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Multi-Residue Pesticide Analysis of Food Matrices using GC/MS/MS and LC/MS/MS

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Overview

- Introduction to pesticide residue analysis
- GC/MS/MS based methods
  - Quattro micro GC for complex matrices
  - 100 pesticide residues in a single analysis
- LC/MS/MS based methods
  - 100 pesticide residues using the Quattro Premier API
  - Enhancing the method
    - ACQUITY UPLC™
- TargetLynx Data Management Software

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GC/MS/MS Analysis of Pesticides in Complex Food Matrices

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Introduction

- Hundreds of Pesticide Compounds in Use
  - Requirement for Surveillance Monitoring of Foodstuffs
  - Permitted Concentrations Regulated by Legislation
- Reporting Levels
  - Limits of Detection below the regulation are Required
    - target LOD of 0.01mg / kg (10 ppb)
- Need for the Targeting of Multiple Analytes per Analysis
  - Wide Range of Analyte Chemistries
    - polarity, thermal stability, volatility
  - Wide Range of Sample Matrices
- Common Sample Matrix Extraction and Minimal Sample Prep
  - Selectivity obtained from Chromatographic and Determinative Step
  - Analyte Specific Multiple Reaction Monitoring (MRM)
    - Triple Quadrupole Mass Spectrometry
  - Combined GC, chromatographic selectivity, and MS/MS is recommended

Method Objective

- Develop a rapid multi-residue pesticide screening method
  - 100 Residue Analytes in a Single Analysis
  - Reduce overall method cycle time, injection to injection
  - To increase efficiency and sample throughput

- Able to quantify and confirm the analyte at MRL tolerance levels
  - Include an additional MRM transition for the most commonly observed residues
    - Confirmatory method
      - Potentially reducing overall analysis time for a batch
    - TargetLynx

GC/MS Based Multi-residue Methods

- Increasing selectivity and/or sensitivity
  - Sample Preparation (unlikely for multi-residue methods)
  - Chemical Ionization (narrows target range)
  - High Resolution MS
    - Magnetic Sector (expensive)
      - Dioxin/Furans, Bromonated Flame Retardants, PCB
      - EPA approved methods
    - Time of Flight (limited dynamic range)
  - Tandem MS/MS
    - Ion Trap (limited dynamic range, complex matrices)
    - Tandem Quadrupole MS/MS

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System Solution

Quattro micro GC/MS/MS

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Method Strategy

- 100 residue compounds in a single analysis
- Multiple compound classes
  - OPs, OCs, Pyrethroids
- Run time approximately <45 minutes
- Chromatographic and mass selectivity to compensate for non-selective sample preparation
- Less than 10 ppb sensitivity to minimize injection volumes of “dirty” matrix

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GC Conditions

- Column
  - DB5 MS, 30m x 0.25mm i.d., 0.25μm
- Injector
  - Cryo cooled PTV in solvent vent mode, 2 μL injected
- Constant flow rate of 1.0 mL/min Helium
- Temperature program

0 min	50°C (2min)
6 min	150°C @ 25 °C/min
32 min	280°C @ 5°C/min
37 min	280°C

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Pesticide Analytes

Dichlorvos
cis-Mevinphos
trans-Mevinphos
Ethoprophos
Chlorpropham
Cadusafos
Phorate
α-HCH
Hexachlorobenzene
Dimethoate
Carbofuran
β-HCH
Dimethipin
Quintozene
Lindane
Diazinone
Chlorothalonil
Pentachloroaniline
Phosphamidon
Chlorpyriphos-methyl
Vinclozolin
Parathion-methyl
Alachlor
Carbaryl
Heptachlor

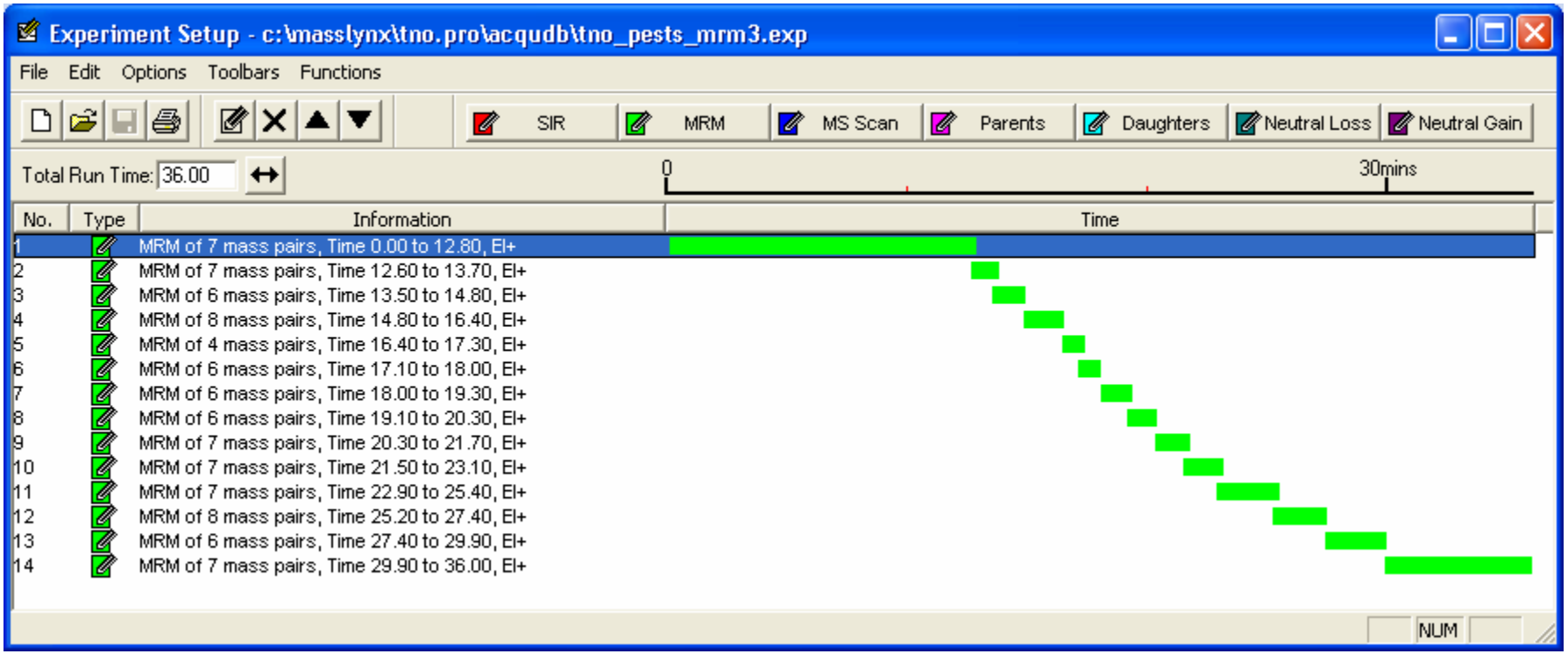
Pirimiphos-methyl
MPCPS
Malathion
Chlorpyriphos
Aldrin
Fenthion
Parathion
Fenpropimorf
Triadimefon
Oxychlordane
Fipronil
Heptachlor epoxide
Chlorfenvinphos
Phenthoate
Quinalphos
Triadimenol
Methidathion
α-Chlordane
Tetrachlorvinphos
Endosulfan α
γ-Chlordane
Prothiofos
Profenofos
p,p'-DDE

Dieldrin
o,p'-TDE
Cinerin I
Endrin
Nitrofen
Endosulfan β
Fensulfothion
p,p'-TDE
o,p'-DDT
Ethion
Jasmolin I
Triazophos
Carbophenothion
Endosulfan sulphate
p,p'-DDT
Propargite
Piperonylbutoxide
Pyrethrin I
Phosmet
Bromopropylate
Fenpropathrin
Tetradifon
Phosalone
Phenothrin

Azinphos-methyl
Cinerin-II
λ-Cyhalothrin
Azinphos-ethyl
Acrinathrin
Jasmolin II
cis-Permethrin
trans-Permethrin
Prochloraz
Cyfluthrin I
Cyfluthrin II
Cyfluthrin III
Cyfluthrin IV
Cypermethrin I
Cypermethrin II
Cypermethrin III
Flucythrinate I
Cypermethrin IV
Etofenprox
Flucythrinate II
Fenvalerate I
Fluvalinate
Fenvalerate II
Deltamethrin

MS/MS Method

- MRM functions arranged into 14 time windows
  - More flexible use of dwell times
  - Improved signal to noise (S/N) ratios for some analytes
  - Overlapping allows for small changes in retention time



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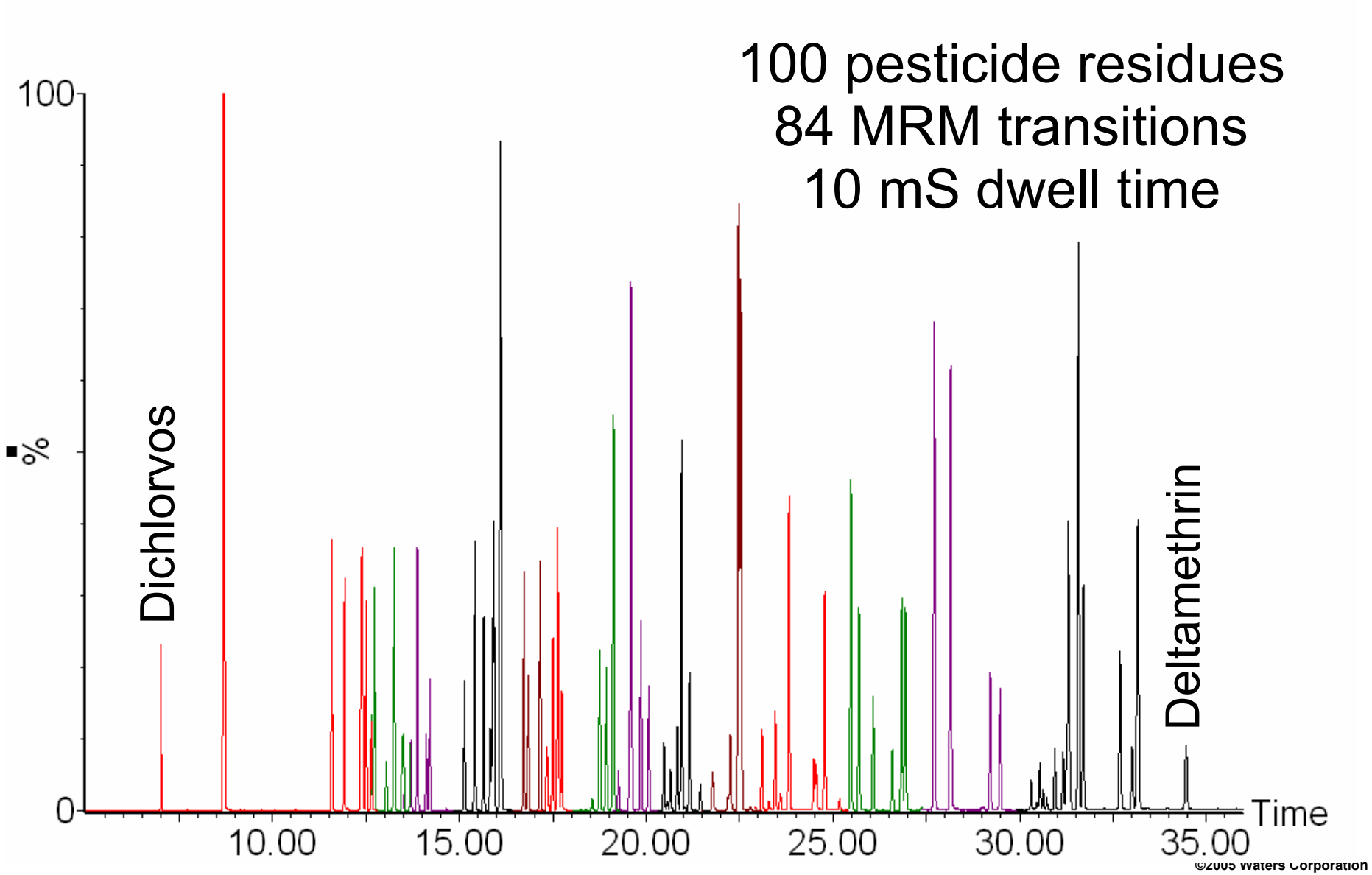
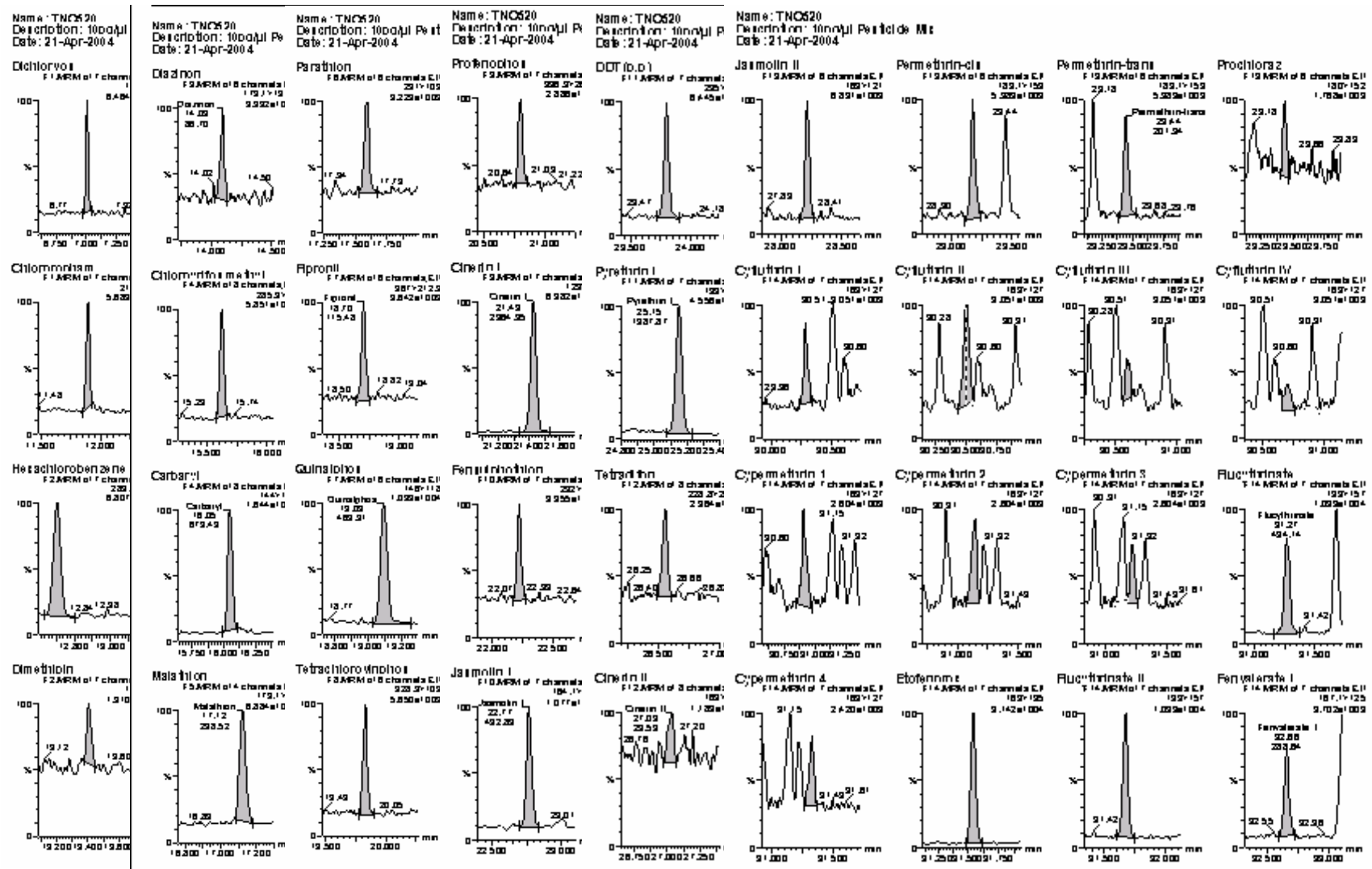
Total Ion Chromatogram (TIC)



