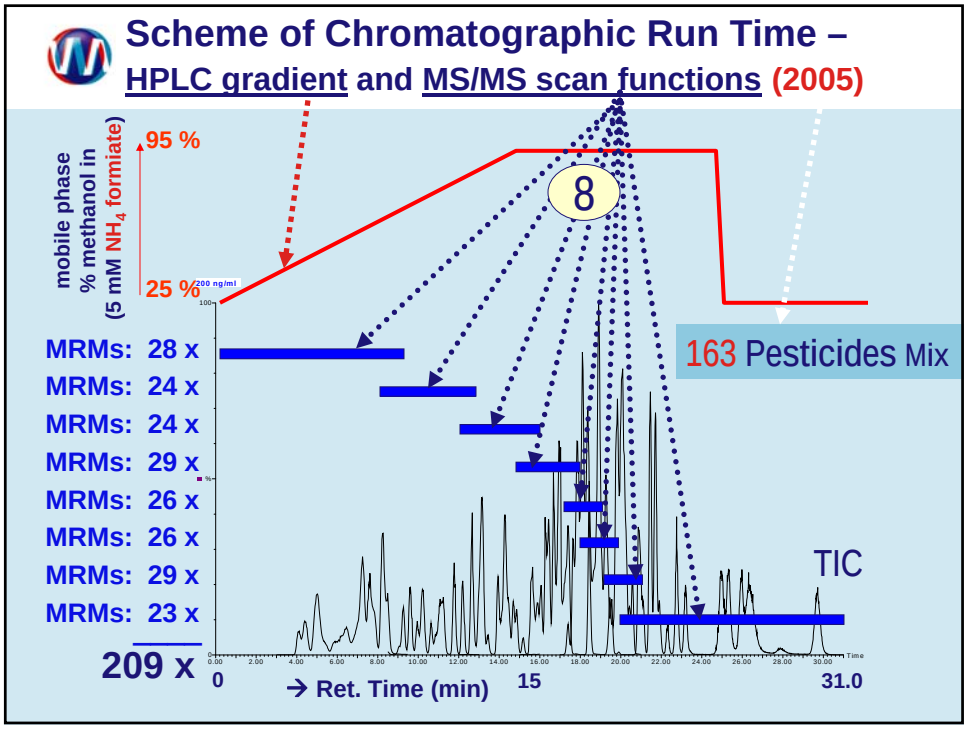
LC-MS/MS



Chromatographic Run Time

	1 sample	1 series (30 samples)
	32 min. (ESI^+)	24 hours
	10 min. (ESI^-)	7.5 hours


Scheme of Chromatographic Run Time –
HPLC gradient and MS/MS scan functions (2004)
Mobile phase B
% methanol
(in 5 mM formic acid)
95 %
25 %
7
MRMs: 26 x
MRMs: 26 x
MRMs: 30 x
MRMs: 26 x
MRMs: 31 x
MRMs: 30 x
MRMs: 13 x
182 x
156 Pesticides Mix
TIC
0
→ Ret. Time (min)
15
27.5
32.5


Scheme of Chromatographic Run Time –
HPLC gradient and MS/MS scan functions (2005)
mobile phase
% methanol in
(5 mM NH4 formiate)
95 %
25 %
8
MRMs: 28 x
MRMs: 24 x
MRMs: 24 x
MRMs: 29 x
MRMs: 26 x
MRMs: 26 x
MRMs: 29 x
MRMs: 23 x
209 x
163 Pesticides Mix
TIC
0
→ Ret. Time (min)
15
31.0

MS/MS Scan parameters

Dwell time: 20 msec → 20
Inter-channel delay time: 20 msec → 10

e.g. 32 MRM's / channels (32x40/30) 1280 msec → 960

Interscan delay time: 20 msec → 10

Duty cycle time: 1300 msec → 970
(2004) ---------- →(2005)

20 (25) data points per HPLC peak
[10 (13) for 2 overlapping scan functions]

Expansion of Scope: *Quattro Ultima → Premier XE*

Limitations:
Acquisition Time: ~ 30 min.
Maximum of 32 "channels" /Scan function
Total number of MS/MS transitions: ~ 200 – 220

Optional Improvements:
1. *Quattro Ultima → Premier XE:*
   Dwell time: *20 msec → 10 (→ 5 ?)*
   Inter-channel delay time: *10 msec → 5 ?*

   Sum: 20 (or 15?)

