



voedsel en waren autoriteit - **VWA**

Food and Consumer Product Safety Authority
Chemistry Laboratory, R&D - Pesticide Analysis Group,
National Reference Laboratory (NRL) – Pesticides/Mycotoxines
Amsterdam, The Netherlands

André de Kok, Peter Rensen and Maurice Hiemstra

**Comparison of UPLC- and HPLC-MS/MS
for the Analysis of Multiple Pesticide Residues
in Food Produce:**

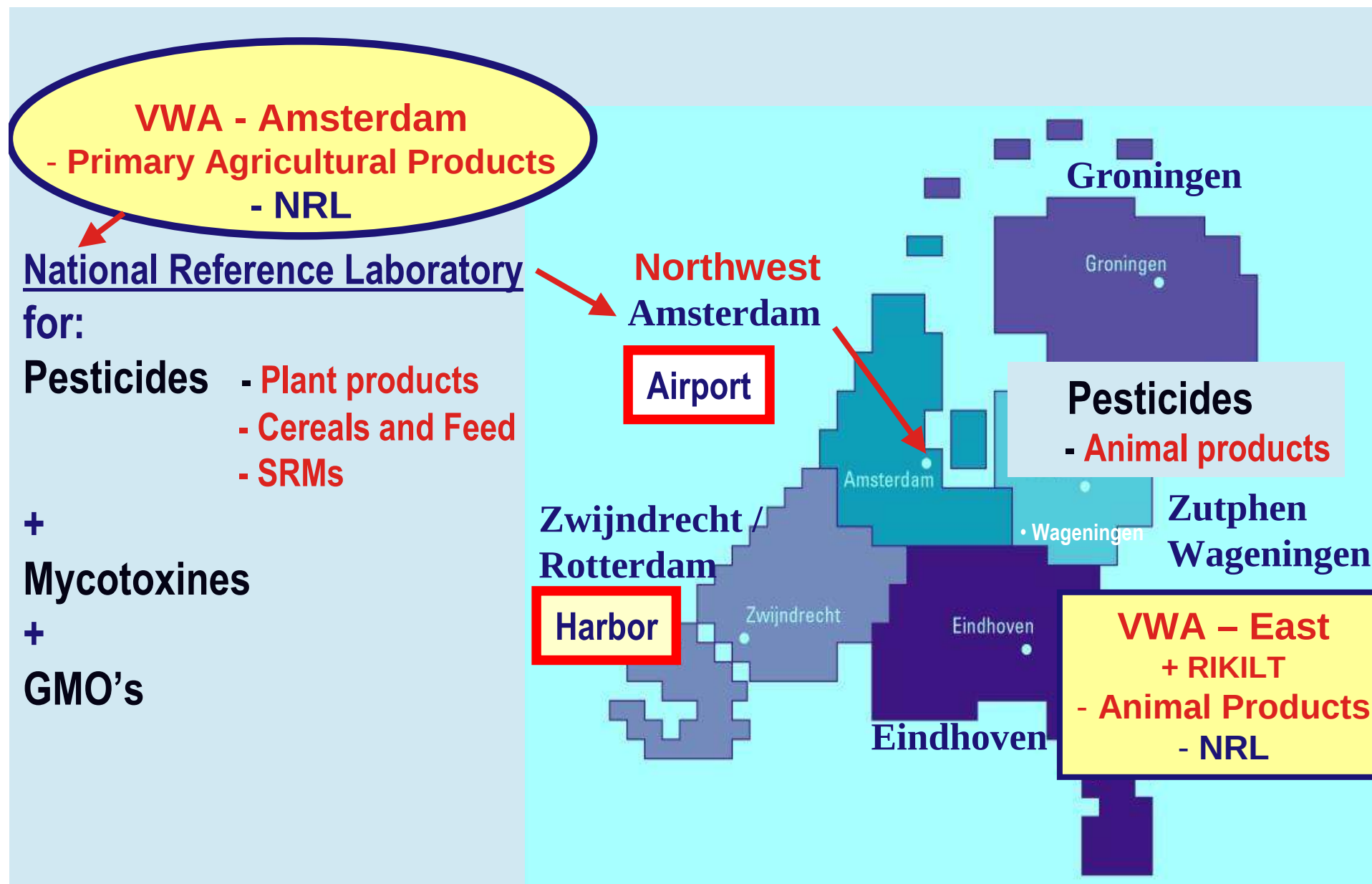
“Extension of Scope vs. Shorter Run Times”

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CRL-Workshop on Pesticide Residues, Stuttgart, Germany, 6-7 Dec. 2006



VWA - Food and Consumer Product Safety Authority





OUTLINE

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



Official Food Control Lab

Pesticides Monitoring/Enforcement Requirements:

❖ Accreditation of laboratory (ISO 17025) – EU Food and Feed Law

- Validated and Accredited methods

(Collaborative Studies ↔ In-house Validation)

- Quality Assurance / Quality Control (QA/QC)

- ✓ (Calibration) Standards stability check

- ✓ Calibration curve check

- ✓ Recovery check

- ✓ Detector Response check (RL, drift)

- Proficiency Tests (EU-PTs)



Official Food Control Lab

Pesticides Monitoring/Enforcement Requirements:

- Up to **1000 pesticides** to measure – wide scope → priorities
- Low MRL's (Baby food Directive, Organically-grown products, EU-forbidden pesticides)
 - Low Reporting Limits (**0.01 mg/kg**)
- High Sample Throughput (**2 weeks → 24-72 hr throughput time**)
 - **Low-cost, efficient, robust methods**



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Multi Residue Methods Schemes and Strategies

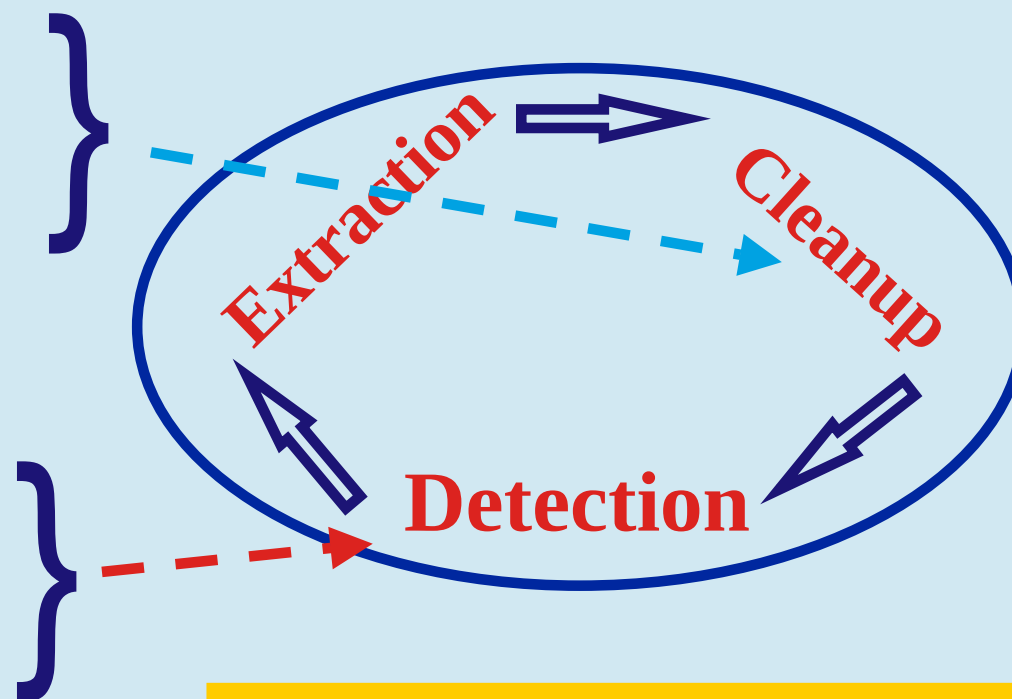
Selection Criteria

SPEED

ROBUSTNESS

SENSITIVITY

SELECTIVITY



→ COST-EFFICIENCY



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Multi Residue Methods Schemes and Strategies

2006

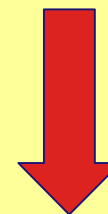
3500 Samples / Year

- Fruits and Vegetables
- Cereals
- Processed foods
- Juices and wines
- Baby foods

2007 (Planning):

4500 Samples / Year

~ 7 FTE's



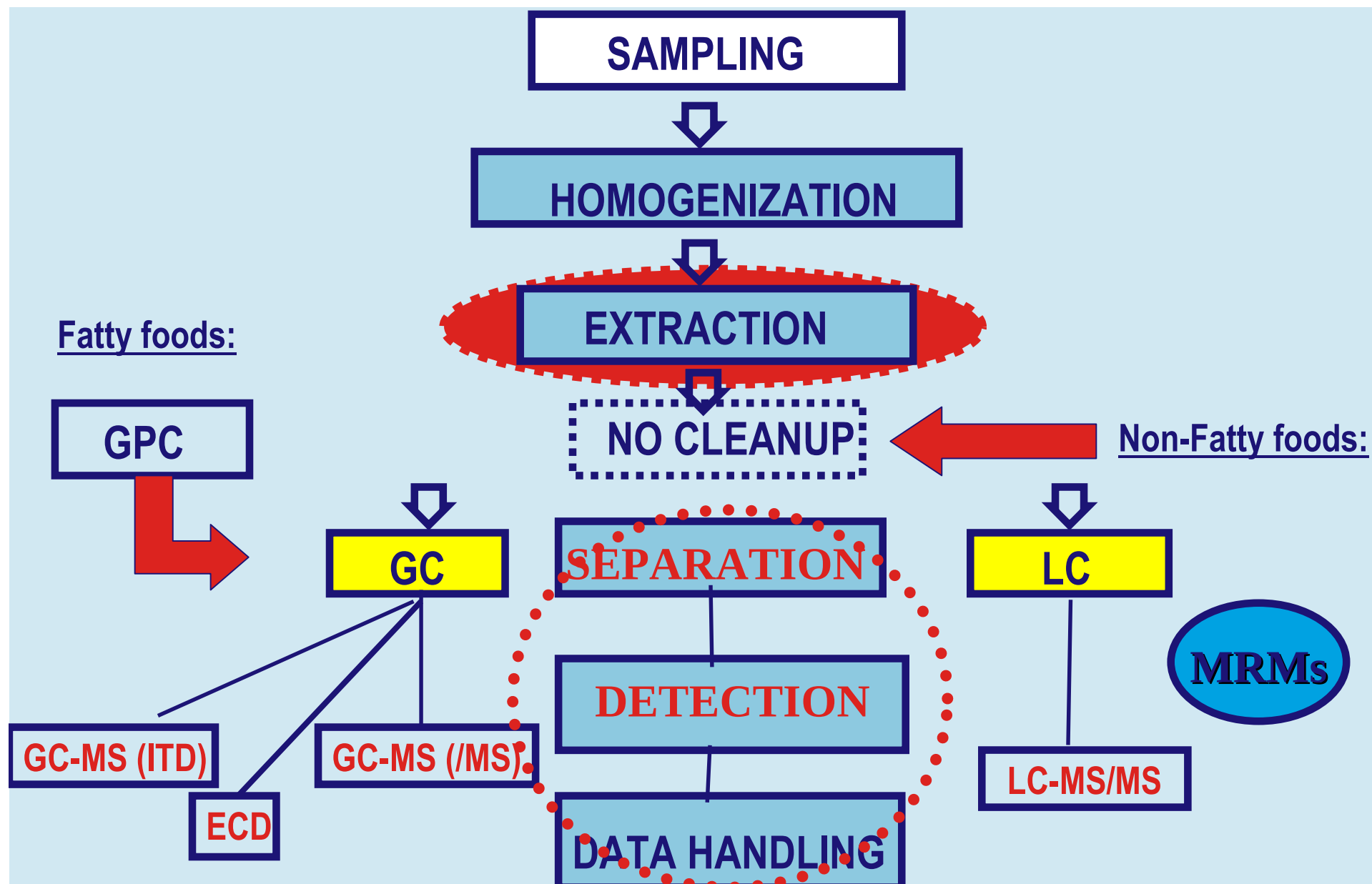
~ € 200.- / sample

GC-MS + LC-MS/MS + GC-ECD
30% GC-MS-Dithiocarbamates
(15% 2nd analyses ~ > MRL)