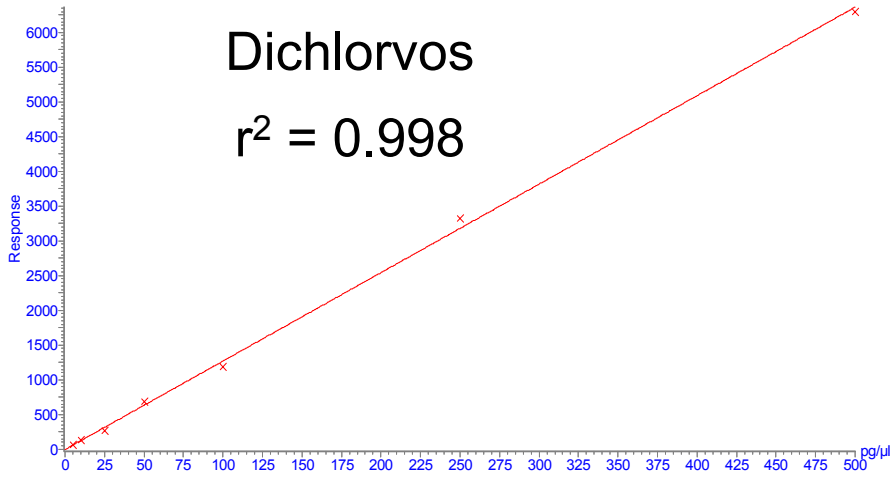
MRM Chromatograms 10 ppb Sensitivity



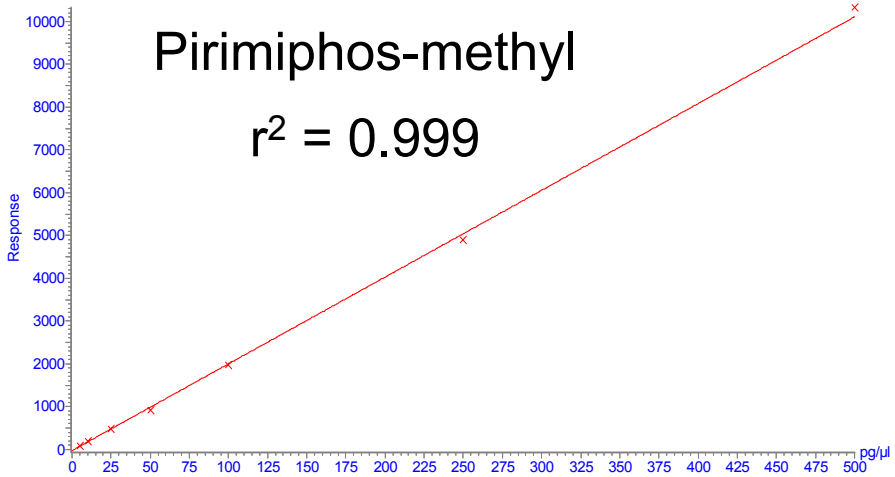
2µl injected = 20 pg on column

Linearity (10-1000pg)

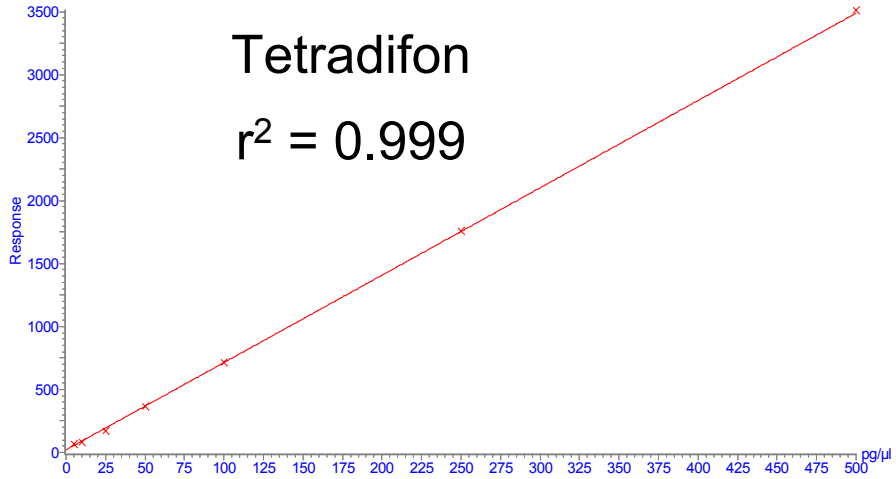
Compound name: Dichlorvos
Correlation coefficient: r = 0.998799, r^2 = 0.997600
Calibration curve: 12.7295 * x + -2.86235
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



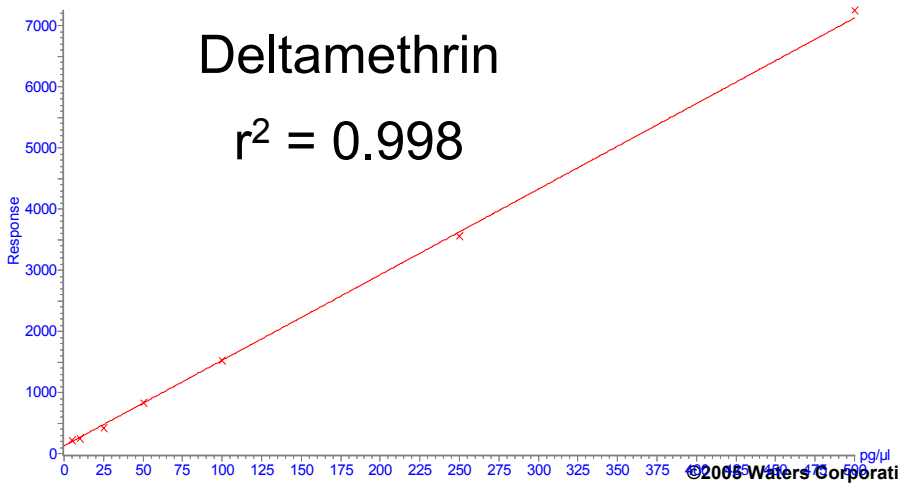
Compound name: Pirimiphos-methyl
Correlation coefficient: r = 0.999511, r^2 = 0.999022
Calibration curve: 20.2691 * x + -27.0132
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



Compound name: Tetradifon
Correlation coefficient: r = 0.999367, r^2 = 0.998735
Calibration curve: 6.93326 * x + 21.1316
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



Compound name: Deltamethrin
Correlation coefficient: r = 0.998918, r^2 = 0.997838
Calibration curve: 14.0112 * x + 125.819
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



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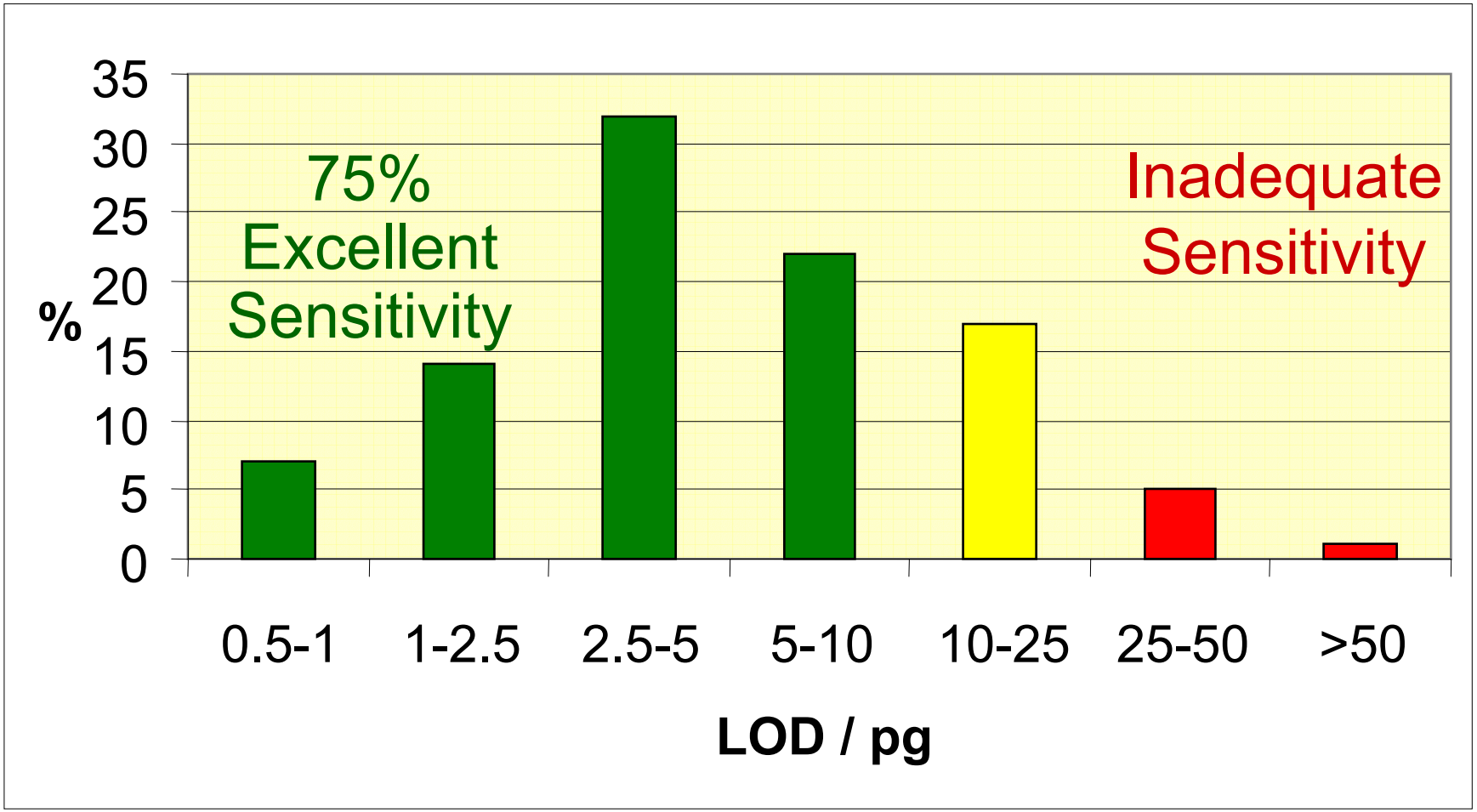
Detection Limit Sensitivity



Limit of Detection = S/N ratio ≥ 3:1

Matrix Extraction Method

- Sample matrix
  - Strawberry, Baby Food, Dried Tea, Tobacco, Spices, Ginko, Cannabis
- Homogenize using high speed blending
- Extraction with ethyl acetate (Turrax)
- Cleanup with dispersive SPE
  - Primary Secondary Amine followed by Graphitised Carbon Black
- Final extract
  - Fresh produce, baby food = 0.5 g/mL
  - Dried herbs, spices, tobacco = 0.1 g/mL