Number of MS/MS transitions: ~ 300 – 330 (or 400 – 440)
3 or 4 overlapping scan functions possible

Scheme of Chromatographic Run Time –
HPLC gradient and MS/MS scan functions (2006)

Quattro Ultima → *Premier XE*

9 15

172 Pesticides Mix

Number of transitions (1st)	: 172	172
Number of transitions (2nd)	: 72	172
Total	: 244	344

15

Number of transitions (1st)	:	220
Number of transitions (2nd)	:	220
Total	:	440

>220 Pesticides

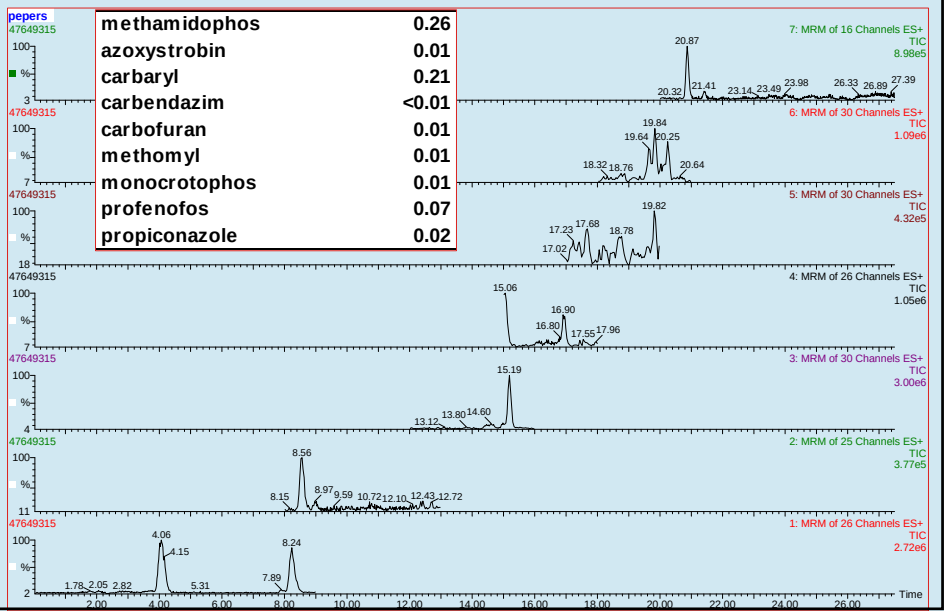
HPLC → UPLC

HPLC-MS/MS: WATERS Quattro Ultima Triple Quad

