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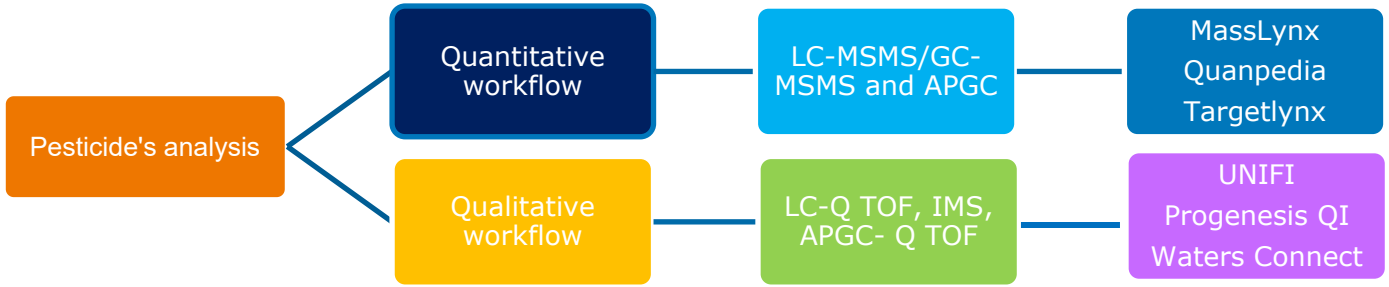
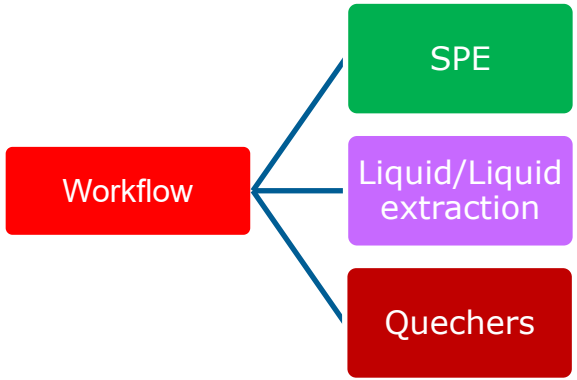
MULTI-RESIDUE PESTICIDE ANALYSIS TECHNIQUES, AN OVERVIEW

PMN Rajesh

Instruments and workflow



SAMPLE EXTRACTION WORKFLOW



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Waters Product line segmentation Food Testing

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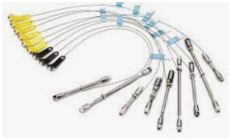
Sample preparation

Oasis

DisQuE



Premier columns

GC-MS,APGC Systems

Application Managers

& Informatics

MassLynx

TargetLynx™

UNIFI

Progenesis QI

Nonlinear dynamiics

APGC-Q TOF

MS Detectors Tandem Quad

XEVO TQD

TQ-S Cronos

TQ-S micro

XEVO TQ-XS

APGC-Tandem Quad

HRMS - Q TOF

XEVO G2 QTof MS

SYNAPT G2 HDMS

SELECT SERIES Cyclic IMS

GC-MSMS

Why mass spectrometry required

Spectrometry - LC/UV/FL, HPTLC

- Insensitive, non- specific
- Rigorous sample prep and derivatization often required
- Difficulty to analyze multiple analytes in a single run

Immunoassay - RIA, EIA (screening only)

- Not specific, issues with cross contamination, false positive
- Expensive and only available for a few compound classes
- No way to analyze multiple analytes in a single run

LC/MS/MS

- Highly selective and accurate
- Reduced sample preparation
- No issue with false positives
- Provides both qualitative and quantitative analysis in a single run
- Meet all requirements for modern residue analysis
- Technique recommended by all food safety guidelines

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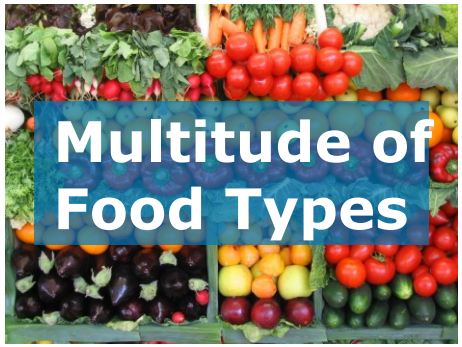
What are the challenges?



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What impact does this have on the laboratory?

- Demand for Flexible methods
  - Many analytes
  - Many matrices
  - Simple set-up / modification
- Method performance
  - High sensitivity
  - Quantitative accuracy
- Data management
  - Automated processing
  - Reporting
  - Archive and back-up

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Harmonisation of analytical quality control and validation

- **SANTE/12682/2019** EU Guidelines on Analytical Quality Control and Validation Procedures for Pesticide Residues Analysis in Food and Feed
- Provides detailed advice on **best laboratory practice** from sample receipt though to reporting of the result(s)
- Includes **identification** criteria (NOT identification points)
  - Allows the use of accurate mass
- Intended as **guidance** for official control purposes
- **Regularly reviewed and updated**

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Selection of representative commodities for validation

- High water content
  - Apples, carrots, lettuce, peaches, tomatoes
- High acidity (and high water content)
  - Berries, citrus, grapes, pineapples
- High sugar (and low water content)
  - Dried fruits, jams
- High starch and/or protein content (low water and fat content)
  - Cereals, dried beans pulses
- High oil (and very low water content)
  - Tree nuts, oil seeds
- High oil (and intermediate water content)
  - Avocados, olives
- Unique/difficult
  - Cocoa, coffee, hops, spices, teas
- Meat and seafood
  - Beef, chicken muscle, liver, kidney, cod, salmon, shrimp
- Milk and milk products
  - Milk, cheese
- Eggs
- Fat from food of animal origin
  - Lard, butter, cod liver oil

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XEVO Tandem Quadrupole

XEVO is

- ✓ Robust, reliable and accessible high performance
- ✓ Innovative, information rich analysis
- ✓ Versatility with engineered simplicity

Xevo TQD

Accessible, reliable and proven

Xevo TQ-S micro

Sensitive, reliable and compact

Xevo TQ-S

High sensitivity and reliability

Xevo TQ-XS

Ultimate sensitivity and robustness

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Key Analytical Challenges

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- **Increasing Instrument Uptime**
- **Reducing Sample Turnaround Time**
- **Easy Method Transfer**
- **Understanding Sample Complexity**

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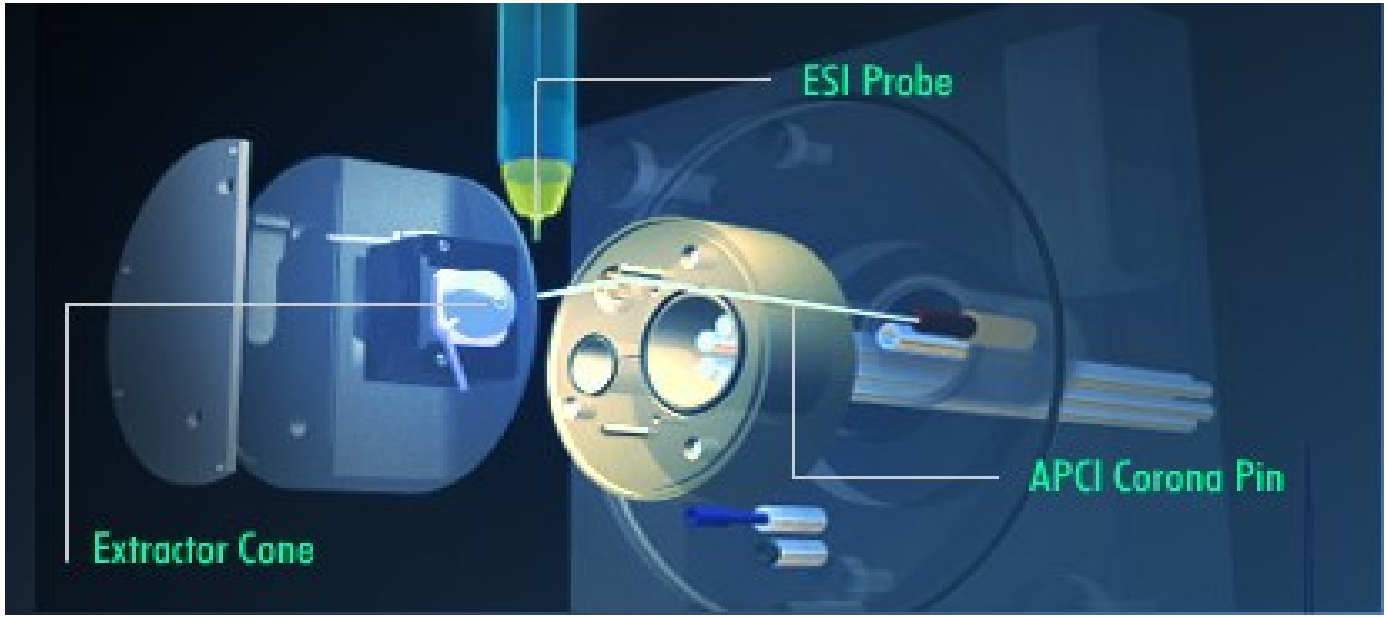
ESCi Multi-mode Ionisation

- ESI and APCI scans in the same acquisition
- Allows wider range of compounds to be ionised



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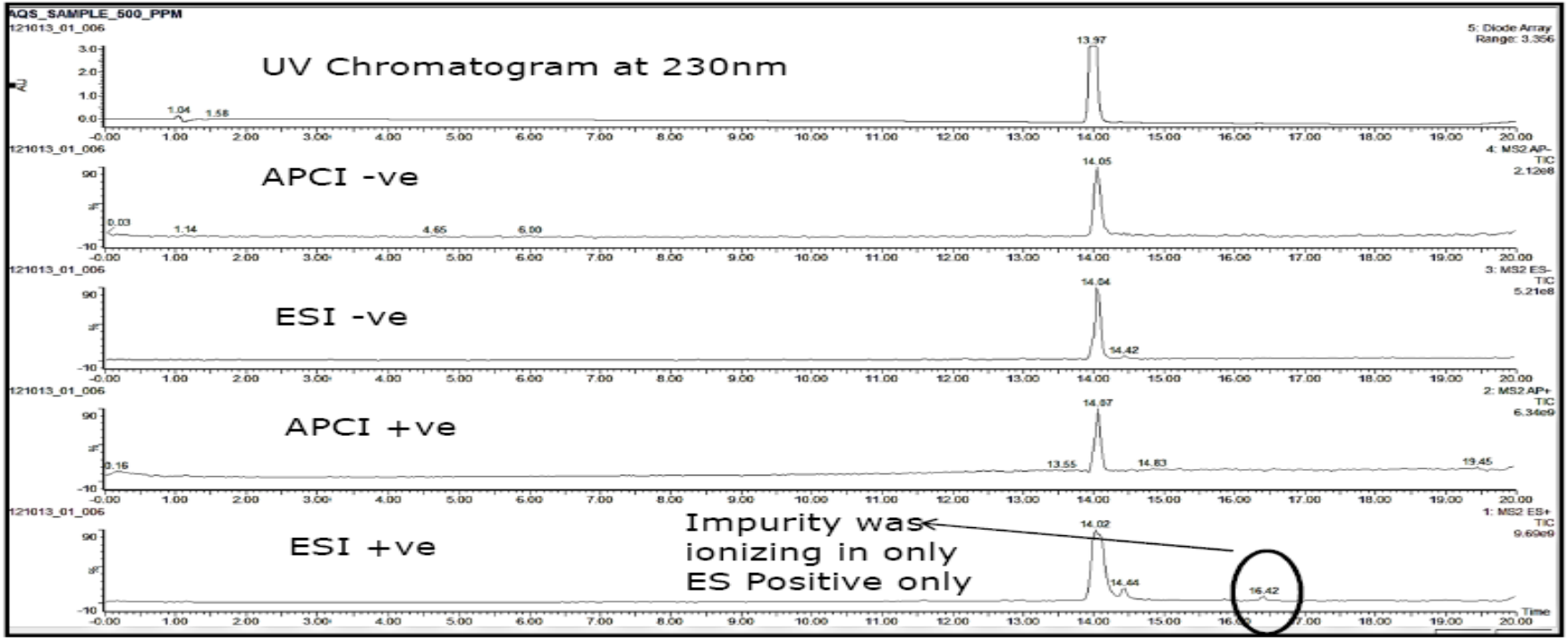
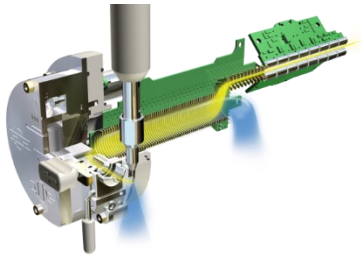
ESCi mode

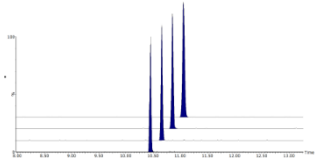

AQS_SAMPLE_500_PPM
121013_01_006
UV Chromatogram at 230nm
5: Diode Array
Range: 3.356
APCI -ve
4: MS2 AP-
TIC
2.12e8
ESI -ve
3: MS2 ES-
TIC
5.21e8
APCI +ve
2: MS2 AP+
TIC
6.34e9
ESI +ve
1: MS2 ES+
TIC
9.69e9
Impurity was
ionizing in only
ES Positive only
Time

Xevo TQ-S micro Innovative Technologies

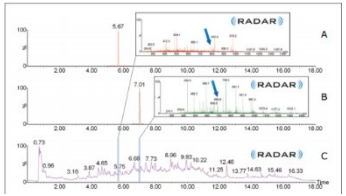
- STEPWAVE ion guide



- SPEED with high quality data



- RADAR and PICs



- Quantitative workflow updates
  - Standard Addition
  - Increased no. compounds