GC Chromatographic Selectivity Examples Food Matrix

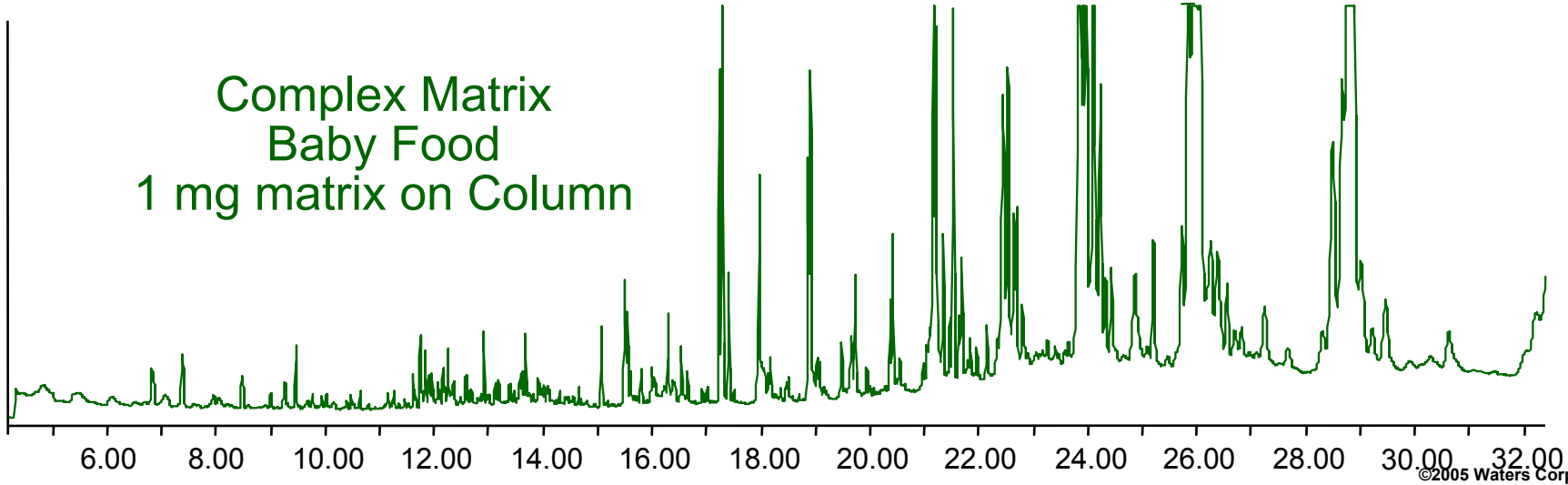
Strawberry in full scan TIC EI+

20 µL of 0.5 g/mL

10 mg matrix on Column

Simple Matrix

Complex Matrix
Baby Food
1 mg matrix on Column



MS/MS Selectivity in Baby Food

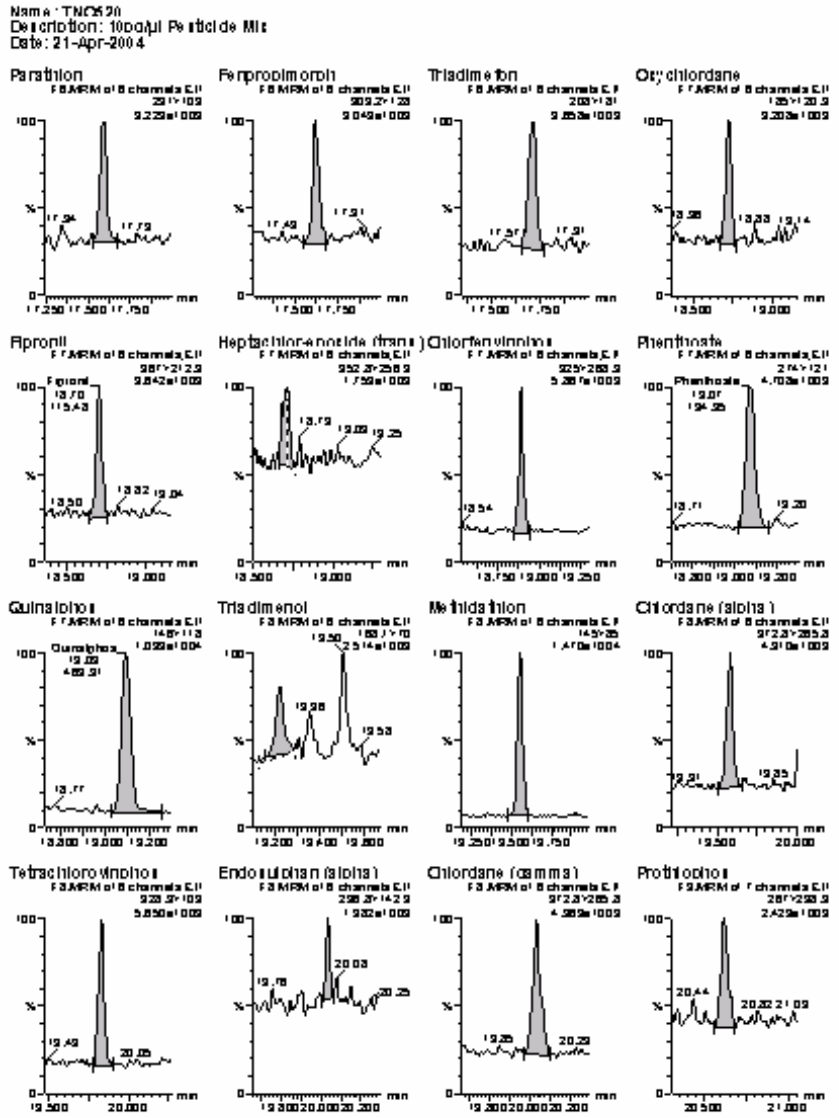
Pesticides Spiked

in Baby Food

Quattro micro GC

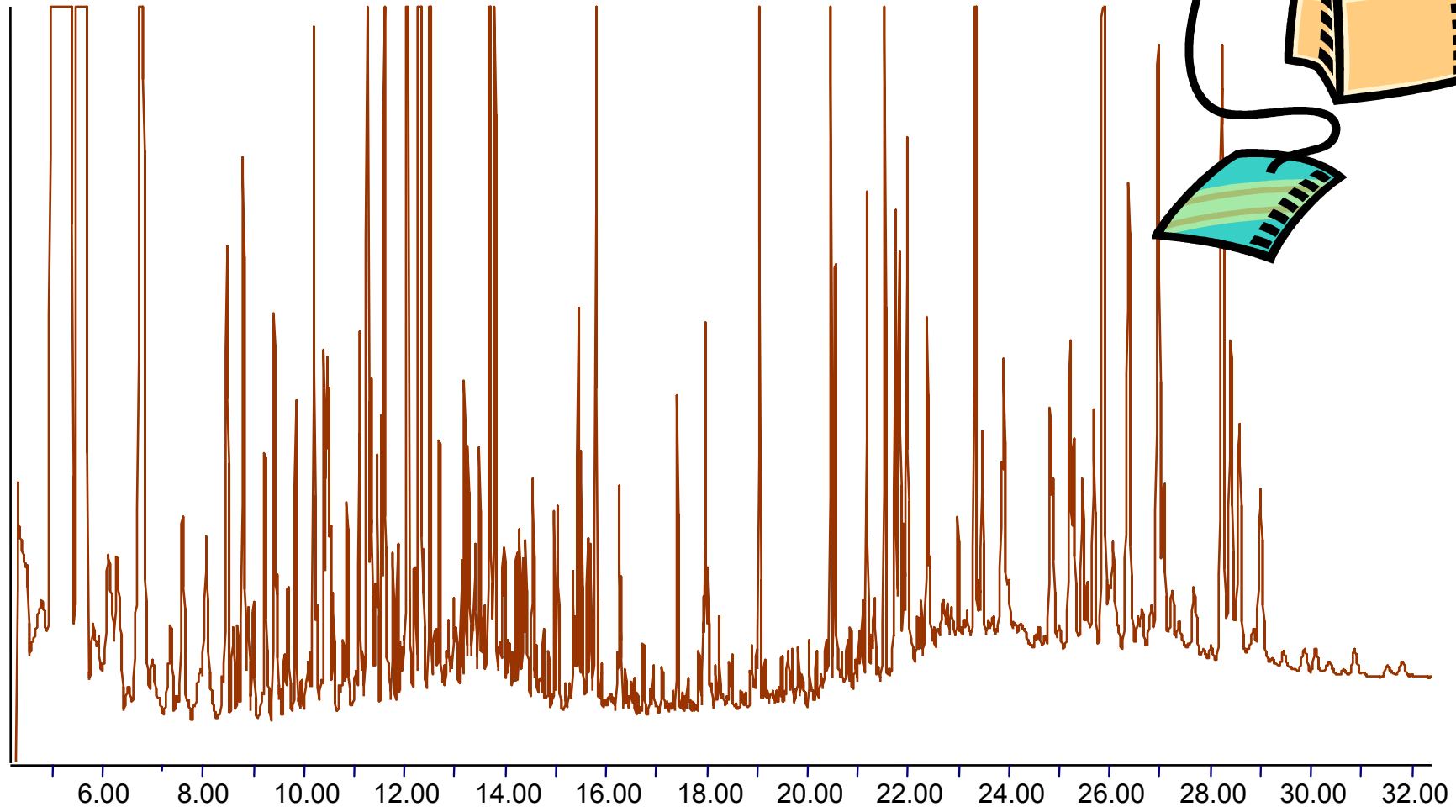
0.01 mg / kg

10 ppb



GC Chromatographic Selectivity in Herbal Tea

Herbal Tea = 0.2 mg matrix



MS/MS Selectivity in Herbal Tea

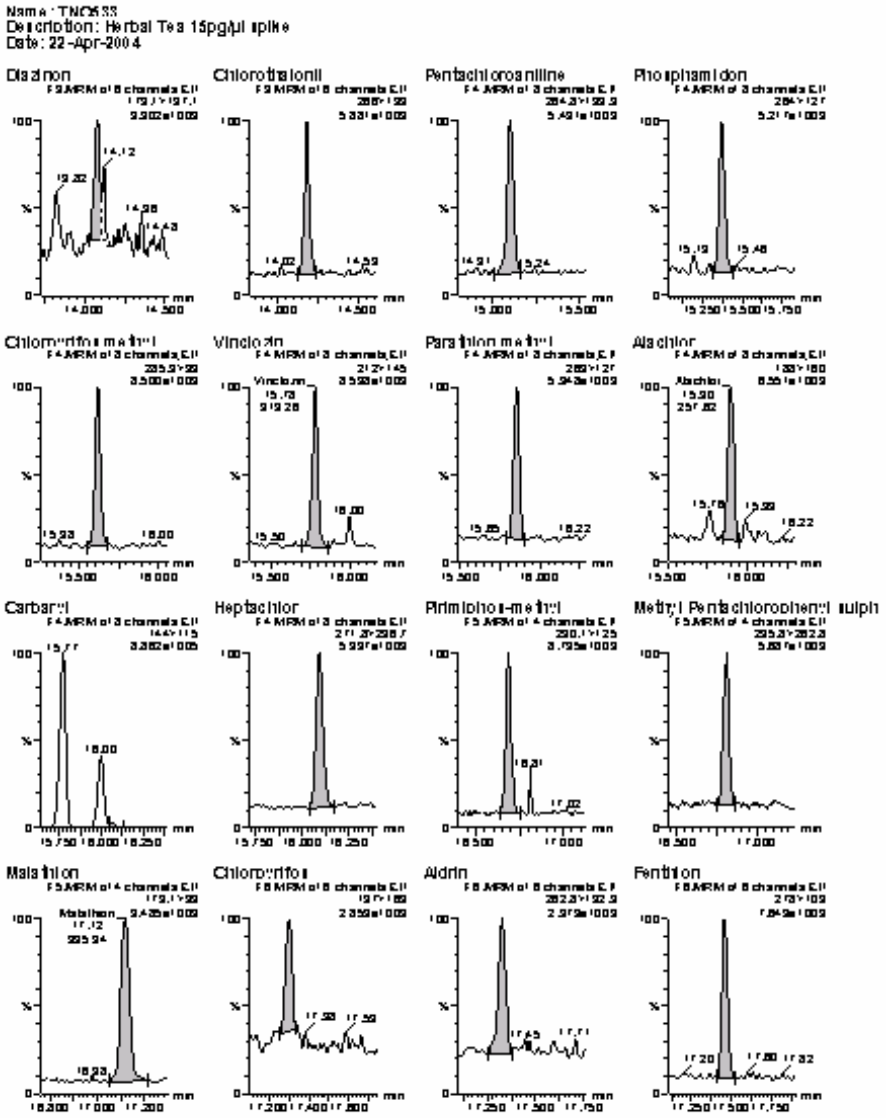
Pesticides Spiked

in Herbal Tea

Quattro micro GC

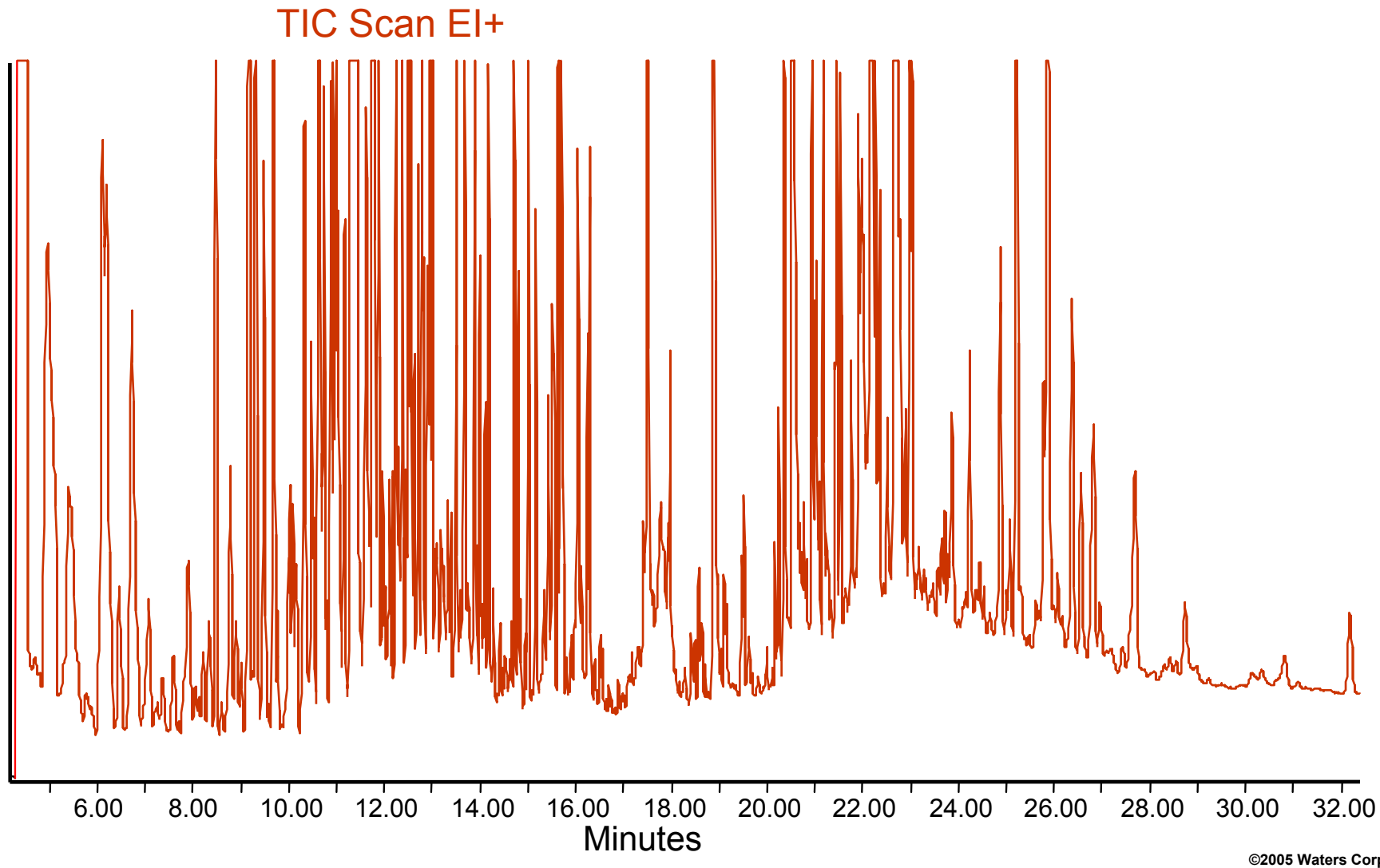
0.3 ppm dry

0.03 ppm fresh



GC Chromatographic Selectivity Curry Spice Mix

Curry Spice Mix = 0.2 mg matrix on column



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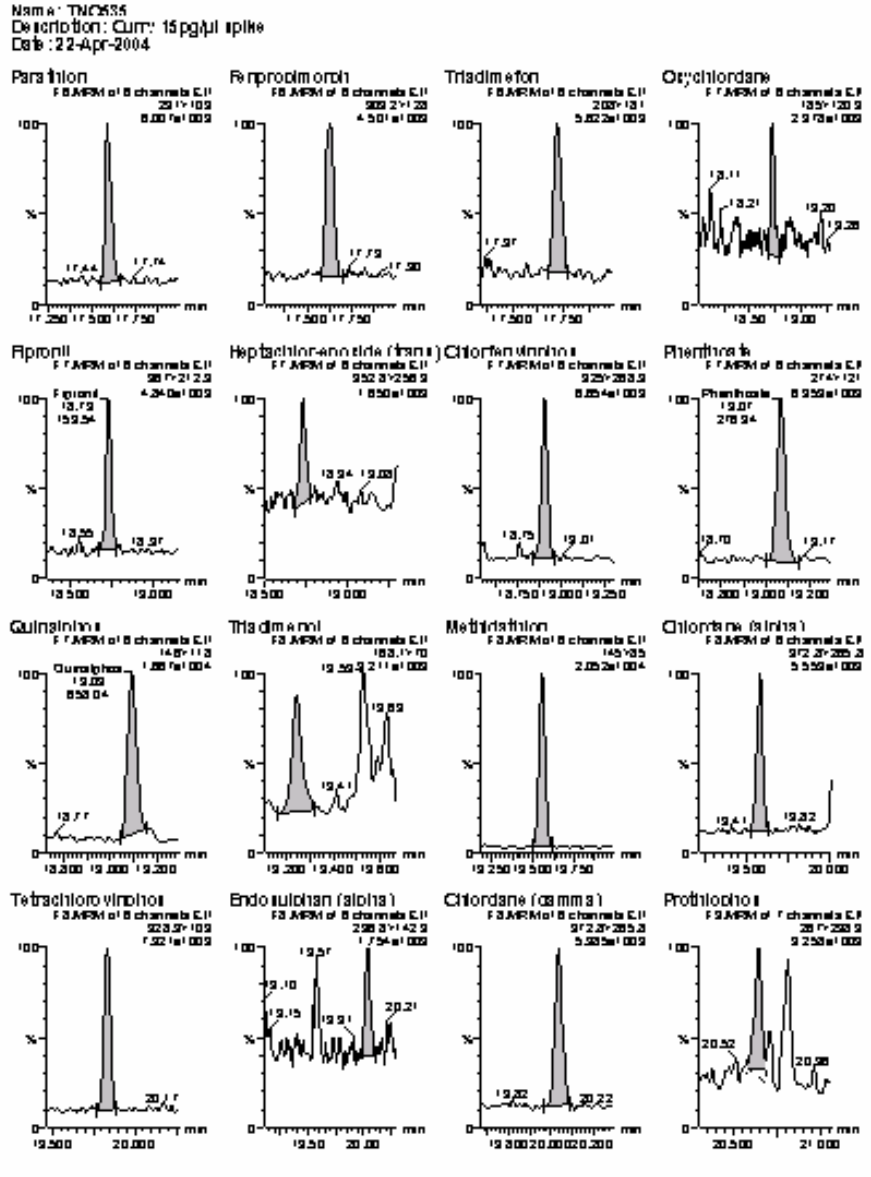
MS/MS Selectivity in Curry Spice Mix