→ COST-EFFICIENCY



VWA-Amsterdam MRM Scheme





EXTRACTION METHOD



Fast Acetone Extraction / CH_2Cl_2 + PE Partitioning (with or without Na_2SO_4)

15 (or 7.5 g) sample in 250-ml centrifuge tube

+ 30 ml acetone

- mix 20 sec. (Polytron homogeniser)

+ 30 ml CH_2Cl_2 + 30 ml PE (+ 7.5 g Na_2SO_4) (Partitioning)

- mix 20 sec. (Polytron homogeniser)

- centrifuge 4 min. at 3500 rpm

- decant supernatant in 100-ml erlenmeyer flask

- take aliquots for multiresidue methods, no cleanup !

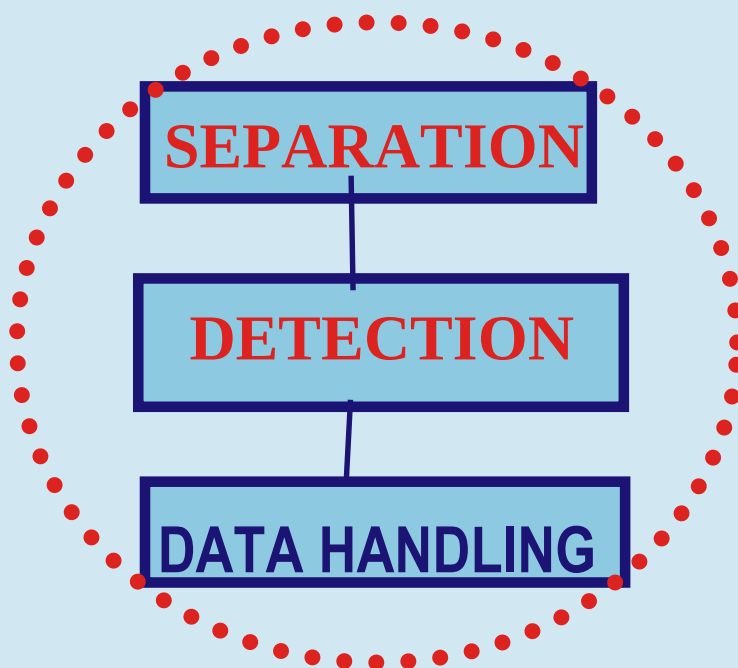
e.g. : LC-MS/MS method: take 1- (or 2-)ml aliquot

- evaporate (*batchwise!*) and redissolve in 1 ml MeOH



Status MRM's in Pesticide Analysis

LC-MS/MS



Chromatographic Run Time

1 sample

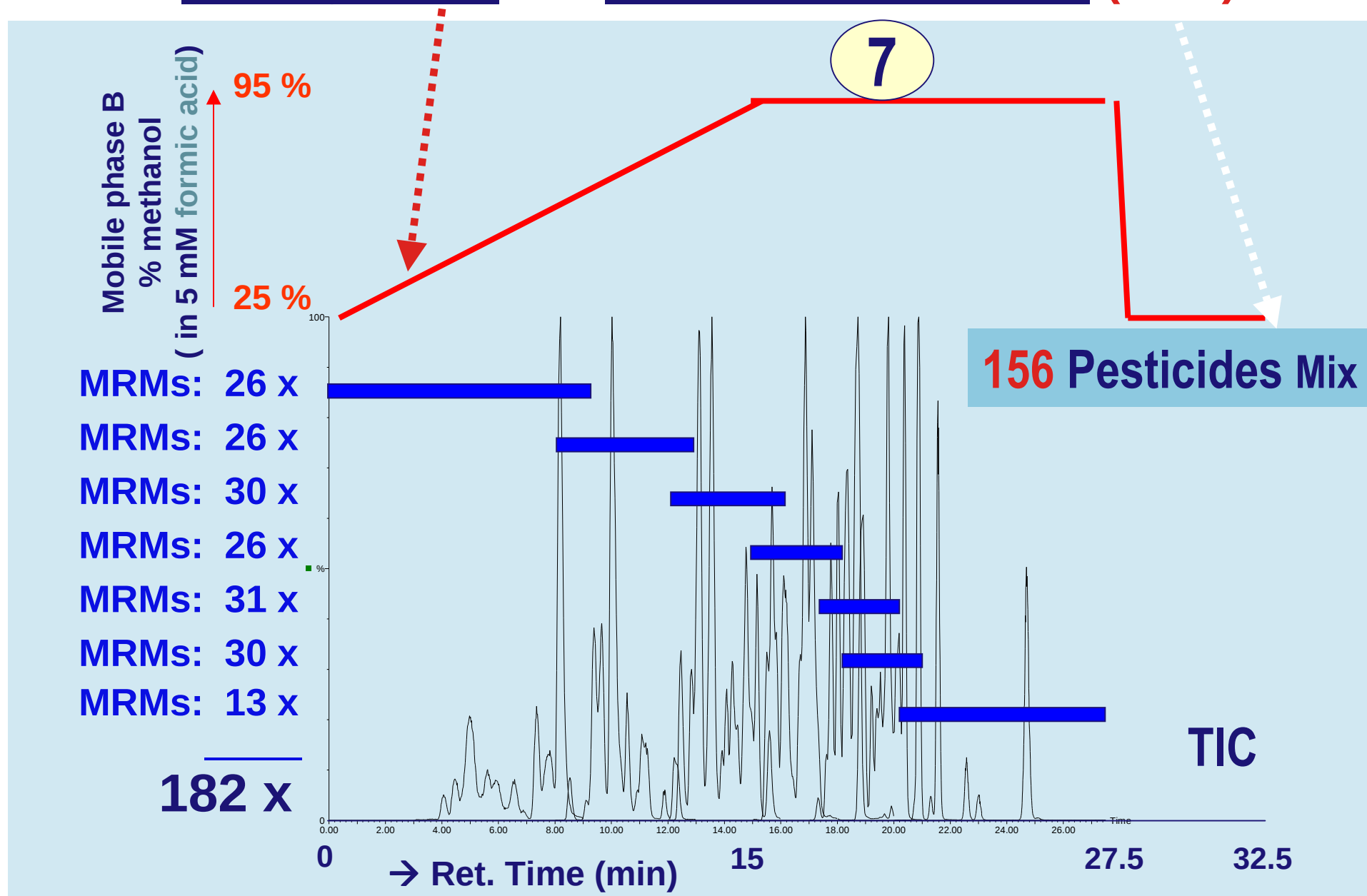
32 min. (ESI⁺)
10 min. (ESI⁻)

1 series

(30 samples)
24 hours
7.5 hours



Scheme of Chromatographic Run Time – HPLC gradient and MS/MS scan functions (2004)





MS/MS Scan parameters

Dwell time: 20 msec → 20

Inter-channel (-MRM) delay time: 20 msec → 10

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

Interscan delay time: 20 msec → 10

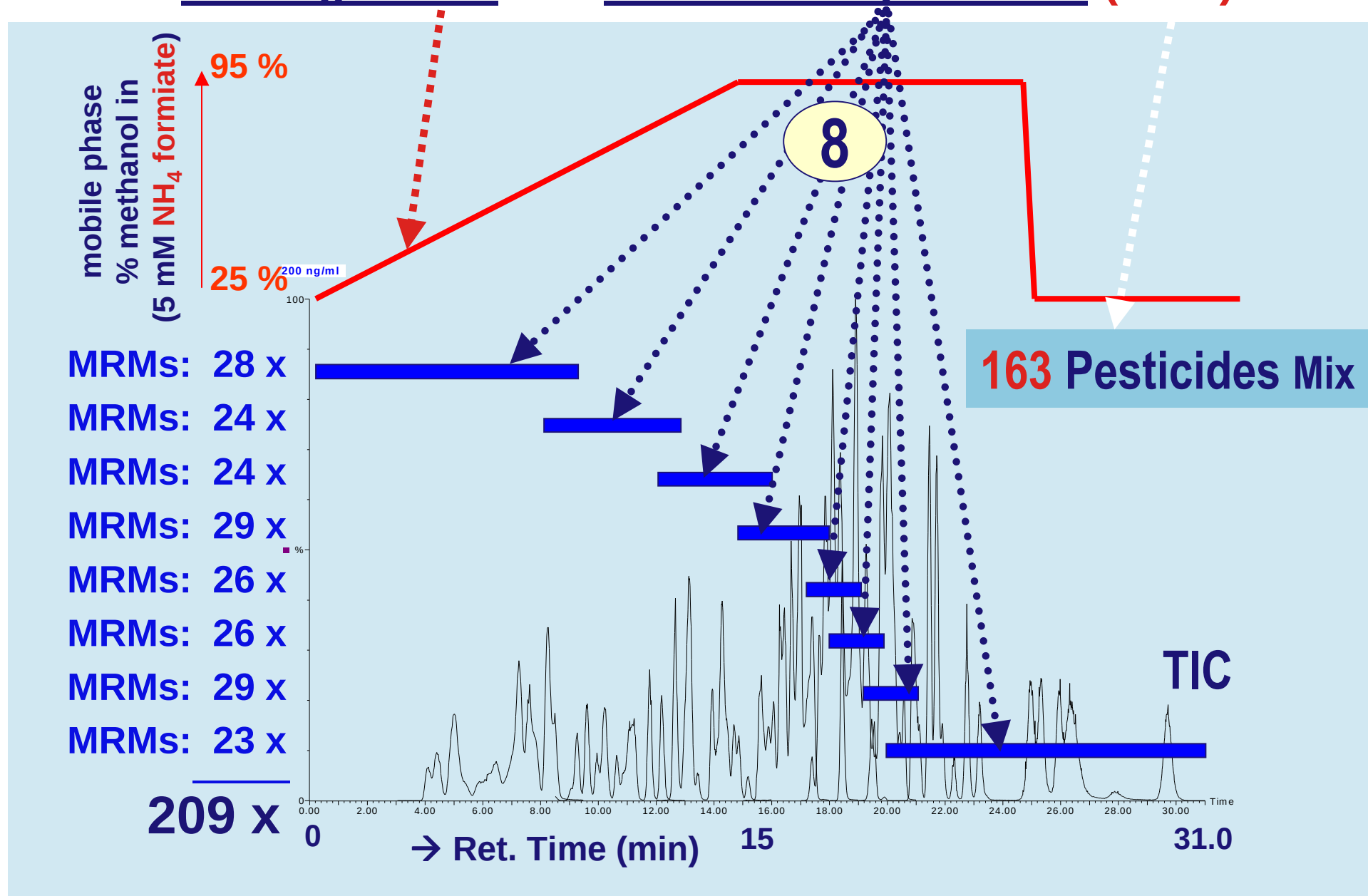
Duty cycle time: 1300 msec → 970
(2004) ----- → (2005)