Pesticide Residues (conc. in mg/kg) in Red hot chili pepper Sample

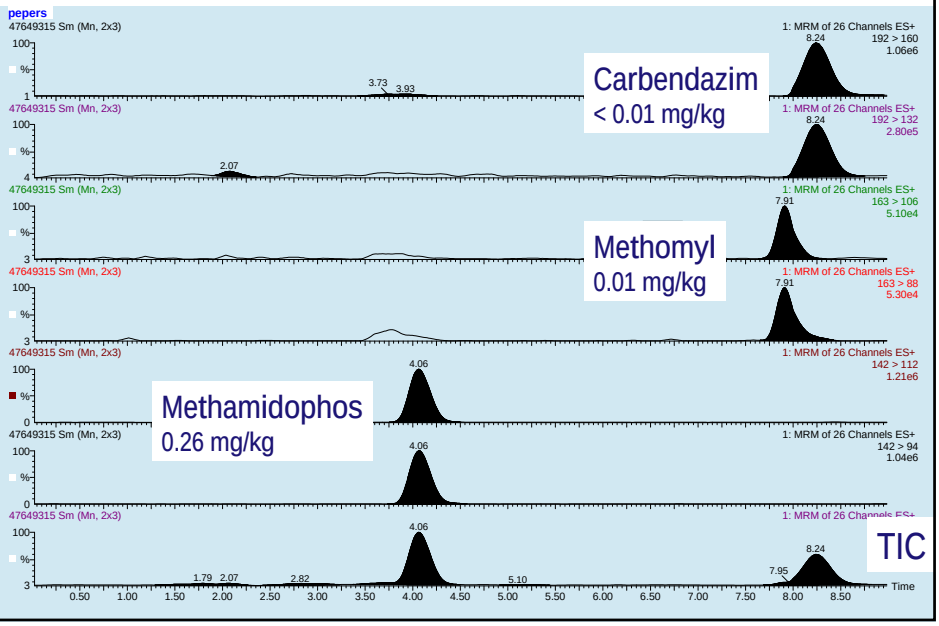
TICs of 7 Scan Functions

methamidophos	0.26
azoxystrobin	0.01
carbaryl	0.21
carbendazim	<0.01
carbofuran	0.01
methomyl	0.01
monocrotophos	0.01
profenofos	0.07
propiconazole	0.02

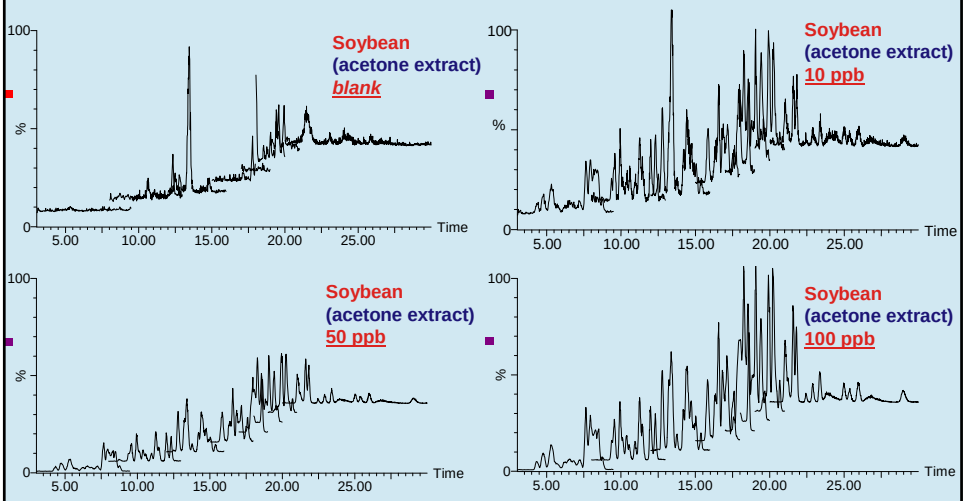


Red hot chili pepper Sample

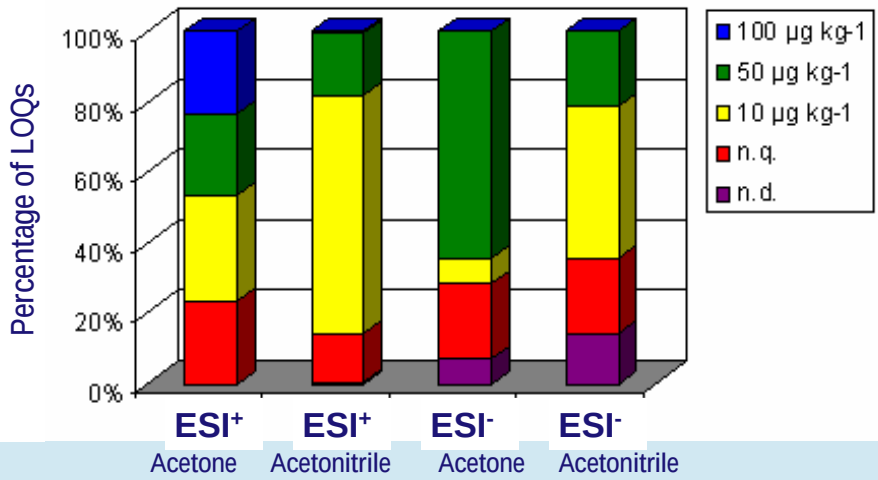
MRM's



Total ion chromatograms (LC-MS/MS, ESI+) of Soybean Sample, spiked with 155 pesticides at 10, 50 and 100 ppb level