Spiked Pesticides in Curry Spice Mix

Quattro micro GC

0.15 ppm



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GC/MS/MS Pesticide Screening Summary

- High % of pesticides correctly identified & quantified
- Highly complex matrices
- Generic, limited sample preparation

Food Matrix	Pesticide Fortification	# Pesticides Spiked	# Pesticides Identified	% Detection
Baby Food	0.01 mg/kg	93	81	87
Baby Food	0.05 mg/kg	93	92	99
Herbal Tea	0.03 mg/kg	93	88	95
Herbal Mix	0.03 mg/kg	93	87	94
Curry	0.15 mg/kg	93	90	97
Massala	0.15 mg/kg	93	87	94
Tobacco	0.15 mg/kg	93	87	94
Ginko	0.02-4.0 mg/kg	52	44	85
Cannabis	0.02-4.0 mg/kg	52	41	79

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A Rapid Multi-Residue LC-MS/MS Method for the Analysis of Pesticides

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Previous Work

- Validated a multi-residue screening method in 2003, suitable for surveillance monitoring of fruits and vegetables using a Waters Alliance 2795 with Quattro Micro API MS
  - 81 Residue Compounds in a Single Analysis
    - Benzimidazoles, Carbamates, Organophosphorous etc.
    - All LODs below those required for monitoring purposes
  - Overall cycle time of the method was 40 min
  - Analyses performed from a variety of food matrices (avocado, tomato, raisin, lemon, flour)
- Method transferred to Waters Alliance 2795 with Waters micromass Quattro Premier API MS
  - Confirmatory method for the 20 most commonly observed residues
  - Overall cycle time of the method was 25 min

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Aims of Project

- Develop a rapid multi-residue pesticide screening method
  - 100 Residue Compounds in a Single Analysis
  - Reduce overall cycle time of the method
    - To increase efficiency and sample throughput
- Able to quantify and confirm at MRL tolerance levels
  - Include an additional transition for a selection of the most commonly observed residues
    - Confirmatory method
      - Potentially reducing overall analysis time for a batch

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System Solution
ACQUITY UPLC/MS/MS

Quattro Premier

UPLC Chromatographic and Mass Spec Method

- Column: BEH C_{18}, 2.1x100 mm, 1.7 μm
- Gradient: 20% to 90% MeOH containing 5 mM NH_4OAc
  Linear for 8.5 mins, hold for 2 min, re-equilibrate
- Flow: 450 μL/min
- Col Temp: 40º C
- Inj Volume: 20 μL

- Waters Micromass Quattro Premier

Ionization mode:	ES+ / ES-
Capillary voltage:	0.8 kV (ES+ and ES-)
Gas Flow:	800 L/hr
Source Temp:	120º C
Desolv'n Temp:	80º C
Cone Voltage:	Analyte specific
MS/MS:	operated in MRM mode
Collision Voltage:	Analyte specific

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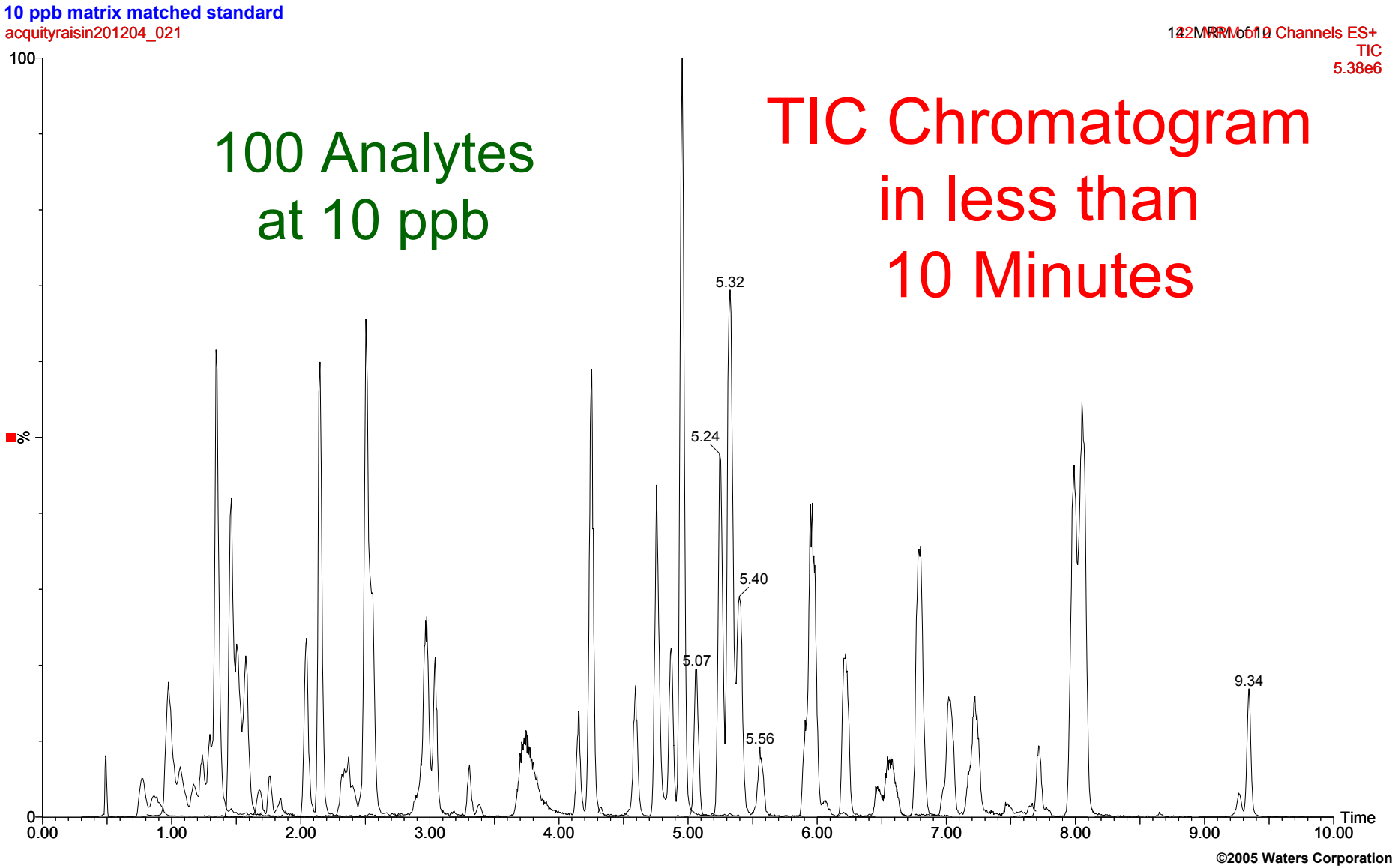
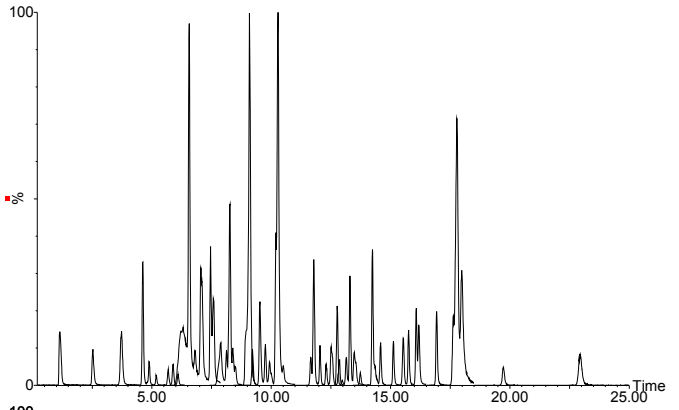
100 Pesticides Analytes

5-Hydroxy-clethodim-sulfon	Clethodim-sulfon	Imazalil	Propoxur
6-chloro-4-hydroxy-3-phenyl-pyridazin	Clethodim-sulfoxid	Imidacloprid	Prosulfuron
Acephate	Cyprodinil	Indoxacarb	Pymetrozin
Aldicarb	Daminozid	Ioxynil	Pyridate
Aldicarb-sulfoxid	Demeton-S-methyl-sulfon	Iprovalicarb	Pyrimethanil
Aldoxycarb	Desmedipham	Isoproturon	Quinmerac
Amidosulfuron	Difenzoquat methylsulfate	Isoxaflutole	Quizalofop-ethyl
Atrazin	Diflubenzuron	Linuron	Rimsulfuron
Azoxystrobin	Dimethoat	Metalaxyl	Spiroxamine
Bendiocarb	Diuron	Metamitron	Tebuconazol
Bensulfuron-methyl	Ethiofencarb	Methamidophos	Tebufenozide
Bromoxynil	Ethiofencarbsulfon	Methiocarb	Teflubenzuron
Butocarboxim	Ethiofencarbsulfoxid	Methomyl	Thiabendazol
Butoxycarboxim	Fenhexamid	Metolachlor	Thiacloprid
Butoxycarboxim-sulfoxid	Fenoxycarb	Metsulfuron-methyl	Thifensulfuron-methyl
Carbaryl	Fenpropimorph	Monocrotophos	Thiodicarb
Carbendazim	Flazasulfuron	Nicosulfuron	Thiofanox
carbofuran	Florasulam	Omethoat	Thiofanox-sulfon
Carbofuran-3-hydroxy	Fluazifop-P-butyl	Oxamyl	Thiofanox-sulfoxid
Chlorsulfuron	Fludioxonil	Oxydemeton-methyl	Triasulfuron
Cinosulfuron	Flufenoxuron	Phenmedipham	Triflumuron
Clethodim	Formetanate	Pirimicarb	Triflusulfuron-methyl
Clethodim-imin-sulfon	Furathiocarb	Primisulfuron methyl	Vamidothion
	Haloxyfop-ethoxyethyl	Promecarb	

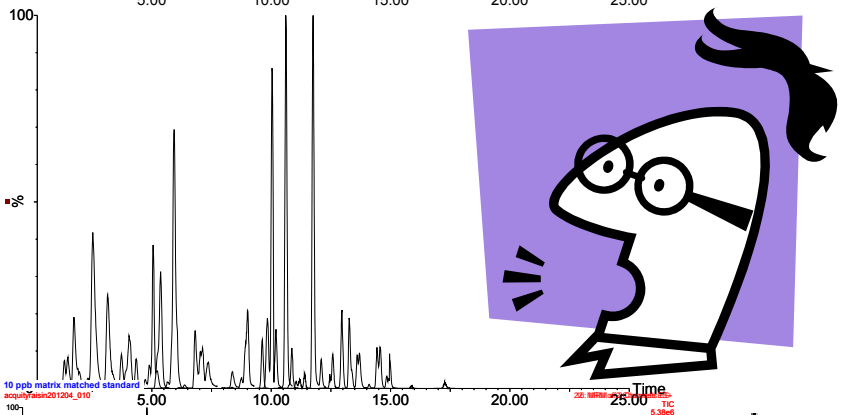
Pesticide Spike in Raisins



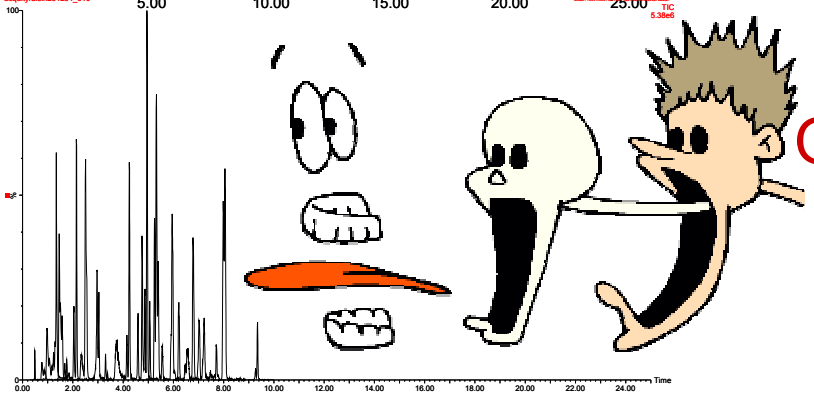
Increasing the Throughput
HPLC vs. ACQUITY UPLC



