Waters

KVCV Studiedag - 14 oktober 2005

“Perspectieven in de Organische Milieu Analyse’

Peter Batjoens

Acquity
Ultra Performance LC™

and its
Powerful Combination with Mass Spectrometry

“Fast Non-Target Screening and Quantitative Monitoring of Residues in Environmental and Food Samples.”

For Complete Confidence

Waters

Outline

- Introduction to Environmental Analysis
- Analytical Requirements
- Waters approach to Complex Mixture Analysis
  - Target Quantitative Monitoring
    - Acquity UPLC – Quattro Premier XE
    - TargetLynx
  - Non-Target Unknown Screening
    - Acquity UPLC - LCT Premier
    - ChromaLynx
- Example data
    - Pesticide residue screening

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EU Maximum Residue Limits Pesticides

- Current Situation
  - MRLs do not always exist and are not harmonised
  - Range from not detectable to 25mg/kg
- EU Proposal 2003/0052 (COD) 14th March 2003
  - Harmonised MRLs for all pesticide/product combinations
  - Not detectable defaults to 0.01mg/kg
- General target LOD = 0.01mg/kg
  - Applies to raw commodities of plant and animal origin
  - Some exceptions, e.g. Baby Food, Medicinal Plants

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Multiresidue screening methods Requirements

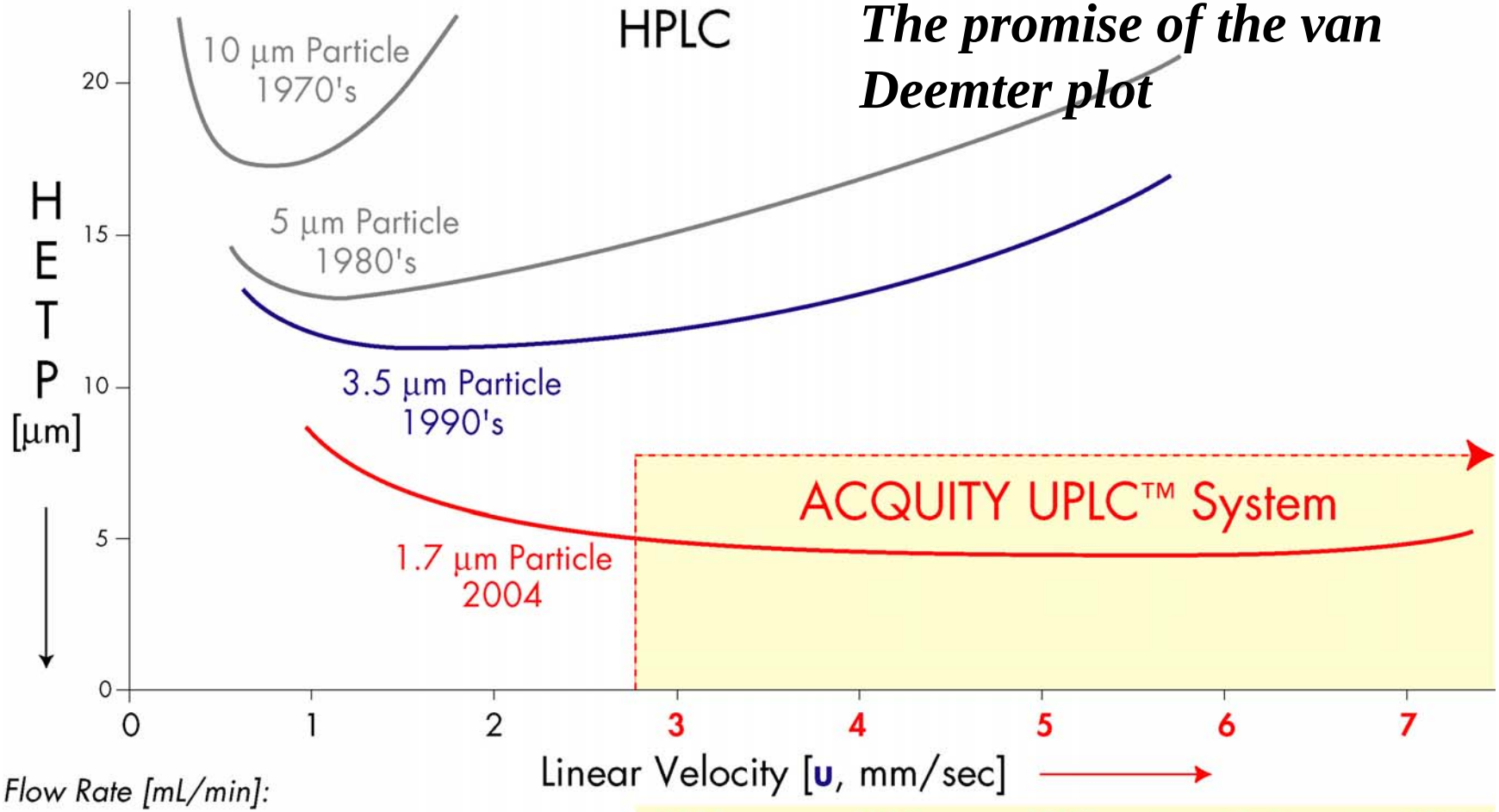
- Requirement for surveillance monitoring of foodstuffs
  - Hundreds of pesticide compounds in use
- Need for targeting multiple compounds per analysis, in a variety of produce
  - Wide range of analyte chemistries
  - Wide range of sample matrices
- Need to decrease overall cycle time
  - High sample throughput
  - Addition of confirmatory transitions
  - Addition of compounds in negative ionisation mode

Multiresidue screening methods Requirements

- High selectivity
  - Reduce or eliminate matrix interferences
- High sensitivity
  - Low reporting limits for individual components of the MRLs
  - Quantitative accuracy
  - Reproducibility, stability and dynamic range
  - Accurate quantification of targets at low levels in matrix
- Ruggedness
  - Complex sample matrices
  - Reduced or no sample clean-up

Smaller Particles
The enabler of productivity

The promise of the van Deemter plot



Flow Rate [mL/min]:							
ID = 1.0 mm	0.04	0.07	0.10	0.13	0.17	0.20	0.24
ID = 2.1 mm	0.15	0.3	0.45	0.6	0.75	0.9	1.05
ID = 4.6 mm	*0.7*	*1.4*	*2.1*	*2.8*	*3.5*	*4.2*	*4.9*

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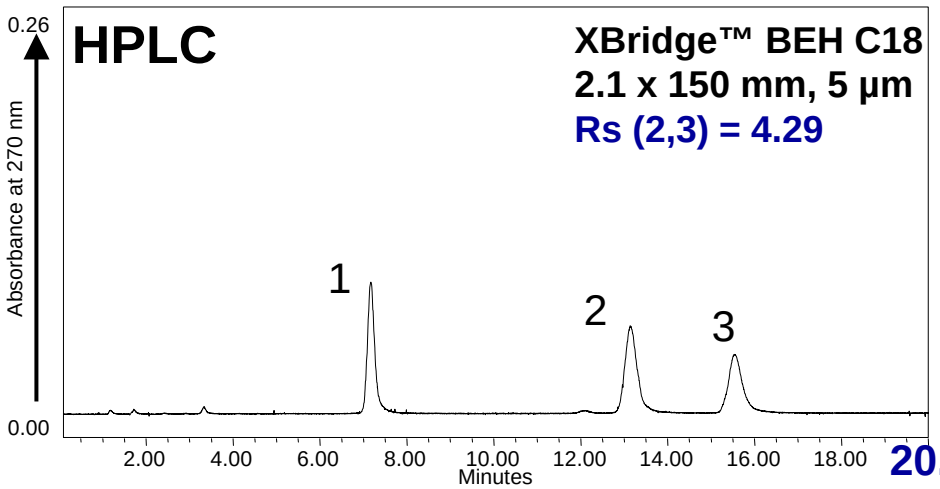
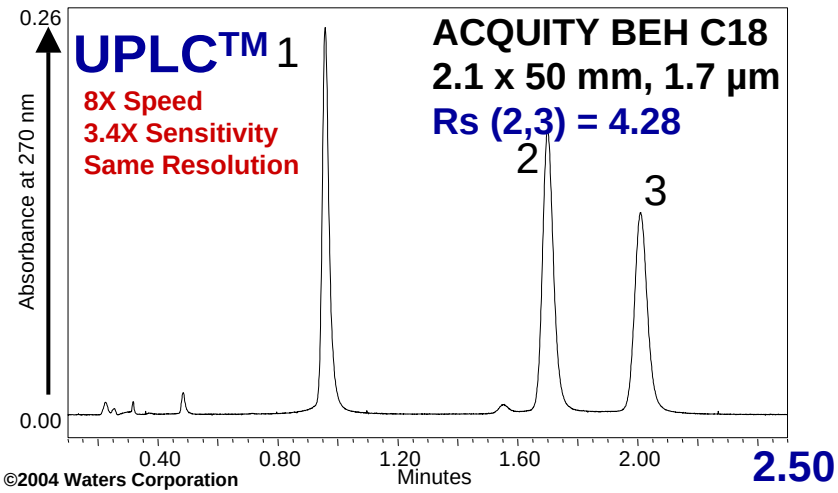
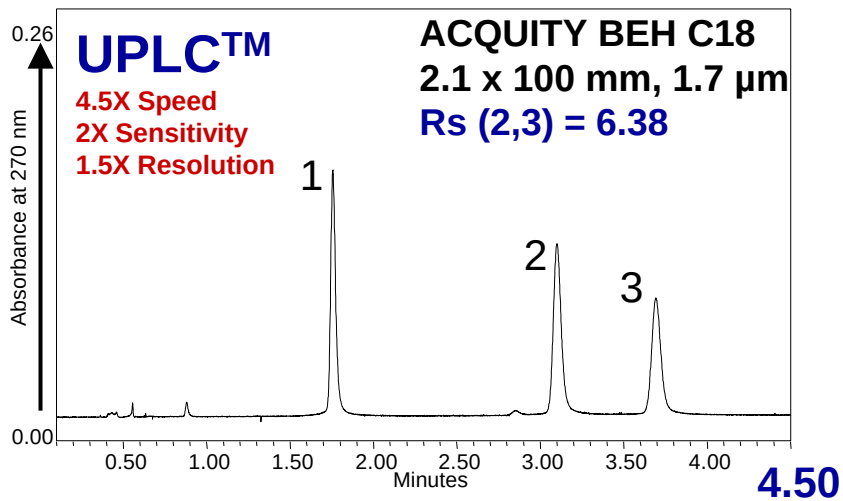
HPLC vs. UPLC™ Speed, Sensitivity and Resolution



Faster, More Sensitive Methods

Faster, More Sensitive, Higher Resolution Methods





Technology Achievements of UPLC™

How can we benefice of the advantages of the 1.7 µm particle ?

- **High pressure fluidic modules (up to15,000 psi)**
  - developped for UPLC, small internal volume
- **Autosampler**
  - reduced cycle time and negligible carryover
- **Tubings**
  - special for UPLC, reduced volume, optimized connections
- **Optical and Mass Detectors**
  - Fast, high acquisition rates, small dispersion flowcell
- **New communication protocols**
  - ethernet technology, more information can be captured, faster
- **Comprehensive diagnostic suite**
  - online diagnostics for all the modules

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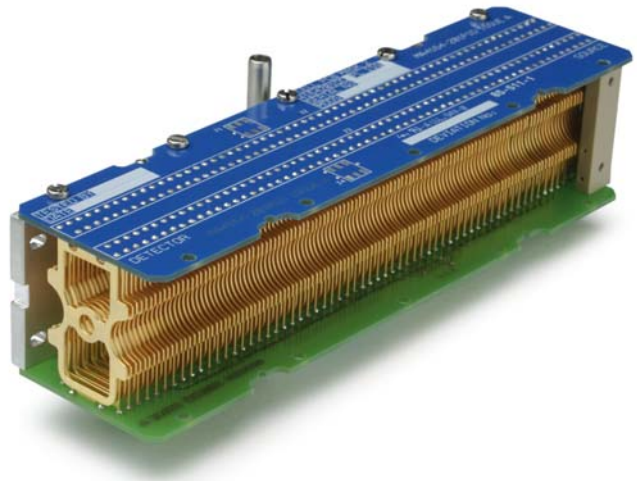
UPLC advantages for MS users?