LC-MS/MS of Soybean Sample, 155 (ESI+) + 14 (ESI-) Pesticides LOQ's of 10, 50 or 100 ppb level



HPLC conditions

HPLC → UPLC

Analytical column:	Alltima C_{18},	5 µm, 150 x 3.2 mm	
Column temp.:	30 °C		
Mobile phase flow:	0.30 mL.min^{-1}		
Injection volume:	5 µL (Std. In 100% MeOH)		
Solvent A:	5 mM $HCOONH_4$ in water		
Solvent B:	Methanol		
Gradient profile:	0 min	75% A	25% B
	15 min	5% A	95% B
	25 min	5% A	95% B
	25.1 min	75% A	25% B
Acquisition time:	31.0 min		
Total run time:	32.5 min		

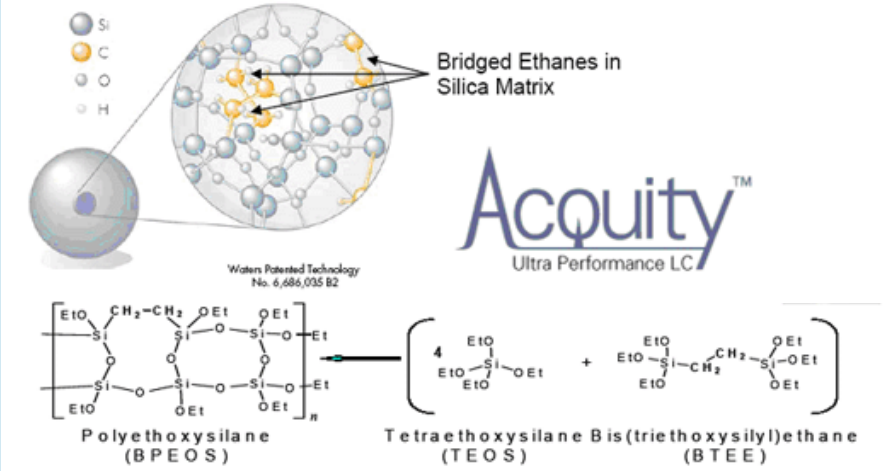
UPLC conditions

Analytical column:	Acquity UPLC BEH C_{18}, 1.7 µm, 150 x 2.1 mm		
Column temp.:	65 °C		
Mobile phase flow:	0.30 mL.min^{-1}		
Injection volume:	2 µL (Std. In 100% MeOH)		
Solvent A:	5 mM $HCOONH_4$ in water		
Solvent B:	Methanol		
Gradient profile:	0 min	75% A	25% B
	15 min	5% A	95% B
	25 min	5% A	95% B
	25.1 min	75% A	25% B
Acquisition time:	31.0 min		
Total run time:	32.5 min		

UPLC-MS/MS:
WATERS Acquity UPLC - Quattro Premier Triple Quad

HPLC → UPLC

New Stationary Phase for UPLC



Van Deemter Equation

A term + B term + C term

$$H = a(dp) + \frac{b}{u} + c(dp)^2u$$

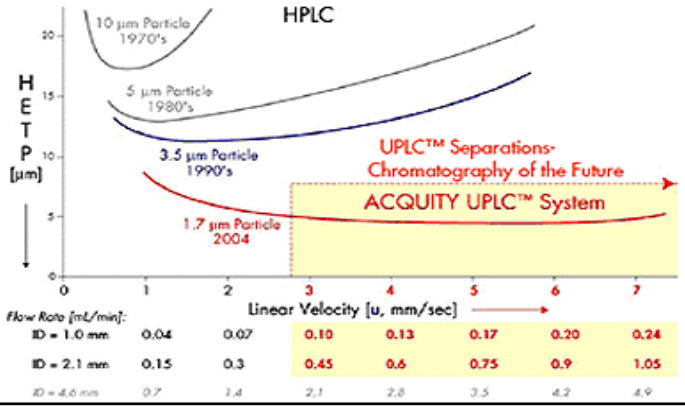
C term is impacted by Both

U = Linear Velocity dp = Particle Size

A term Eddy Diffusion/ Interparticle channels. It is particle size dependant

B term Molecular Diffusion (Axially). It is inversely proportional to velocity and insignificant in LC