Quattro micro API, Alliance 2695
81 residues
40 min cycle time

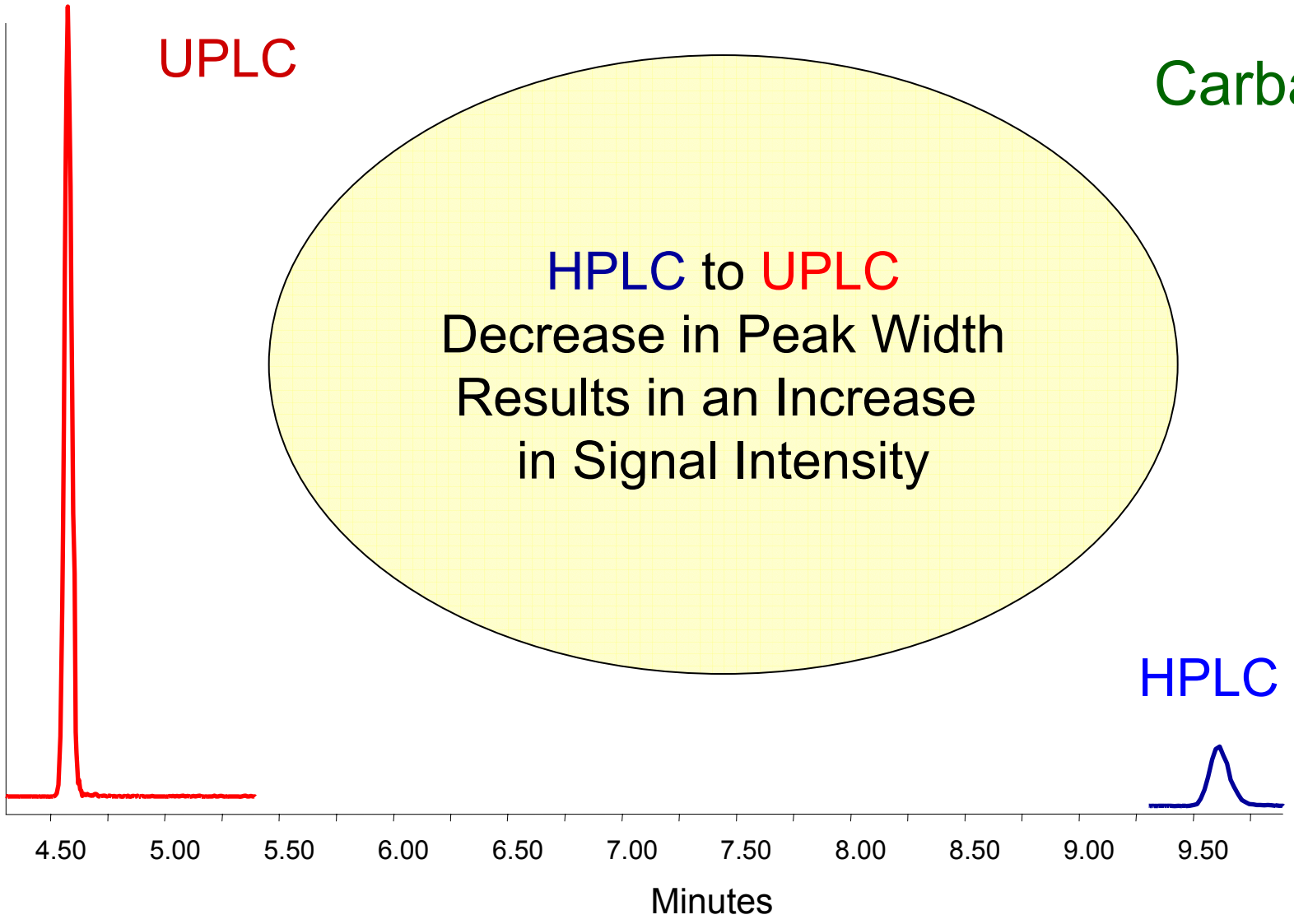


Quattro Premier, Alliance 2695
100 residues
25 min cycle time

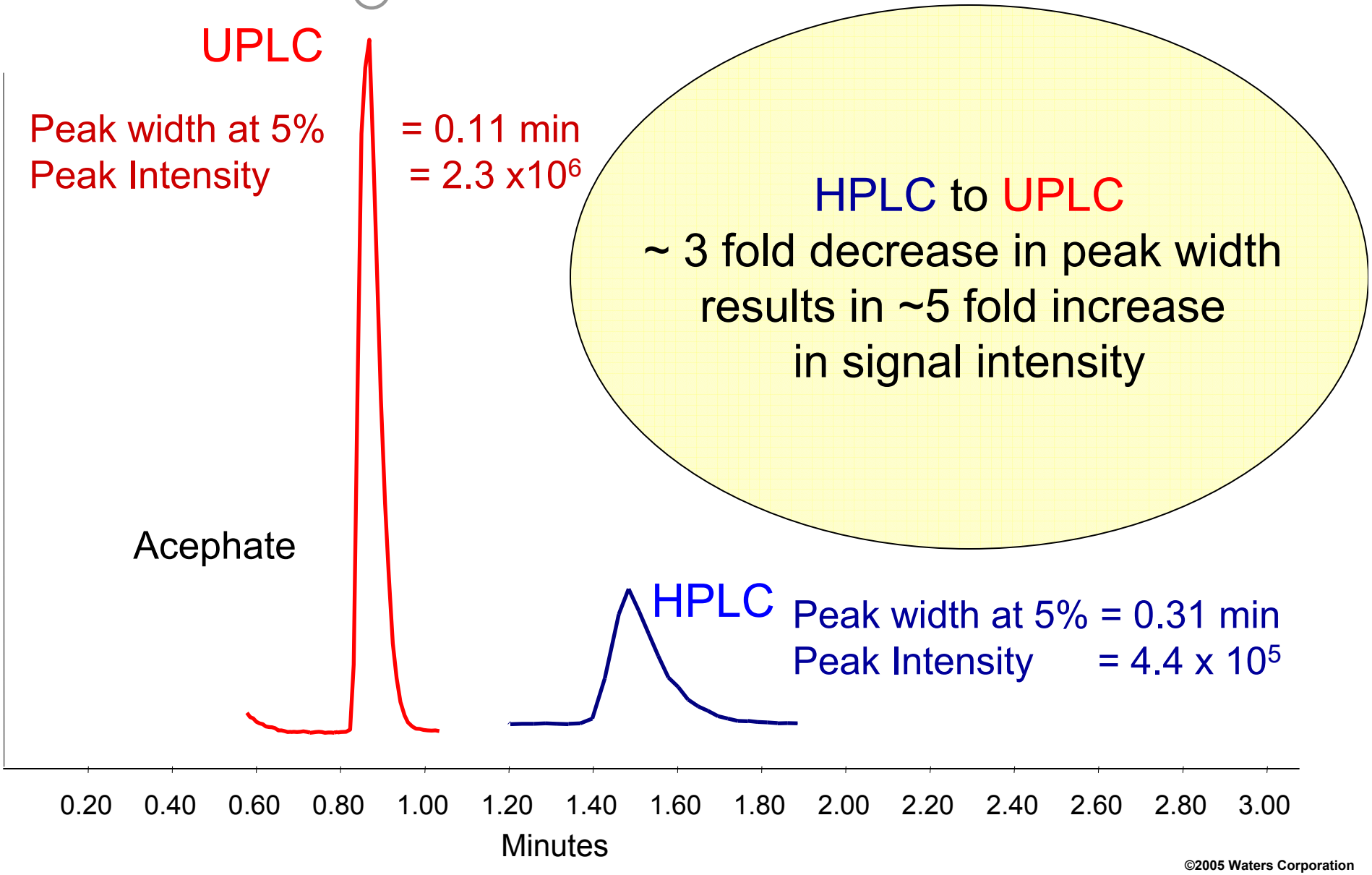


Quattro Premier, ACQUITY UPLC
100 residues
14.5 min cycle time

Comparison of HPLC and UPLC



Comparison of HPLC and UPLC



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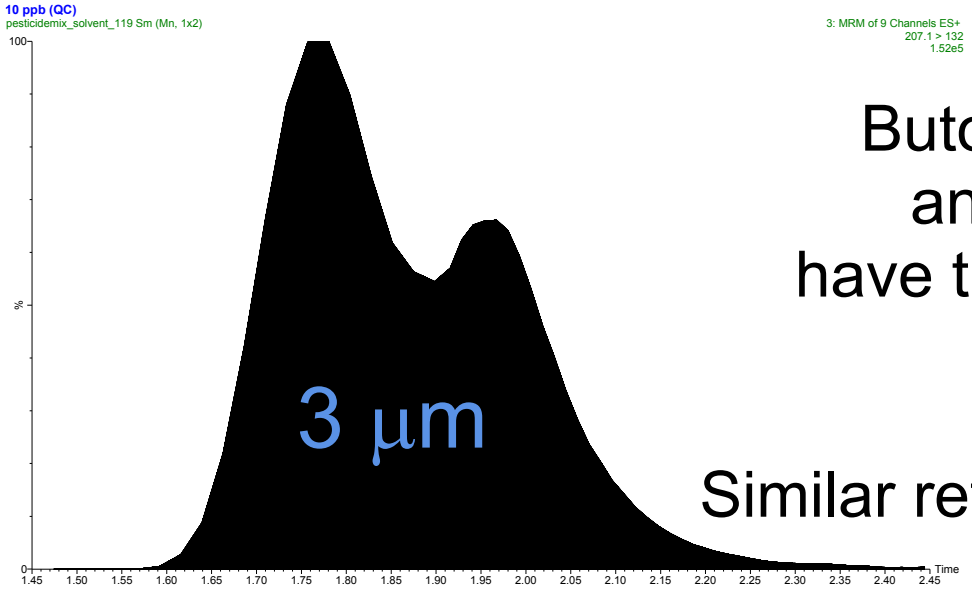
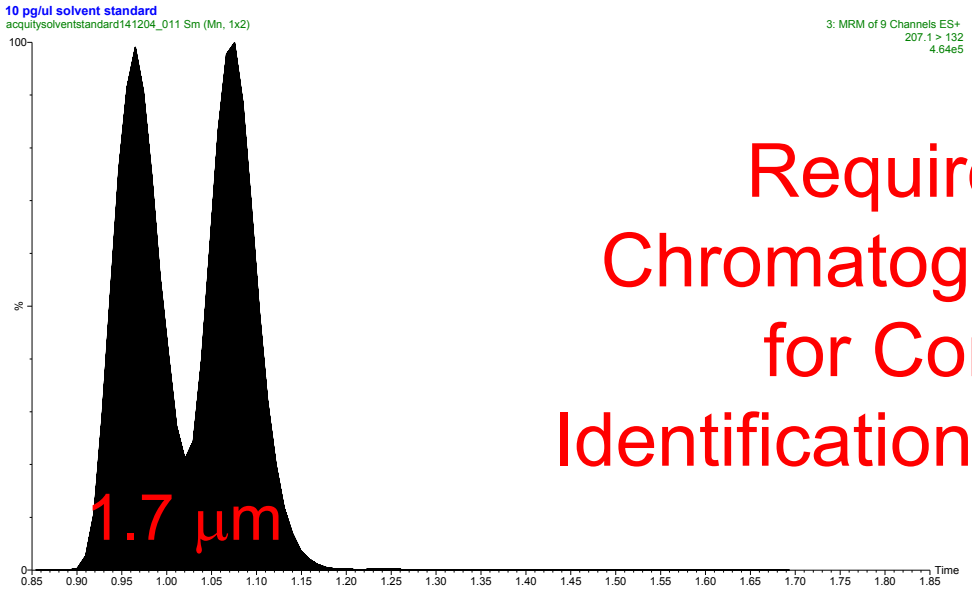
Resolution Comparison HPLC and UPLC



Butoxycarboxim Sulfoxide
and Aldicarb Sulfoxide
have the same MRM transition
m/z 207.1 > 89
And
Similar retention time: Δ =12 seconds



Requires Enhanced
Chromatographic Resolution
for Correct Analyte
Identification and Quantification

Demands on the mass spectrometer

For accurate quantitation a minimum of 10 data points are needed across each peak.

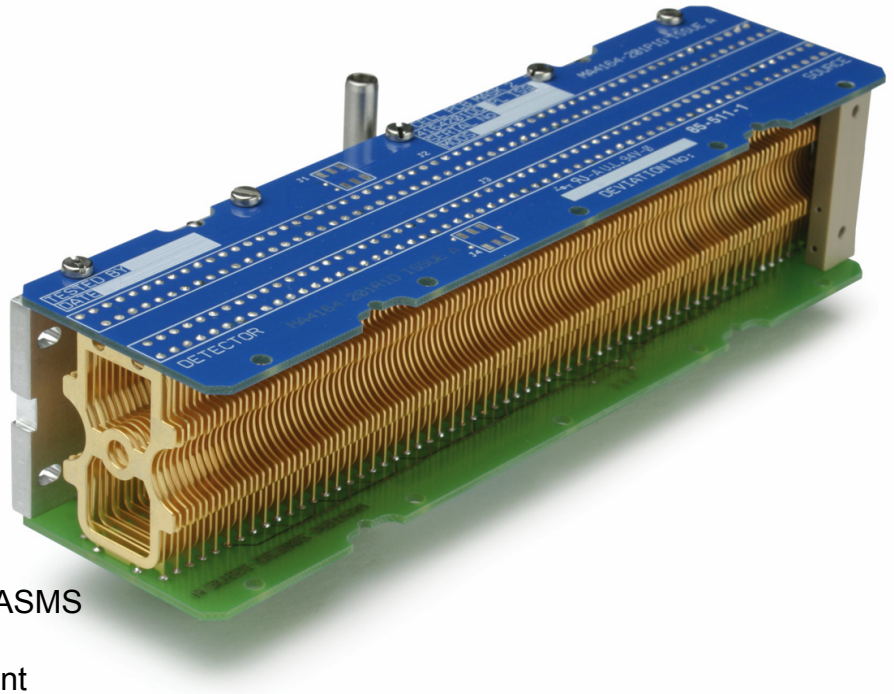
Due to the

- Narrow peak widths
- Number and type of compounds to be analysed
  - Multi-analyte method
  - Inclusion of confirmatory transitions for some analytes
  - Some analytes ionise preferentially in negative ion mode