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



Scheme of Chromatographic Run Time – HPLC gradient and MS/MS scan functions (2005)





(HP)LC-MS/MS

HPLC-MS/MS: WATERS Quattro Ultima Triple Quad





HPLC conditions

Analytical column: Alltima C₁₈, **5 μ m, 150 x 3.2 mm**
Column temp.: 30 °C
Mobile phase flow: 0.30 mL.min⁻¹

Injection volume: **5 μ L** (in 100% MeOH)

Solvent A: 5 mM HCOO**NH₄** 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** ← (incl. Equilibration)



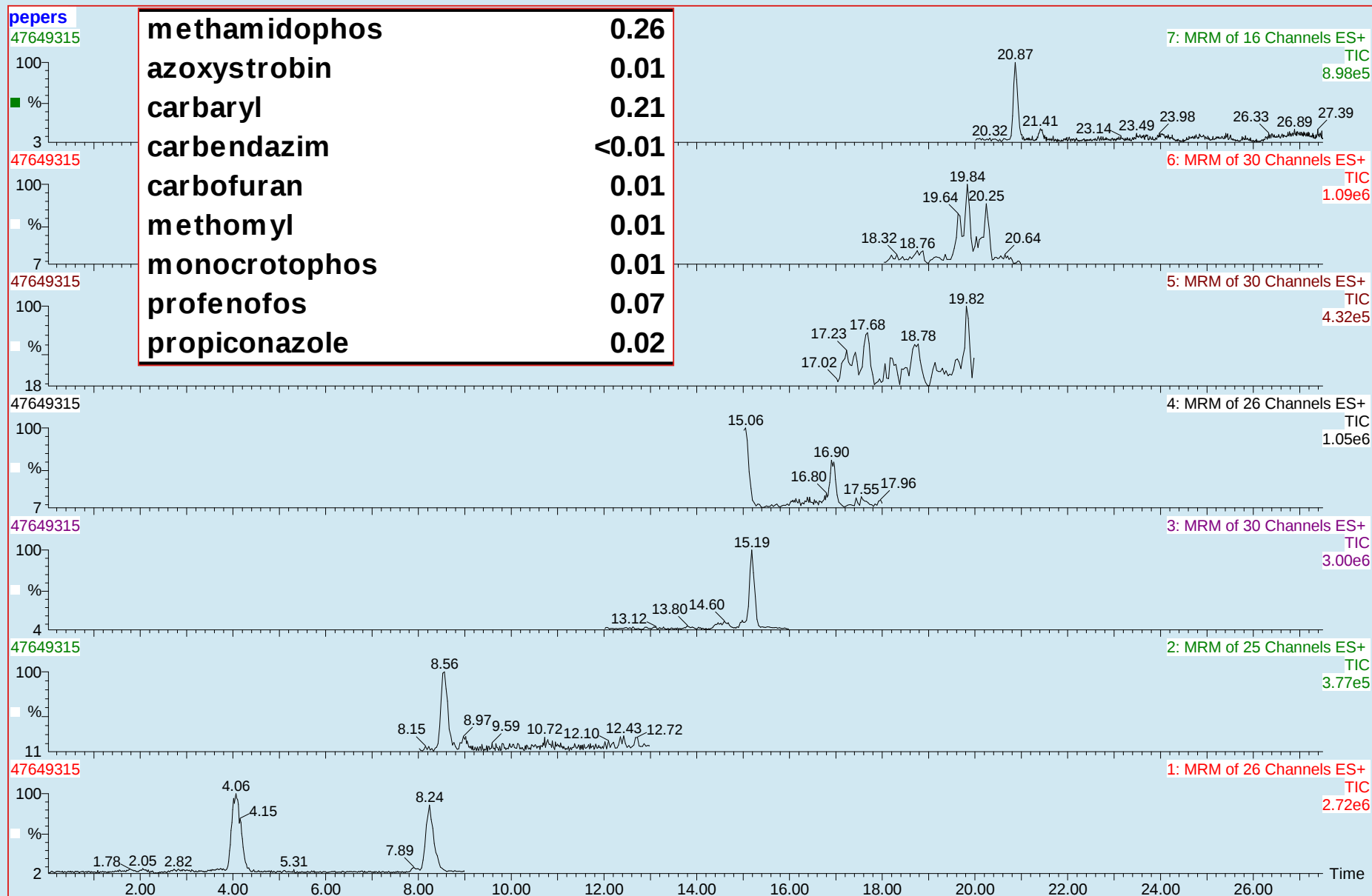
OUTLINE

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- **LC-MS/MS Applications**
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Pesticide Residues (conc. in mg/kg) in Red hot chili pepper Sample

TICs of 7 Scan Functions



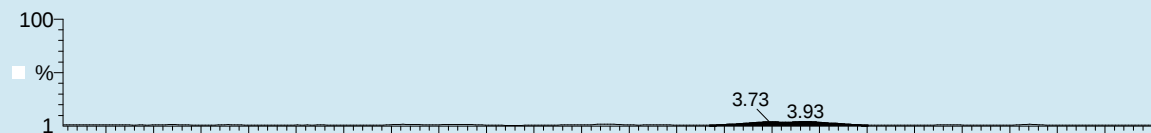


Red hot chili pepper Sample

MRM's

pepers

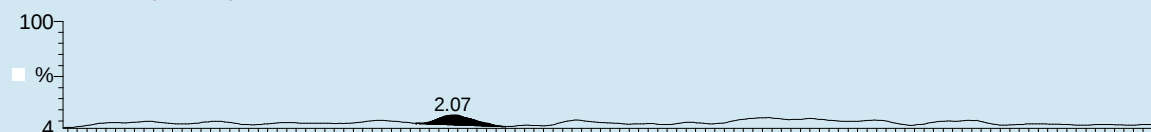
47649315 Sm (Mn, 2x3)



Carbendazim
< 0.01 mg/kg

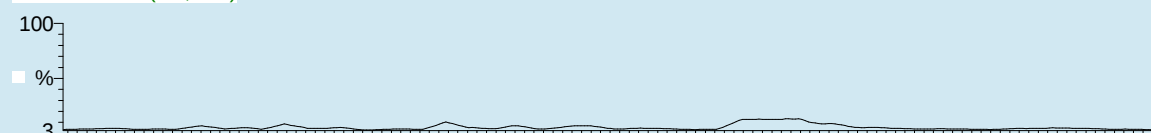
1: MRM of 26 Channels ES+
8.24 192 > 160
1.06e6

47649315 Sm (Mn, 2x3)



1: MRM of 26 Channels ES+
8.24 192 > 132
2.80e5

47649315 Sm (Mn, 2x3)



Methomyl
0.01 mg/kg

1: MRM of 26 Channels ES+
7.91 163 > 106
5.10e4

47649315 Sm (Mn, 2x3)



1: MRM of 26 Channels ES+
7.91 163 > 88
5.30e4

47649315 Sm (Mn, 2x3)



Methamidophos
0.26 mg/kg

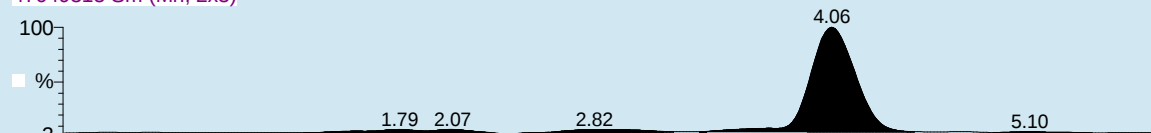
1: MRM of 26 Channels ES+
4.06 142 > 112
1.21e6

47649315 Sm (Mn, 2x3)



1: MRM of 26 Channels ES+
4.06 142 > 94
1.04e6

47649315 Sm (Mn, 2x3)



1: MRM of 26 Channels ES+

TIC

Time



- **Validation of methods**

Comparison of 3 extraction methods for 2 crops, 3 levels (n=6):

Acetone / CH₂Cl₂ + PE

Acetone / CH₂Cl₂ + PE + Na₂SO₄

Acetonitrile (QuEChERS)

*[Ref.: S. Lehotay, A. de Kok, M. Hiemstra and P. van Bodegraven,
J. AOAC Int., 88 (2005) 595-614.]*

Recoveries for Orange spiked at **10 ppb-level**: 3 Extraction methods

Acetone extraction

Acetone + Na₂SO₄

QuEChERS

	10 ppb level		10 ppb level		10 ppb level	
	recovery	RSD	recovery	RSD	recovery	RSD
acephate	37%	9.7%	114%	4,8%	62%	11,4%
acetamiprid	105%	4.7%	99%	6,1%	95%	8,0%
aldicarb	109%	3.7%	100%	4,9%	99%	6,0%
aldicarb sulfone	72%	3.0%	106%	3,8%	68%	9,0%
aldicarb sulfoxide	28%	4.2%	100%	1,6%	101%	2,9%
asulam	67%	7.0%	70%	8,4%	50%	8,5%
azaconazole	100%	3.3%	91%	5,4%	94%	1,2%
azamethiphos	101%	3.0%	108%	7,5%	88%	9,3%
azinphos-methyl	101%	2.4%	101%	2,6%	61%	2,4%
azoxystrobin	104%	3.4%	103%	3,0%	105%	3,2%
bitertanol	98%	14.9%	99%	8,4%	92%	15,2%
bromuconazole	92%	6.6%	85%	10,8%	37%	5,9%
buprofezin	91%	2.8%	98%	2,3%	96%	2,4%
butocarboxim	104%	1.5%	99%	3,5%	93%	1,6%
butocarboxim sulfone	88%	1.7%	107%	1,8%	87%	3,4%
butocarboxim sulfoxide	23%	7.9%	92%	3,8%	90%	10,5%
carbaryl	102%	7.5%	101%	12,0%	87%	11,1%