C term Mass Transfer Kinetics. It is directly proportional to velocity, and the particle size squared.

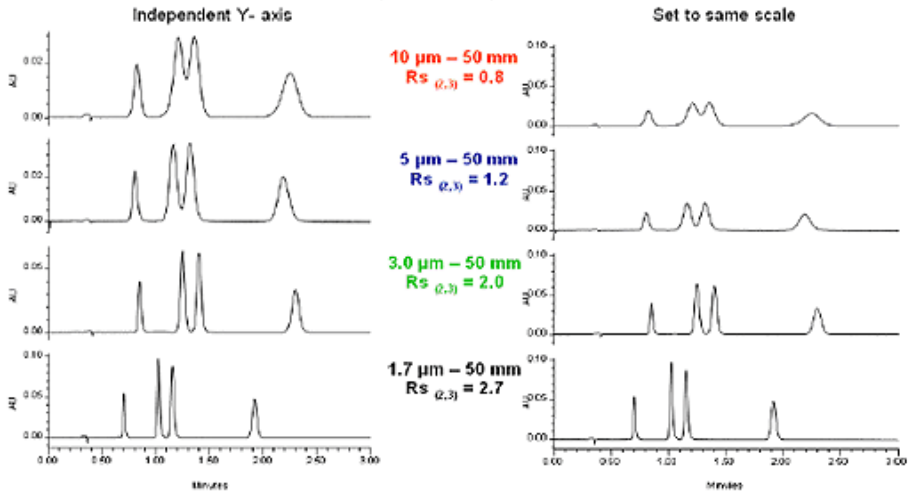


$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k}{k+1}\right)$$

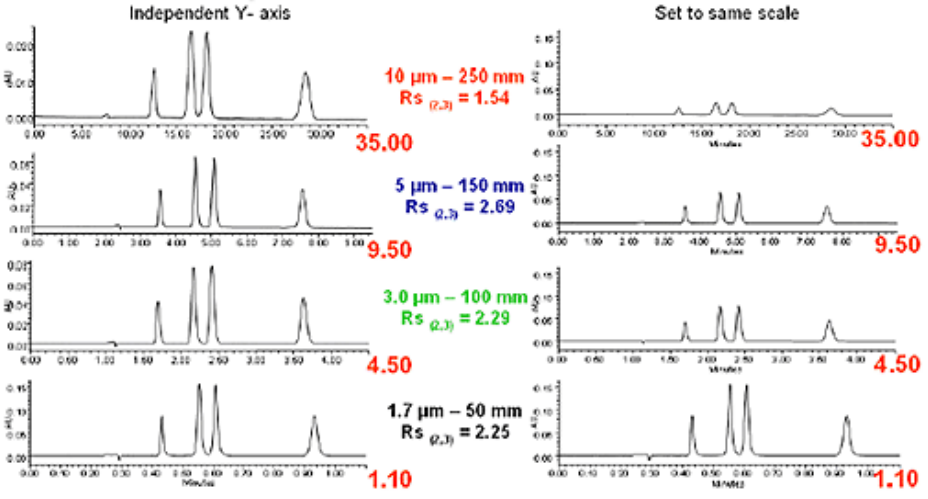
System Efficiency Selectivity Retentivity

If N ↑ 3X, Rs ↑ 1.7X

Keeping Column Length the Same



N = 1X, Rs = 1X
dp ↓ 3X, L ↓ 3X, , F ↑ 3X, T ↓ 9X
Sensitivity ↑ 3X



HPLC → UPLC

Quattro Ultima → Premier XE

Objectives

- **1 Extention of the scope of the routine LC-MS/MS multiresidue method**
- **2 At least two transitions per pesticide for confirmation**
- **3 Reduction of analytical run time by a factor of two**