Significant demands are placed on the mass spectrometer

Quattro Premier and T-Wave Technology

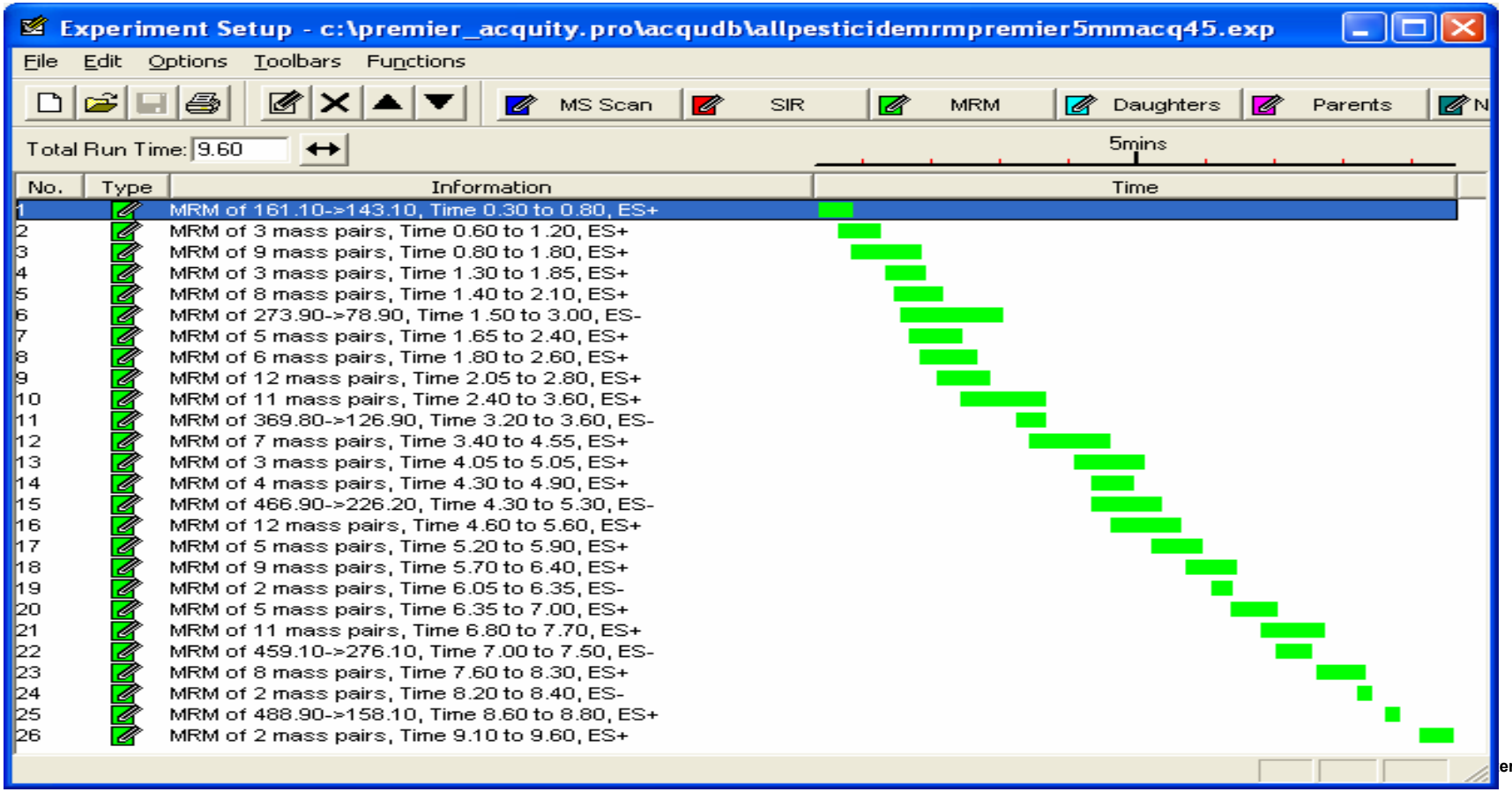
- The T-Wave is used to rapidly clear the cell between transitions, even when using a very short inter-channel delay (5ms) – minimising crosstalk.
- The T-Wave also ensures that the cell is completely refilled before acquisition commences allowing very short dwell times without loss of intensity.
- This enables quantification of narrower chromatographic peaks or quantification of more MRM transitions in the same time window.



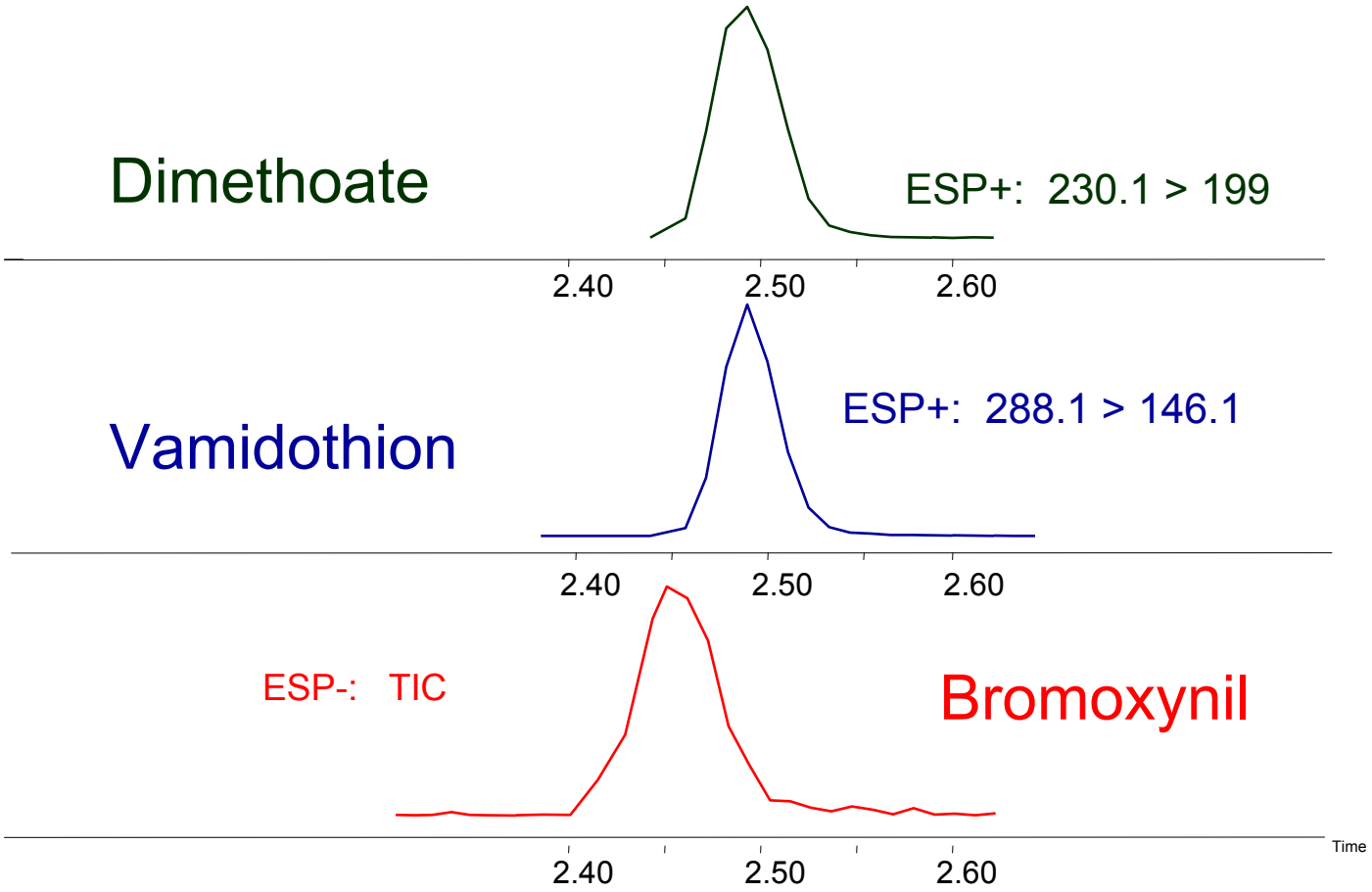
"Travelling Wave Ion Propulsion in Collision Cells" K. Giles, S. Pringle, K. Worthington and R. Bateman—Presented at the 51st ASMS Conference, Montreal, Canada 2003. The travelling wave device described here is similar to that described by Kirchner in US Patent 5,206,506 (1993).

MRM Time Windows to Maximize Sensitivity

- MRM functions arranged into 26 retention time windows
  - More flexible use of dwell times
  - Positive and negative switching
  - Improved signal to noise (S/N) ratios



ESP+/ESP- Mode Switching



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

Analyte Limit of Detection

Pesticide Residue	Retention Time (min)	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Cone Voltage (V)	Collision Voltage (V)	Dwell Time (ms)	LOD (pg/uL)
Daminozid	0.50	161.1	143.1	18	12	200	0.01
Methamidophos	0.79	141.8	93.8	22	14	80	0.02
			124.9	22	13	80	
Acephate	0.89	184.1	143.0	16	8	40	0.04
Butoxycarboxim-sulfoxide	1.00	207.1	132.1	17	6	30	0.05
Omethoate	1.01	214.0	183.0	20	12	30	0.01
			154.9	20	15	30	
Aldicarb-sulfoxide	1.11	207.1	132.0	16	10	30	0.1
			89.0	16	14	30	
Butoxycarboxim	1.20	240.1	106.1	10	14	30	0.04
Aldoxycarb	1.26	240.1	86.0	15	20	30	0.005
Oxamyl	1.32	237.1	71.9	12	10	30	0.003
Propamocarb	1.36	189.1	102.0	25	17	30	0.01
			144.0	25	12	30	
Oxydemeton-methyl	1.49	247.0	169.0	20	13	10	0.002
Pymetrozin	1.57	218.0	105.0	25	17	10	0.02
6-chloro-4-hydroxy-3-phenyl-pyridazin	1.60	207.1	77.0	35	30	10	0.04
			104.0	35	21	10	
Methomyl	1.60	162.9	87.8	15	8	10	0.01
			105.9	15	10	10	
Demeton-S-methyl-sulfon	1.61	263.1	169.1	28	16	10	0.02
			121.2	28	16	10	
Quinmerac	1.69	222.0	141.0	22	33	10	0.008
Monocrotophos	1.78	224.0	126.9	20	15	10	0.005
Bendiocarb	1.78	224.1	109.0	18	18	10	0.01
			167.1	18	9	10	
Nicosulfuron	1.80	411.0	182.1	22	18	10	0.05
Amidosulfuron	1.84	370.0	261.2	18	14	10	0.02
Metsulfuron-methyl	2.00	382.0	167.0	22	15	10	0.02
Thifensulfuron-methyl	2.00	388.0	167.1	22	15	10	0.02
Ethiofencarbsulfon	2.04	275.1	107.1	10	20	10	0.006
Rimsulfuron	2.05	431.9	182.1	30	22	10	0.02
Ethiofencarbsulfoxide	2.13	242.1	107.0	18	18	10	0.003
Thiofanox-sulfoxide	2.14	252.1	104.0	10	12	10	0.3
Imidacloprid	2.14	256.1	209.2	22	16	10	0.02
			175.1	22	20	10	
Florasulam	2.28	360.1	129.0	30	20	10	0.09
5-Hydroxy-clethodim-sulfon	2.29	408.2	204.2	22	16	10	0.1
Thiofanox-sulfon	2.32	268.1	76.0	10	10	10	0.02
Clethodim-imin-sulfon	2.35	302.2	98.1	35	30	10	0.04
Metamitron	2.37	203.0	175.1	28	16	10	0.02
Cinosulfuron	2.42	414.1	183.1	25	18	10	0.05
Chlorsulfuron	2.43	358.1	141.1	25	16	10	0.08
			167.1	25	16	10	
Bromoxynil*	2.45	273.9	78.9	40	25	30	0.2

LOD at 3x S/N
in the sub-ppb range
0.003 to 2 ppb

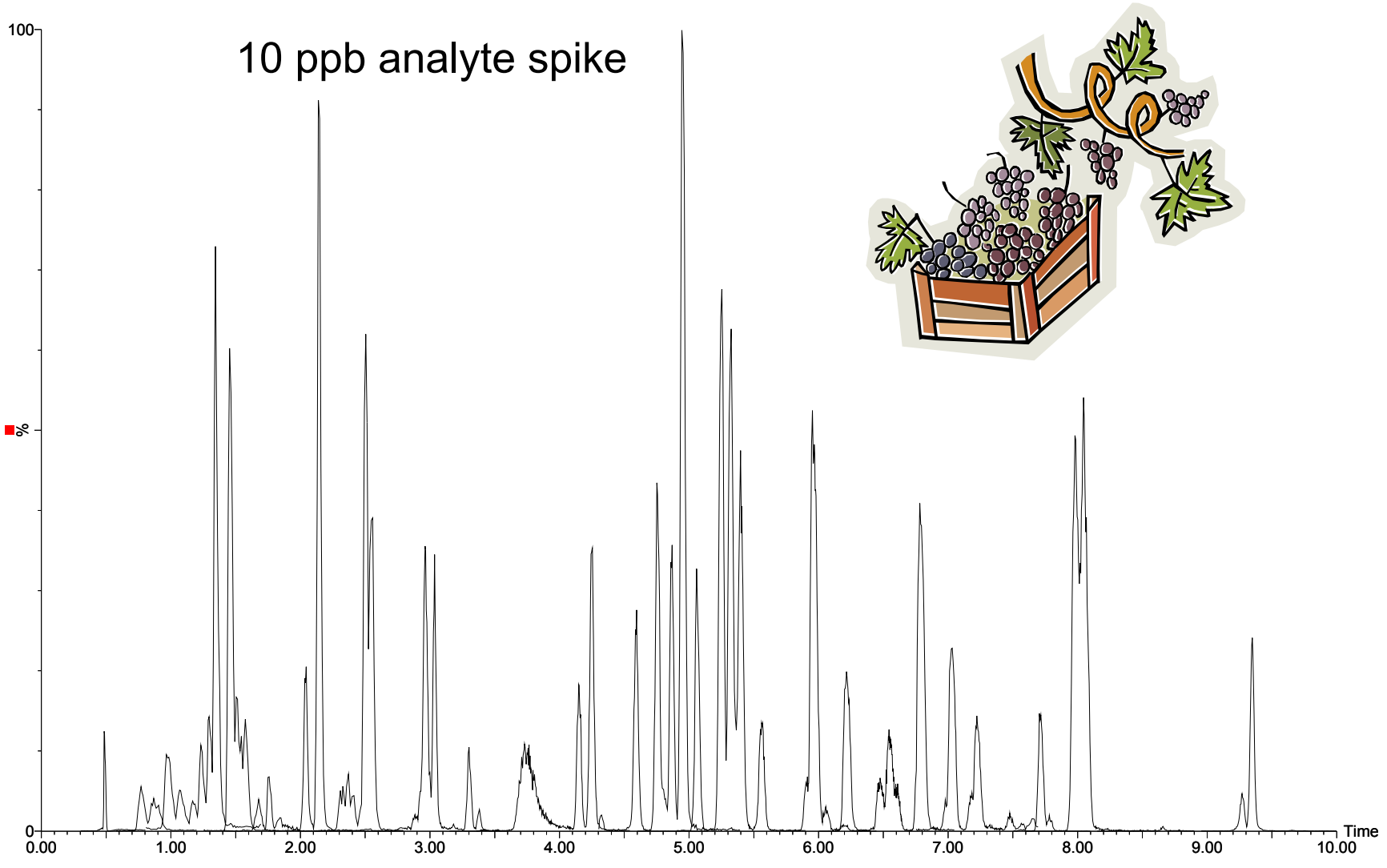
Sample Preparation

- Sample: Californian Sun-dried Seedless Raisins (Thompson variety)
- Chopped
- 5g Aliquot homogenised
- 9 mL water added
- After 10 min, 20 mL of methanol added
- 6mL of extract was mixed with 2mL of sodium chloride solution
- 5mL aliquot of the mixture was transferred to a ChemElut column containing 5 mL of diatomaceous earth
- After 5min the column was eluted with 16 mL DCM
- Elute evaporated
- Reconstituted in methanol (250 μL)
- Addition of 1 mL water and filtering into glass vial