- Narrower chromatographic peaks effectively increase concentration of analytes entering the MS source – increasing signal intensity and improving detection limits.
- Improved resolution can reduce MS ion suppression by separating species that may co-elute in conventional HPLC
  - improved detection limits for SIR/MRM quantitation
  - Improved spectral quality
  - Improved mass accuracy for Tof MS
- Improved resolution: separation of isomers
- Improved resolution for complex mixture analysis
  - More information, obtained more easily
- Shorter analytical run times without compromising chromatographic resolution – increasing sample throughput

Coupling MS to UPLC

- Sharp peaks, fast separations, increased chromatographic resolution requires
  - Sufficient acquisition rate (>10 points/peak)
    - Short dwell time and inter channel delay
  - Fast positive/negative switching (when relevant)
  - Software tools to handle increased number of results

T-Wave devices:
Designed as a means of propelling ions through rf-only collision cells and transfer optics to enhance the fast acquisition performance of a tandem quadrupole mass spectrometer.



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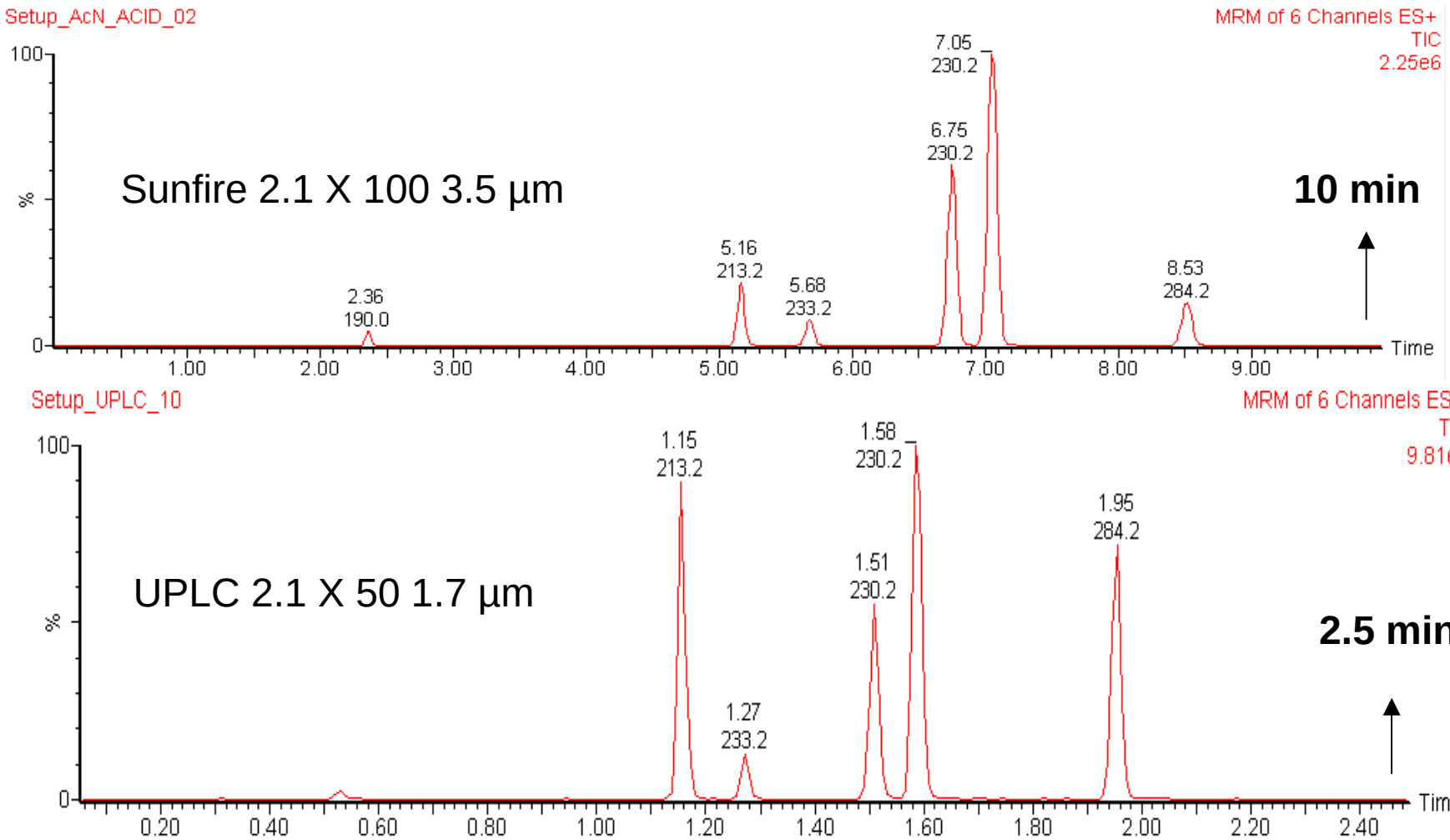
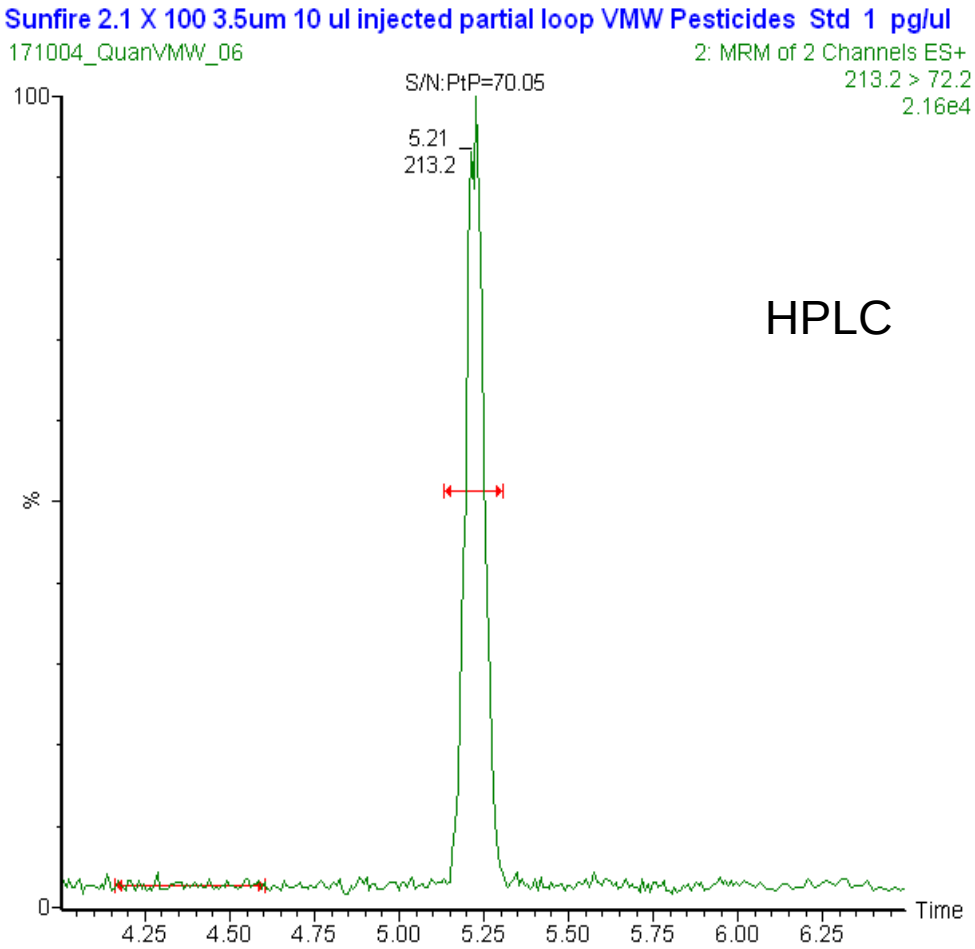
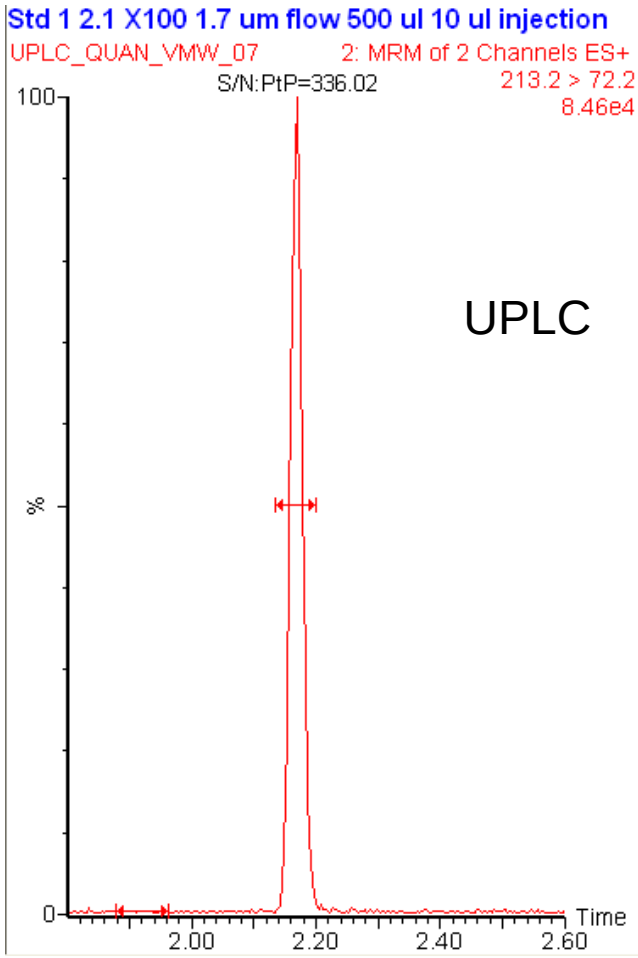
Compare HPLC 15 min <> UPLC 5 min



HPLC/UPLC S/N ratios 10 pg on column
Chlorotoluron



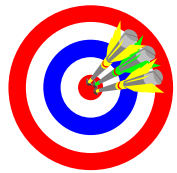
HPLC



UPLC

The Daily Challenge: Analysis of Complex Mixtures

- The "KNOWN" (or suspected residue)
  - Routine monitoring program
  - SIM or MRM normally used
  - Single quad, Tandem quad, Sector



Targeted analysis

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Waters approach of Targeted Analysis