Tuning

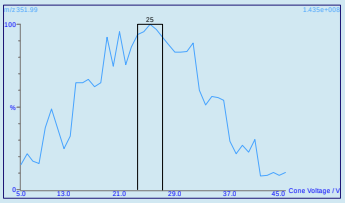
Quattro Premier XE

Optimisation of MS/MS parameters for 235 pesticides

- 3 MS/MS transitions / pesticide
- cone-voltage and collision energy
- QuanOptimize feature of Masslynx software
- Individual pesticides: new autotune wizard

Optimisation

example : Chlorpyrifos



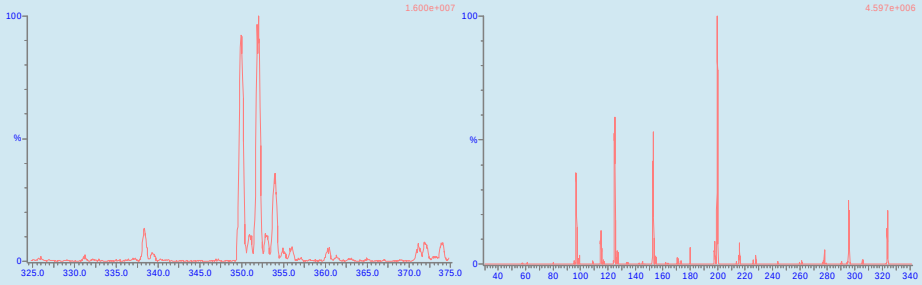
Cone voltage

Collision Energy

Tuning

Quattro Premier XE

Mass spectrum



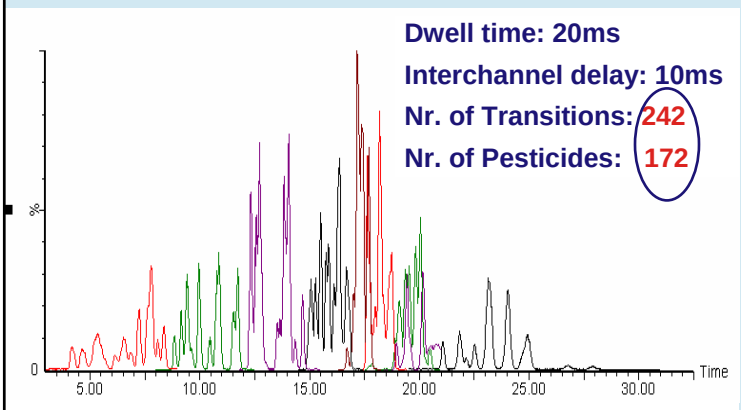
Optimisation Parent ion

Optimisation Daughter ion

Influence of Dwell time and Inter-channel delay time on Repeatability (% RSD_r)

Pesticide	Quattro Premier			Quattro Ultima
	dwell-time/interchannel delay (ms)			
	5 / 10	10 / 10	10 / 5	20 / 10
	% RSD_r	% RSD_r	% RSD_r	% RSD_r
methamidophos	6.4	3.5	14.6	3.3
azinphos-methyl	9.5	6.0	15.6	8.8
azoxystrobin	1.6	2.6	5.7	6.3
carbendazim	1.7	6.2	14.0	5.5
cyproconazole	8.8	7.8	15.8	15.2
dimethoate	2.5	3.7	15.2	7.1
omethoate	2.3	3.0	16.4	6.4
dimethomorph	9.5	4.1	13.1	5.1
fenarimol	13.1	8.3	20.6	13.0
fenhexamid	14.4	9.6	20.9	9.1
fenthion sulfoxide	5.0	2.7	21.9	7.2
imazalil	3.8	3.1	15.5	11.7
imidacloprid	9.1	6.4	16.3	15.9
indoxacarb	21.1	9.5	18.2	10.4
linuron	10.3	3.4	10.8	5.1
methomyl	3.8	4.3	12.7	9.4
methoxyfenozide	4.5	3.3	16.9	11.4
monocrotophos	4.8	3.2	18.5	3.6
oxamyl	8.9	5.3	28.3	4.4
prochloraz	6.0	4.4	8.1	9.5
thiabendazole	2.1	8.3	17.6	4.0
tolylfluanid	7.2	4.5	28.0	8.6
triforine	8.8	13.8	17.3	4.6
hexythiazox	7.8	6.8	6.6	15.7