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Informatics solutions

INTELLISTART™

QUANPEDIA™

QCMonitor

TargetLynx™ XS

Trendplot

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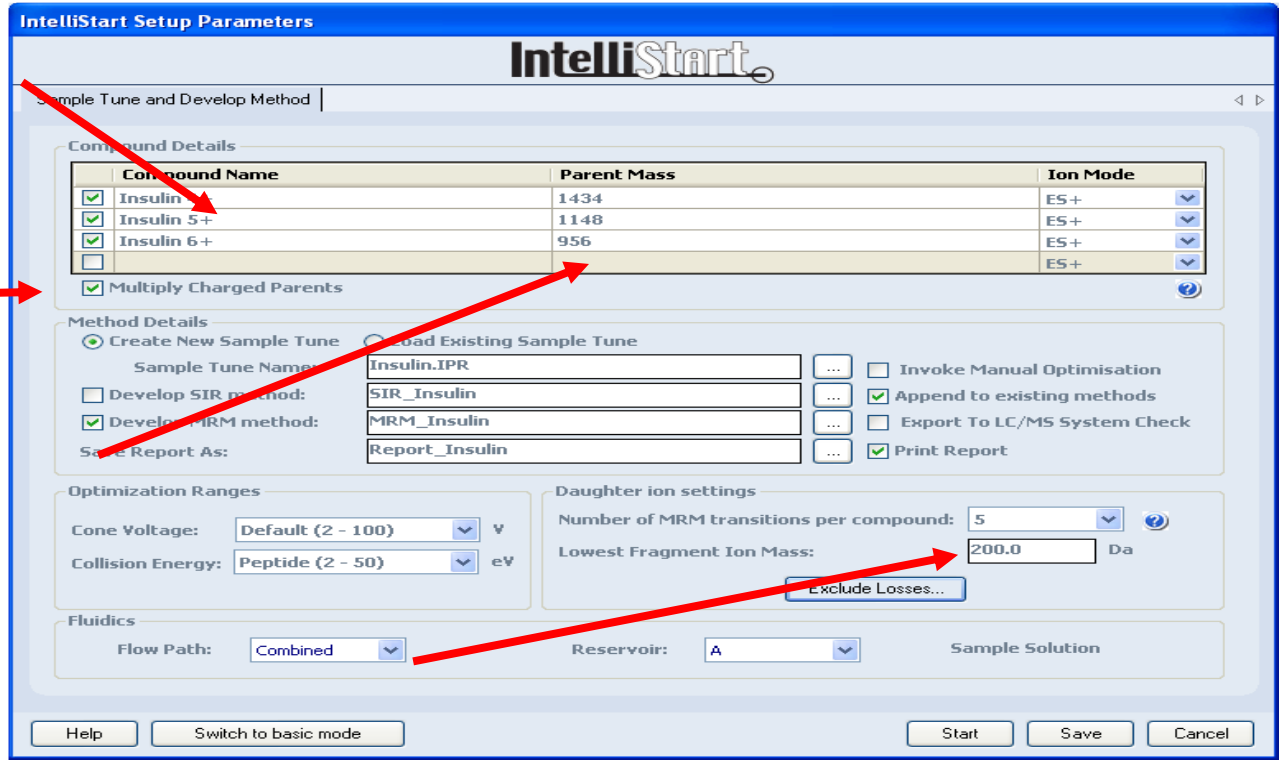
Intellistart™

- Input the compound name
- Calculate potential precursor masses
- Select Multiply Charged Parents
- Input the multiply charged precursor mass(es) from MW calculator
- Select advanced mode
- Limit low mass fragments
- Click Start



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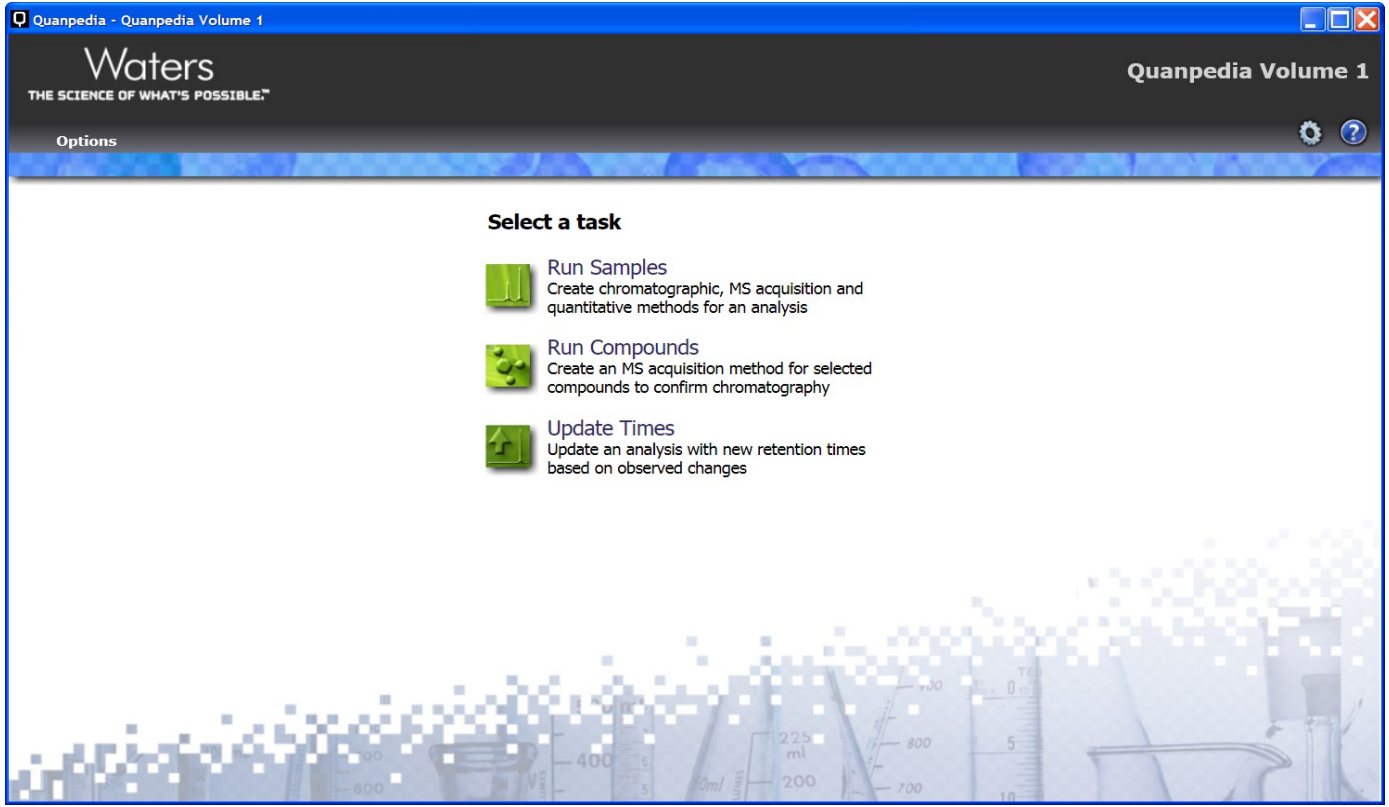
Quanpedia



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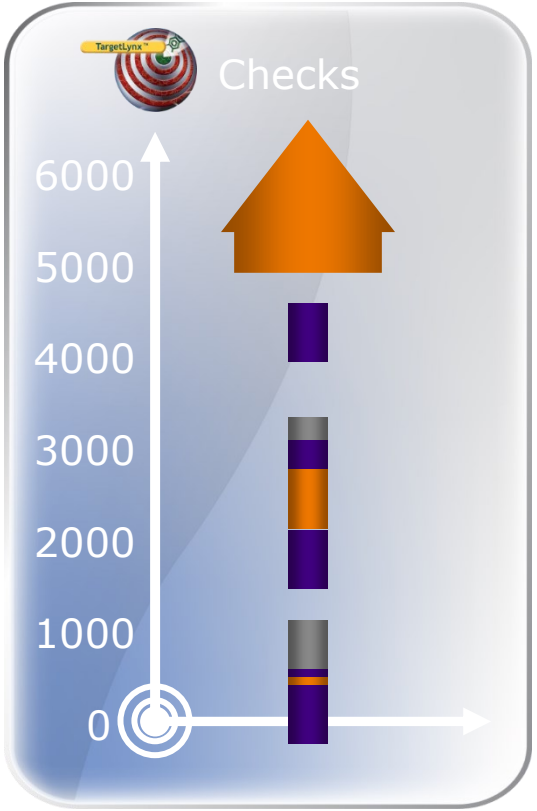
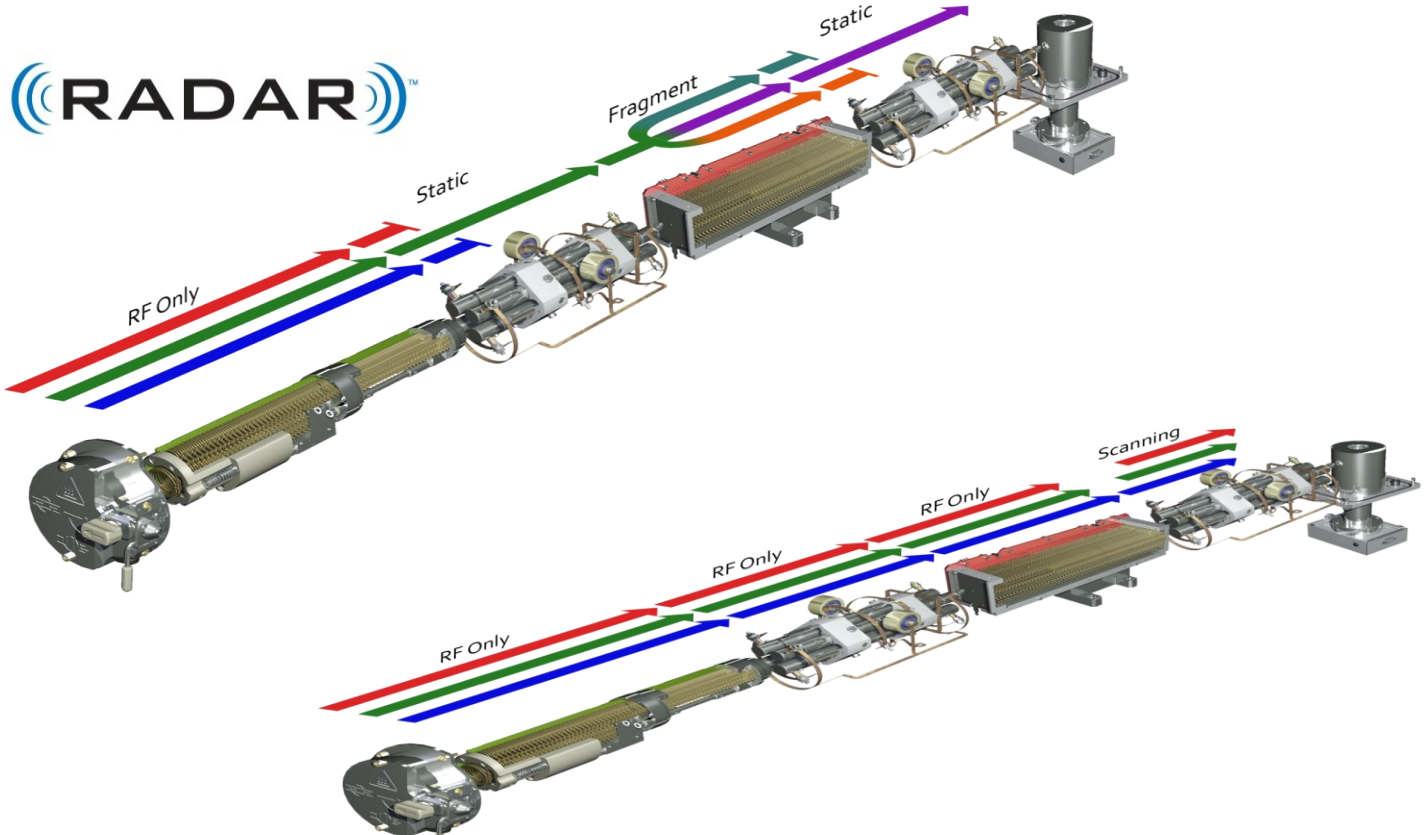
TargetLynx