Matrix effects, Fruits & Vegetables, 3 Extraction methods

Acetone extraction

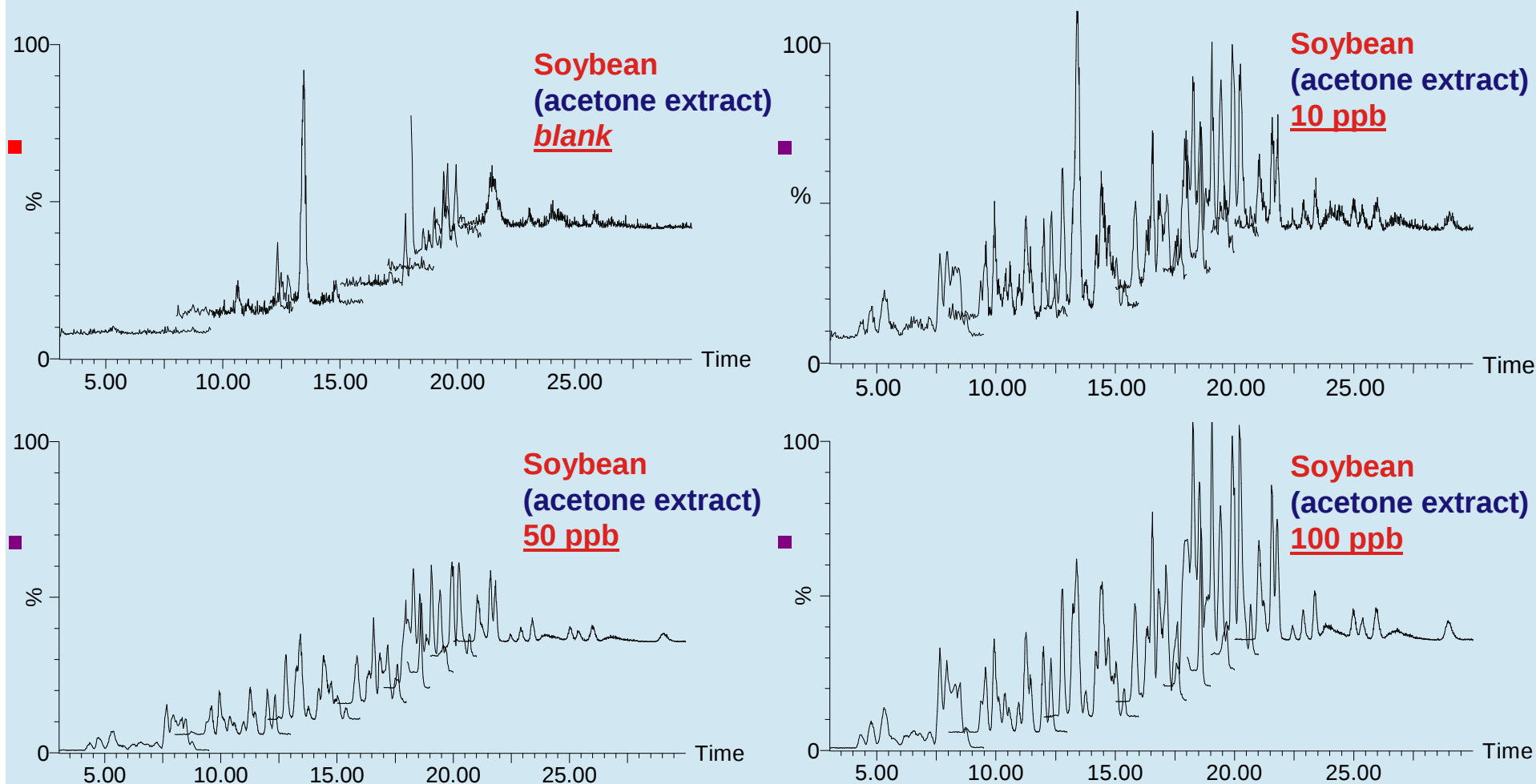
Acetone + Na₂SO₄

QuEChERS

Pesticide	matrix effect				matrix effect			
	orange	lettuce	cabbage	apple	orange	lettuce	orange	lettuce
acephate	-1%	-1%	-2%	-2%	0%	-5%	5%	2%
acetamiprid	-1%	5%	0%	4%	6%	2%	5%	1%
aldicarb	-2%	0%	-4%	3%	-3%	-1%	2%	0%
aldicarb sulfone	-1%	0%	-1%	2%	-3%	3%	-25%	2%
aldicarb sulfoxide	-2%	-2%	-5%	-4%	-1%	-2%	2%	0%
asulam	-2%	-1%	-7%	-3%	-3%	-1%	-1%	3%
azaconazole	0%	6%	-2%	0%	0%	4%	-2%	3%
azamethiphos	-2%	0%	-5%	-5%	-5%	1%	-9%	1%
azinphos-methyl	-25%	-51%	-7%	-4%	-30%	-47%	-29%	-4%
azoxystrobin	-1%	8%	1%	-2%	2%	17%	-5%	2%
bitertanol	-7%	0%	-2%	-9%	-12%	-4%	-1%	-4%
bromuconazole	-40%	2%	0%	0%	-55%	0%	-59%	1%
buprofezin	-7%	-2%	-6%	-4%	-7%	-4%	-4%	-2%
butocarboxim	0%	3%	-5%	5%	-8%	-1%	1%	0%
butocarboxim sulfone	0%	1%	-2%	-2%	-4%	-1%	-14%	0%
butocarboxim sulfoxide	-1%	-1%	-4%	-2%	-2%	-1%	2%	1%
carbaryl	-8%	1%	-4%	-4%	-10%	-1%	-10%	-4%



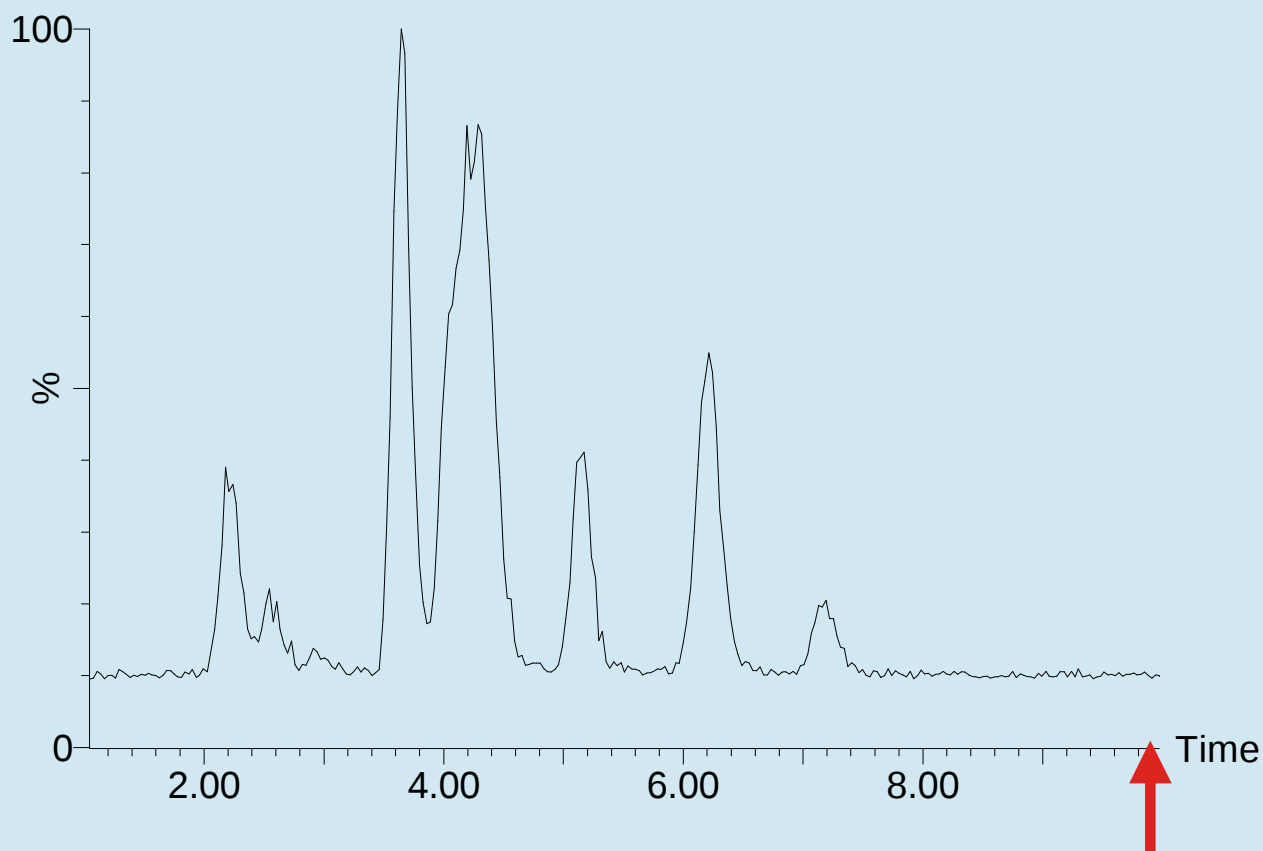
Total ion chromatograms (LC-MS/MS, ESI⁺) of **Soybean Sample**, spiked with 155 pesticides at 10, 50 and 100 ppb level





Routine LC-MS/MS Method on Quattro Ultima

Total ion chromatogram (LC-MS/MS, ESI-) of
Soybean Sample (acetone extract),
spiked with 14 pesticides at 100 ppb level



→ Total Run time: 10 min.



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- Conclusion



Expansion of Scope: *Quattro Ultima* → *Premier XE*

Limitations:

Acquisition Time: ~ 32 min.

Maximum of 32 “channels”(MRMs) / Scan function (time-window)

→ Total number of MS/MS transitions limit: ~ 220

Optional Improvements:

1. *Quattro Ultima* → *Premier XE*:

Dwell time: 20 msec → 10 (→ 5 ?)

Inter-channel delay time: 10 msec → 5 ?

Sum: 30 → 20 (or 15)?

Number of MS/MS transitions: 220 → ~ 330 (or 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

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

15

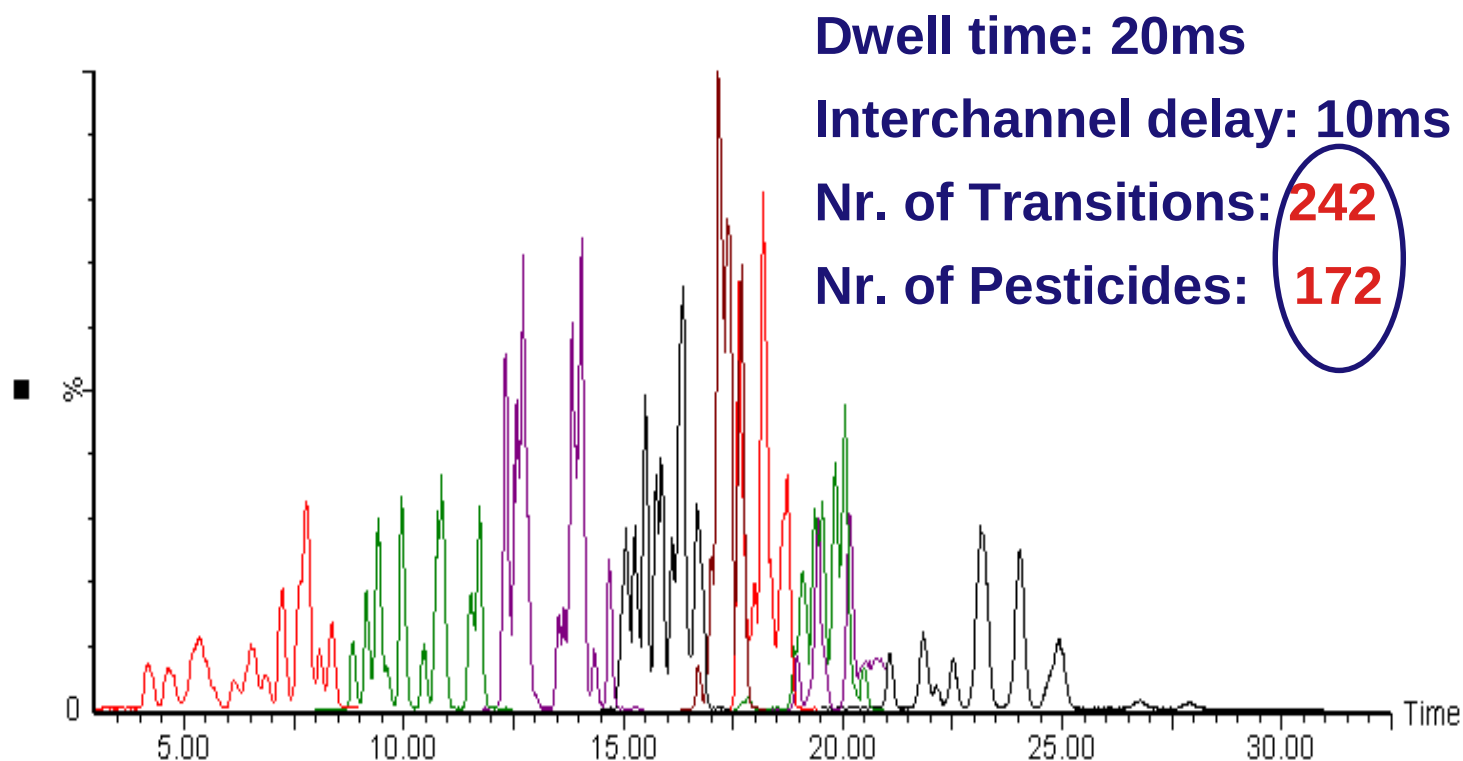
Number of transitions (1 st)	:	220
Number of transitions (2 nd)	:	220
Total	:	440