Quattro Premier XE LC/MS/MS

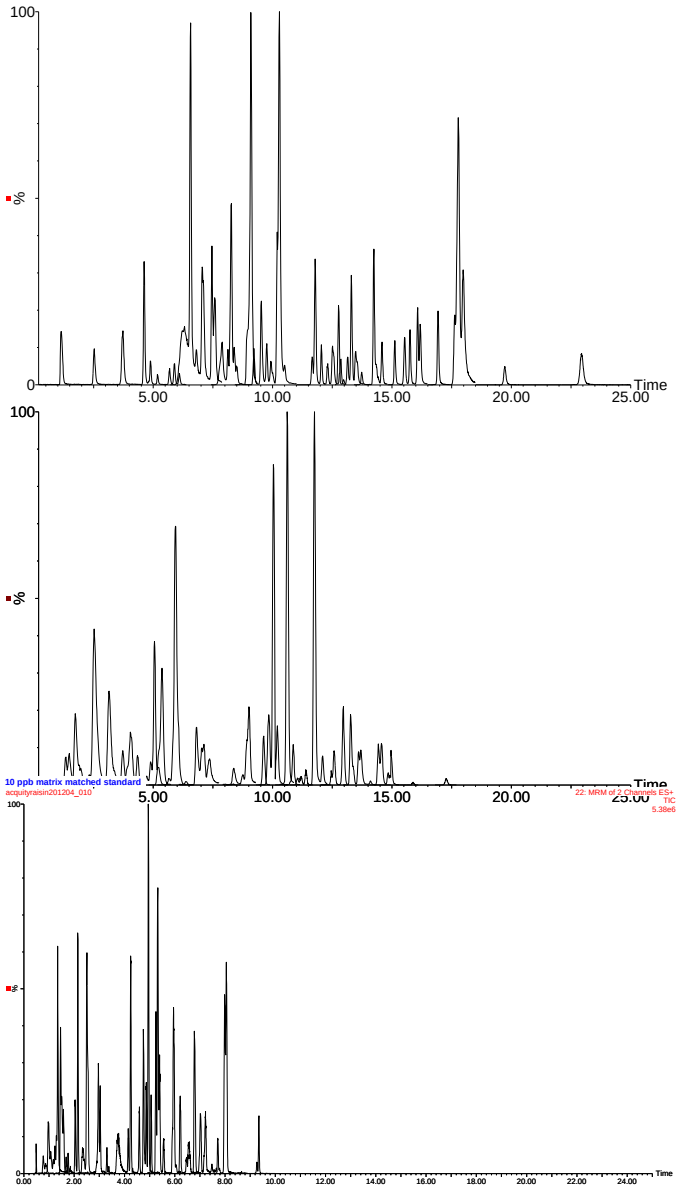
Acquity UPLC

TargetLynx

Advanced quantitation with a full range of automatic quality control checks

Waters

Multiresidue screening method development
HPLC vs. ACQUITY UPLC

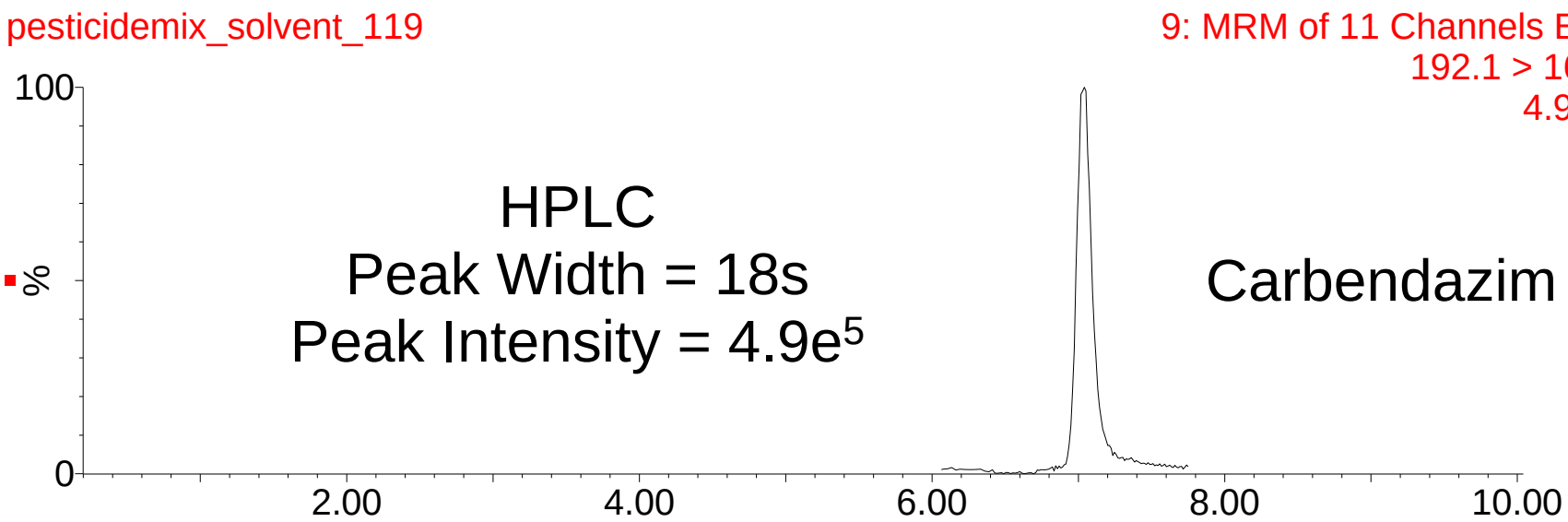


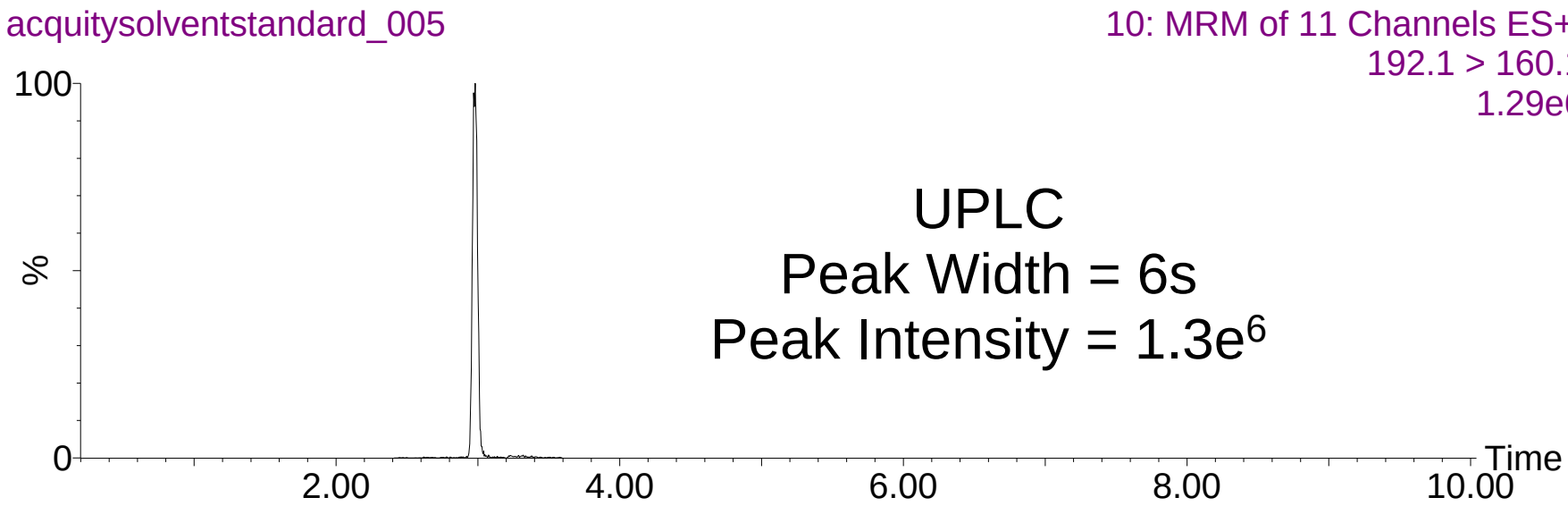
Quattro micro API, Alliance 2795
81 pesticide residues
40min cycle time

Quattro Premier, Alliance 2795
100 pesticide residues
25min cycle time

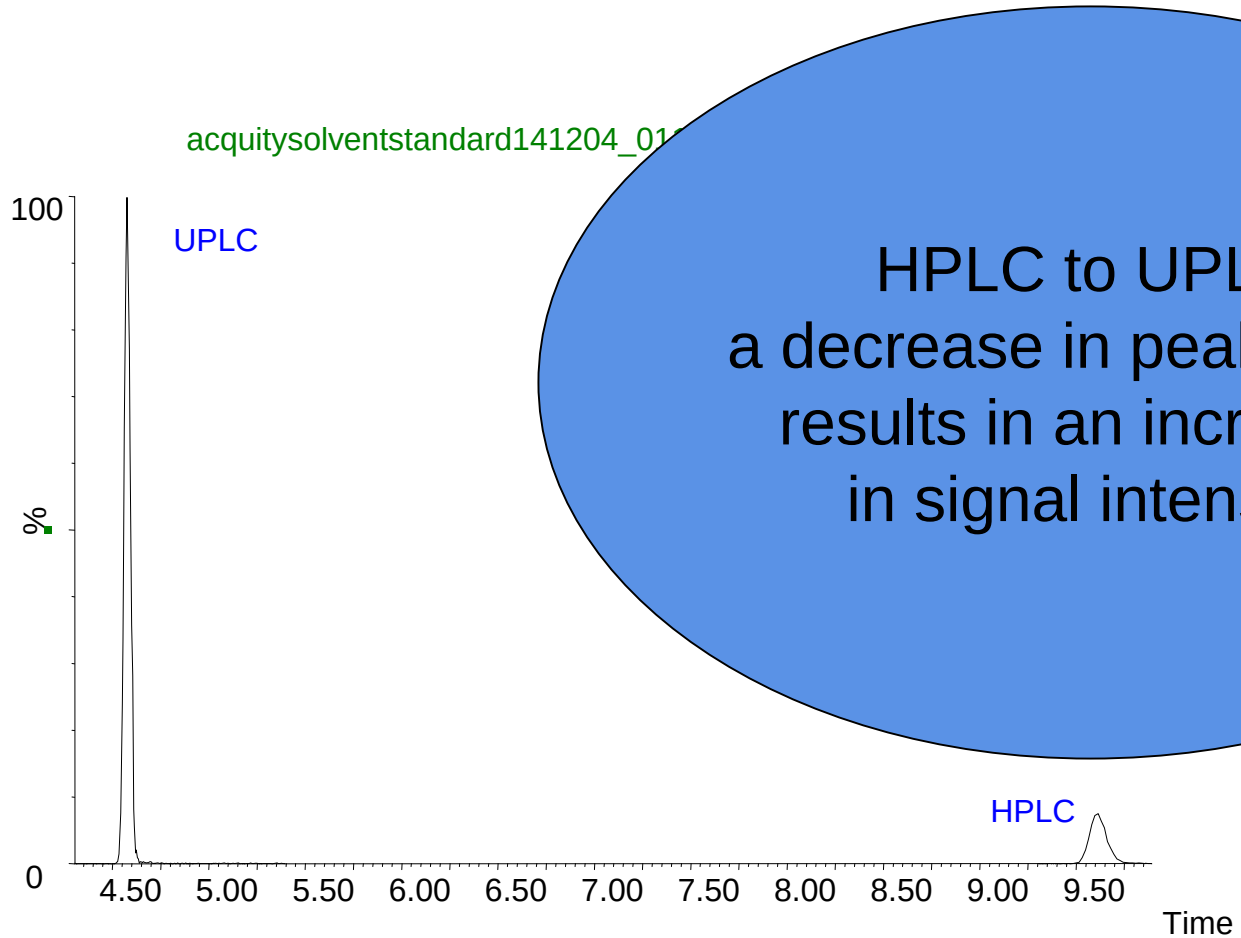
Quattro Premier XE, ACQUITY UPLC
100 pesticide residues,
positive and negative ionisation mode,
confirmation
13.5min cycle time