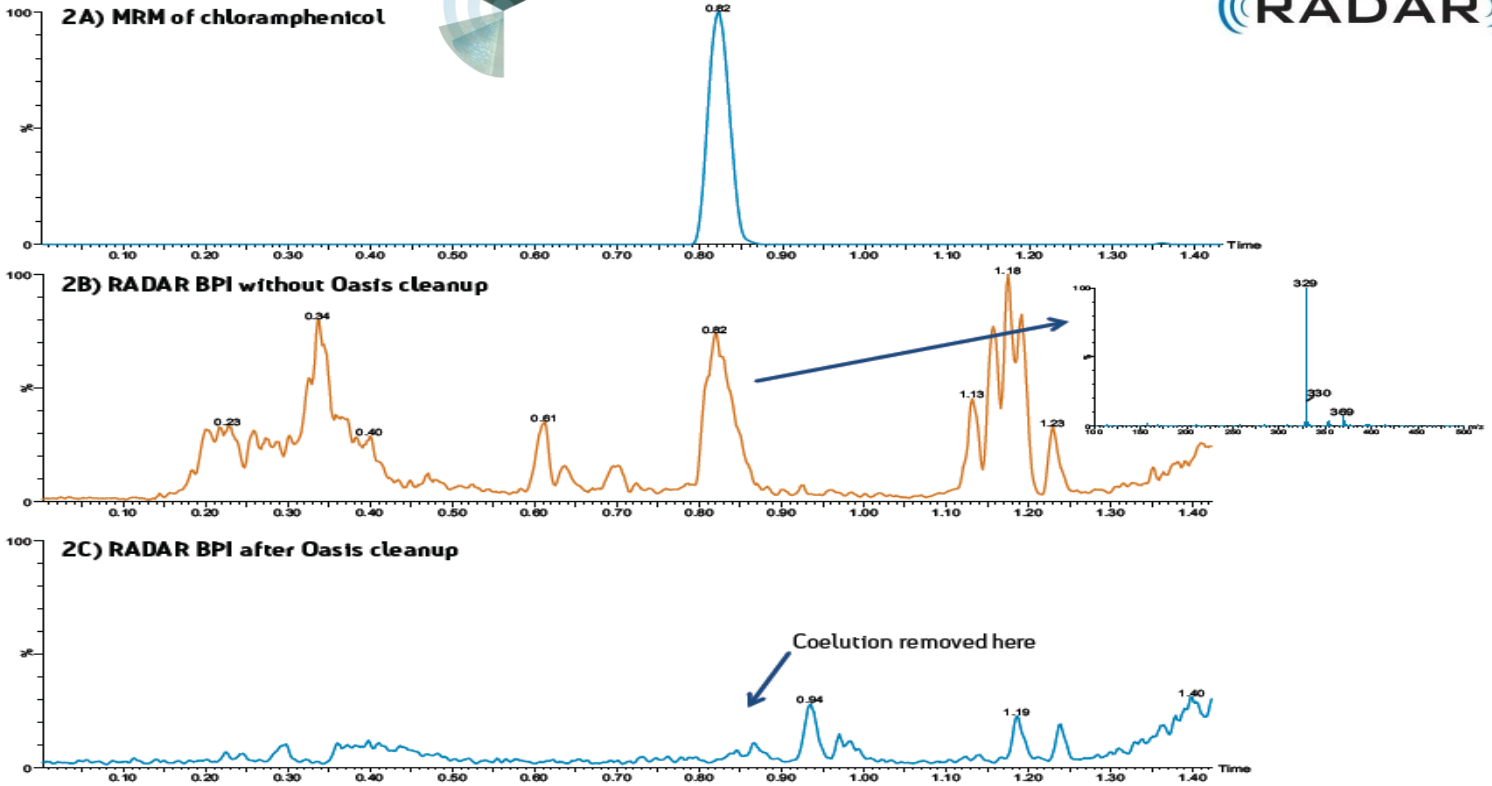

Checks
6000
5000
4000
3000
2000
1000
0


Correct RT and ion ratios
Analytes above RL/MRL
Recoveries
TargetLynx™
RL standar
Ion ra
Within RT
Ion ratios cha
RT shifted
Blank response OK

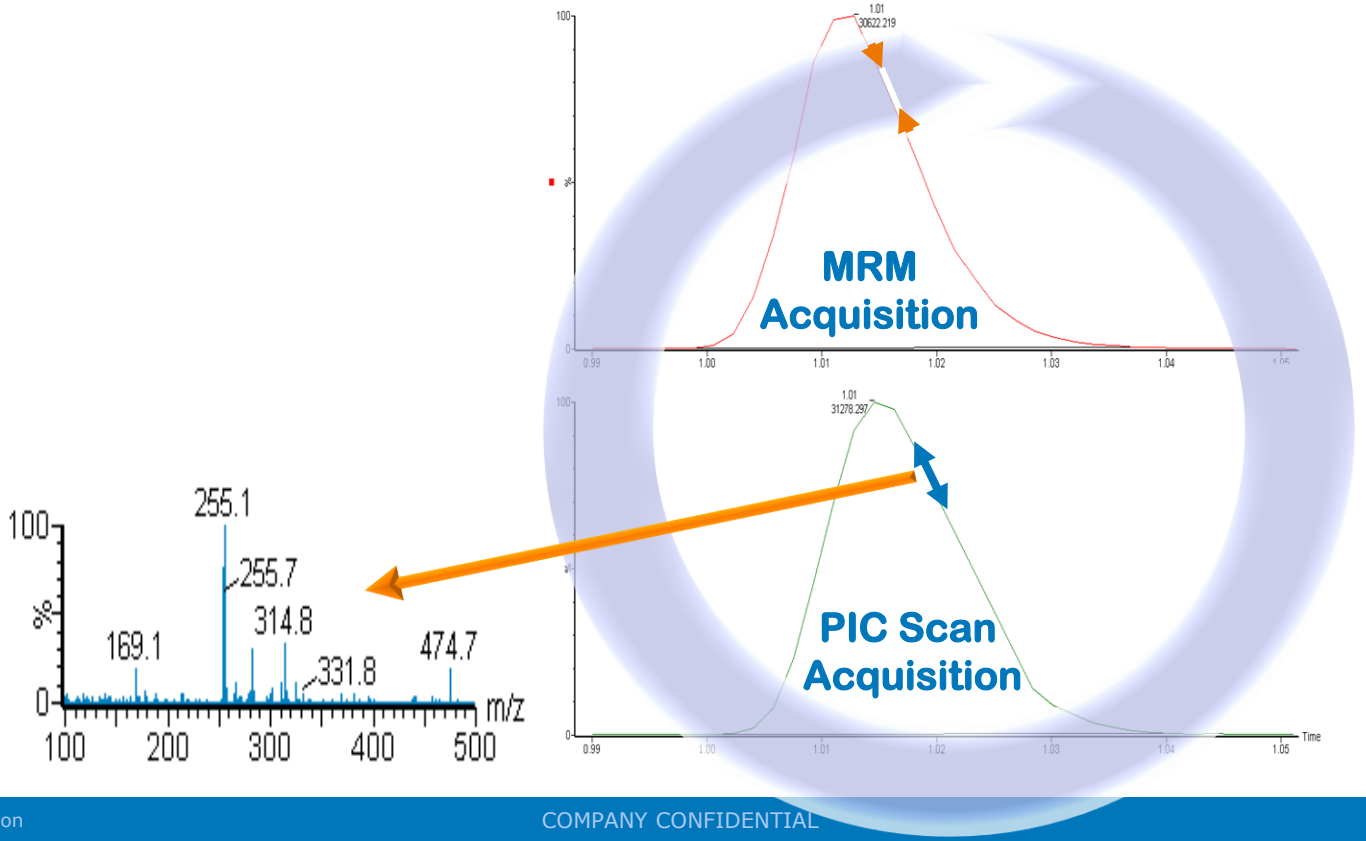
Rapid MS to MRM Switching



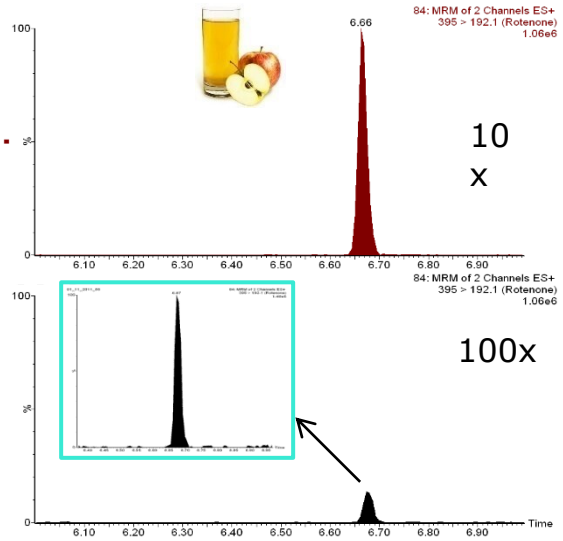
Knowing more about your samples
RADAR



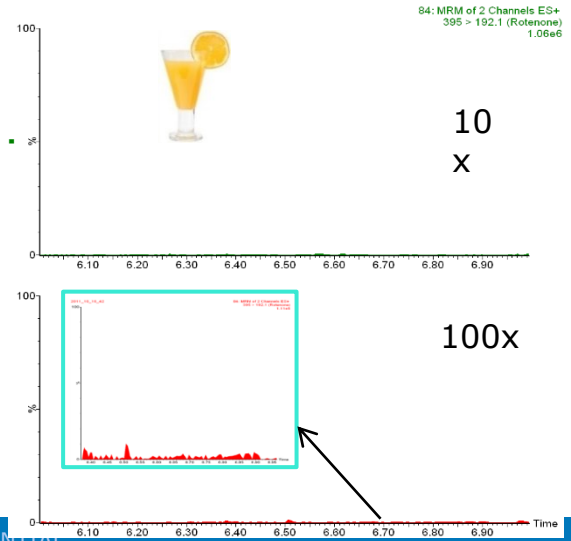
Information rich data
Precursor Ion Confirmation Scan



“Dilute and shoot” 10 ppb pesticides



ROTENONE



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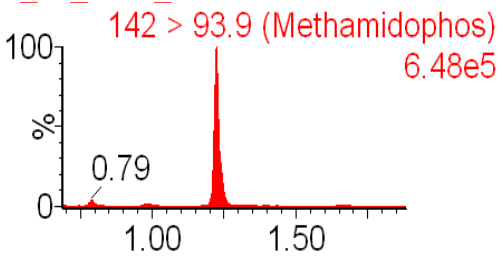
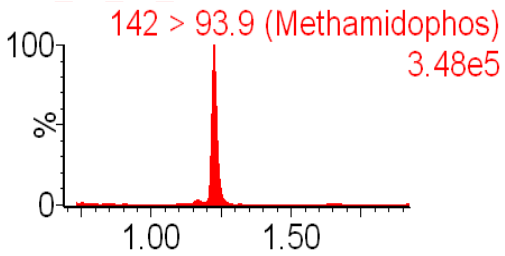
Sensitivity and Matrix effect

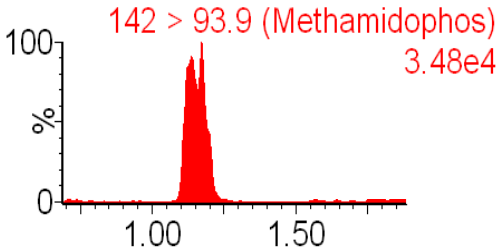
Methamidophos @ 10 ppb

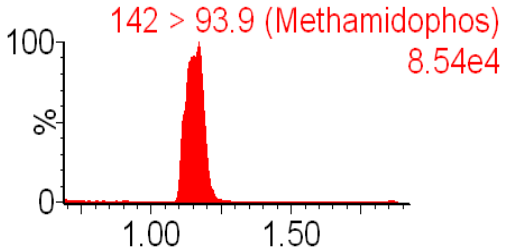
10x dilution





Quechers extract
10 % CH3CN





TargetLynx XS Standard Addition

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TargetLynx XS - Rhodamine B in chilli.qld

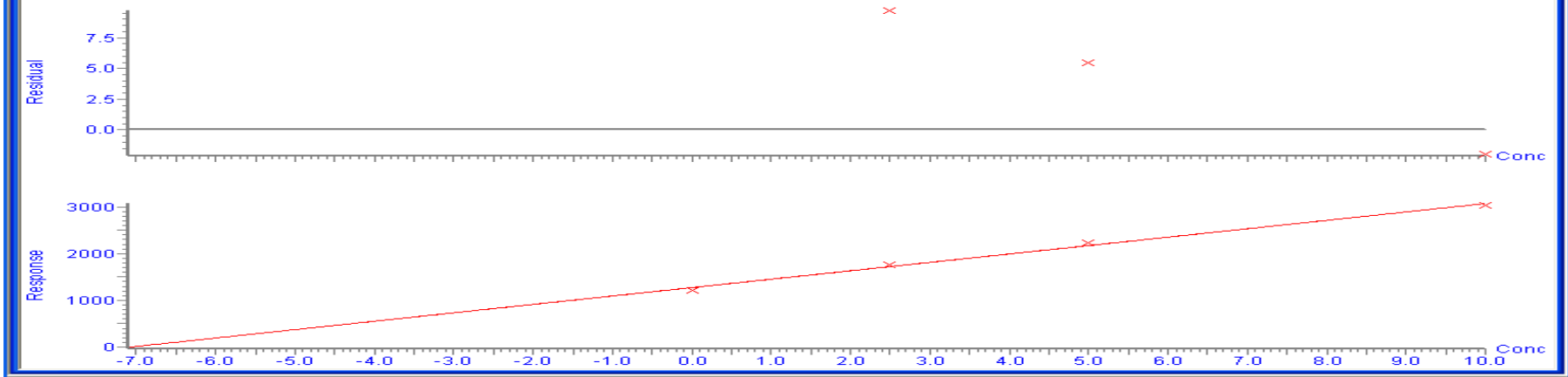
File Edit View Display Processing Window Help

Rhodamine B — Group 1 - SAMPLE ID 1

	#	Name	Type	Conc. Added	RT	Area	Response	Primary Flags	Conc.	%Dev	Std. Add. Conc
1	1	14_04_2014_10	Standard	0.000	2.08	1215.999	1215.999	dd	0.0		7.078
2	2	14_04_2014_16	Standard	2.500	2.07	1766.899	1766.899	bd	2.7	9.7	7.078
3	3	14_04_2014_19	Standard	5.000	2.07	2222.451	2222.451	bd	5.3	5.5	7.078
4	4	14_04_2014_22	Standard	10.000	2.07	3036.976	3036.976	bd	9.8	-2.0	7.078

Calibration: 21 May 2014 10:10:04

Compound name: Rhodamine B
Correlation coefficient: r = 0.997490, r^2 = 0.994987
Calibration curve: 179.915 * x + 1273.45
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: Null, Axis trans: None
Standard Addition Concentration : 7.07809