Routine HPLC Method on Premier XE



Dwell time: 20ms

Interchannel delay: 10ms

Nr. of Transitions: 242

Nr. of Pesticides: 172

Column: Alltima C_{18}; 150mm x 3.2mm; 5µm

Flow rate: 0.3 ml/min

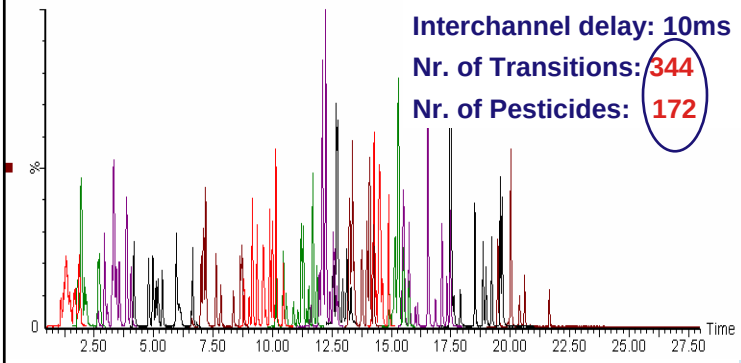
Temperature: 30°C

Repeatability
Level 10 ng/ml

Pesticide	RSDr (%)
methamidophos	1.5
azinphos-methyl	6.7
azoxystrobin	2.7
carbendazim	4.9
cyproconazole	3.0
dimethoate	3.2
omethoate	1.8
dimethomorph	3.6
fenarimol	11.7
fenhexamid	5.2
fenthion sulfoxide	4.7
imazalil	3.5
imidacloprid	11.0
indoxacarb	11.1
linuron	4.1
methomyl	3.9
methoxyfenozide	4.7
monocrotophos	5.6
oxamyl	3.5
prochloraz	3.6
spinosad A/D	*
thiabendazole	3.4
tolylfluanid	4.3
triforine	6.5
hexythiazox	5.3

UPLC- Method on Premier XE

2 transitions / pesticide



Dwell time: 10ms

Interchannel delay: 10ms

Nr. of Transitions: 344

Nr. of Pesticides: 172

Column: Acquity UPLC™ BEH C_{18}, 2.1 x 150mm, 1.7µm

Flow rate: 0.45 ml/min

Temperature: 65°C

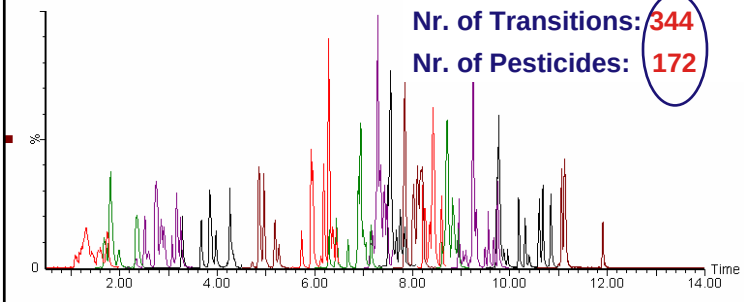
Repeatability
Level 10 ng/ml

Pesticide	RSDr (%)
methamidophos	5.5
azinphos-methyl	3.8
azoxystrobin	3.8
carbendazim	3.8
cyproconazole	6.4
dimethoate	5.1
omethoate	7.1
dimethomorph	4.9
fenarimol	11.0
fenhexamid	8.7
fenthion sulfoxide	4.7
imazalil	7.2
imidacloprid	4.8
indoxacarb	4.8
linuron	7.9
methomyl	9.8
methoxyfenozide	5.4
monocrotophos	9.3
oxamyl	25.0
prochloraz	5.2
spinosad A/D	2.9
thiabendazole	4.8
tolylfluanid	8.5
triforine	28.4
hexythiazox	8.0

UPLC- Method on Premier XE

Total Run time: 16 min. !!!