Increasing the Sensitivity HPLC vs. Acquity UPLC



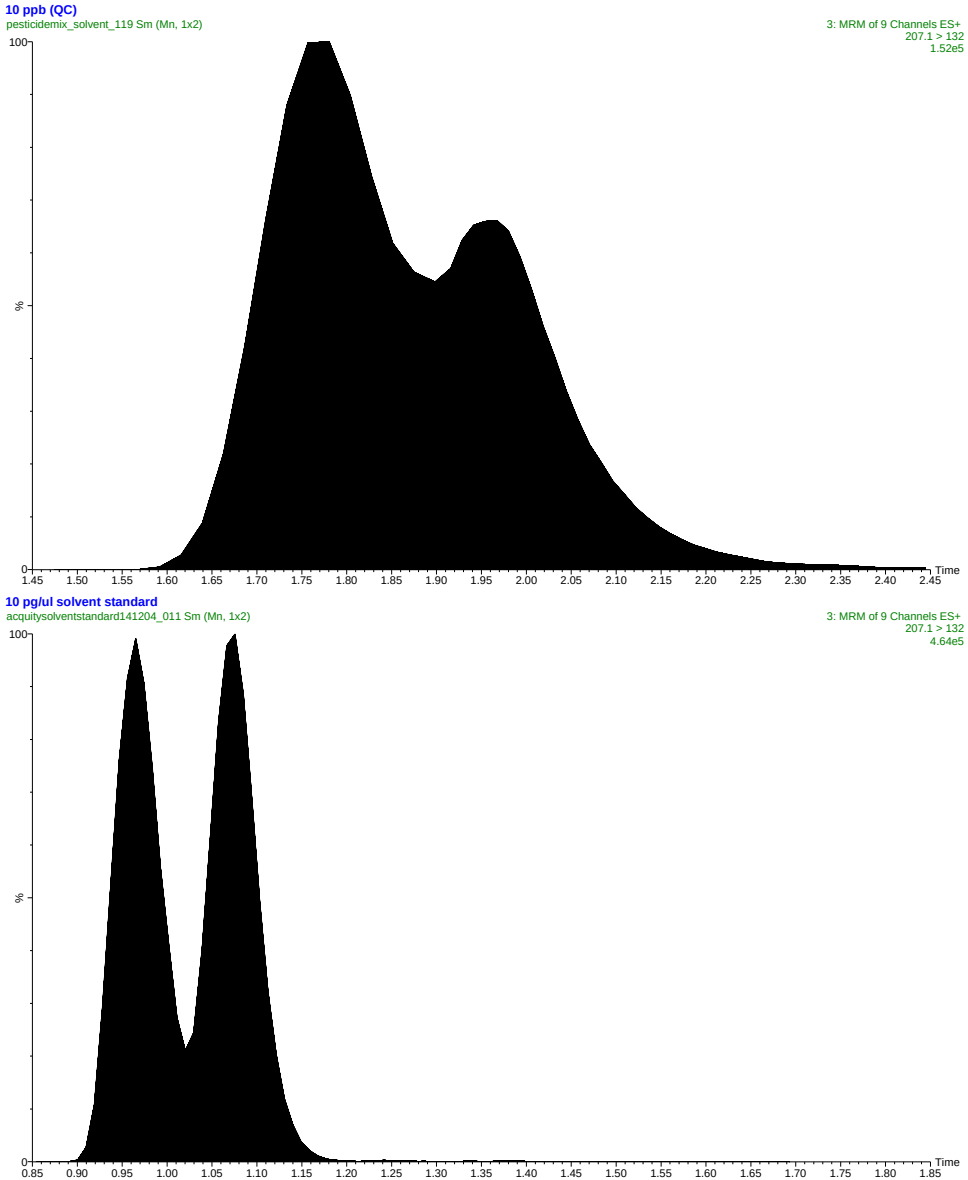


Comparison of HPLC vs UPLC carbaryl



HPLC to UPLC
a decrease in peak width
results in an increase
in signal intensity

Increasing the Resolution HPLC vs. ACQUITY UPLC



Butoxycarboxim Sulfoxide and Aldicarb Sulfoxide have the same MRM transition
m/z 207.1 > 89

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MS Parameters

Pesticide Residue	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Cone Voltage (V)	Collision Voltage (V)	Dwell Time (ms)
Daminozid	161.1	143.1	18	12	200
Methamidophos	141.8	93.8	22	14	80
		124.9	22	13	80
Acephate	184.1	143.0	16	8	40
Butoxycarboxim-sulfoxide	207.1	132.1	17	6	30
Omethoate	214.0	183.0	20	12	30
		154.9	20	15	30
Aldicarb-sulfoxide	207.1	132.0	16	10	30
		89.0	16	14	30
Butoxycarboxim	240.1	106.1	10	14	30
Aldoxycarb	240.1	86.0	15	20	30
Oxamyl	237.1	71.9	12	10	30
Propamocarb	189.1	102.0	25	17	30
		144.0	25	12	30
Oxydemeton-methyl	247.0	169.0	20	13	10
Pymetrozin	218.0	105.0	25	17	10
6-chloro-4-hydroxy-3-phenyl-pyridazin	207.1	77.0	35	30	10
		104.0	35	21	10
Methomyl	162.9	87.8	15	8	10
		105.9	15	10	10
Demeton-S-methyl-sulfon	263.1	169.1	28	16	10
		121.2	28	16	10
Quinmerac	222.0	141.0	22	33	10
Monocrotophos	224.0	126.9	20	15	10
Bendiocarb	224.1	109.0	18	18	10
		167.1	18	9	10

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MS Parameters

Pesticide Residue	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Cone Voltage (V)	Collision Voltage (V)	Dwell Time (ms)
Nicosulfuron	411.0	182.1	22	18	10
Amidosulfuron	370.0	261.2	18	14	10
Metsulfuron-methyl	382.0	167.0	22	15	10
Thifensulfuron-methyl	388.0	167.1	22	15	10
Ethiofencarbsulfon	275.1	107.1	10	20	10
Rimsulfuron	431.9	182.1	30	22	10
Ethiofencarbsulfoxide	242.1	107.0	18	18	10
Thiofanox-sulfoxide	252.1	104.0	10	12	10
Imidacloprid	256.1	209.2	22	16	10
		175.1	22	20	10
Florasulam	360.1	129.0	30	20	10
5-Hydroxy-clethodim-sulfon	408.2	204.2	22	16	10
Thiofanox-sulfon	268.1	76.0	10	10	10
Clethodim-imin-sulfon	302.2	98.1	35	30	10
Metamitron	203.0	175.1	28	16	10
Cinosulfuron	414.1	183.1	25	18	10
Chlorsulfuron	358.1	141.1	25	16	10
		167.1	25	16	10
Bromoxynil*	273.9	78.9	40	25	30
Dimethoate	230.1	125.1	17	20	10
		199.1	17	10	10
Clethodim-imin-sulfoxide	286.2	208.2	25	17	10
Vamidothion	288.1	146.1	17	12	10
Carbofuran-3-hydroxy	220.1	163.1	25	10	10
Flazasulfuron	408.1	182.1	25	22	10

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MS Parameters

Pesticide Residue	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Cone Voltage (V)	Collision Voltage (V)	Dwell Time (ms)
Nicosulfuron	411.0	182.1	22	18	10
Amidosulfuron	370.0	261.2	18	14	10
Metsulfuron-methyl	382.0	167.0	22	15	10
Thifensulfuron-methyl	388.0	167.1	22	15	10
Ethiofencarbsulfon	275.1	107.1	10	20	10
Rimsulfuron	431.9	182.1	30	22	10
Ethiofencarbsulfoxide	242.1	107.0	18	18	10
Thiofanox-sulfoxide	252.1	104.0	10	12	10
Imidacloprid	256.1	209.2	22	16	10
		175.1	22	20	10
Florasulam	360.1	129.0	30	20	10
5-Hydroxy-clethodim-sulfon	408.2	204.2	22	16	10
Thiofanox-sulfon	268.1	76.0	10	10	10
Clethodim-imin-sulfon	302.2	98.1	35	30	10
Metamitron	203.0	175.1	28	16	10
Cinosulfuron	414.1	183.1	25	18	10
Chlorsulfuron	358.1	141.1	25	16	10
		167.1	25	16	10
Bromoxynil*	273.9	78.9	40	25	30
Dimethoate	230.1	125.1	17	20	10
		199.1	17	10	10
Clethodim-imin-sulfoxide	286.2	208.2	25	17	10
Vamidothion	288.1	146.1	17	12	10
Carbofuran-3-hydroxy	220.1	163.1	25	10	10
Flazasulfuron	408.1	182.1	25	22	10

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MS Parameters

Pesticide Residue	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Cone Voltage (V)	Collision Voltage (V)	Dwell Time (ms)
Triasulfuron	402.0	167.1	25	17	10
		141.0	25	20	10
Clethodim-sulfon	392.1	300.2	20	12	10
Clethodim-sulfoxide	376.1	206.2	22	15	10
Carbendazim	192.1	160.1	25	18	10
		132.1	25	30	10
Thiacloprid	253.0	126.0	28	22	10
Difenzoquat methylsulfate	249.2	193.1	45	28	10
Butocarboxim	213.1	75.0	20	14	10
Aldicarb	208.1	116.0	7	7	10
Ioxynil*	369.8	126.9	40	30	20
Carbofuran	222.3	165.2	25	15	10
Iodosulfuron	508.2	167.2	25	18	30
Thiabendazol	202.0	175.1	40	25	20
		131.0	40	32	20
Propoxur	210.1	111.0	14	15	10
Formetanate	222.1	165.2	20	12	10
Prosulfuron	420.0	141.1	25	20	10
		167.0	25	18	10
Carbaryl	202.1	145.0	18	10	10
Bensulfuron-methyl	411.1	149.1	25	22	10
Ethiofencarb	226.1	107.1	15	15	10
		164.1	15	8	10
Primisulfuron methyl*	466.9	226.2	20	15	10
Triflusulfuron-methyl	493.0	264.2	28	20	10
Thiodicarb	355.1	87.9	15	16	10
Thiofanox	219.0	56.9	15	18	10

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MS Parameters

Pesticide Residue	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Cone Voltage (V)	Collision Voltage (V)	Dwell Time (ms)
Pirimicarb	239.1	72.0	28	18	10
		182.1	28	15	10
Atrazin	216.1	174.1	30	17	10
Isoproturon	207.1	72.1	25	18	10
Isoxaflutole	377.1	251.2	15	20	10
Metalaxyl	280.1	220.2	20	13	10
		192.2	20	17	10
Diuron	233.1	72.1	25	18	10
3,4,5-Trimethacarb	194.1	137.1	18	10	10
Clethodim	360.2	164.1	20	19	10
Desmedipham	318.2	182.2	17	12	10
Phenmedipham	301.1	168.0	25	10	10
Linuron	249.1	160.0	28	16	10
		182.1	28	15	10
Pyrimethanil	200.1	107.0	42	22	10
		82.0	42	25	10
Azoxystrobin	404.1	372.2	22	15	10
		329.2	22	30	10
Methiocarb	243.1	121.0	10	22	10
Fludioxonil*	247.0	180.1	45	28	20
		126.1	45	35	20
Promecarb	208.1	151.0	20	9	10
		109.0	20	15	10
Iprovalicarb	321.2	119.1	15	18	10
Fenhexamid	302.1	97.0	35	25	10
		55.1	35	35	10