Ready — Rhodamine B — NUM

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SAMPLE EXTRACTION

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Multi Residue Analysis for Food Safety

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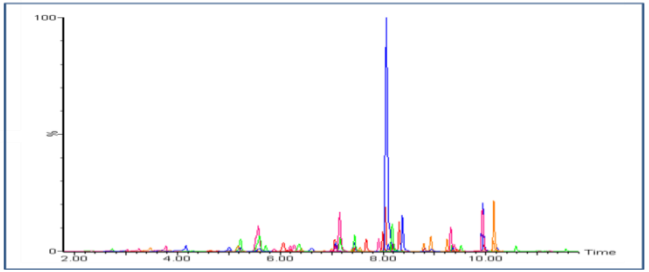
Whole product

Representative extract

Ultra trace detail targeted analysis (low ppb)



Typical challenges include

- ? Sample throughput: >1000 pesticides commercially availabl foods and feeds
- ? Regulatory requirements: Approx 740 compounds regulatec concentrations
- ? Accessibility of analysis: LC and GC amenable pesticides
- ? Nontargeted analysis: Metabolites or unknown contaminants

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DisQuE Products

Liquid-extraction and partition cleanup

Dispersive SPE cleanup

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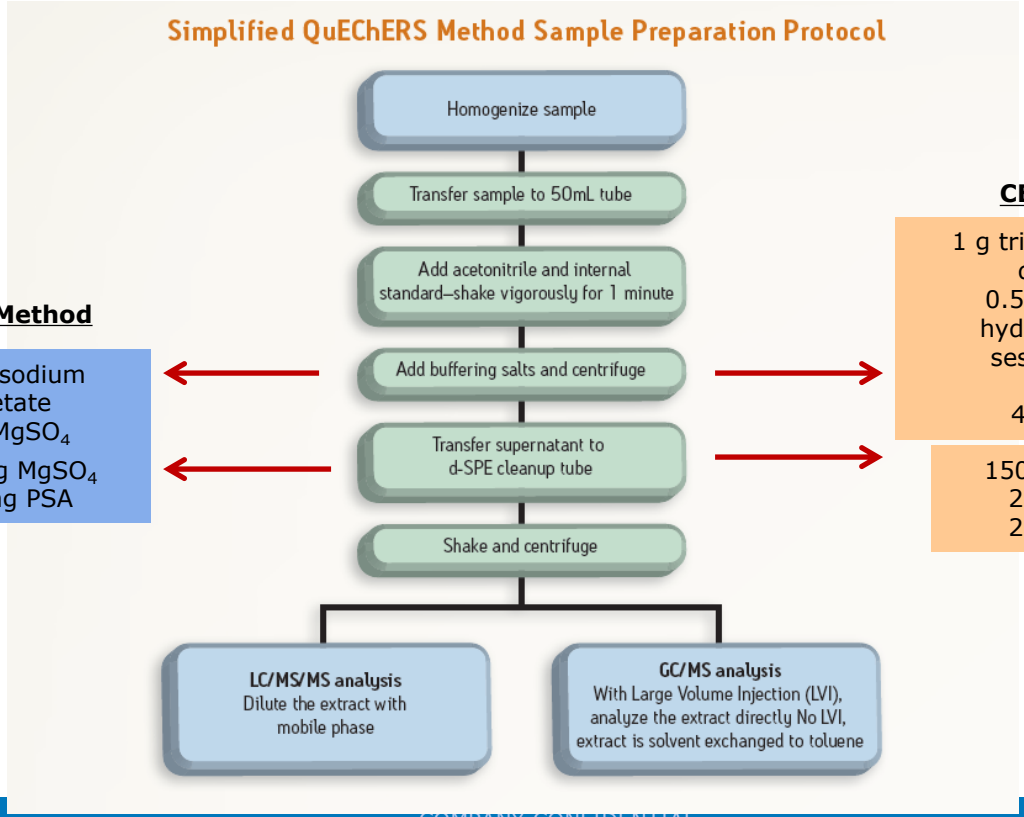
Basic QuEChERS Procedure



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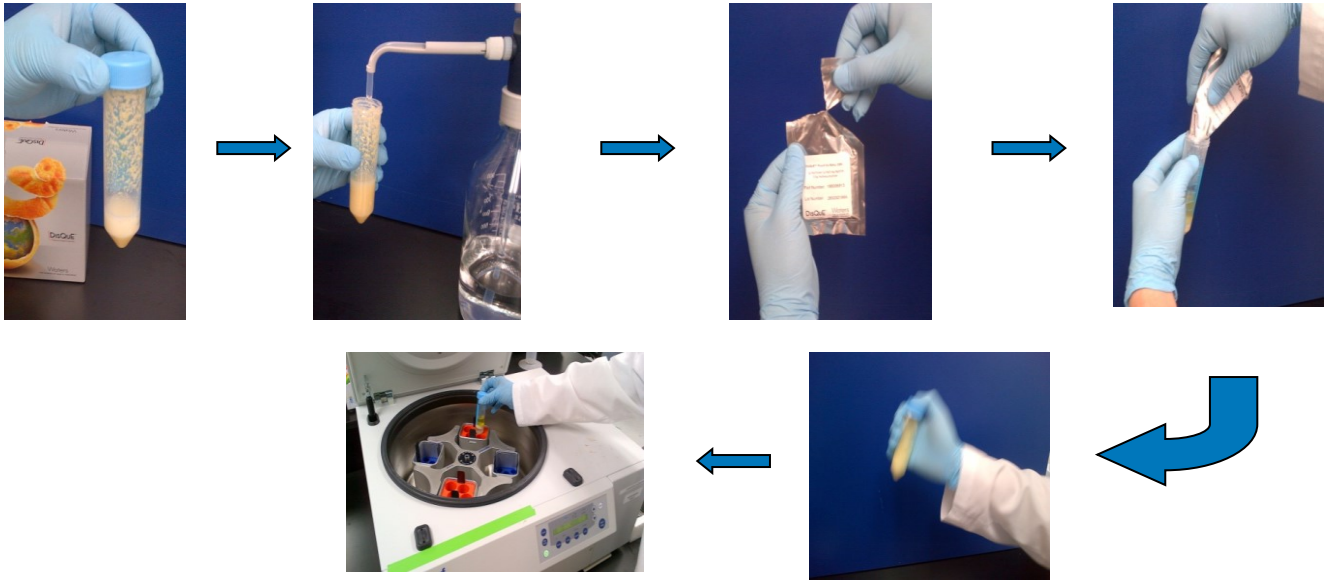
Extraction/Partitioning
CEN 15662 Method



- Transfer 10 g of sample to a 50 mL extraction tube
- Add 10 mL acetonitrile. Shake for 1 minute
- Add contents of DisQuE pouch
- Shake vigorously for 1 minute
- Centrifuge, take aliquot of top layer for cleanup by dSPE or other methods

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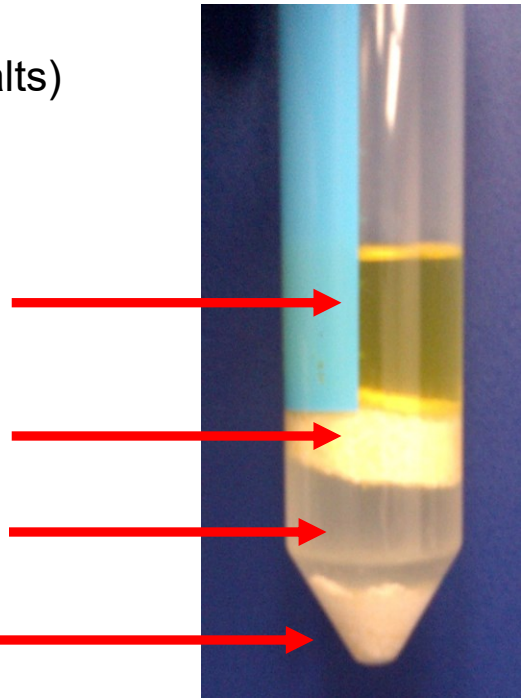
QuEChERS Extraction: 50 mL Tube

- Water is IMPORTANT!!
  - Produces liquid/liquid partition cleanup after DisQuE salts are added
    - Removes unwanted polar matrix components (sugars, salts)
    - Makes acetonitrile extraction more effective
  - If water not present in matrix, **then add it**



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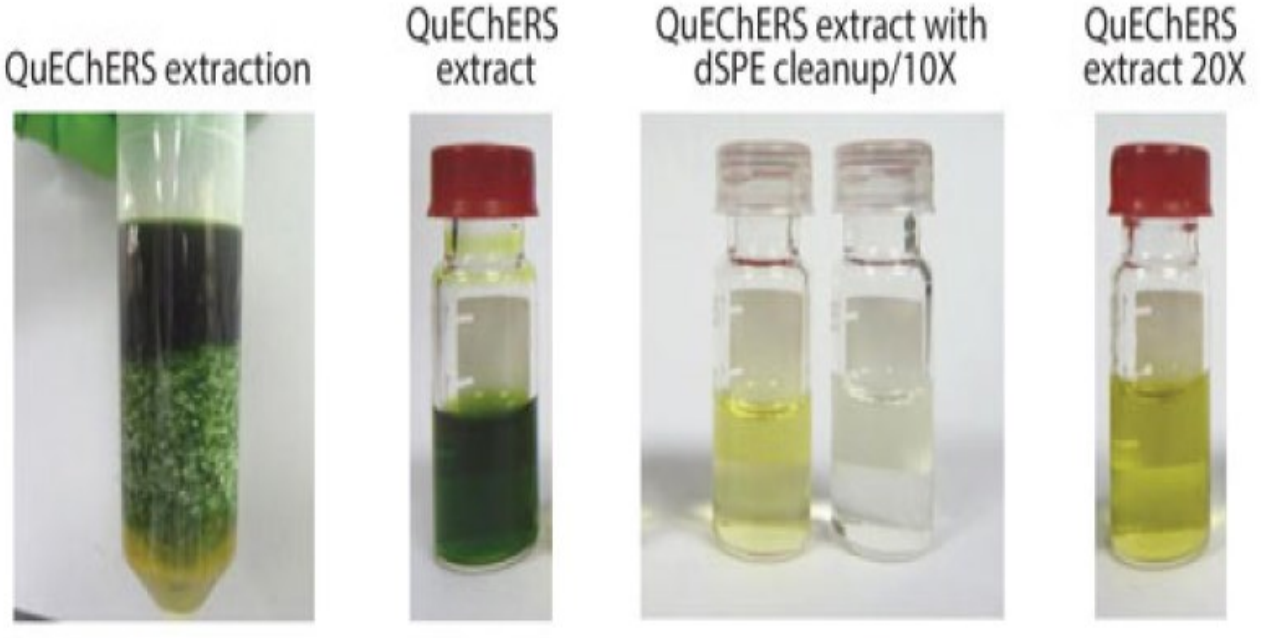
Quechers



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New DisQuE Cleanup Tubes - Available Now

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Tube Size	Contents	Description
2 mL	150 mg MgSO4, 25 mg PSA, 25 mg C18, 7 mg GCB	**General purpose cleanup**
15 mL	1200 mg MgSO4, 400 mg PSA	AOAC Method
15 mL	1200 mg MgSO4, 400 mg PSA, 400 mg C18	AOAC Method, fatty matrix √
15 mL	1200 mg MgSO4, 400 mg PSA, 400 mg C18, 400 mg GCB	AOAC Method, fatty & pigment matrix √
2 mL	150 mg MgSO4, 50 mg C18	Acidic pesticides
2 mL	150 mg MgSO4, 25 mg PSA, 2.5 mg GCB	CEN Method, pigment matrix
15 mL	900 mg MgSO4, 300 mg PSA	High fatty acids samples
15 mL	900 mg MgSO4, 300 mg PSA, 300 mg C18	Fatty produce/cereal
15 mL	900 mg MgSO4, 450 mg PSA, 300 mg C18, 50 mg GCB	Fatty and pigment matrix, Tea
15 mL	750 mg MgSO4, 250 mg PSA, 150 mg C18, 150 mg Al-N	Multi-residue mycotoxins
2 mL	150 mg MgSO4, 50 mg PSA, 30 mg C18, 30 mg Al-N	Multi-residue mycotoxins

√ Consider Oasis PRiME HLB pass-thru cleanup

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Other Cleanup Options - by Cartridge SPE

- Sep-Pak Vac 6 cc (500mg) PSA/Carbon cartridges
  - Used for APGC-MS cleanup of spices
- Oasis PRiME HLB for **pass-thru** cleanup
  - As effective as dSPE for removal of
    - Fats
    - Phospholipids
    - Chlorophyll
- Acidic pesticides – PSA cleanup is not suitable
  - Diluted with mobile phase and analyzed directly
  - If cleanup is required, e.g. tea
    - Use Oasis MAX 3 cc 60 mg for acidic pesticides cleanup in tea

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SPE Procedure

Step1
- Condition HLB and MCX the cartridges with 1.5mL of methanol and 1.5mL of water

Step 2
- 10mL of centrifuged wastewater is passed through the cartridges

Step 3
- Wash HLB with 3mL of water
- Wash MCX with 3mL of 2% Formic acid in water

Step 4
- Elute with HLB with 2mL methanol
- Elute with MCX with 2mL methanol with 2% NH4OH

Step 4
- Pool all elations and evaporate to dry ness and reconstitute with 1mL of Mobile phase

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Experimental Overview

Sample Preparation

- CEN QuEChERS, tube 1

Liquid Chromatography Conditions

- ACQUITY UPLC H-Class
- Column: ACQUITY UPLC BEH C_{18} 100 mm x 2.1 mm, 1.7 μm
- Column temperature: 45 ° C
- MP A: 10 mM ammonium acetate (pH 5) in water
- MP B: 10 mM ammonium acetate (pH 5) in acetonitrile
- Flow rate: 450 μl
- Injection volume: 10 μl