172 Pesticides Mix

>220 Pesticides



Routine HPLC Method on Premier XE



Column: Alltima C₁₈; 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
tolyfluanid	4.3
triforine	6.5
hexythiazox	5.3



HPLC → UPLC

UPLC-MS/MS:

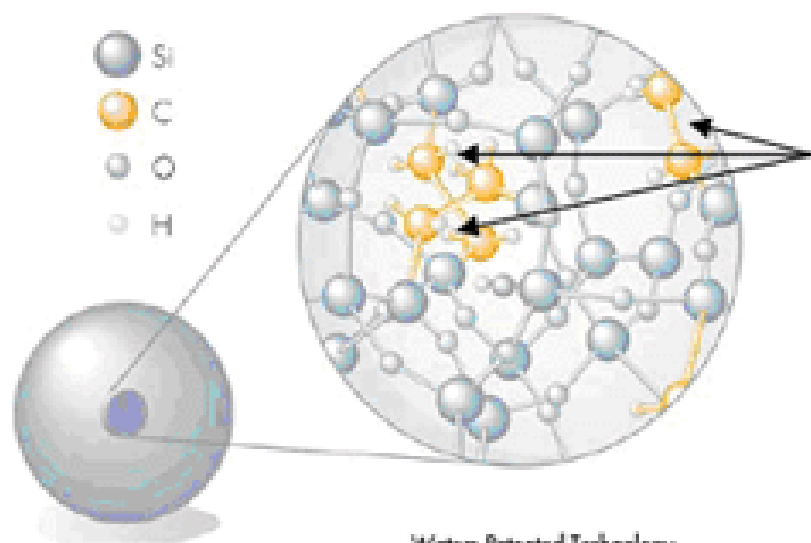
WATERS Acquity UPLC - Quattro Premier Triple Quad





HPLC → UPLC

New Stationary Phase for UPLC

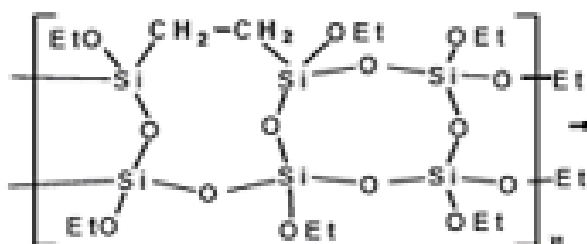


1.7 µm particle size

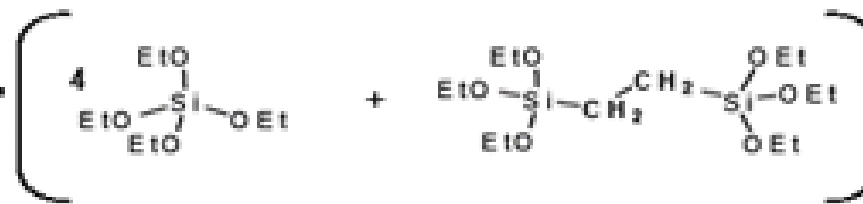
Bridged Ethanes in
Silica Matrix

Acquity™
Ultra Performance LC

Waters Patented Technology
No. 6,686,035 B2



Polyethoxysilane
(BPEOS)



Tetraethoxysilane Bis(triethoxysilyl)ethane
(TEOS) (BTEE)



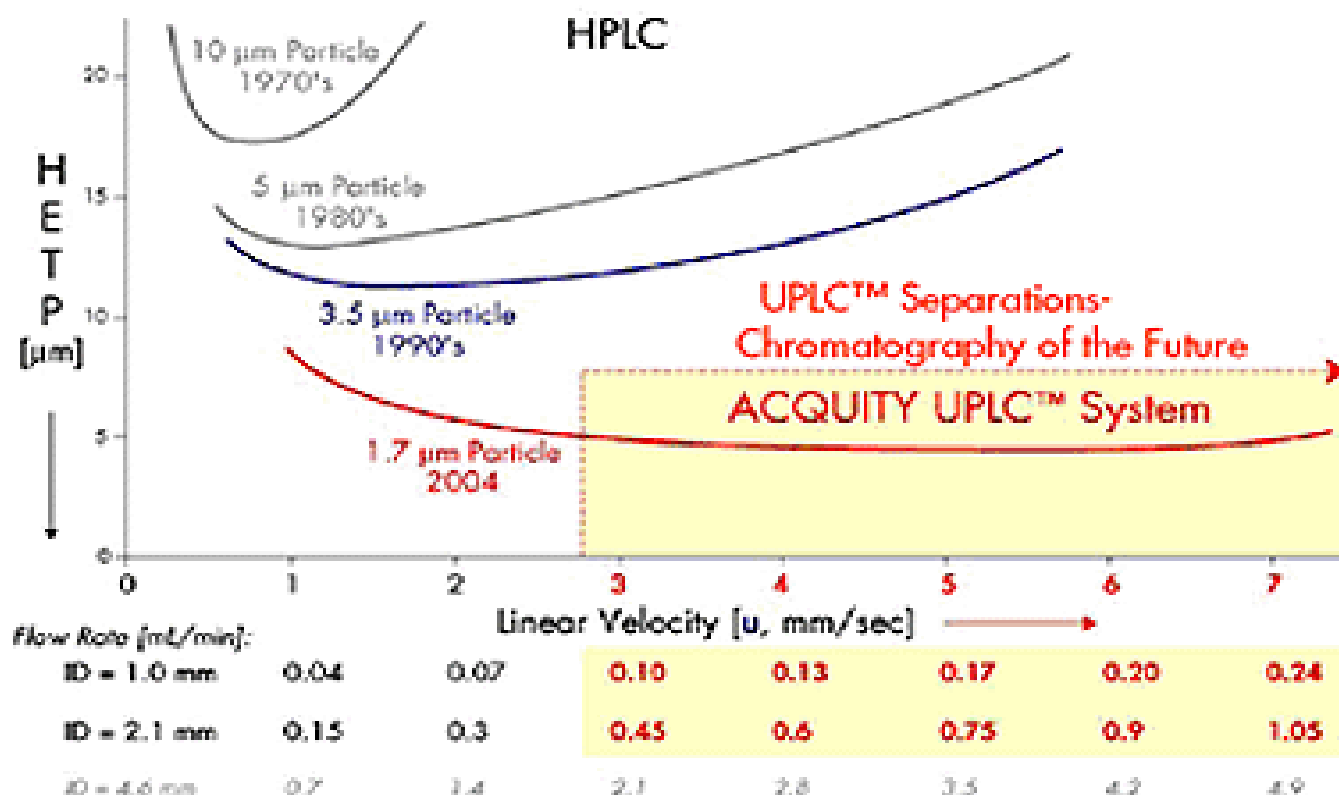
Van Deemter Equation

$$H = \overset{\text{A term}}{a(dp)} + \overset{\text{B term}}{\frac{b}{u}} + \overset{\text{C term}}{c(dp)^2 u}$$

$U = \text{Linear Velocity}$ $dp = \text{Particle Size}$

C term is impacted by Both

- 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.





$R_s =$

$$\frac{\sqrt{N}}{4}$$

System
Efficiency

$$\left(\frac{\alpha - 1}{\alpha} \right) \left(\frac{k}{k + 1} \right)$$

Selectivity

Resolution

If $N \uparrow 3X$, $R_s \uparrow 1.7X$

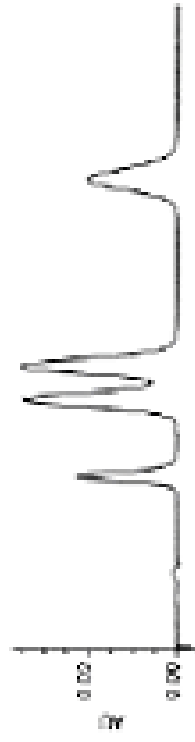
Keeping Column Length the Same

Independent Y-axis

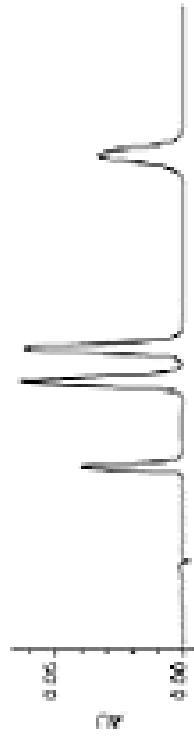
Set to same scale



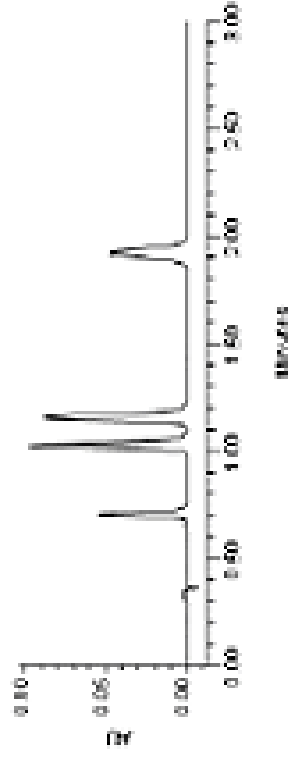
10 µm - 50 mm
 $R_s \phi_{2,3} = 0.8$



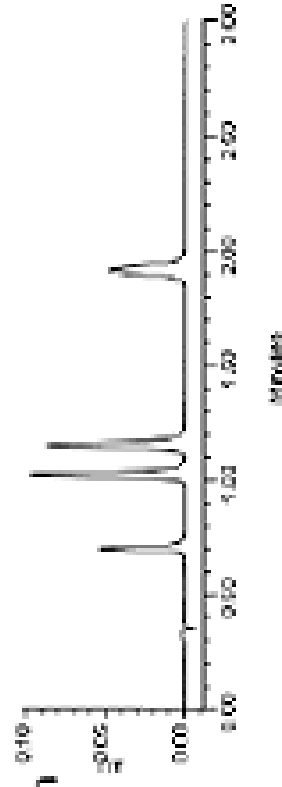
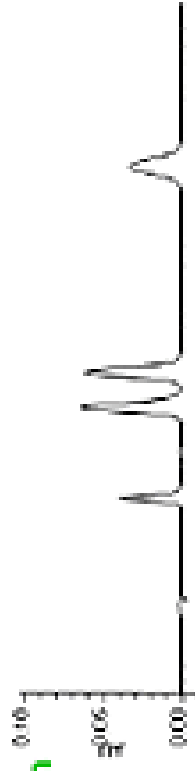
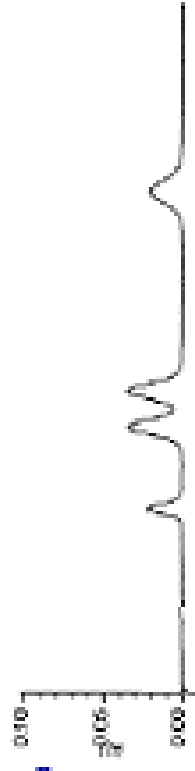
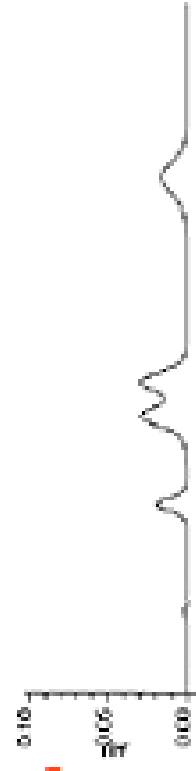
5 µm - 50 mm
 $R_s \phi_{2,3} = 1.2$