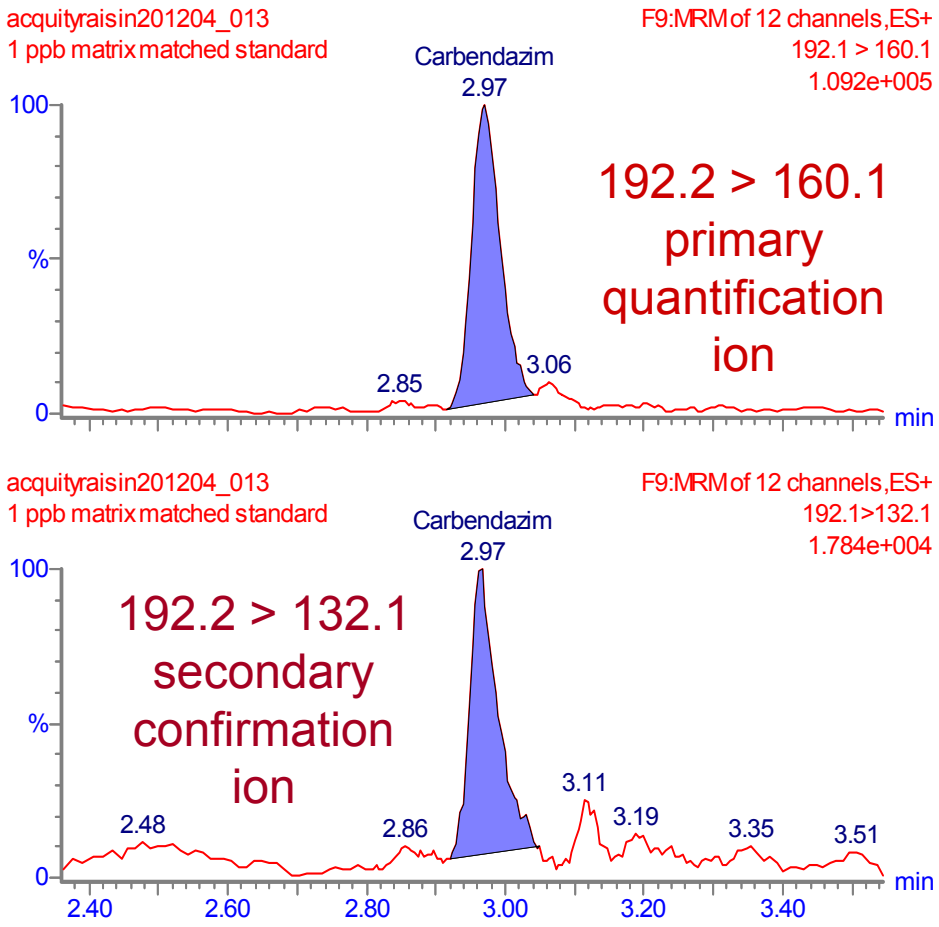
Preparation of Matrix Matched Standards

- Blank matrix: organically grown Sun-dried Seedless Raisins (Thompson variety)
- Same sample preparation, extraction and clean-up procedure as for sample
- All analytes spiked into the matrix at a range of concentrations equivalent to 1, 2, 5, 10, 20 ppb

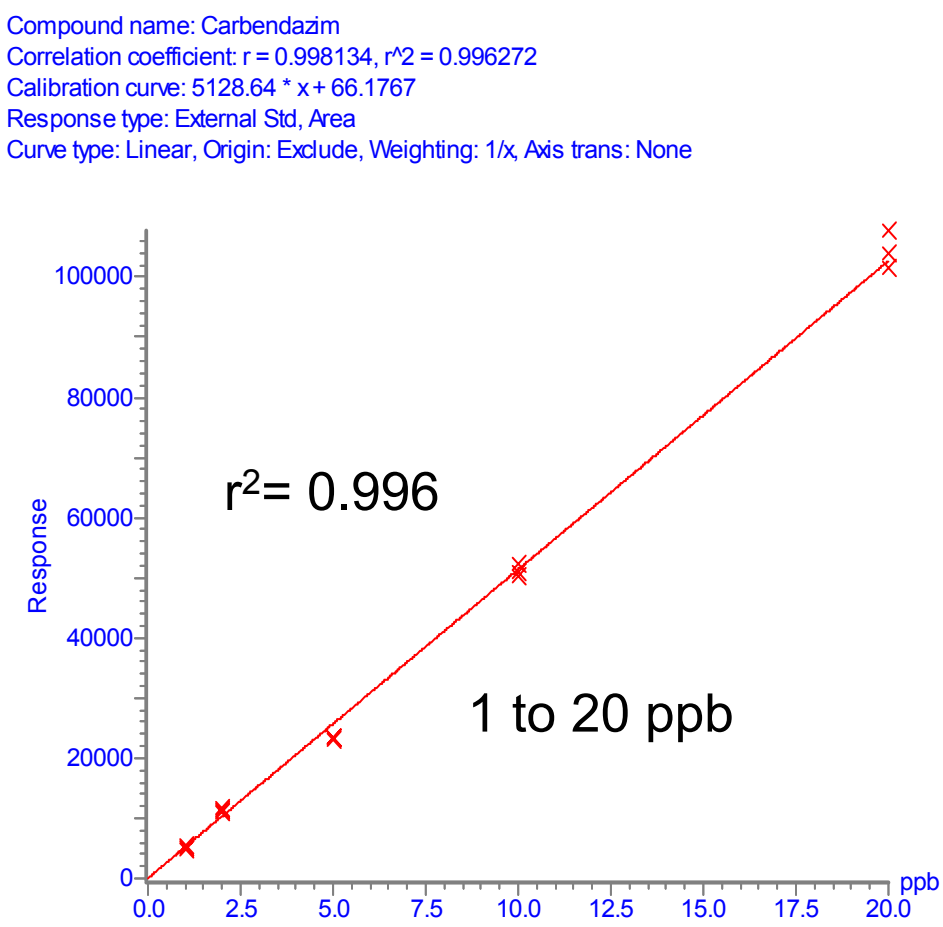
Spiked Raisin Matrix



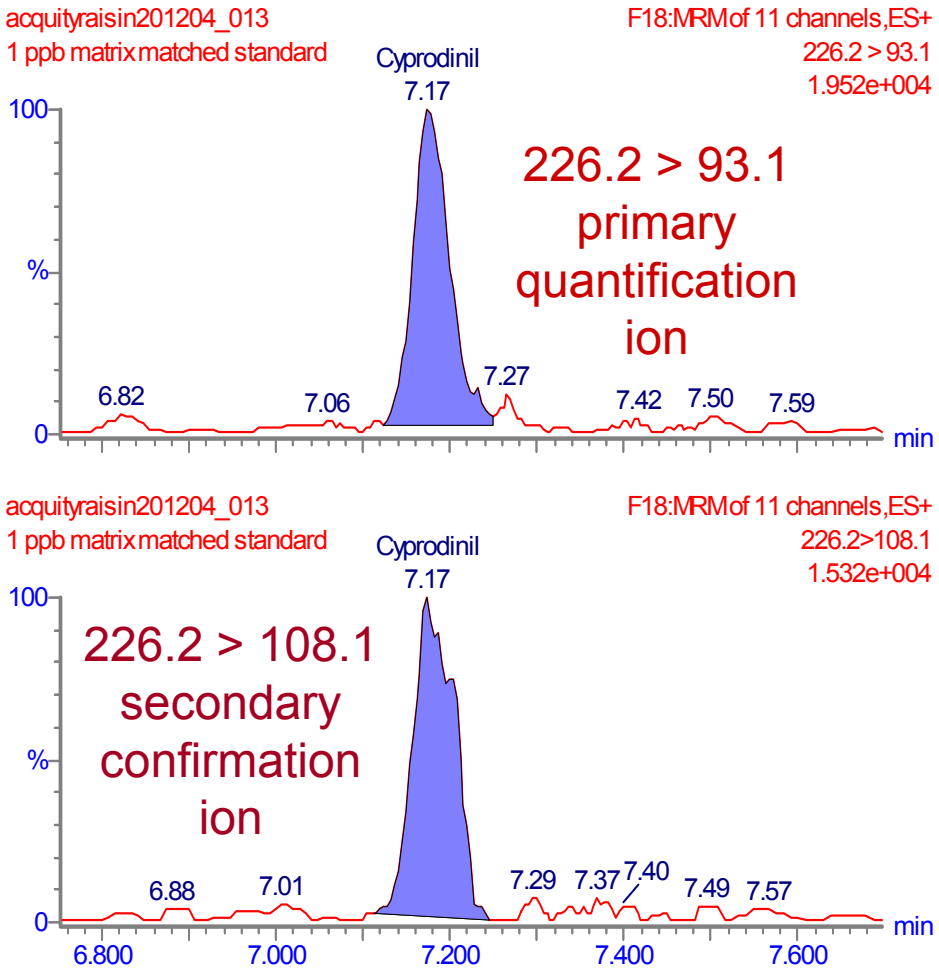
Carbendazim in Raisin (ESP+)



1 ppb Chromatogram

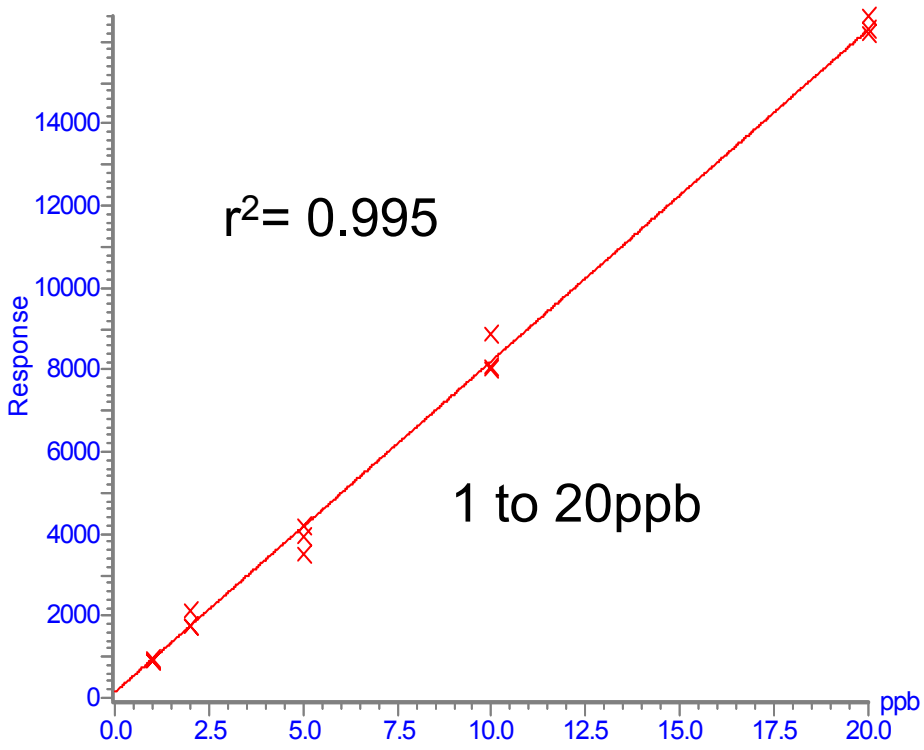


Cyprodinil in Raisin (ESP+)

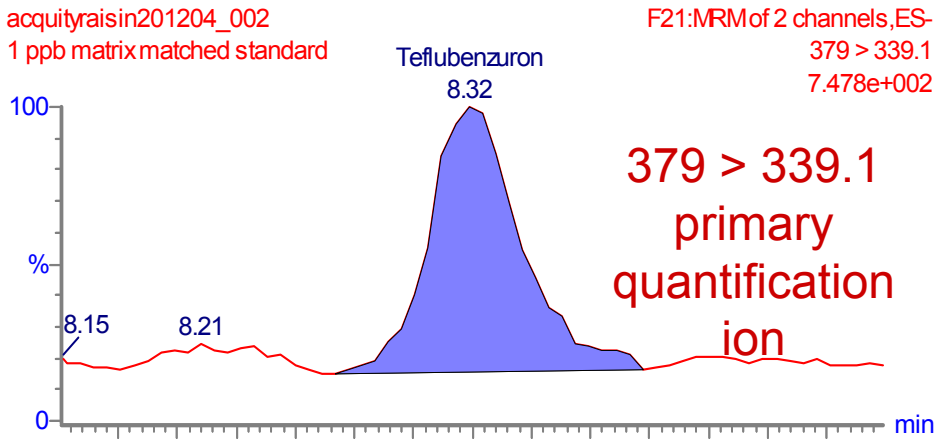


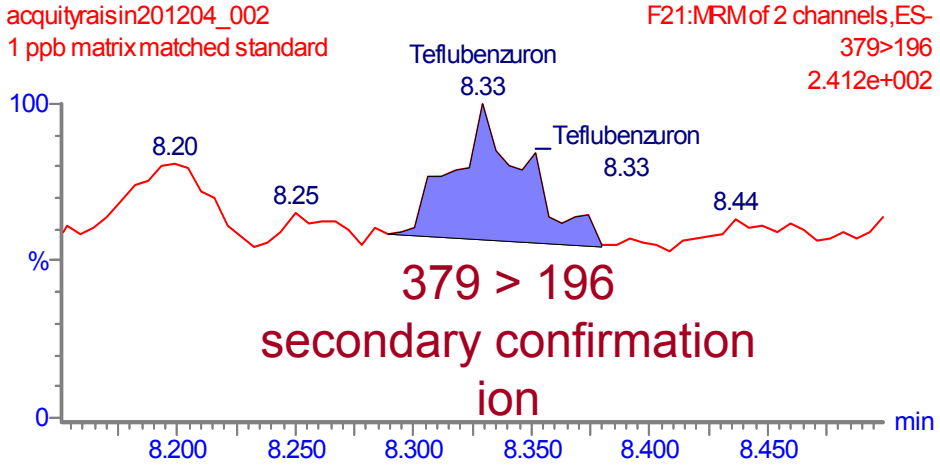
1 ppb Chromatogram

Compound name: Cyprodinil
Correlation coefficient: r = 0.997715, r^2 = 0.995435
Calibration curve: 807.68 * x + 136.057
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



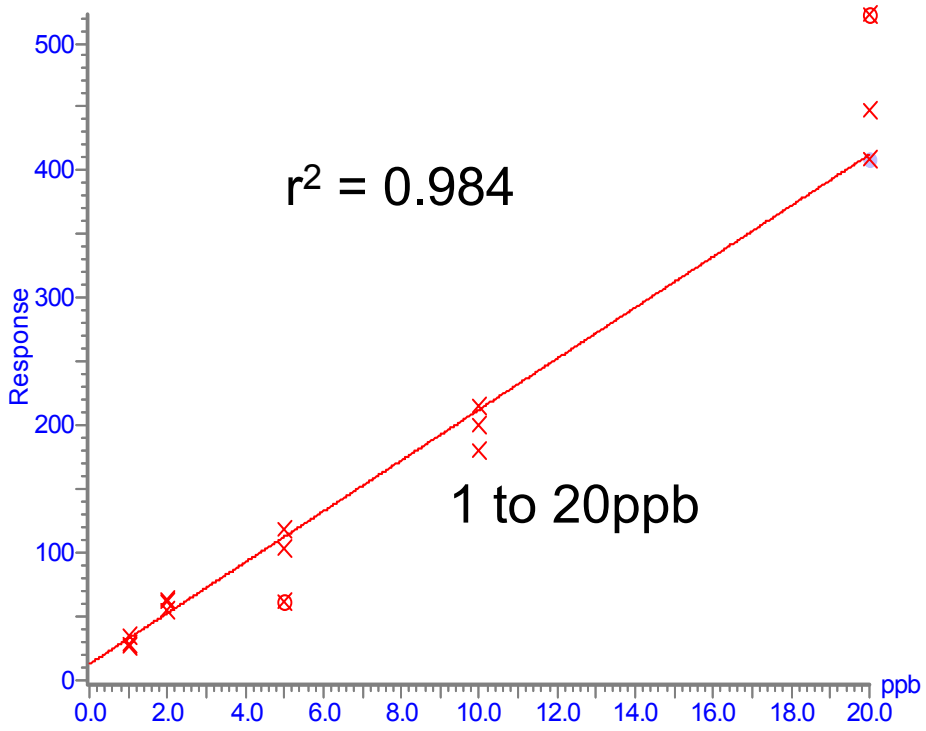
Teflubenzuron in Raisin (ESP-)