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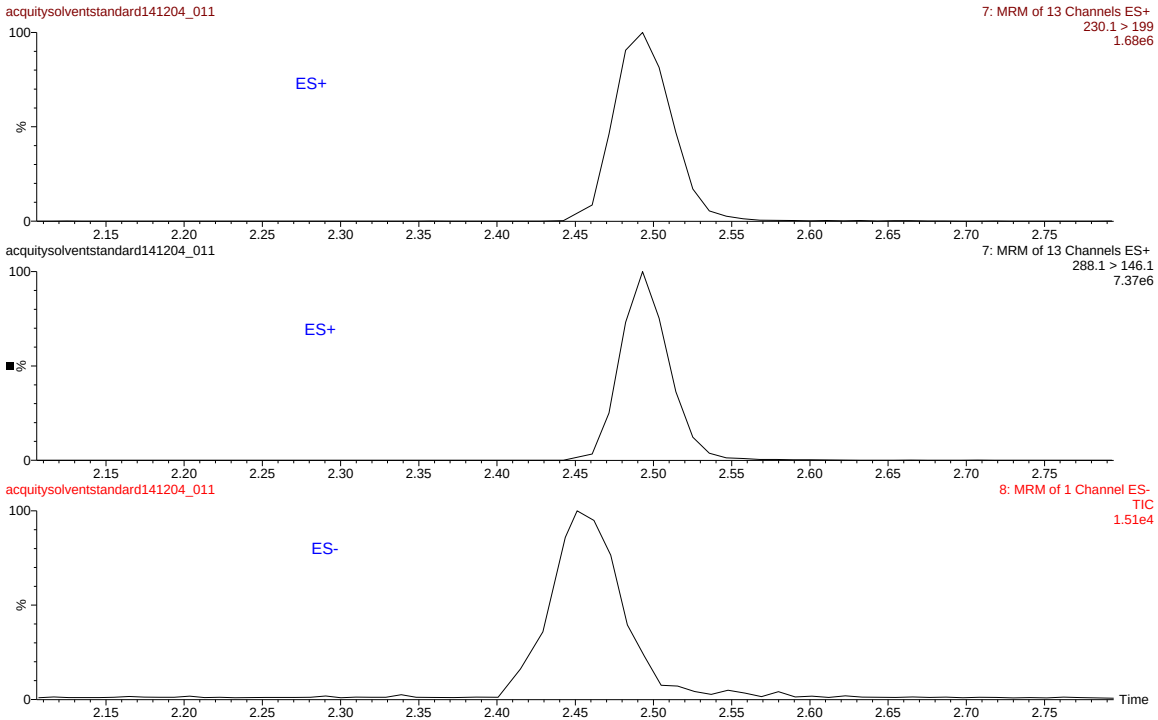
MS Parameters

Pesticide Residue	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Cone Voltage (V)	Collision Voltage (V)	Dwell Time (ms)
Metolachlor	284.1	176.1	20	25	10
		252.1	20	15	10
Tebufenozide	353.2	133.0	13	20	10
		297.2	13	8	10
Fenoxycarb	302.1	88.0	20	20	10
Cyprodinil	226.2	93.1	45	33	10
		108.1	45	25	10
Tebuconazol	308.1	70.0	30	20	10
Imazalil	297.1	159.0	30	20	10
		69.1	30	20	10
Triflumuron	359.1	156.0	25	18	10
		139.0	25	37	10
Haloxyfop-methyl	376.1	316.2	30	18	10
Indoxacarb	527.9	218.1	28	20	10
Hexaflumuron*	459.1	276.1	22	22	30
Quizalofop-ethyl	373.1	299.2	30	19	10
Fluazifop-P-butyl	384.1	282.2	32	22	10
		328.2	32	16	10
Haloxyfop-ethoxyethyl	434.0	316.2	25	20	10
Spiroxamine	298.3	144.1	30	20	10
Furathiocarb	383.1	195.1	20	16	10
Diflubenzuron	311.0	158.1	30	14	10
Teflubenzuron*	379.0	196.0	18	25	10
		339.1	18	15	10
Flufenoxuron	488.9	158.1	25	18	10
Pyridate	379.1	207.1	25	16	120
Fenpropimorph	304.2	147.2	45	30	120

Pos/Neg switching



- Dimethoate and vamidothion determined under positive ion conditions
- Bromoxynil determined under negative ion conditions

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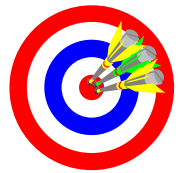
Summary

- Improved efficiency and increased sample throughput has been realised through the combination of new technologies which offer
  - Enhanced chromatographic resolution and short analysis times
  - Ability to group MRM functions into time windows, enabling the incorporation of confirmatory MRM traces
  - Ability to switch rapidly between MRM channels and between positive and negative ionisation modes.
- The sensitivity achieved for the majority of pesticide residues indicates that this method could be applied to the analysis of pesticides in different matrices.

The Daily Challenge: Analysis of Complex Mixtures

- The "KNOWN" (or suspected residue)
  - Routine monitoring program
  - SIM or MRM normally used
  - Single quad, Tandem quad, Sector

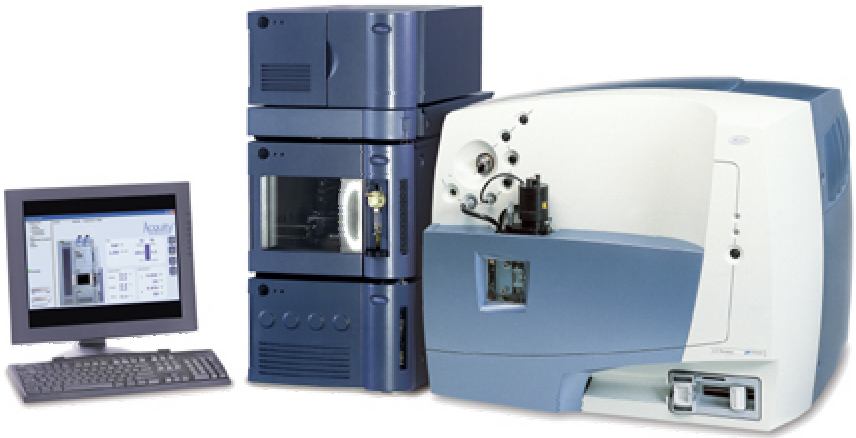


Targeted analysis

- The "UNKNOWN"
  - Environmental contamination
  - Full spectrum will have to be used
  - Time of Flight (TOF)?

Screening analysis

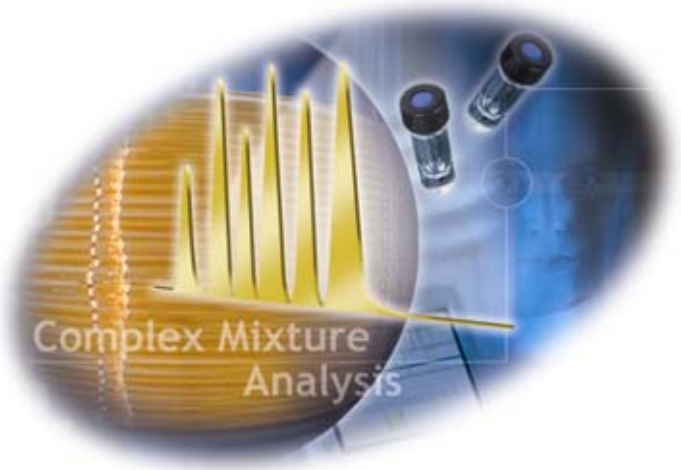
Waters Approach for Non-Target Screening



Acquity UPLC - LCT Premier

- **Optimal full scan sensitivity**
- **Specificity from exact mass (<3ppm)**
- **Fast analysis time**
- **High quality data**

ChromaLynx



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Complex Mixture Analysis Requirements

- Extract, detect and locate all components
  - Minimal non-selective sample preparation
  - Maximise detectable compounds (+/- switching)
  - Automated deconvolution and chromatographic peak detection of all components
- Clean, individual mass spectrum for each component in the sample
  - High resolution LC to minimise ion suppression/enhancement from co-eluting peaks
  - Automatic production of background subtracted spectra
  - Exact mass measurement is retained from TOF

Waters

Complex Mixture Analysis Requirements

- Identify all components in the sample
  - Automated library search and identification
  - Append spectra to existing libraries, especially for LC where commercial libraries are not available
- Estimate the concentrations of all the components on the sample
  - Semi-quantitative determinations, calculation and reporting of peak areas and relative peak areas
- Compare the sample to a ‘control’ sample
  - Comparison of chromatograms from single analyses to identify differences

Waters

ToF MS Method

- Waters Micromass LCT Premier
  - Ionisation mode — ES+/ES-
  - Capillary voltage — 1.5 kV (+ and -)
  - Gas flow — 600 L/hr
  - Source temperature — 120ºC
  - Desolvation temperature — 400ºC
  - Cone voltage — 50V
  - LockSpray reference — Leucine enkephalin

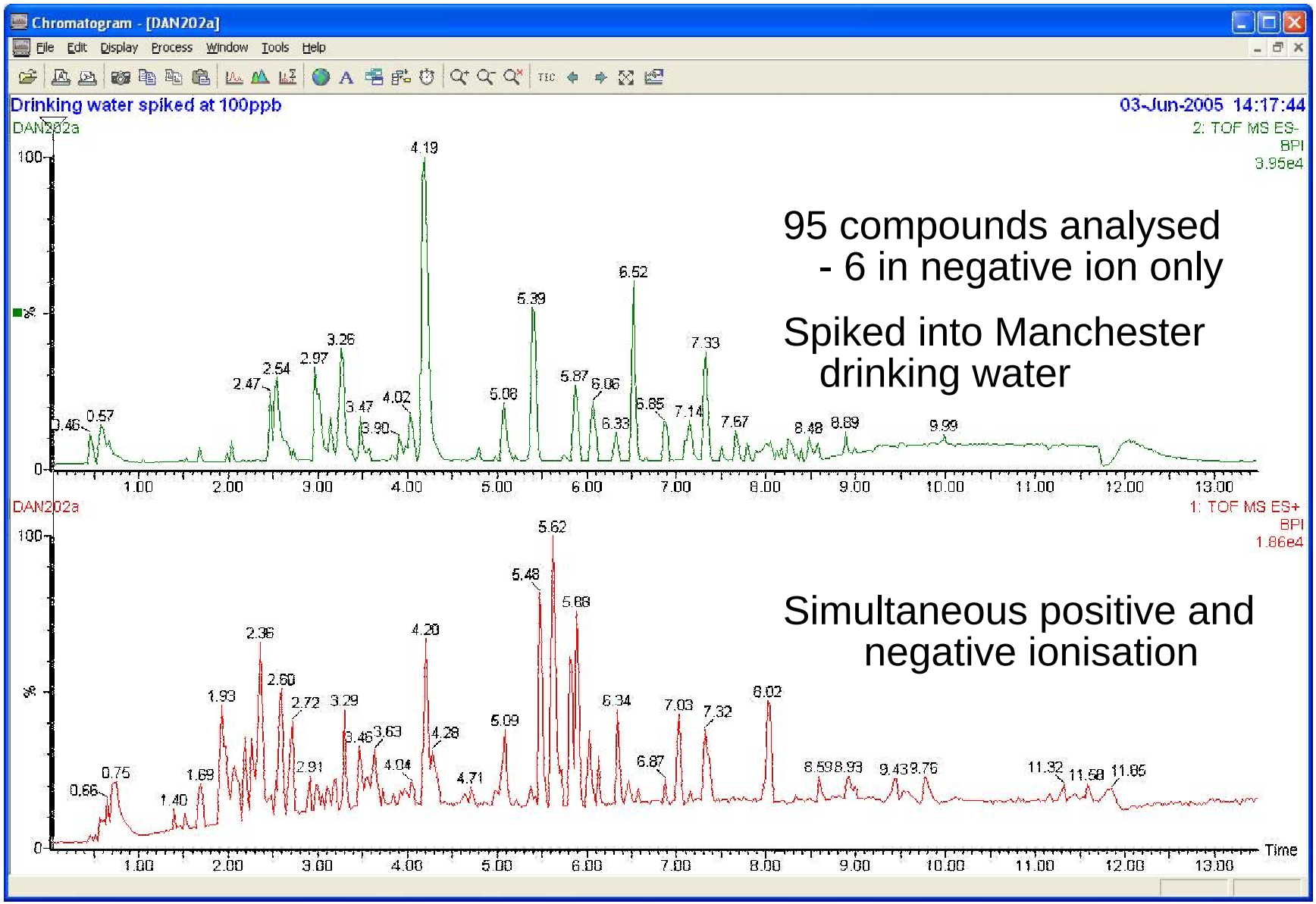
UPLC Method

- ACQUITY UPLC
  - Mobile phase A, MeOH/H_2O (1:19) + 2mM $CH_3CO_2NH_4$
  - Mobile phase B, MeOH/H_2O (19:1) + 2mM $CH_3CO_2NH_4$
  - Waters ACQUITY UPLC BEH C_{18} 2.1 × 100 mm, 1.7μm
  - Flow rate 0.45 mL/min
  - Injection volume 20 μL
  - Column temperature 40°C
  - Overall cycle time 13.5 min