MS Conditions

- Ionisation: ES±
- Capillary: 1 kV (+) and 0.5 kV (-)
- Source Temperature : 150 °C
- Desolvation Temperature : 1000 °C
- Cone Gas flow: 120 $l.hr^{-1}$
- Desolvation Gas flow: 1000 $l.hr^{-1}$

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Chromatogram of 400 pesticides

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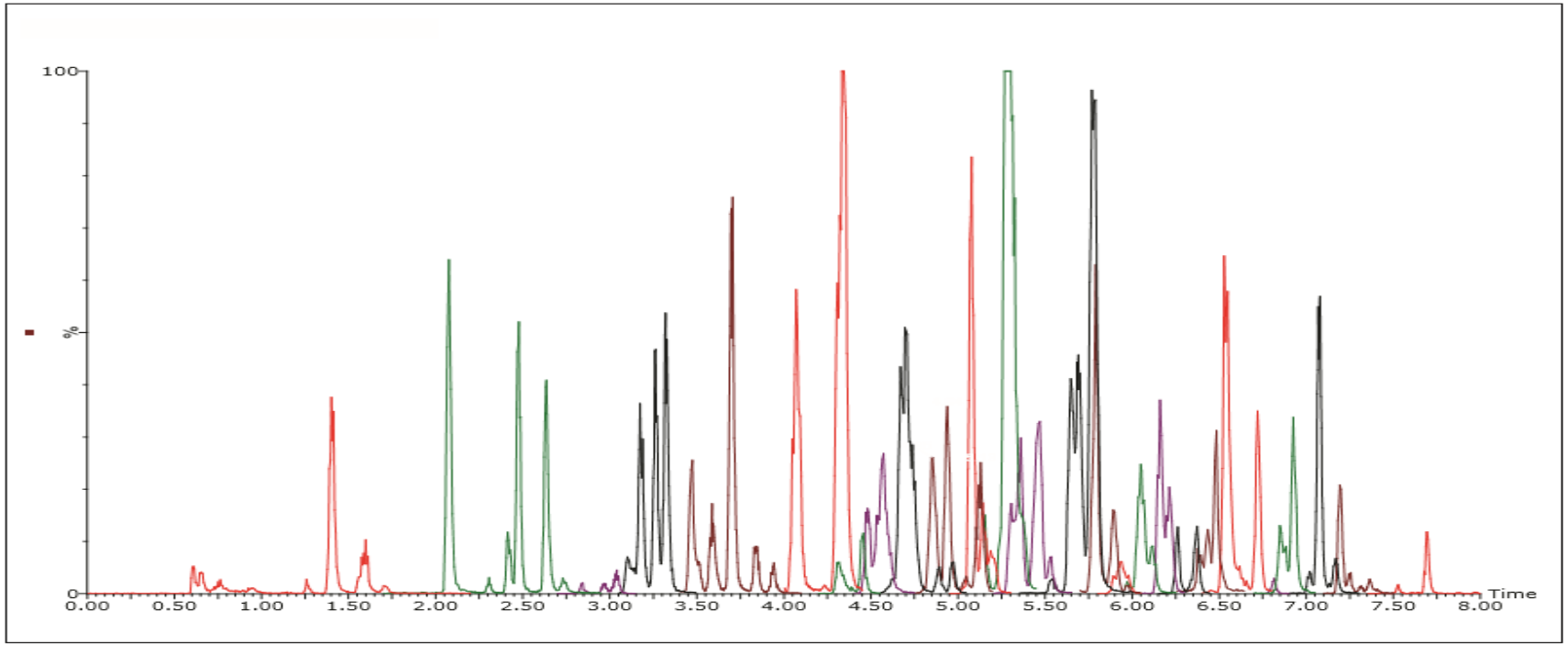

Figure 1. Chromatogram showing all 402 pesticide residues in one 10 minute run in injection solvent.

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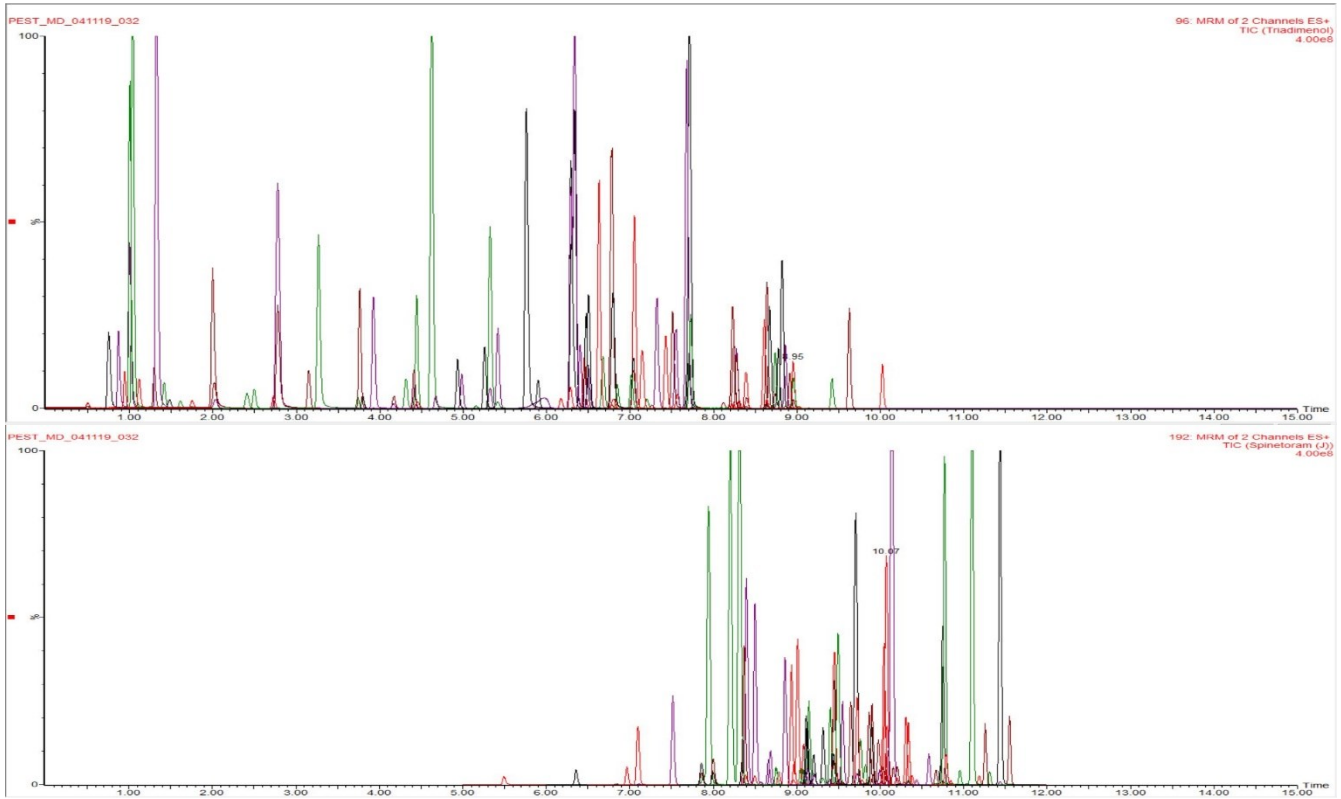
200 Pesticides with two transitions in 12 min on Xevo TQ-S micro



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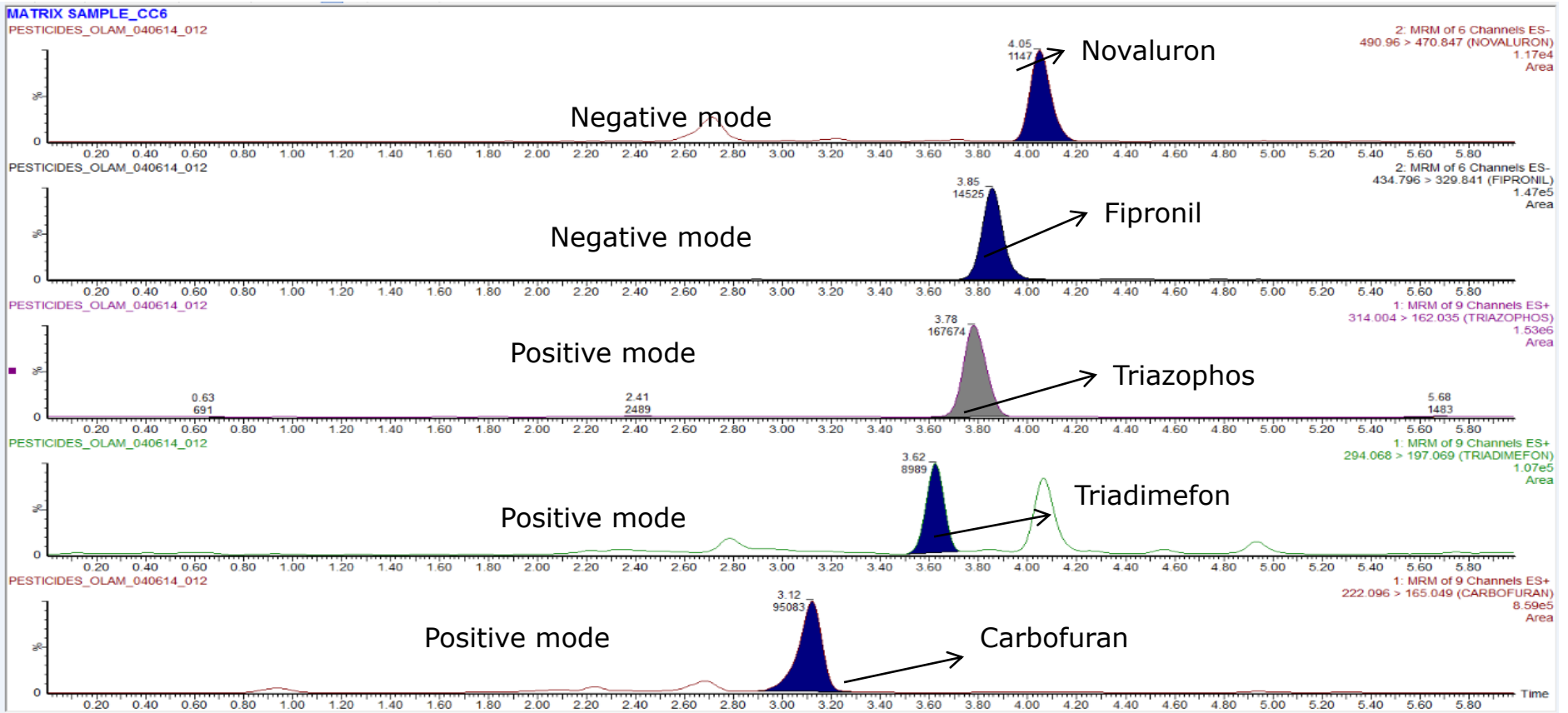
Chromatogram of Chilli extracted sample for five pesticides in dual mode



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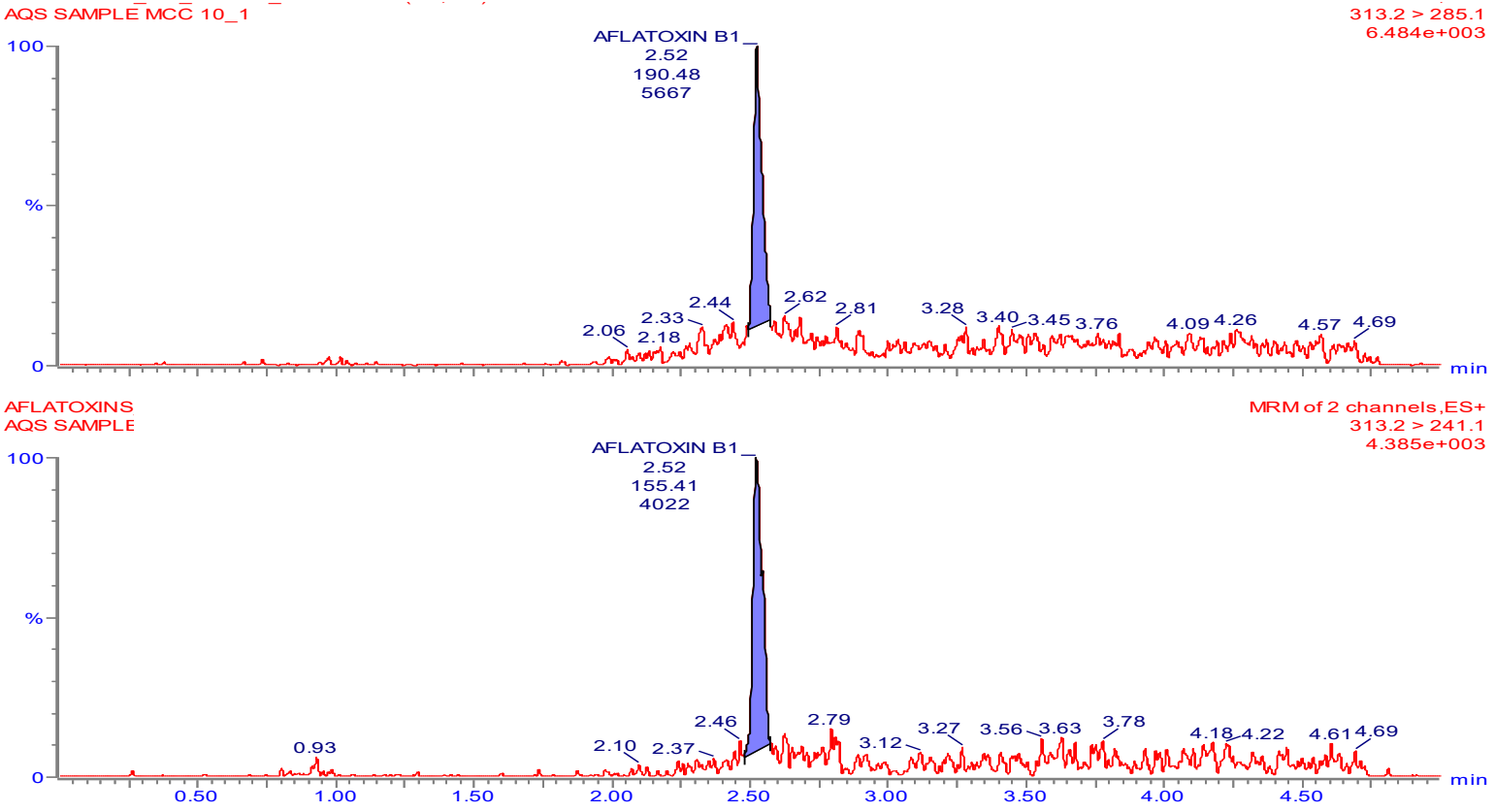
Chromatogram for Aflatoxin B1 (0.015ng/mL)