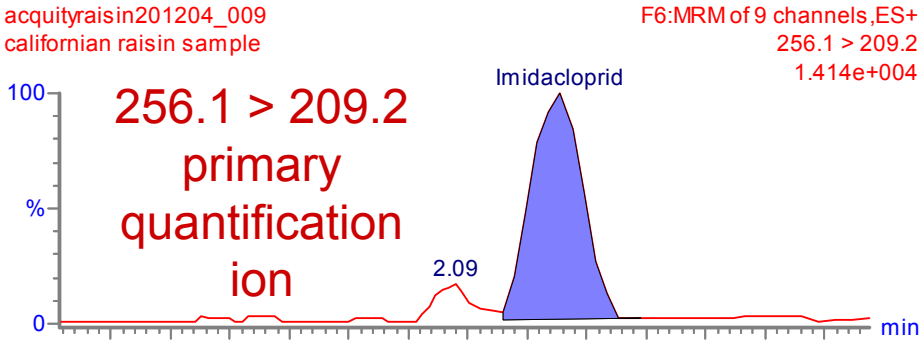
1 ppb chromatogram

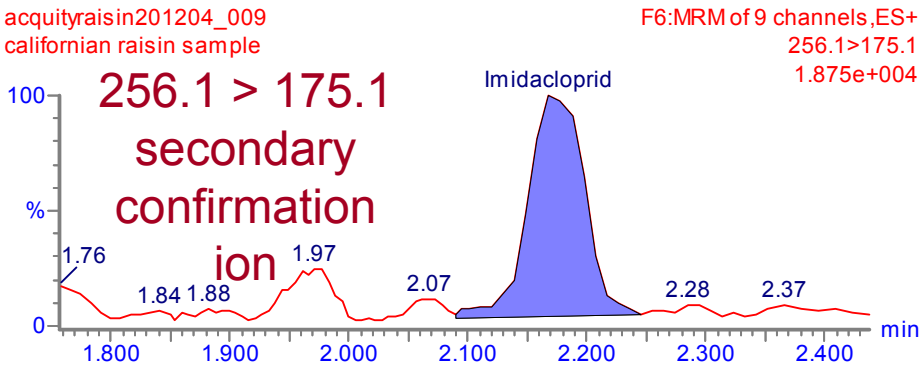
Compound name: Teflubenzuron
Correlation coefficient: r = 0.992141, r^2 = 0.984344
Calibration curve: 19.9838 * x + 12.801
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



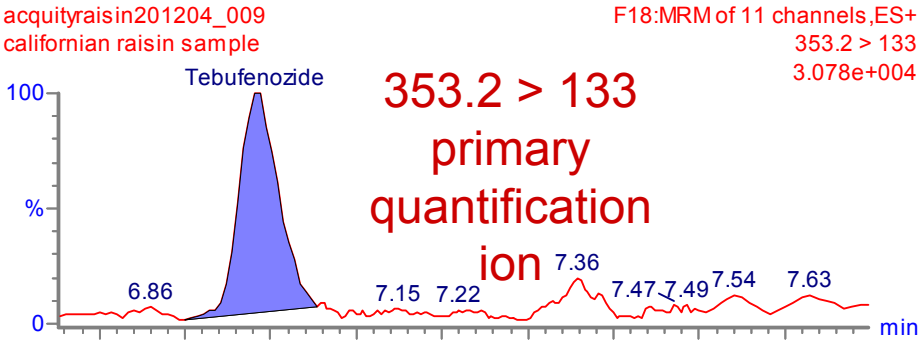
Analysis of Californian Raisins

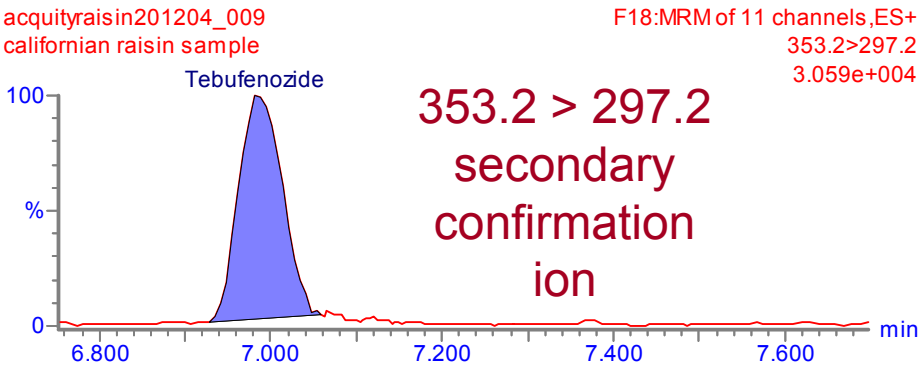
Imidacloprid
2.2 ppb





Tebufenozide
1.7 ppb





The TargetLynx Data Manager

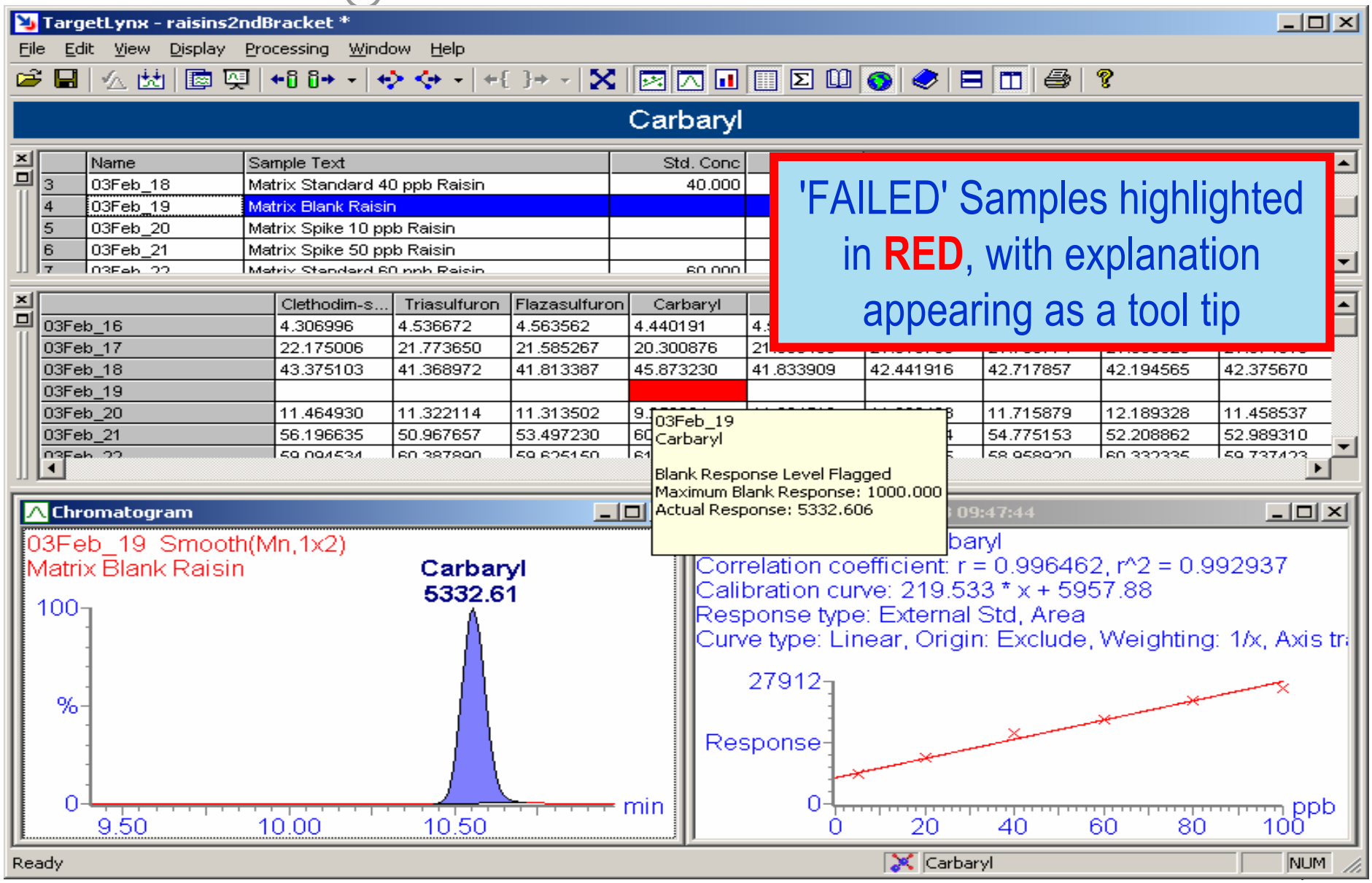
- Comprehensive Tool for Data Processing
  - Quantitative Data Generation and Evaluation
  - QA/QC System and Method suitability
- When injecting a series of samples, the analyte concentrations are reported as follows:
  - Analytes which are below the reporting limit are not reported
    - user defined values, i.e. MRL, LOQ, S/N, etc
  - Analytes between the reporting limit and the maximum allowed MRL are reported
  - Analytes **above** the MRL are reported in **red Bold**
  - The method QA/QC parameters are flagged in red if exceeds user defined limits, and the explanation appears as tool tip
    - RT, S/N, r^2, plate count, calib checks, spiked duplicates, etc

TargetLynx Application Manager Example

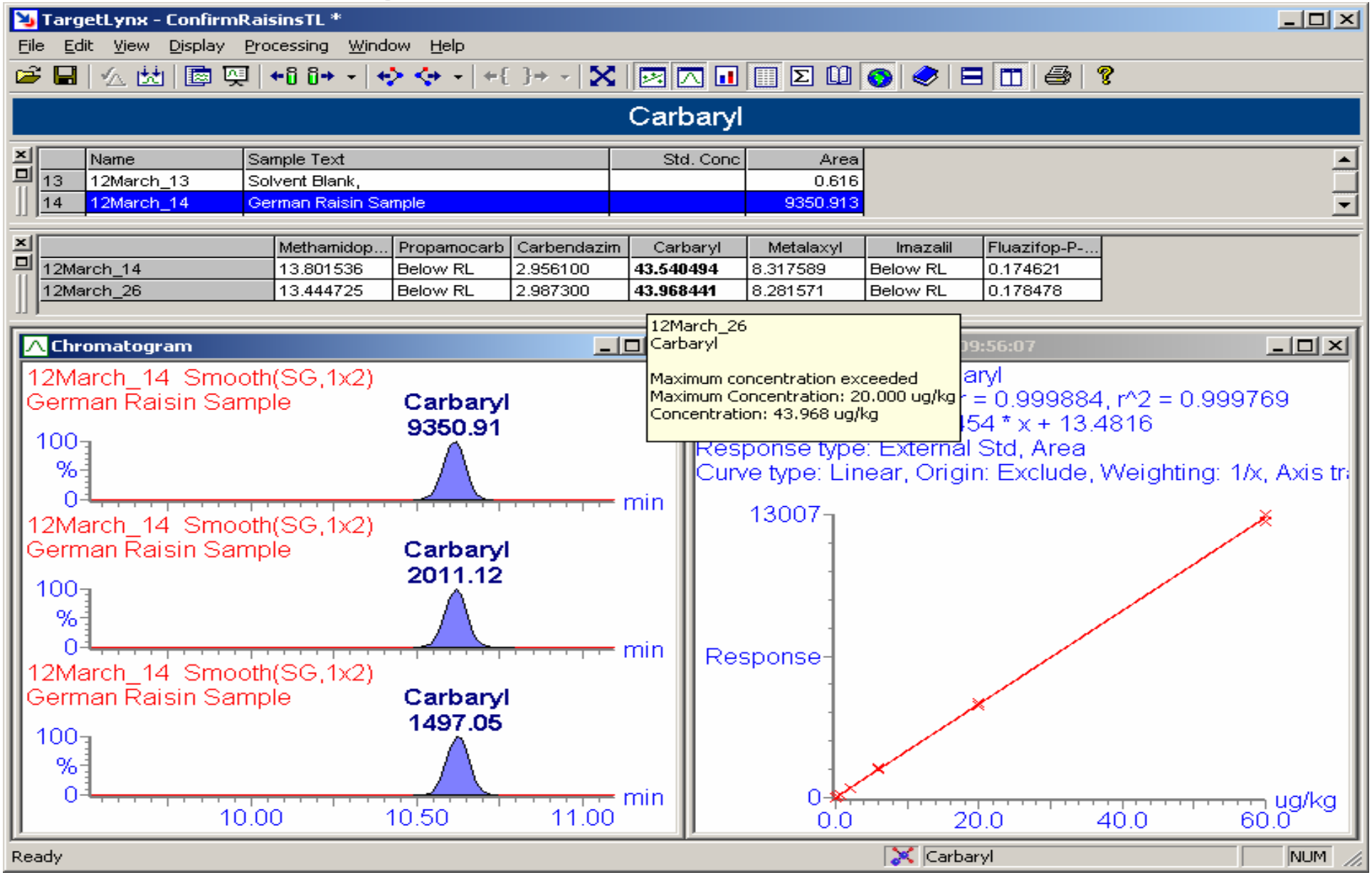
- Carbaryl detected as positive in a raisin matrix blank using the pesticide multi-residue screening method

- 6 additional analytes also detected in matrix blank
  - Confirmatory method created, monitoring 3 MRM transitions per compound
  - TargetLynx method created by exporting the compounds from the screening method
  - Matrix blank, matrix spiked and ‘real’ raisin samples acquired and processed

TargetLynx Detection of Residue in Raisin Blank



TargetLynx Processing of Confirmatory Analysis



Waters Application Solution

- Multi-residue method for surveillance monitoring of 100 pesticide residues in food and natural products, complex matrices
  - GC/MS/MS and UPLC/MS/MS
- Chromatographic and MS/MS Selectivity to allow generic sample preparation
  - Enhanced chromatographic resolution and shorter 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 ionization modes
- Good sensitivity (<5 ppb generally) for pesticide residues
  - Sufficient to limit the amount of matrix introduced into the chromatographic inlet

Acknowledgments

- Waters Corporation, Manchester, UK
  - David Douce
  - Gordon Kearney
  - Peter Hancock
  - Catherine Ryan
- TNO, Zeist, The Netherlands
  - Hans Mol
- FIRA, Berlin, Germany
  - Lutz Alder
  - Jeannette Klein