Time/min	% B
0	0
8.5	100
11.0	100
11.1	0

Waters

Chromatogram



95 compounds analysed
- 6 in negative ion only

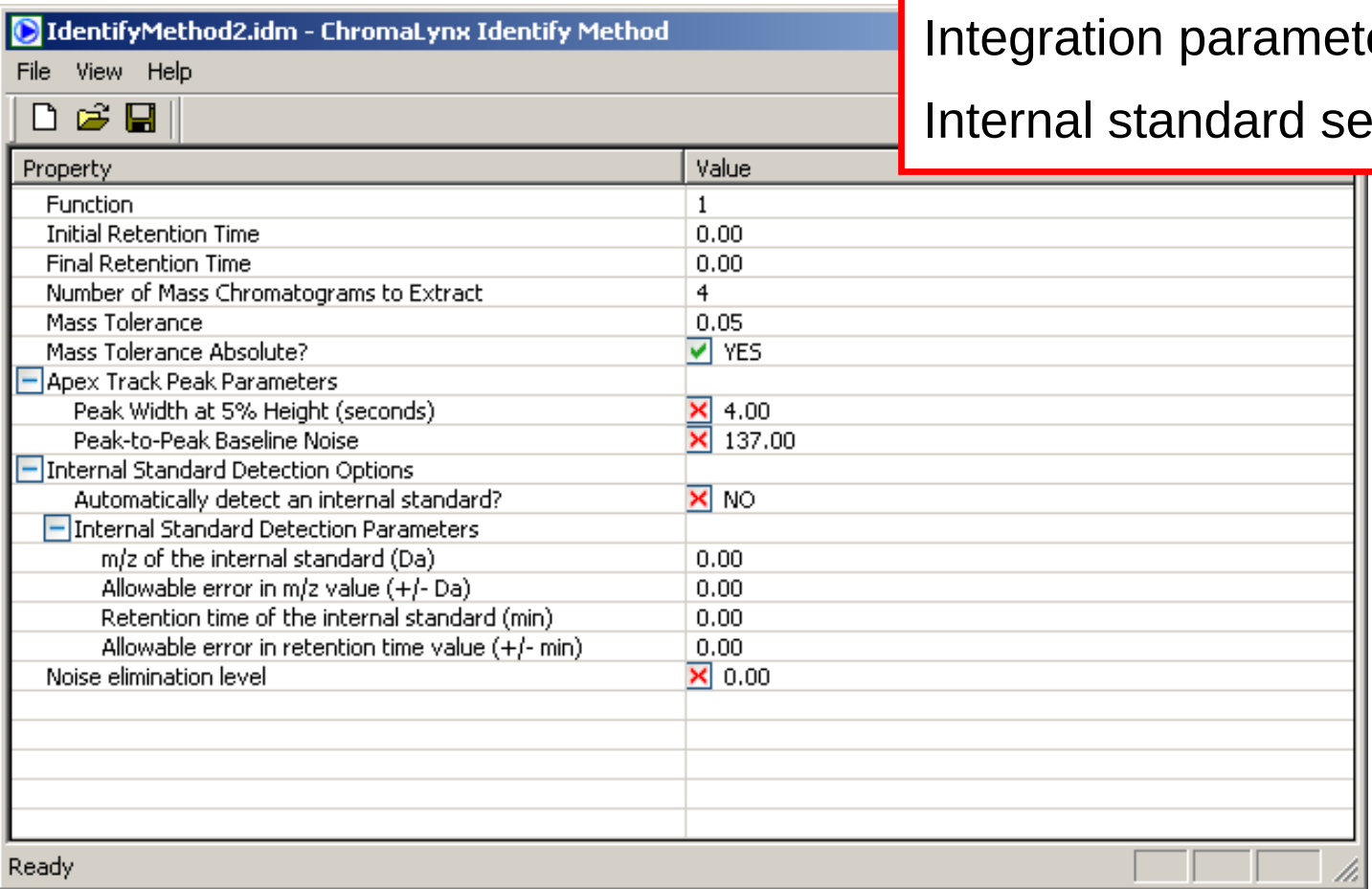
Spiked into Manchester drinking water

Simultaneous positive and negative ionisation

Waters

Identify Method

- Retention time range for processing
- Number of chromatograms to extract
- Mass tolerances
- Integration parameters
- Internal standard selection