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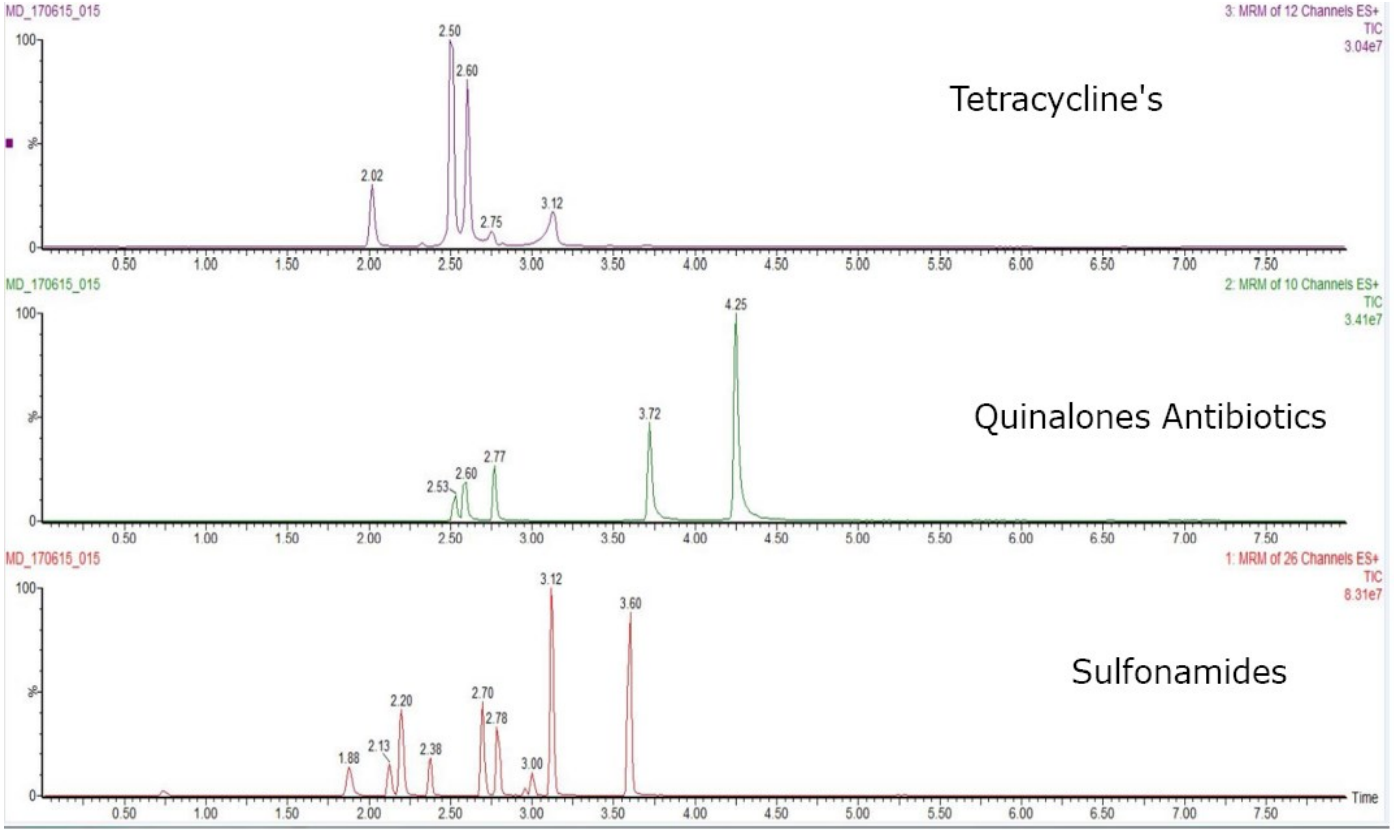
Chromatograms of Tetracycline's, Quinolone antibiotics and Sulfonamides in Single run



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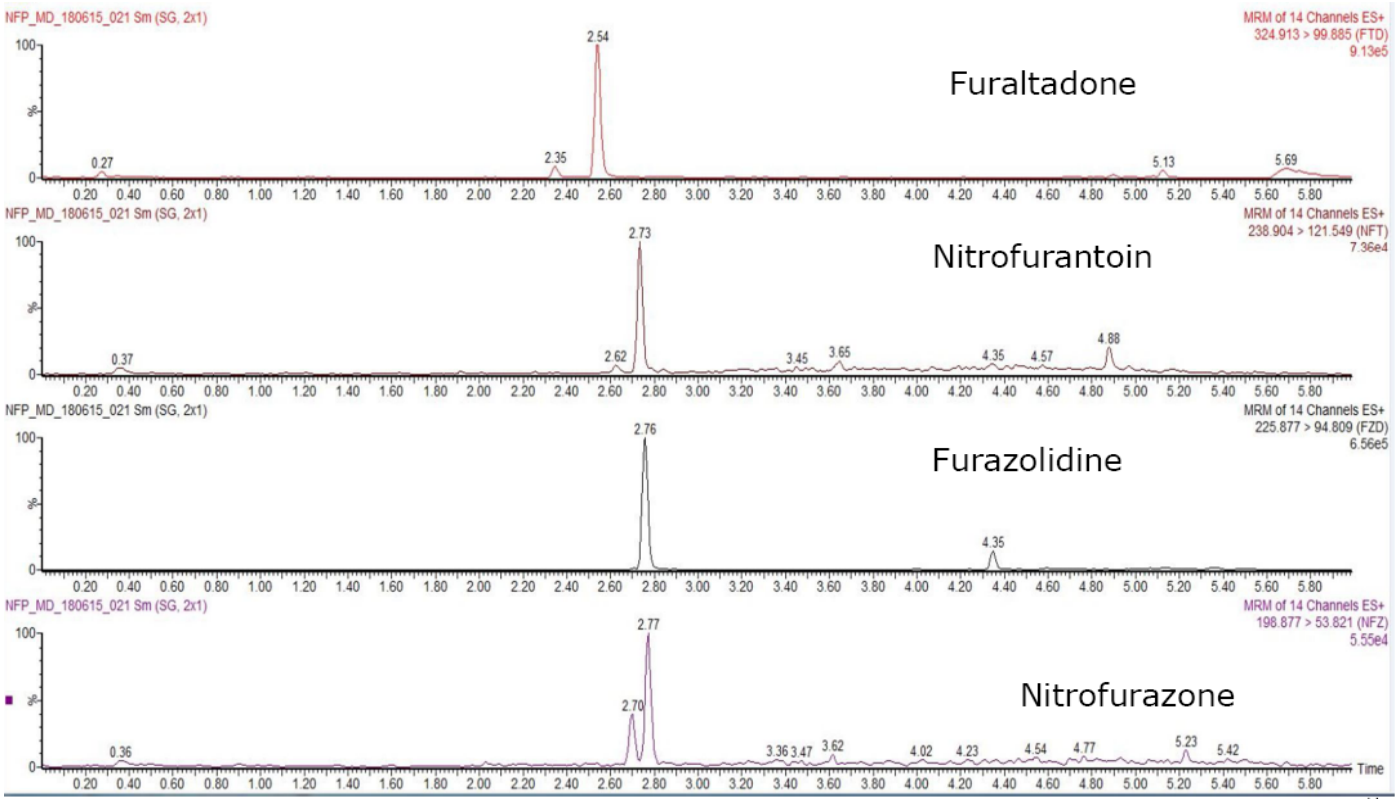
Nitro furan parent compounds



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POLAR PESTICIDES

Sample Preparation for Polar pesticides

Approaching multi residue extraction with QuPPe method

Weigh homogenized sample (10g) into centrifuge tube

(adjust for water content)

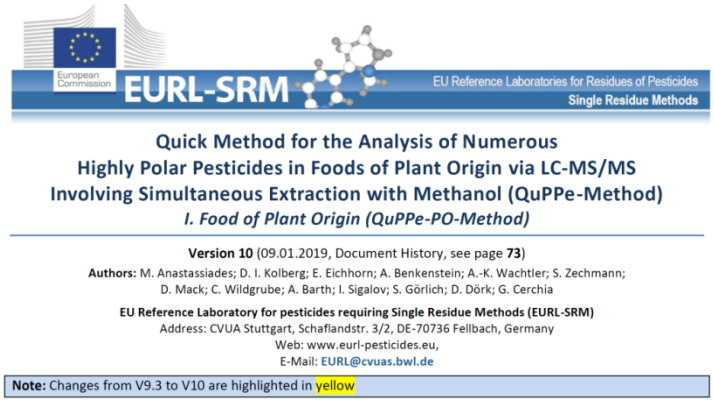
spike commodity with labelled IS or reference material

Add methanol (10 ml) containing 1 % formic acid

Vortex thoroughly for 1 minutes

Centrifuge at 5000 rpm for 5 minutes

Filter supernatant (0.45 μm, PVDF, filter) into a plastic vial



EURL-SRM

EU Reference Laboratories for Residues of Pesticides

Single Residue Methods

Quick Method for the Analysis of Numerous Highly Polar Pesticides in Foods of Plant Origin via LC-MS/MS Involving Simultaneous Extraction with Methanol (QuPPe-Method)

I. Food of Plant Origin (QuPPe-PO-Method)

Version 10 (09.01.2019, Document History, see page **73**)

Authors: M. Anastassiades; D. I. Kolberg; E. Eichhorn; A. Benkenstein; A.-K. Wachtler; S. Zechmann; D. Mack; C. Wildgrube; A. Barth; I. Sigalov; S. Görlich; D. Dörk; G. Cerchia

EU Reference Laboratory for pesticides requiring Single Residue Methods (EURL-SRM)

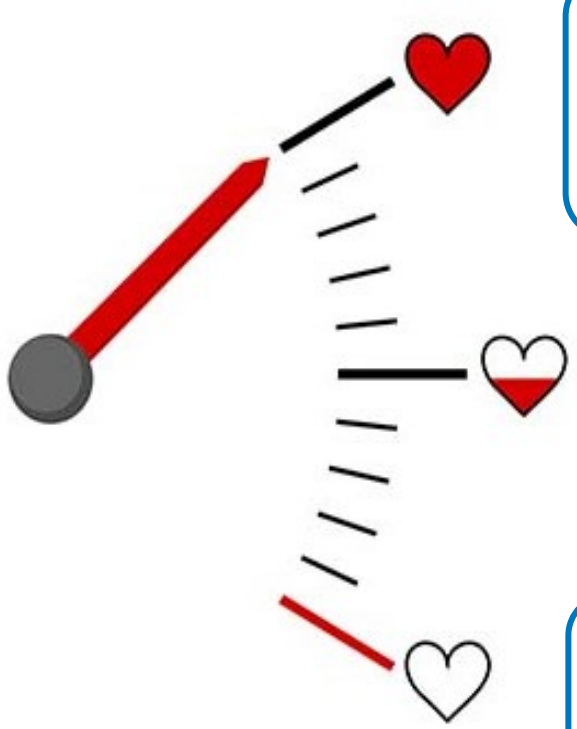
Address: CVUA Stuttgart, Schaflandstr. 3/2, DE-70736 Fellbach, Germany

Web: www.eurl-pesticides.eu,

E-Mail: EURL@cvuas.bwl.de

Note: Changes from V9.3 to V10 are highlighted in yellow

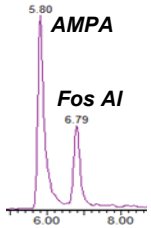
Waters' polar pesticide journey simplified



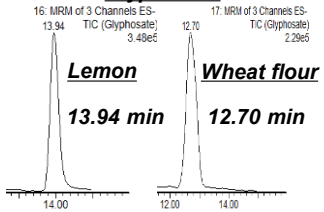
Torus Technology

- ➢ HILIC type: DEA
- ☑ Retention
- ☑ Excellent separation of critical pairs
- ☑ Retention time stable
- ☑ Perchlorate compatible

Separation



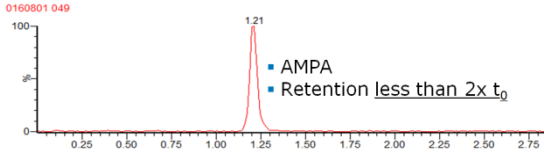
Retention time stability Glyphosate



- ➢ HILIC type (polyvinyl alcohol)
- ☑ Retention
- ☑ Acceptable separation of critical pairs
- ☒ Retention time unstable
- ☒ Perchlorate incompatible (5 min wide peak)