3.0 µm - 50 mm
 $R_s \phi_{2,3} = 2.0$



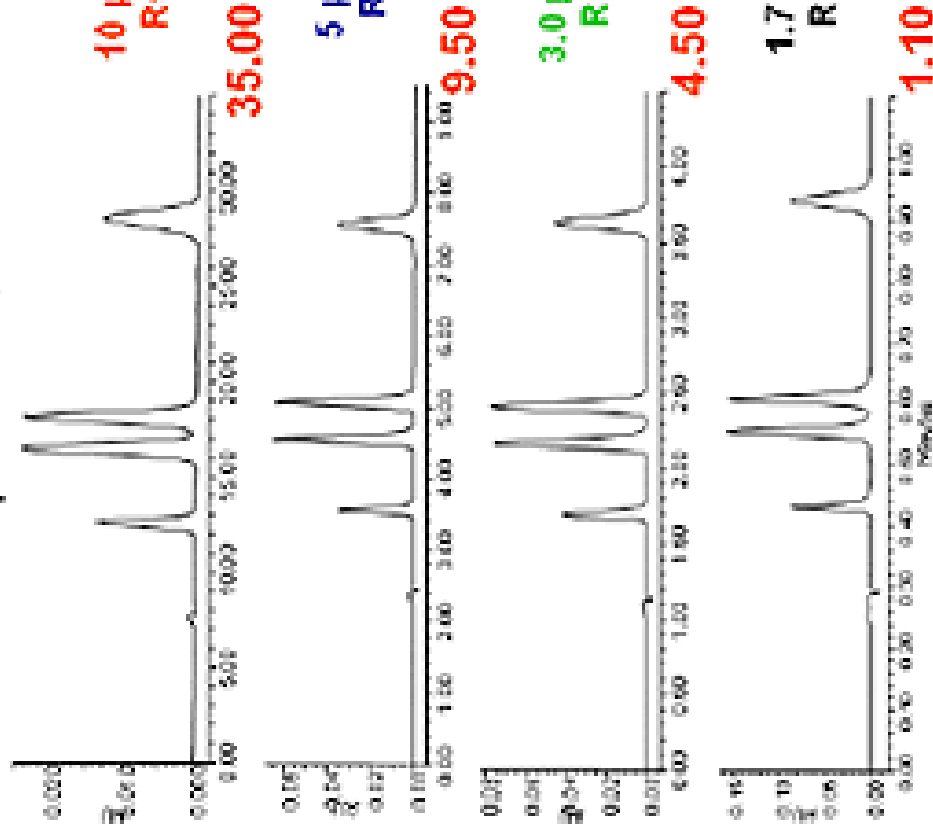
1.7 µm - 50 mm
 $R_s \phi_{2,3} = 2.7$



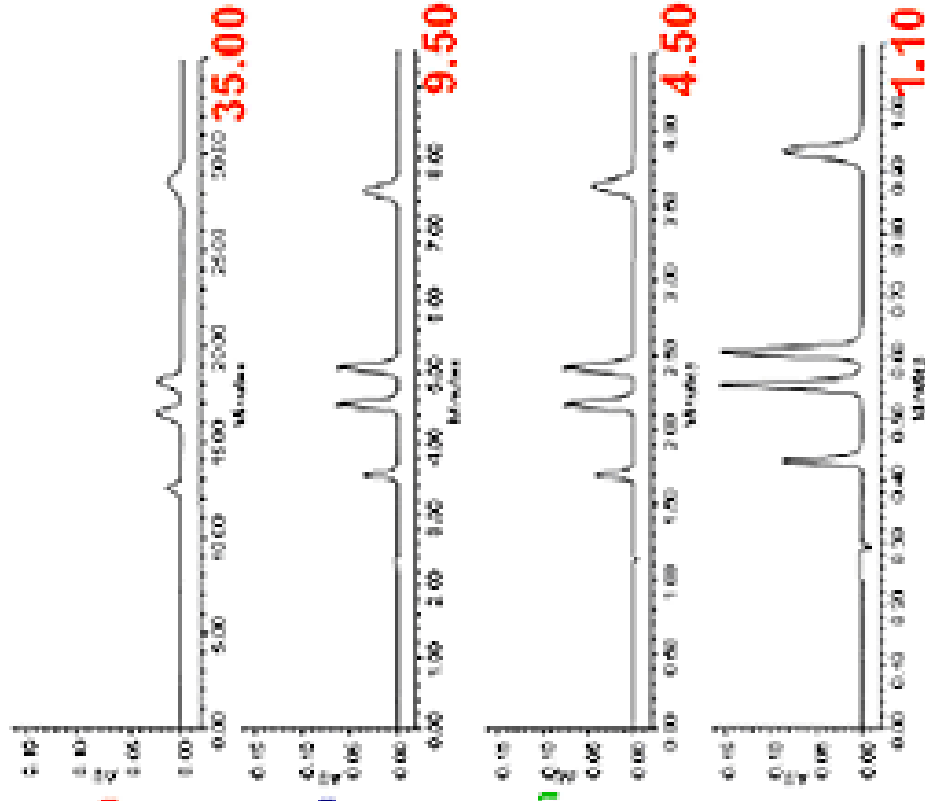


$N = 1X$, $R_s = 1X$
 $dp \downarrow 3X$, $L \downarrow 3X$, $F \uparrow 3X$, $T \downarrow 9X$
 Sensitivity $\uparrow 3X$

Independent Y-axis



Set to same scale





HPLC → UPLC
Quattro Ultima → Premier XE

Objectives

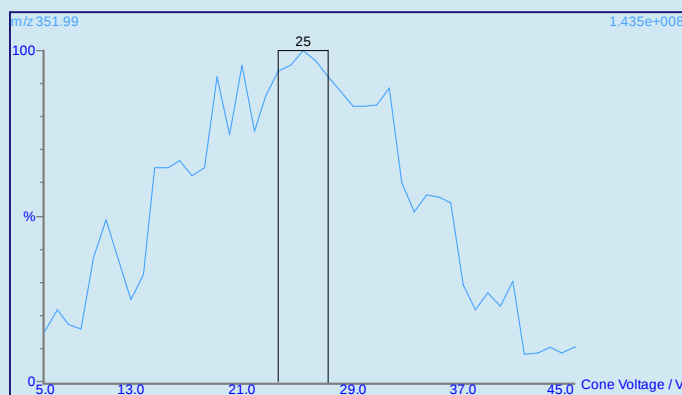
- **1 Extention of the scope of the routine**
LC-MS/MS multiresidue method
- **2 At least two transitions per pesticide**
(quantitation/confirmation)
- **3 Reduction of analytical run time by a factor of two**

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

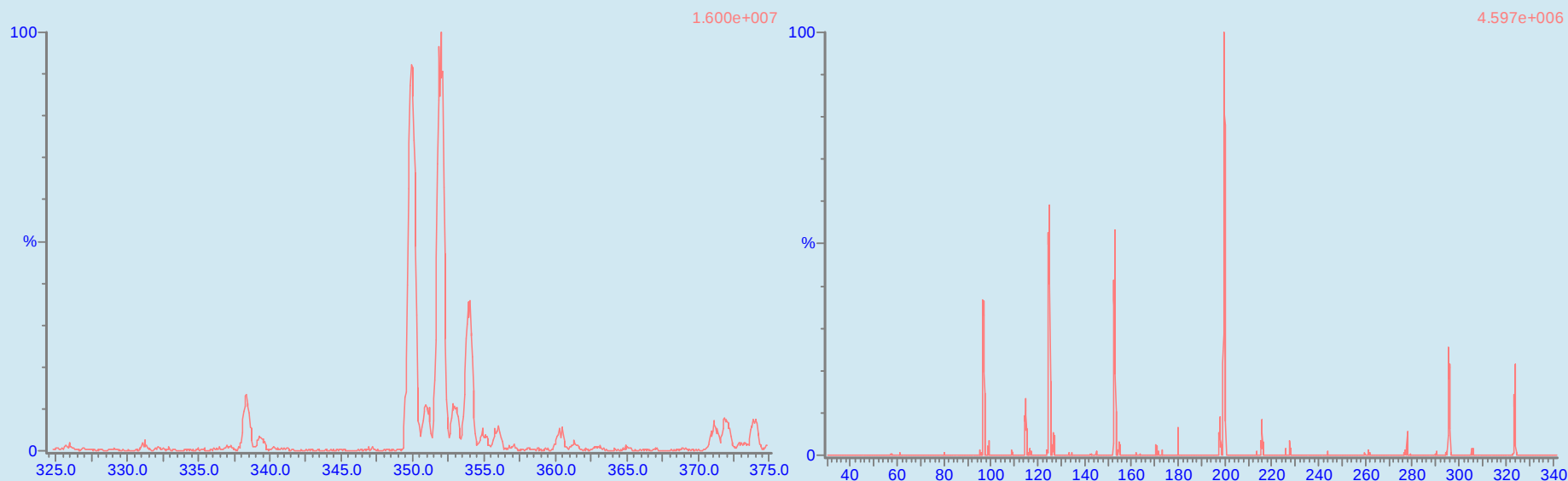


Cone voltage



Collision Energy

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
tolyfluanid	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

UPLC conditions

Analytical column: Acquity UPLC BEH C₁₈, 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 HCOONH₄ 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- Method on Premier XE