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Library Search Method

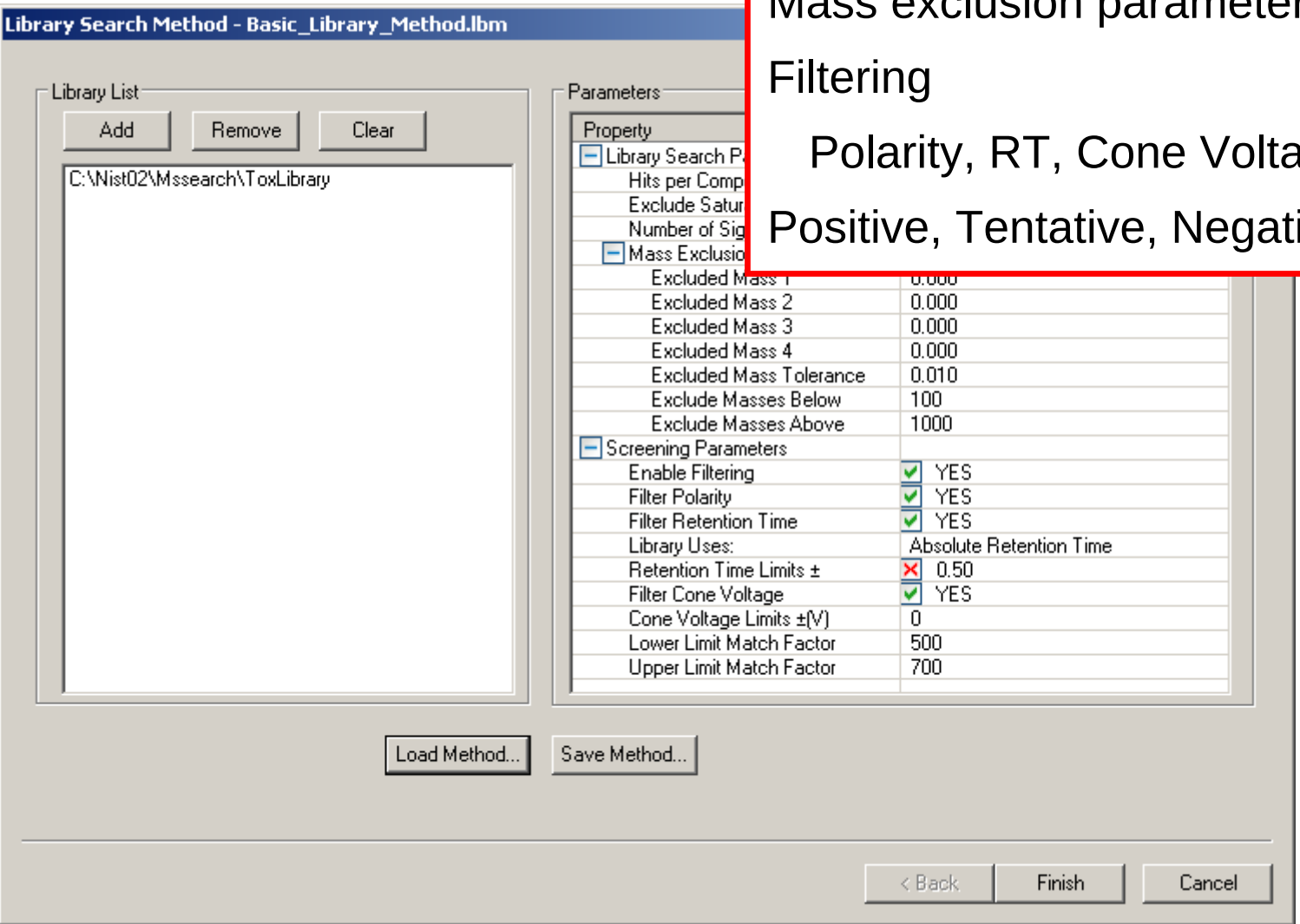
Number of significant ions to search

Mass exclusion parameters

Filtering

Polarity, RT, Cone Voltage

Positive, Tentative, Negative Thresholds



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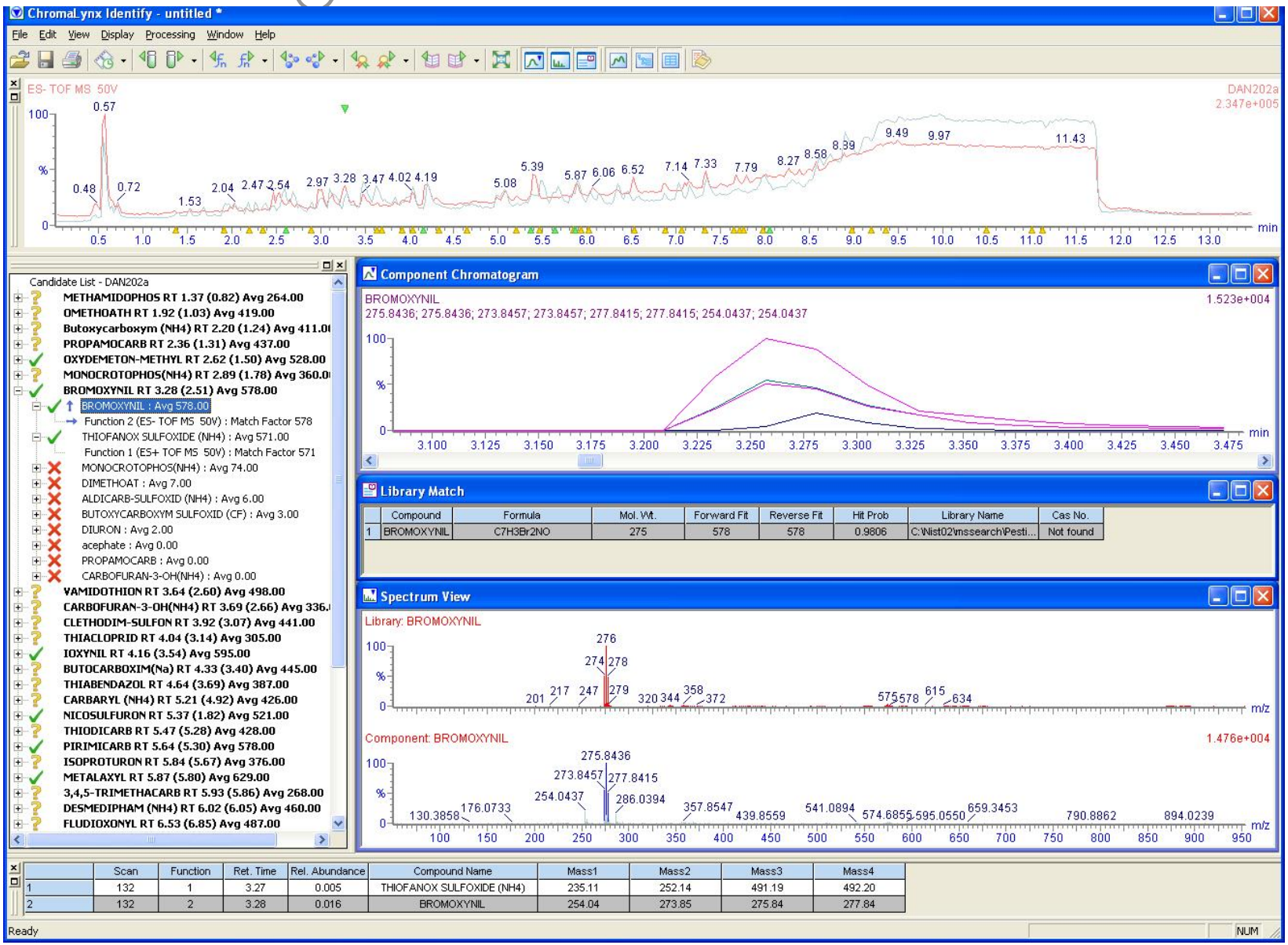
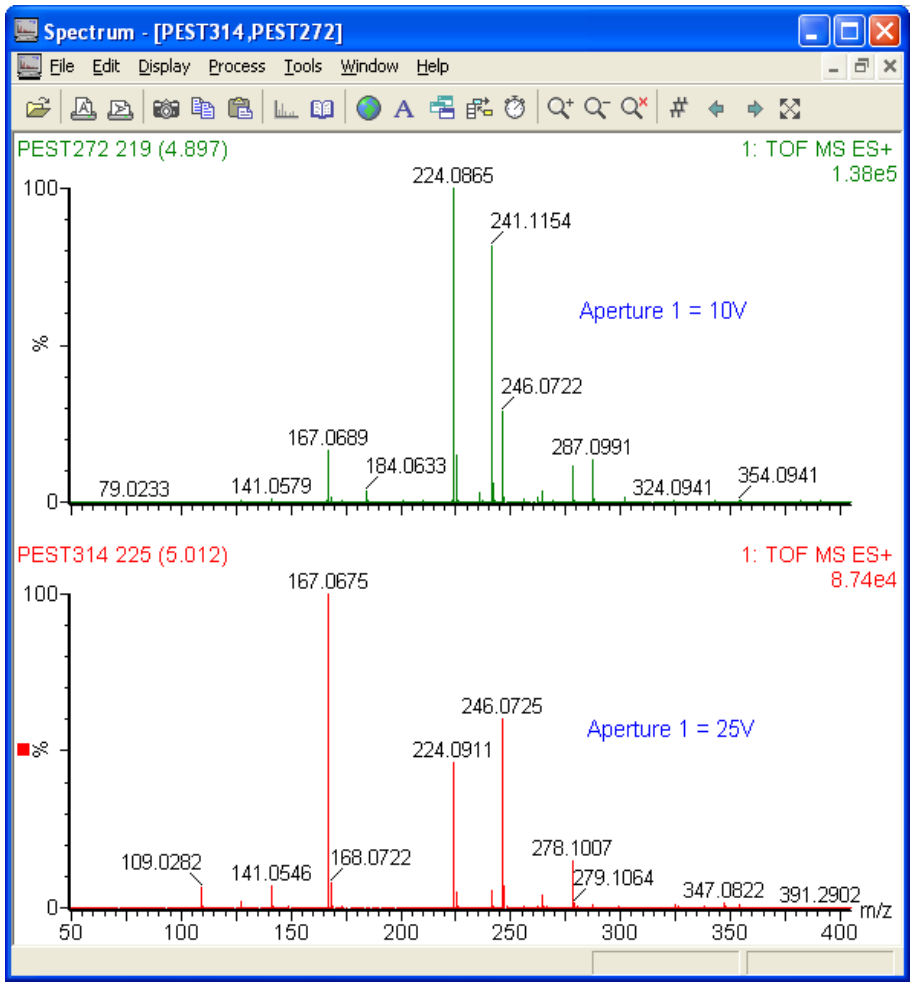
ChromaLynx Screening



In-Source Collisionally Induced Dissociation (CID)

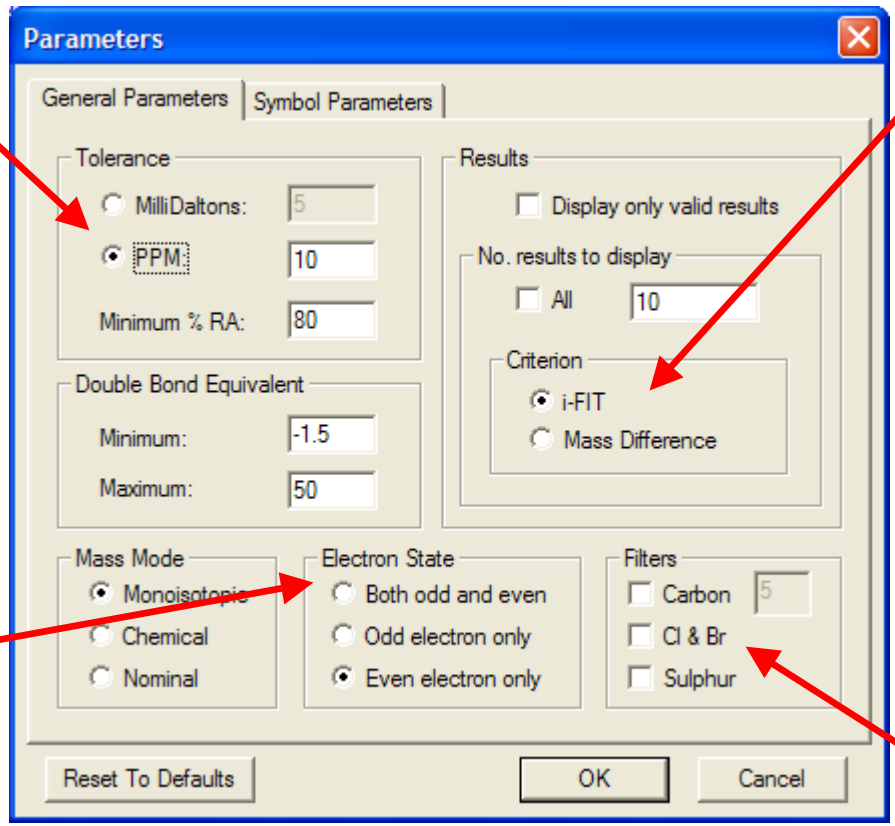


- Source is operated in low and high voltage modes
- High voltage energises neutral gas molecules
- Energy is transferred by collisions with analyte ions and fragments are generated
- Fragments are also measured to < 3ppm mass accuracy
- Not ‘true’ MS/MS, but can be used for confirmation

Exact Mass Parameters

Set a mass measure tolerance

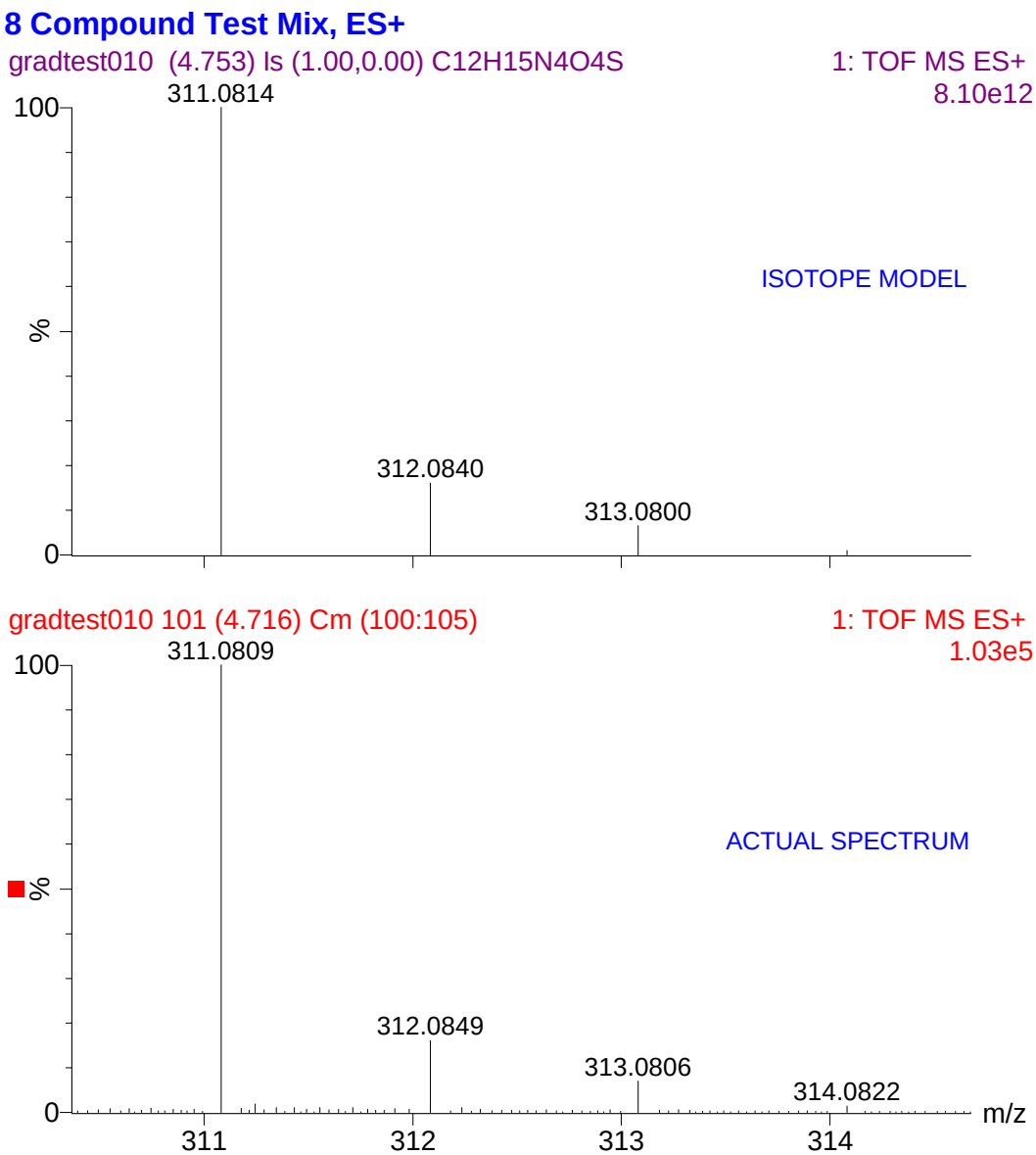
Rank results on Mass Difference or i-FIT™