- ➢ Mixed mode
- ☒ Retention
- ☒ Separation of critical pairs

Retention



RSDs > 20% in different commodities

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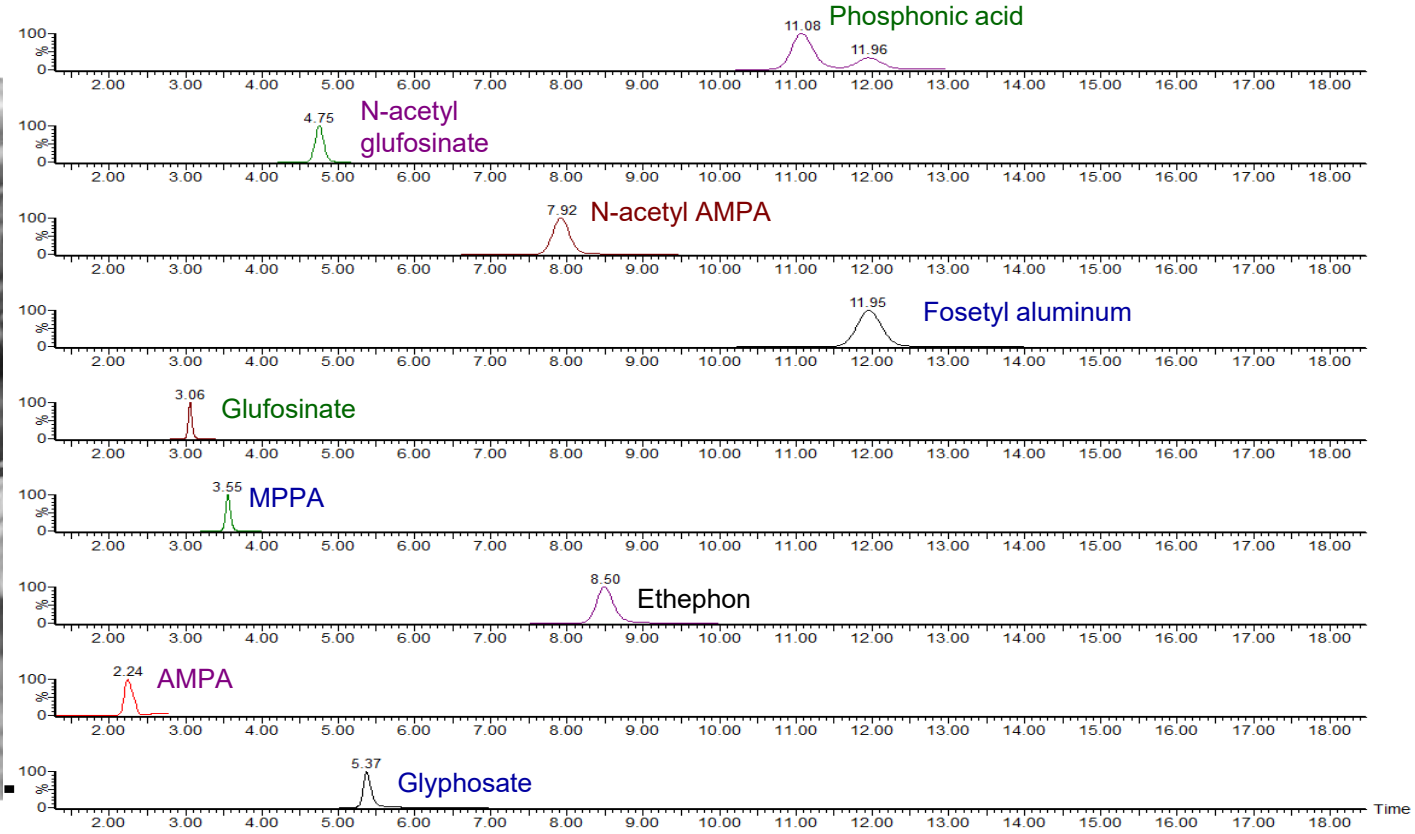
Chromatogram for Anionic polar pesticides analysis



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Atmospheric Pressure Gas Chromatography

- APGC is an inlet option of the Xevo universal source, readily interchangeable with UPLC, *etc*.
- Complementary technique to GC-EI
- Operates
  - At atmospheric pressure, thus allowing higher gas flows to be applied
  - By APCI like ionisation using a corona discharge pin, thus providing 2 types ionisation

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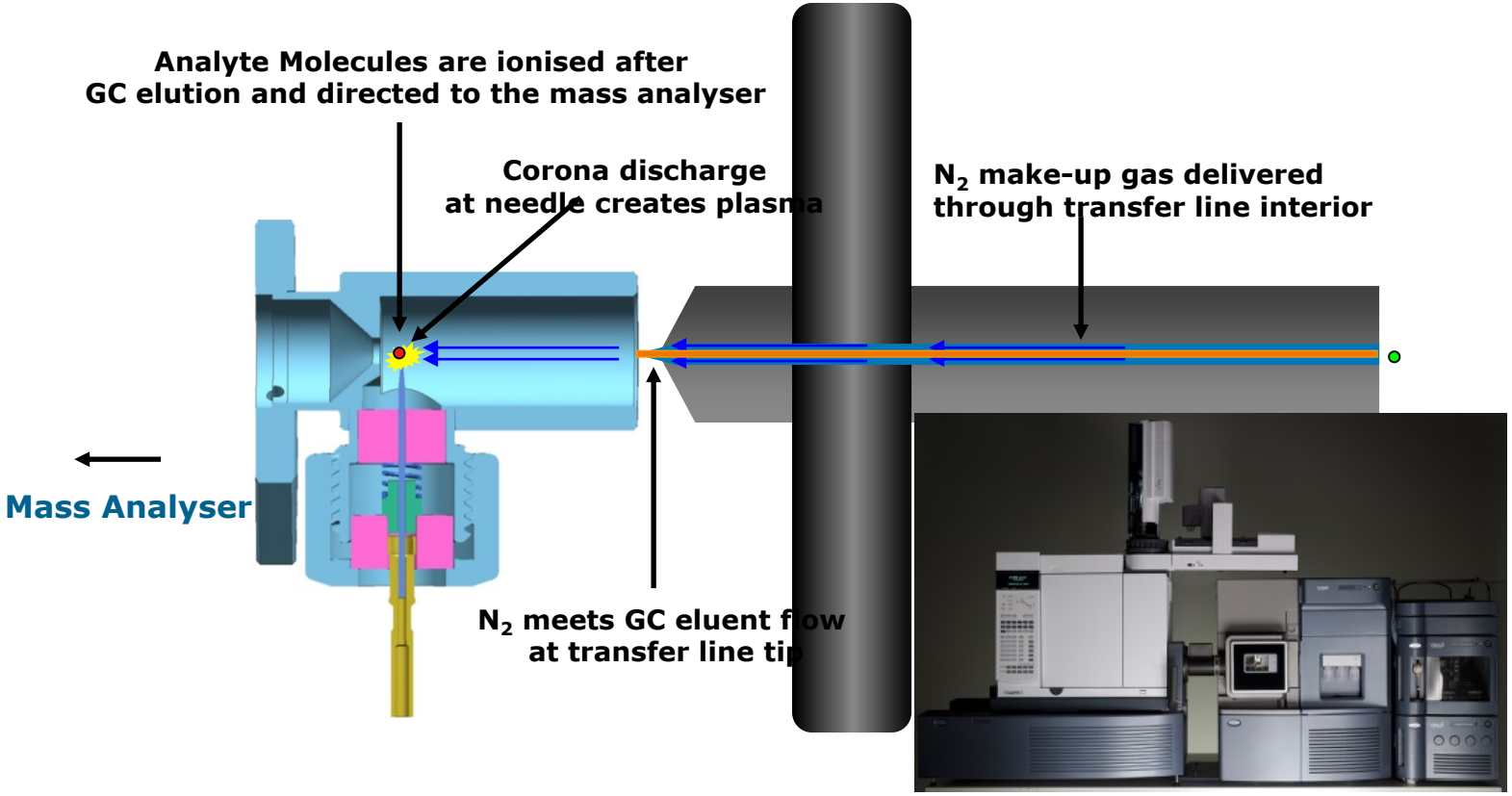
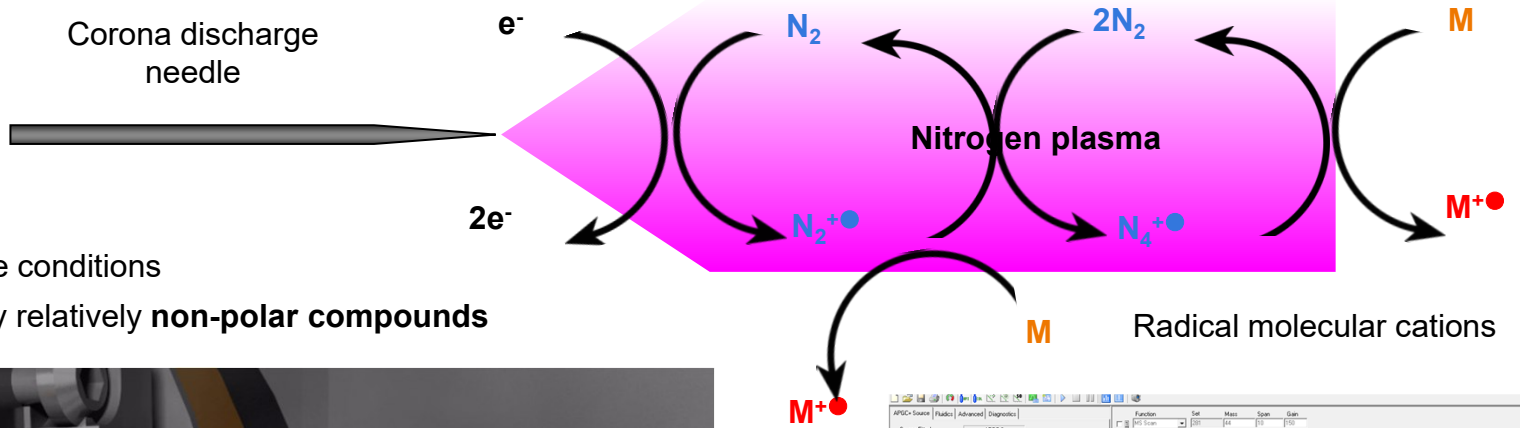
APGC – How It Works