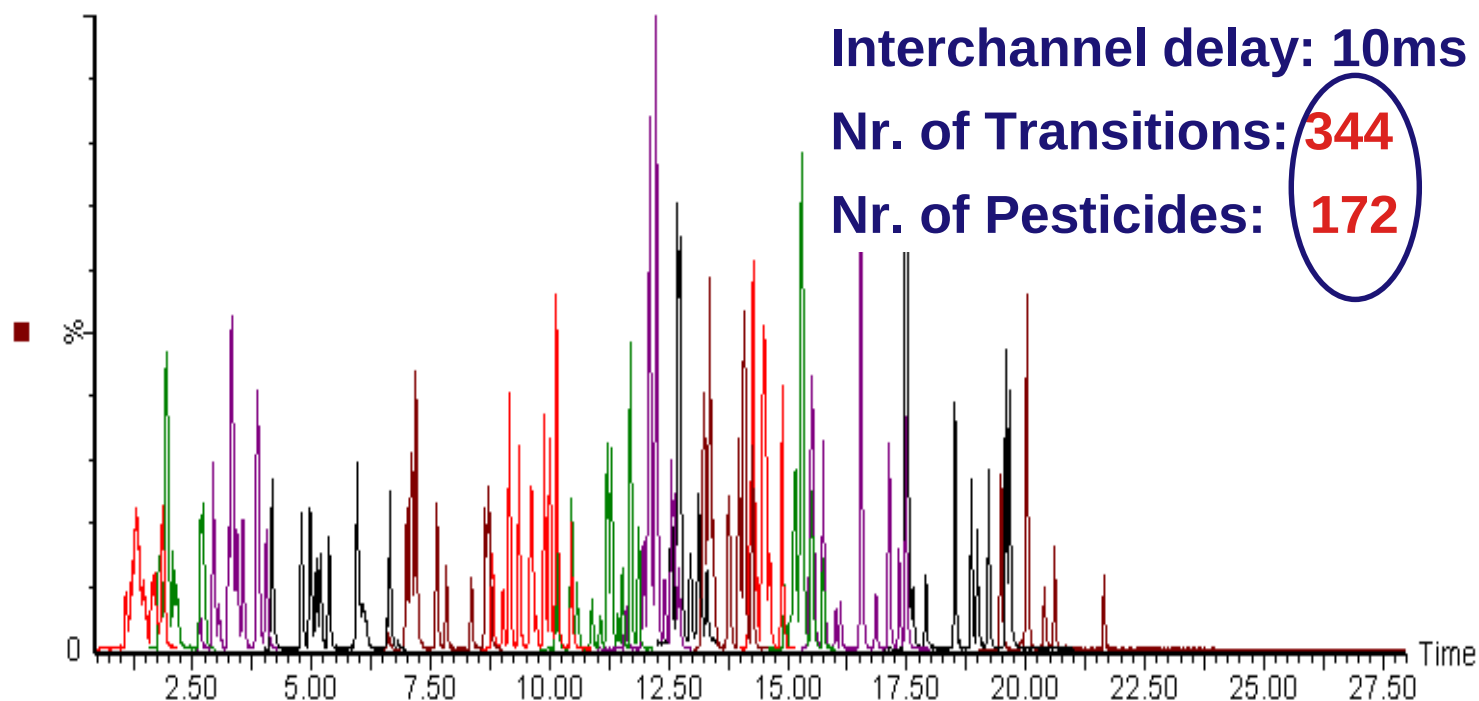
Total Run time: **2 transitions / pesticide**
32 min.

Dwell time: 10ms

Interchannel delay: 10ms

Nr. of Transitions: **344**

Nr. of Pesticides: **172**



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
tolyfluanid	8.5
triforine	28.4
hexythiazox	8.0

Column: Acquity UPLC™ BEH C₁₈, 2.1 x 150mm, 1.7μm

Flow rate: 0.45 ml/min

Temperature: 65°C



UPLC- Method on Premier XE

Experimental UPLC-Method 28 min

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
	23	5	95
	24	80	20
	28	80	20
	Run time: 32 min		
flow rate:	0.45 ml.min-1		
injection volume:	2 µl		
Detection:	Micromass, Quattro Premier XE, 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	1.0 - 4.0	24	32
2	3.0 - 5.5	24	32
3	4.0 - 7.0	24	32
4	6.5 - 10.0	24	32
5	9.5 - 12.0	24	32
6	11.5 - 13.7	24	32
7	13.0 - 15.0	24	32
8	14.5 - 16.0	24	32
9	15.0 - 16.6	24	32
10	15.6 - 17.5	24	32
11	16.5 - 18.0	24	32
12	17.2 - 19.0	24	32
13	17.9 - 20.5	24	32
14	18.5 - 23.0	24	32
15	21.0 - 28.0	8	32
Total Transitions:		344	480
Nr. of pesticides:		172	240

Possible Maximum Scope

Recent method:
220 pesticides in 32 min.

200-240 with **2** transitions / pesticide

or ~ 400 with **1** transition / pesticide



UPLC- Method on Premier XE

Pos./Neg. Ionisation in 1 Run

Total Run time:

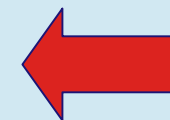
32 min.

Interscan delay time: 20 ms

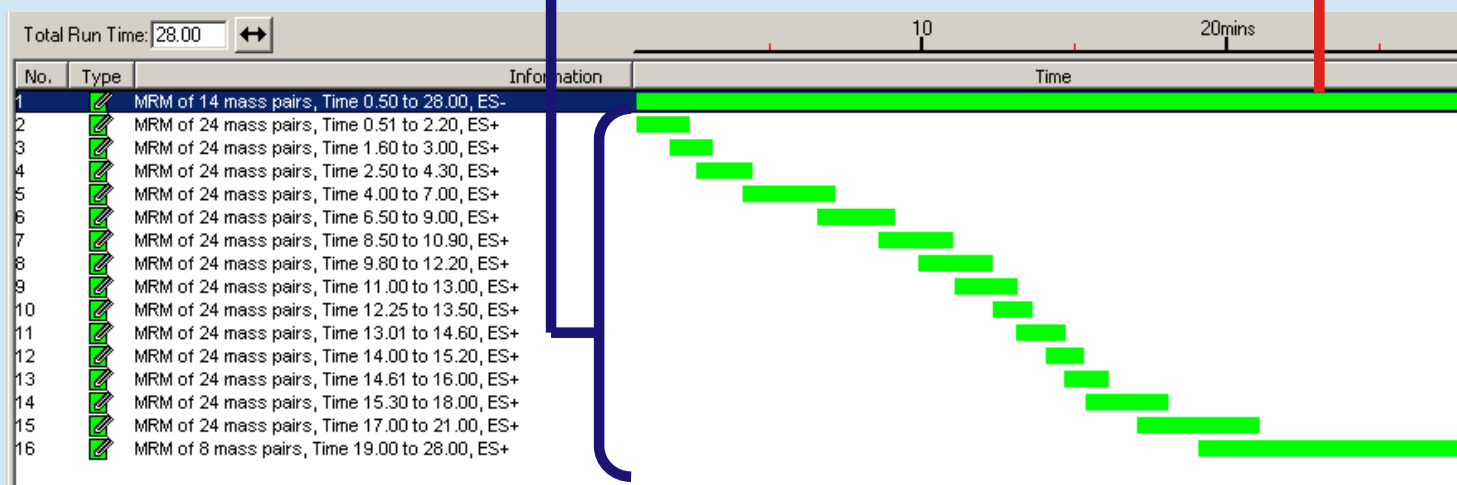


ESI⁺

ESI⁻



Dwell time:	10 ms	10 ms
Interchannel delay:	10 ms	10 ms
Nr. of Transitions:	344	14
Nr. of Pesticides:	172	14
Nr. of Functions:	15	1

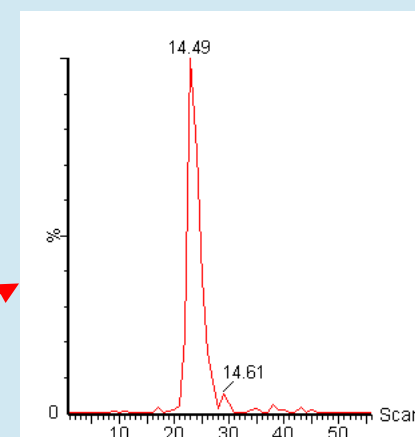
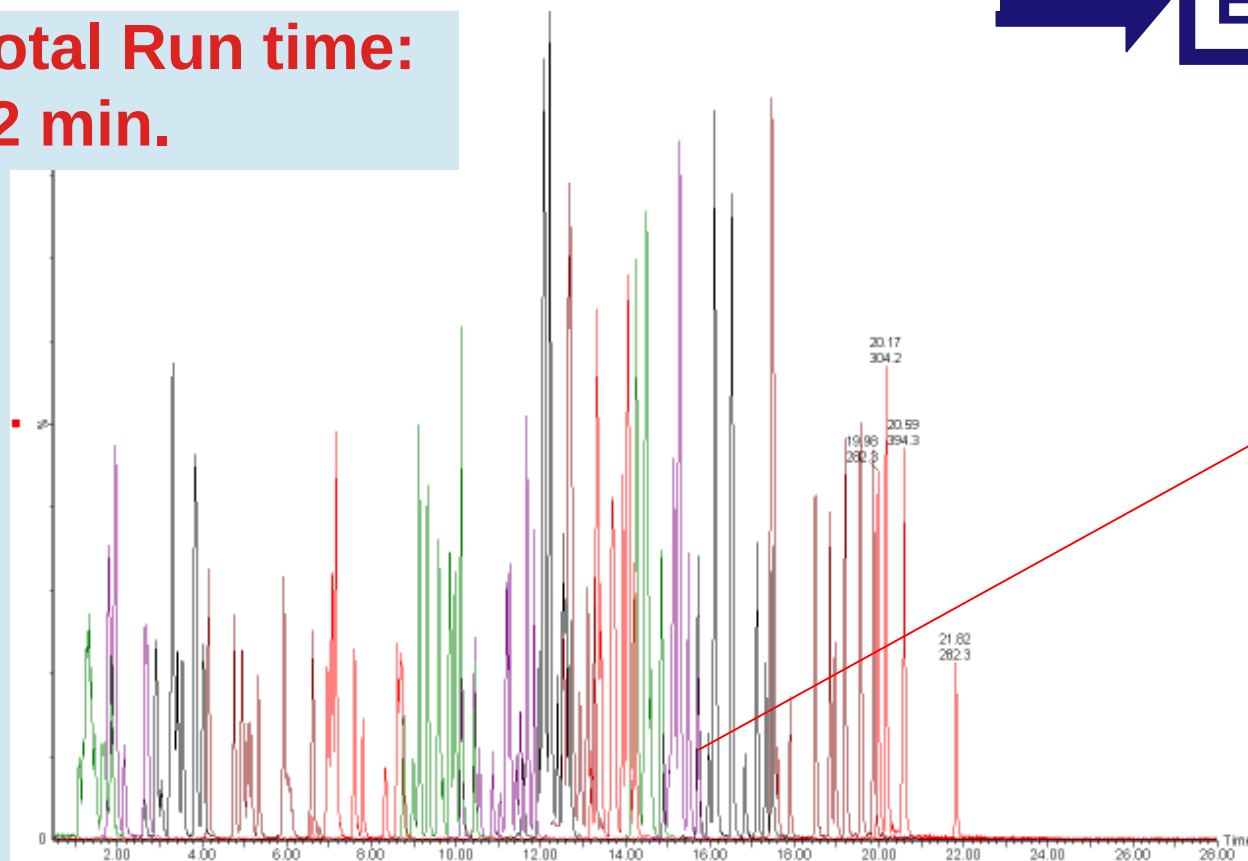
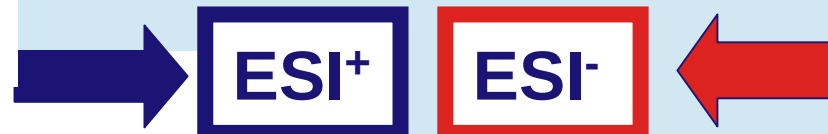




UPLC- Method on Premier XE

Pos./Neg. Ionisation in 1 Run

**Total Run time:
32 min.**



Imazalil

297.1 > 69.1

Nr of scans: 8

Column: Acquity UPLC™ BEH C₁₈, 2.1 x 150mm, 1.7μm

Flow rate: 0.45 ml/min

Temperature: 65°C



UPLC- Method on Premier XE

Shorter Run times:

Total Run time: 16 min. !!!