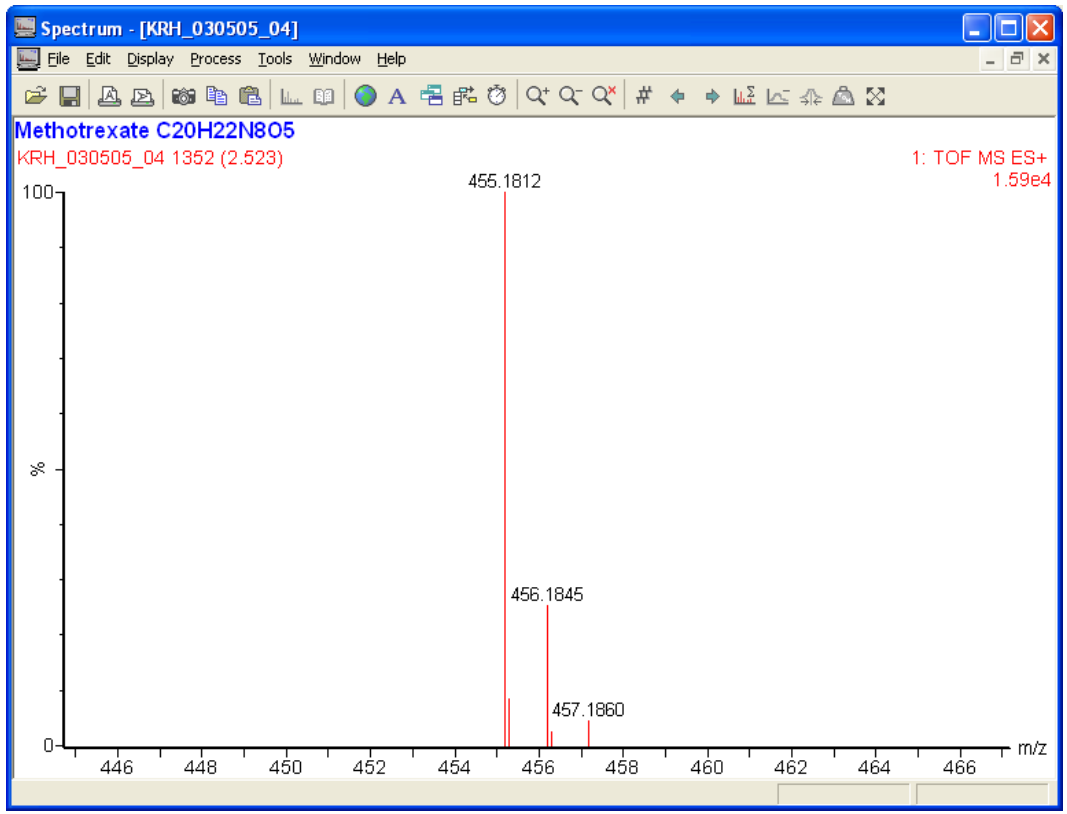
Automatically assign the valency state based on data type

Apply filtering technology to aid simplification of the results list

Isotope Patterns?

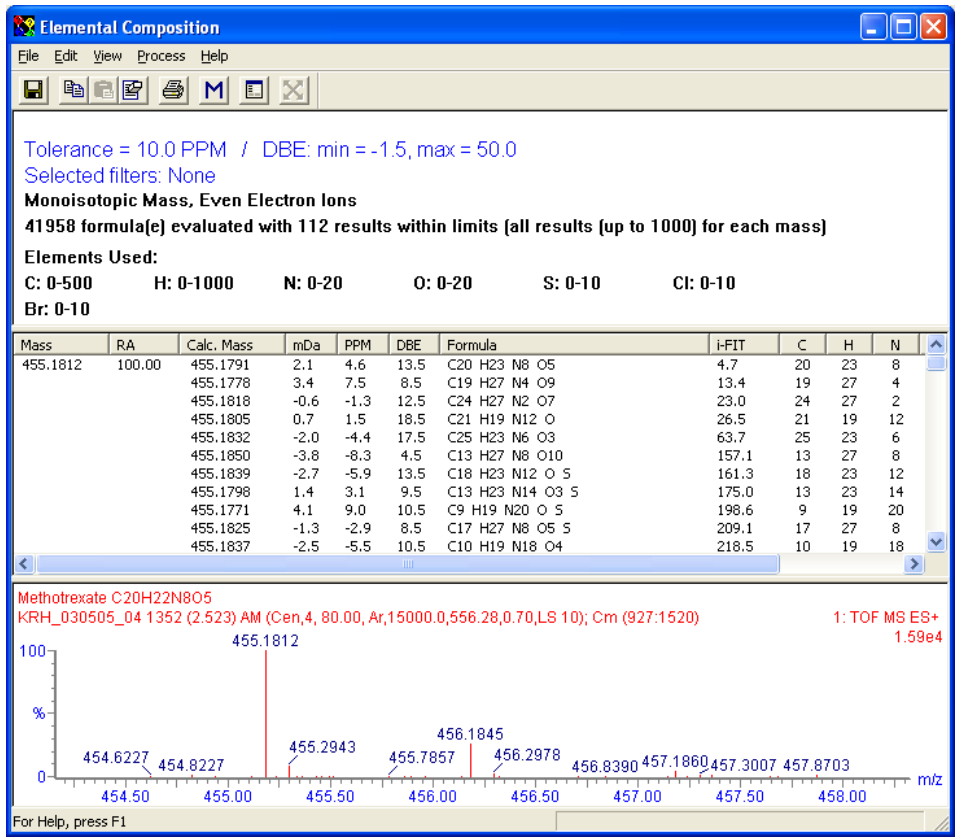


Does Exact Mass Help?



- The monoisotopic peak is selected for elemental composition calculation (m/z 455.1791, $C_{20}H_{23}N_8O_5$)
- The error on the mass measurement is 4.6ppm

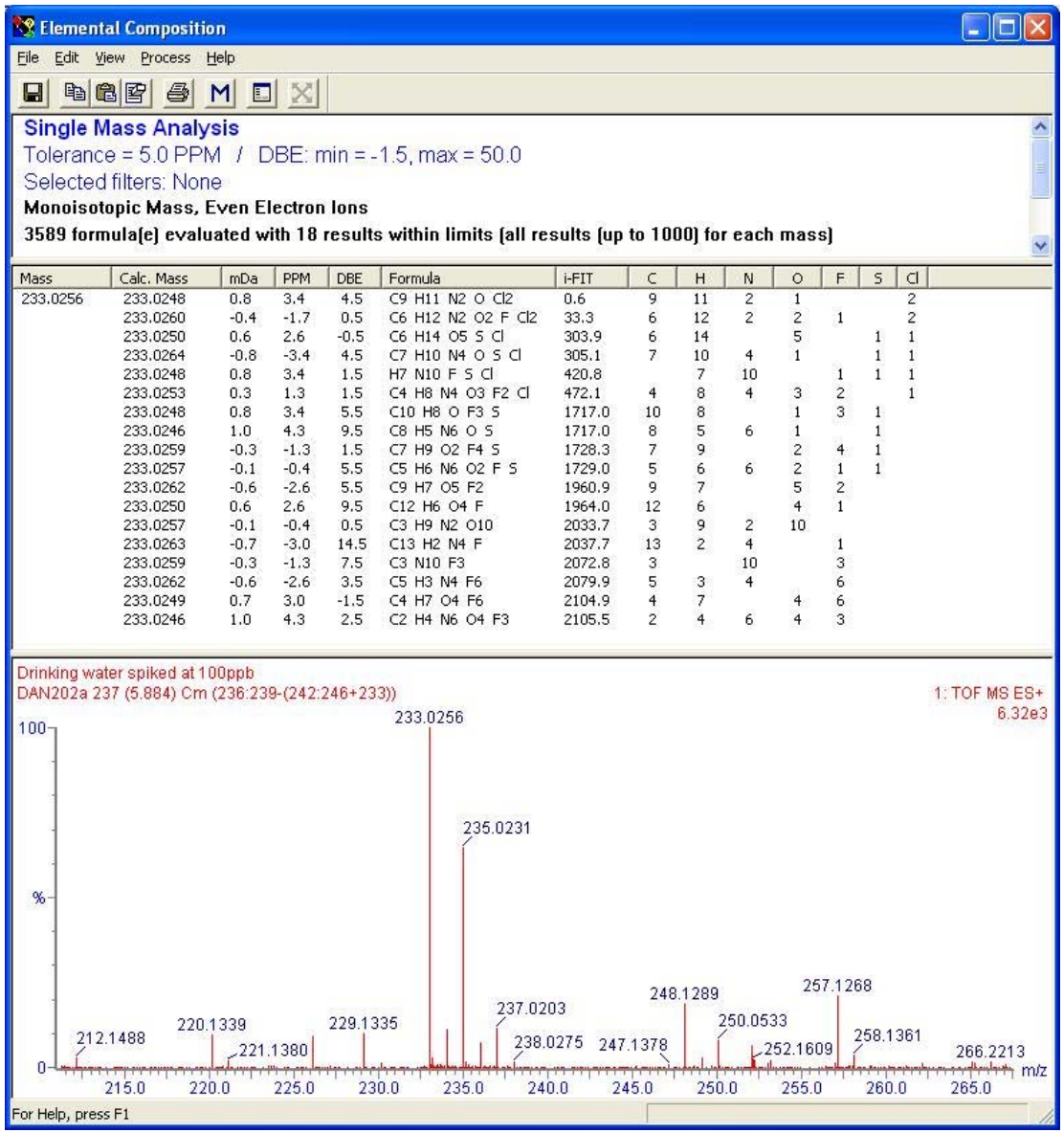
112 Results with No Filters



- 112 results within 10ppm tolerance (using $C_{500}H_{1000}N_{20}O_{20}S_{10}Cl_{10}Br_{10}$)
- Correct answer top with i-FIT of 4.7

Waters

Application of i-FIT



Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	C	H	N	O	F	S	Cl
233.0256	233.0248	0.8	3.4	4.5	C9 H11 N2 O Cl2	0.6	9	11	2	1			2
	233.0260	-0.4	-1.7	0.5	C6 H12 N2 O2 F Cl2	33.3	6	12	2	2	1		2
	233.0250	0.6	2.6	-0.5	C6 H14 O5 S Cl	303.9	6	14		5		1	1
	233.0264	-0.8	-3.4	4.5	C7 H10 N4 O S Cl	305.1	7	10	4	1		1	1
	233.0248	0.8	3.4	1.5	H7 N10 F S Cl	420.8		7	10		1	1	1
	233.0253	0.3	1.3	1.5	C4 H8 N4 O3 F2 Cl	472.1	4	8	4	3	2		1
	233.0248	0.8	3.4	5.5	C10 H8 O F3 S	1717.0	10	8		1	3	1	
	233.0246	1.0	4.3	9.5	C8 H5 N6 O S	1717.0	8	5	6	1		1	
	233.0259	-0.3	-1.3	1.5	C7 H9 O2 F4 S	1728.3	7	9		2	4	1	
	233.0257	-0.1	-0.4	5.5	C5 H6 N6 O2 F S	1729.0	5	6	6	2	1	1	
	233.0262	-0.6	-2.6	5.5	C9 H7 O5 F2	1960.9	9	7		5	2		
	233.0250	0.6	2.6	9.5	C12 H6 O4 F	1964.0	12	6		4	1		
	233.0257	-0.1	-0.4	0.5	C3 H9 N2 O10	2033.7	3	9	2	10			
	233.0263	-0.7	-3.0	14.5	C13 H2 N4 F	2037.7	13	2	4		1		
	233.0259	-0.3	-1.3	7.5	C3 N10 F3	2072.8	3		10		3		
	233.0262	-0.6	-2.6	3.5	C5 H3 N4 F6	2079.9	5	3	4		6		
	233.0249	0.7	3.0	-1.5	C4 H7 O4 F6	2104.9	4	7		4	6		
	233.0246	1.0	4.3	2.5	C2 H4 N6 O4 F3	2105.5	2	4	6	4	3		

Waters

Summary

- UPLC-ToF MS offers a fast, sensitive screening method for targeted and unknown compounds
- ChromaLynx software efficiently de-convolutes complex chromatograms, and delivers a reliable dataset based on nominal mass library matching
- Full-scan MS, so even compounds not in the library may be identified by exact mass
- i-FIT increases the probability of assigning the correct elemental composition to an “unknown”

Waters

Summary

- Ultra Performance Liquid Chromatography offers:
  - Speed (Through-put)
  - Sensitivity (Lower detection limits)
  - Resolution (more and higher quality data)
- UPLC enhances the performance of fast scanning mass spectrometers for both quantitative and qualitative environmental screening /confirmation analysis
- Dedicated software solutions enhance the fast and accurate handling and reporting of the ever expanding data