2 transitions / pesticide



Dwell time: 10ms

Interchannel delay: 10ms

Nr. of Transitions: 344

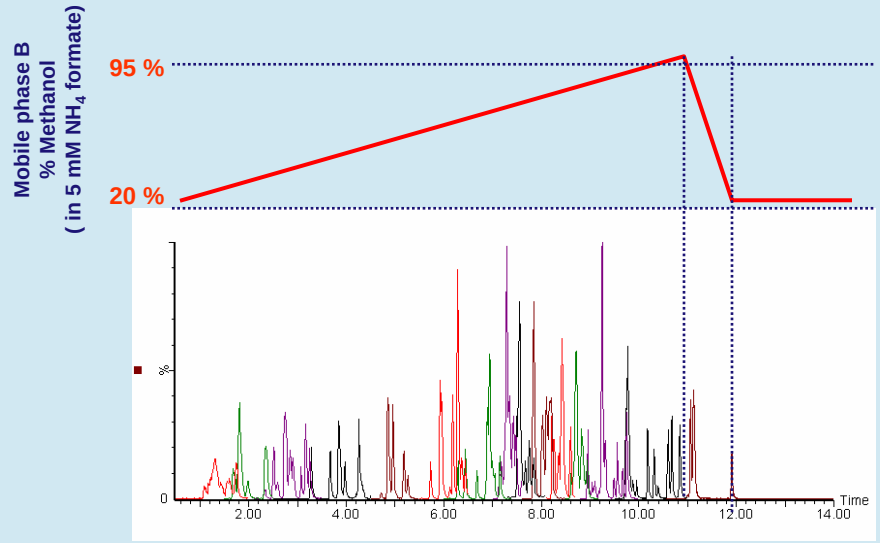
Nr. of Pesticides: 172

Column: Acquity UPLC™ BEH C_{18}, 2.1 x 150mm, 1.7µm

Flow rate: 0.45 ml/min

Temperature: 65°C

Scheme of Chromatographic Run Time – HPLC gradient (2006)



UPLC-MS/MS (Premier XE) conditions

Experimental UPLC-Method, 16 min.-Run time	
Column:	Acquity UPLC BEH C18, 2.1 x 150mm, 1.7µm
Temp. Column:	65°C
Mobile phase:	A: 5 mM Ammonium Formiate
	B: Methanol

Gradient:

Time (min)	A %	B %
0	80	20
11	5	95
12	80	20
16	80	20

Run time: 16 min

Flow rate:	0.45 ml.min-1
Injection volume	2 µl
Detection:	Waters, Quattro Premier, ESI positive mode
	15 functions, dwell time 10 ms
Capillary voltage	1 kV
Cone voltage:	dependable
Collision energy:	5 - 50 eV

Time-scheduled Multiple Reaction Monitoring

Function	Time (min)	Transitions	Max.
1	0.5 - 2.0	24	32
2	1.5 - 2.5	24	32
3	2.1 - 3.5	24	32
4	2.5 - 4.5	24	32
5	4.5 - 5.5	24	32
6	5.5 - 6.5	24	32
7	6.0 - 7.4	24	32
8	7.0 - 7.6	24	32
9	7.3 - 8.1	24	32
10	7.6 - 8.5	24	32
11	8.1 - 8.7	24	32
12	8.5 - 9.2	24	32
13	8.8 - 10.0	24	32
14	9.5 - 11.0	24	32
15	10.5 - 14	8	32
	Total Transitions:	344	480
	Nr. of pesticides:	172	240

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CONCLUSIONS

UPLC-MS/MS

- expands scope
- increases sensitivity
- decreases run time, maintaining resolution
- increases sample throughput

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Waters

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Sandra Rontree, Waters

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