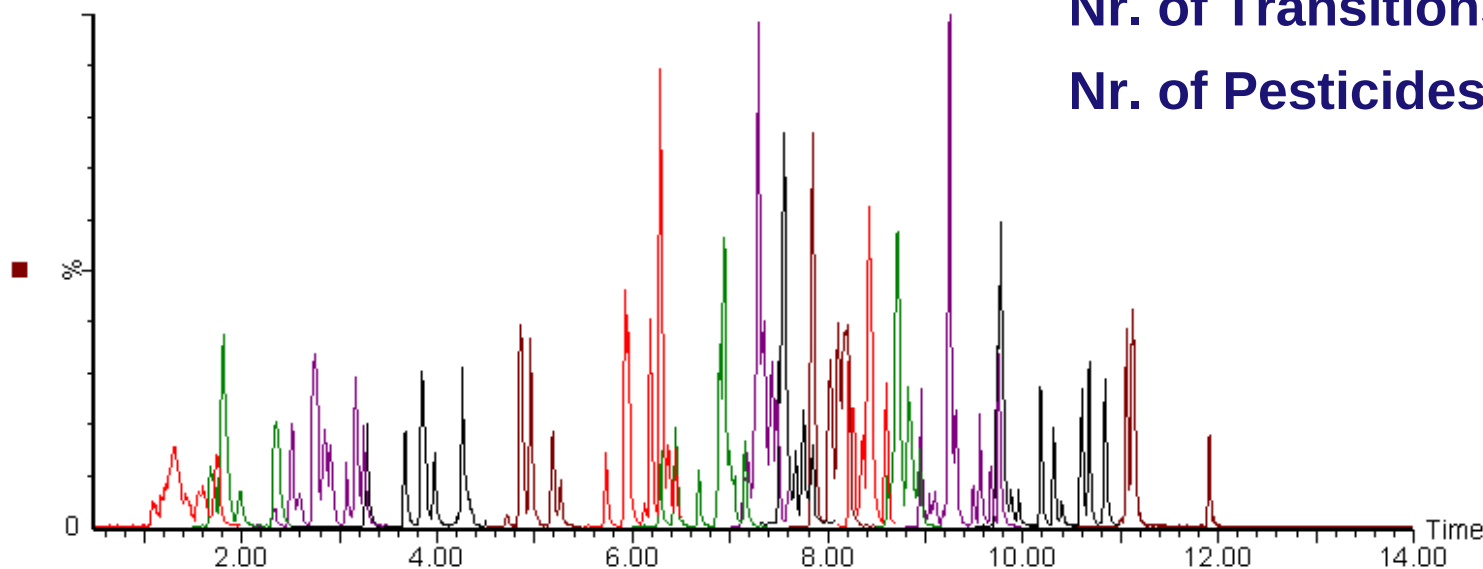
2 transitions / pesticide

Dwell time: 10ms

Interchannel delay: 10ms

Nr. of Transitions: **344**

Nr. of Pesticides: **172**



Column: Acquity UPLC™ BEH C₁₈, 2.1 x 150mm, 1.7μm

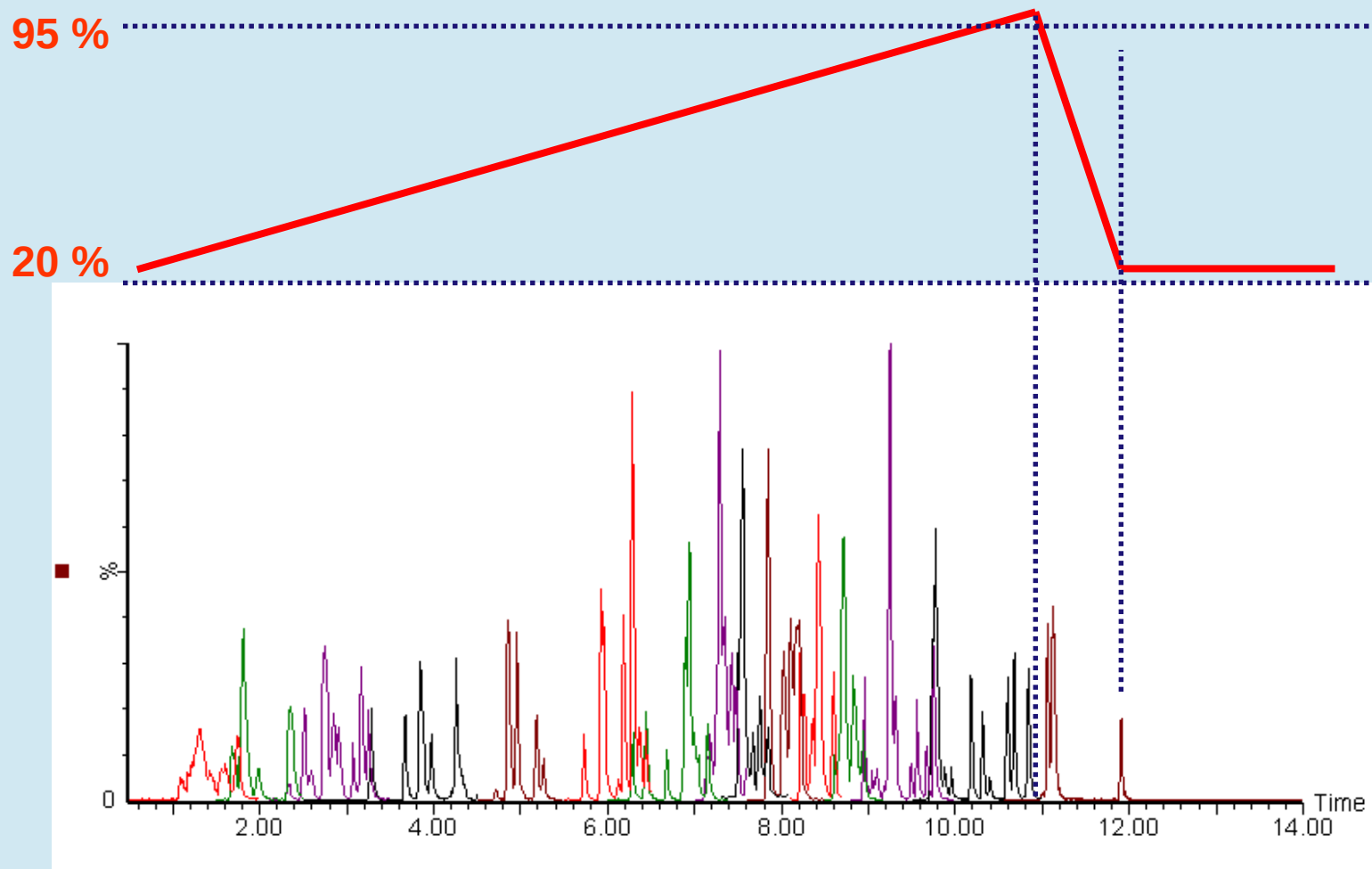
Flow rate: 0.45 ml/min

Temperature: 65°C



Scheme of Chromatographic Run Time – HPLC gradient (2006)

Mobile phase B
% Methanol
(in 5 mM NH_4 formate)



Total Run time: 16 min. !!!

UPLC-MS/MS (Premier XE) conditions

Experimental UPLC-Method, 16 min.-Run time

Column: Acquity UPLC BEH C18, 2.1 x 150mm, 1.7µm

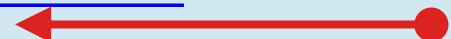
Temp. Column: 65°C

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

Nr of scans: ~ 8
Limit !!

~ 6
Too low !!



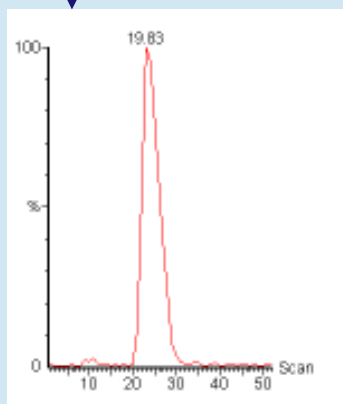
Number of data points (scans) / HPLC peak

Routine Method on Quattro Ultima 2006

Routine Method on Premier XE

UPLC-Method on Premier XE
with two transitions/Pesticide

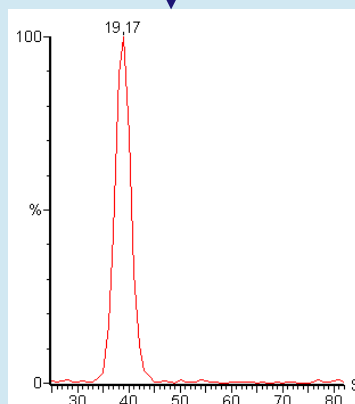
UPLC-Method on Premier XE



Imazalil

297 > 255

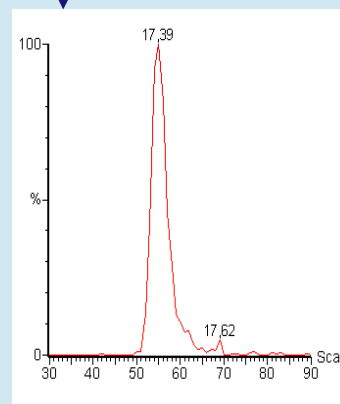
Nr of scans: 10



Imazalil

297.1 > 69.1

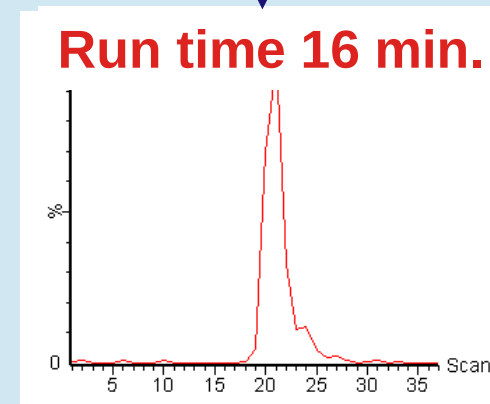
Nr of scans: 10



Imazalil

297.1 > 69.1

Nr of scans: 10



Imazalil

297.1 > 69.1

Nr of scans: 8



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CONCLUSIONS

UPLC-MS/MS

- Expands scope of Multiresidue Methods
- Increases sensitivity
- Decreases run time, maintaining resolution
- Increases sample throughput
- **Implementation in routine: 2007**