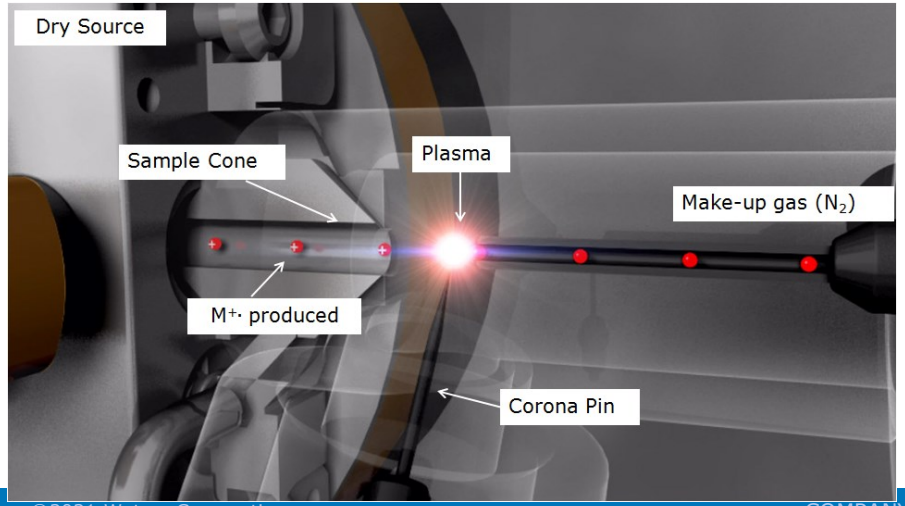
Charge Transfer

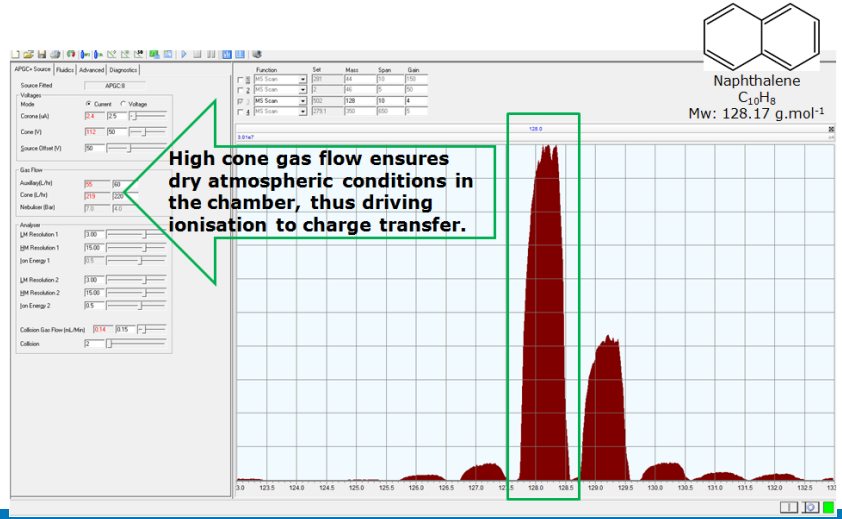


"**Dry**" source conditions

Favoured by relatively **non-polar compounds**

Radical molecular cations





Increased precursor ion providing confidence

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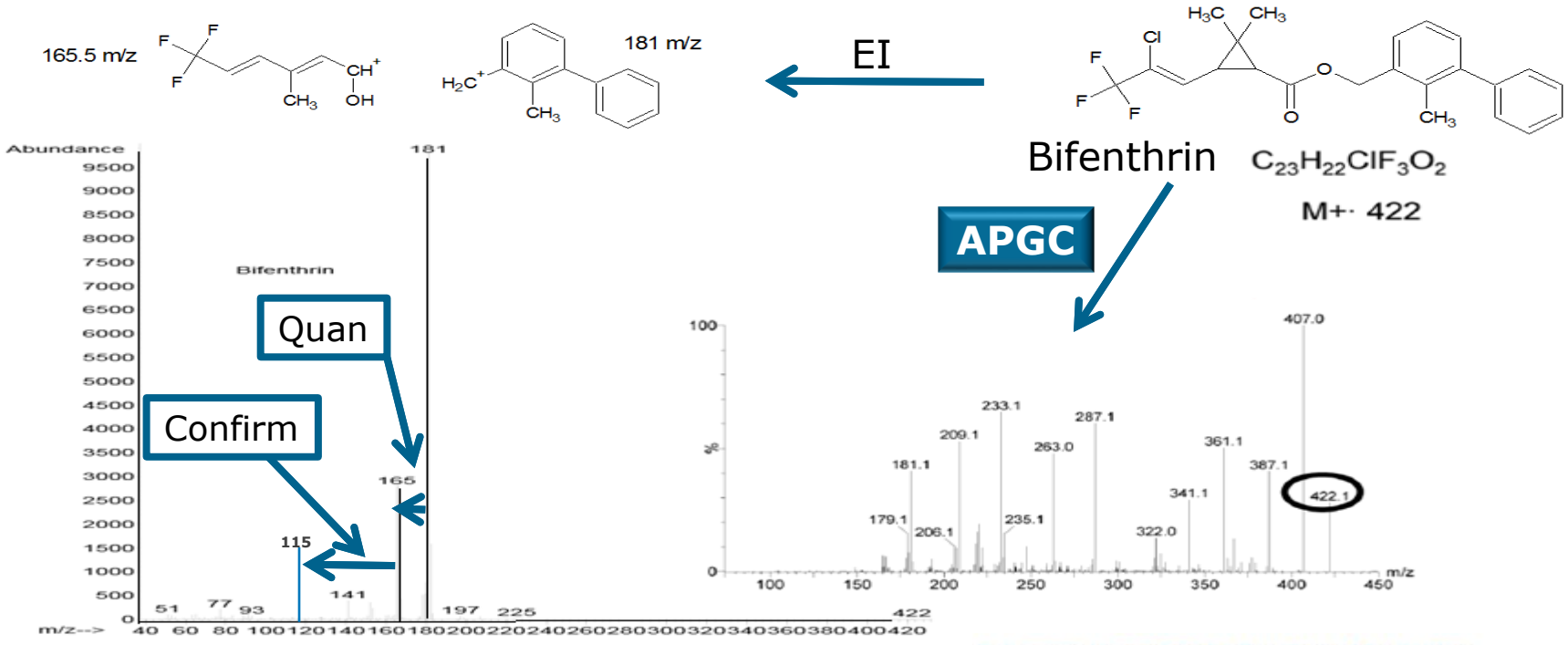

Reference Laboratory for Residues of Pesticides

EURL European Union Reference Laboratory for Pesticide Residues in Fruits & Vegetables

Swedish National Food Administration

Pesticides in Fruits and Vegetables

http://www.crl-pesticides.eu/library/docs/fv/Ethyl_acetate_extraction.pdf

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Softer ionisation, improving selectivity: GC-EI-MS vs GC-APCI-MS/MS

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Fipronil

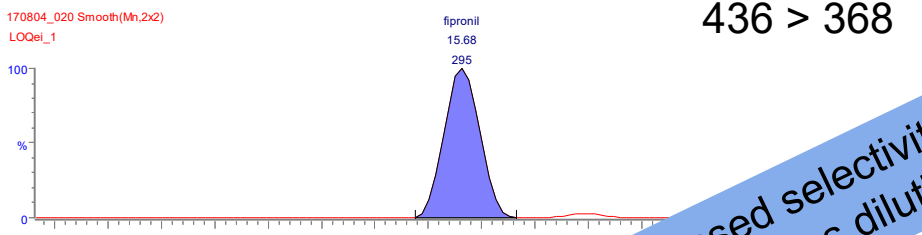
Fipronil	EI	APGC
Quan	367 > 255	436 > 368
Qual	367 > 213	369 > 215

Fipronil sulfone

Sulfone	EI	APGC
Quan	383 > 335	451 > 415
Qual	383 > 255	451 > 282

0.005 mg/kg in egg

436 > 368



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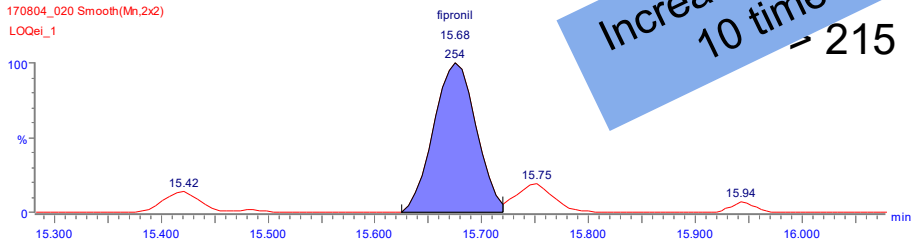
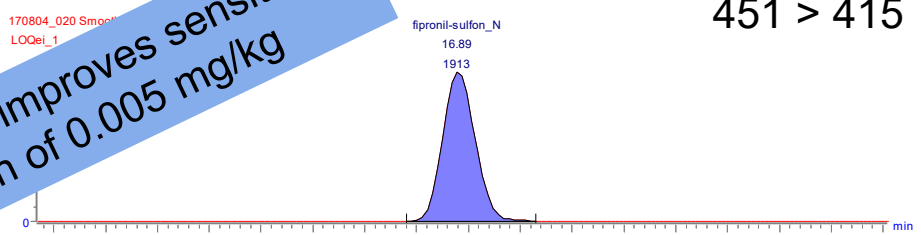


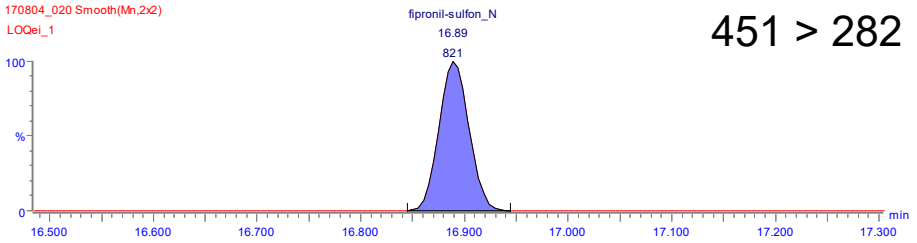
Increased selectivity improves sensitivity
10 times dilution of 0.005 mg/kg

.005 mg/kg in egg

451 > 415



451 > 282



APGC provides softer ionisation and thus more confidence with molecular ion $[M+H]^+$ information

Courtesy of

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Experimental:
Xevo G2 XS QToF Parameters

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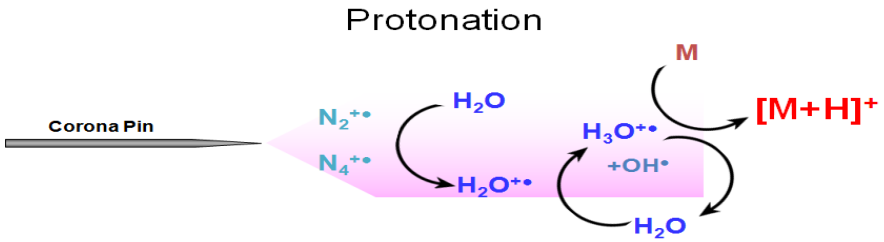
GC-MS Ionisation

Atmospheric pressure (API)

Corona current (µA)	3.0
Sampling Cone	20.0
Source Temperature	120° C
Source Offset	80
Cone Gas Flow	175 L/Hr
Auxiliary Gas Flow	50 L/Hr
Acquisition range	50-1200 m/z
Scan time	0.25 sec
Lockmass	Siloxane Bleed



LC-MS Ionisation

Electrospray (ESI)

Capillary (kV)	0.8
Sampling Cone	20.0
Source Temperature	120° C
Source Offset	80
Desolvation Temperature	550° C
Cone Gas Flow	50 L/Hr
Desolvation Gas Flow	1000 L/Hr
Acquisition range	50-1200 m/z
Scan time	0.25 sec
Lockmass	LeuEnk (556.2771m/z)

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Targeted Screening

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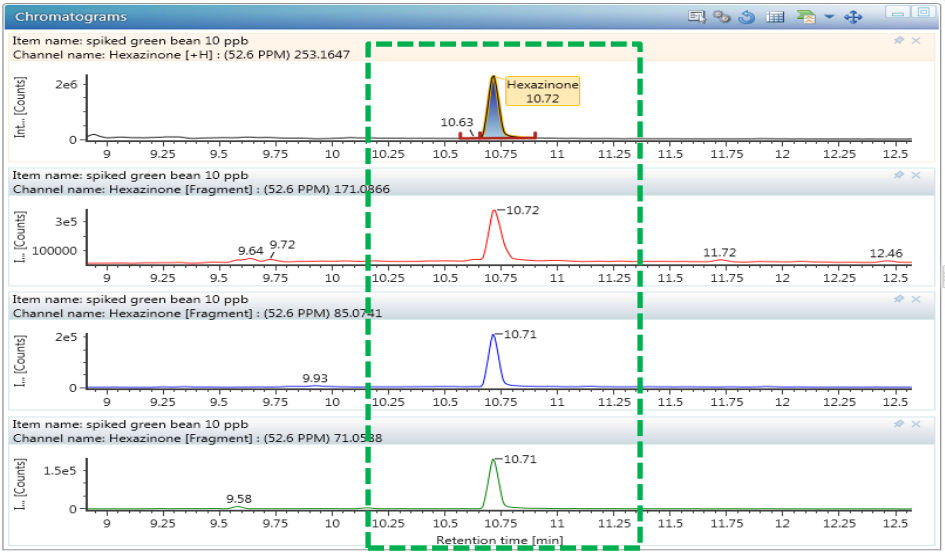
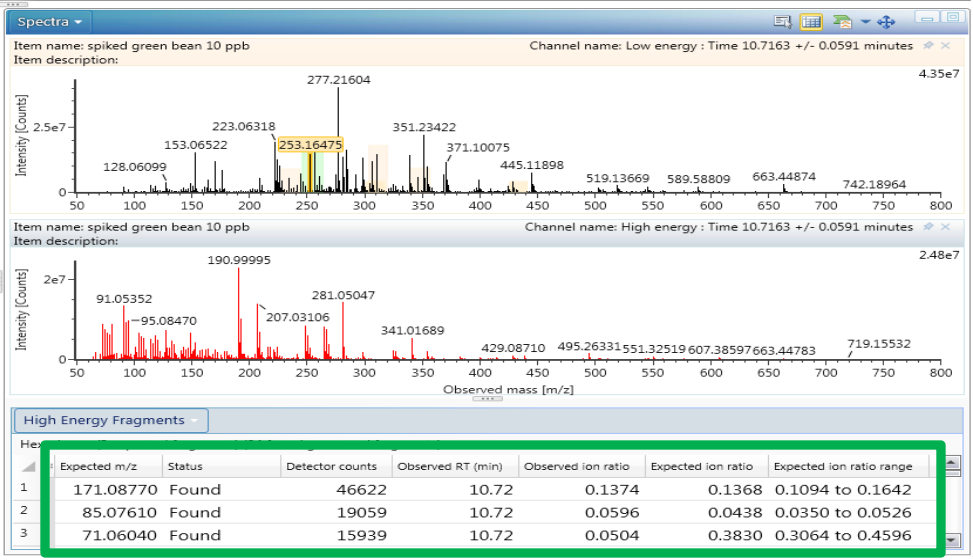
Component Summary

	Component name	Identification status	Formula	Neutral mass (Da)	Observed neutral mass (Da)	Observed m/z	Mass error (mDa)	Mass error (ppm)	Observed RT (min)	esponse	Adducts	Isotope Match Mz RMS PPM
148	Hexazinone	Identified	C12H20N4O2	252.15863	252.1575	253.1647	-1.2	-4.6	10.72	304933	+H	4.54
149	Iodofenfos	Identified	C8H8Cl2IO3PS	411.83535	411.8352	412.8424	-0.2	-0.5	10.12	352525	+H	3.93
150	Iprodione	Identified	C13H13Cl2N3O3	329.03340	329.0341	330.0413	0.7	2.0	10.10	2360	+H	5.93
151	Isazophos	Identified	C9H17ClN3O3PS	313.04168	313.0401	313.0396	-1.5	-4.9	10.91	9993	-e	





	Expected m/z	Status	Detector counts	Observed RT (min)	Observed ion ratio	Expected ion ratio	Expected ion ratio range
1	171.08770	Found	46622	10.72	0.1374	0.1368	0.1094 to 0.1642
2	85.07610	Found	19059	10.72	0.0596	0.0438	0.0350 to 0.0526
3	71.06040	Found	15939	10.72	0.0504	0.3830	0.3064 to 0.4596

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Accurate Mass HRMS:
Suspect to unknown screening

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Are these compounds in my sample?

Screening

How much is in my sample?

What else is in my sa

What is the difference between my sample and another one?

Comparison

Elucidation Toolset

Discovery

- Halogen Match
- mmon gment arch
- Neutral Loss
- Mass Defect Filter

Identification

- Library Search (UNIFI Scientific Library)
- Chemspider
- Isotope Match
- Isotope Model
- Elemental Composition

UNIFI™

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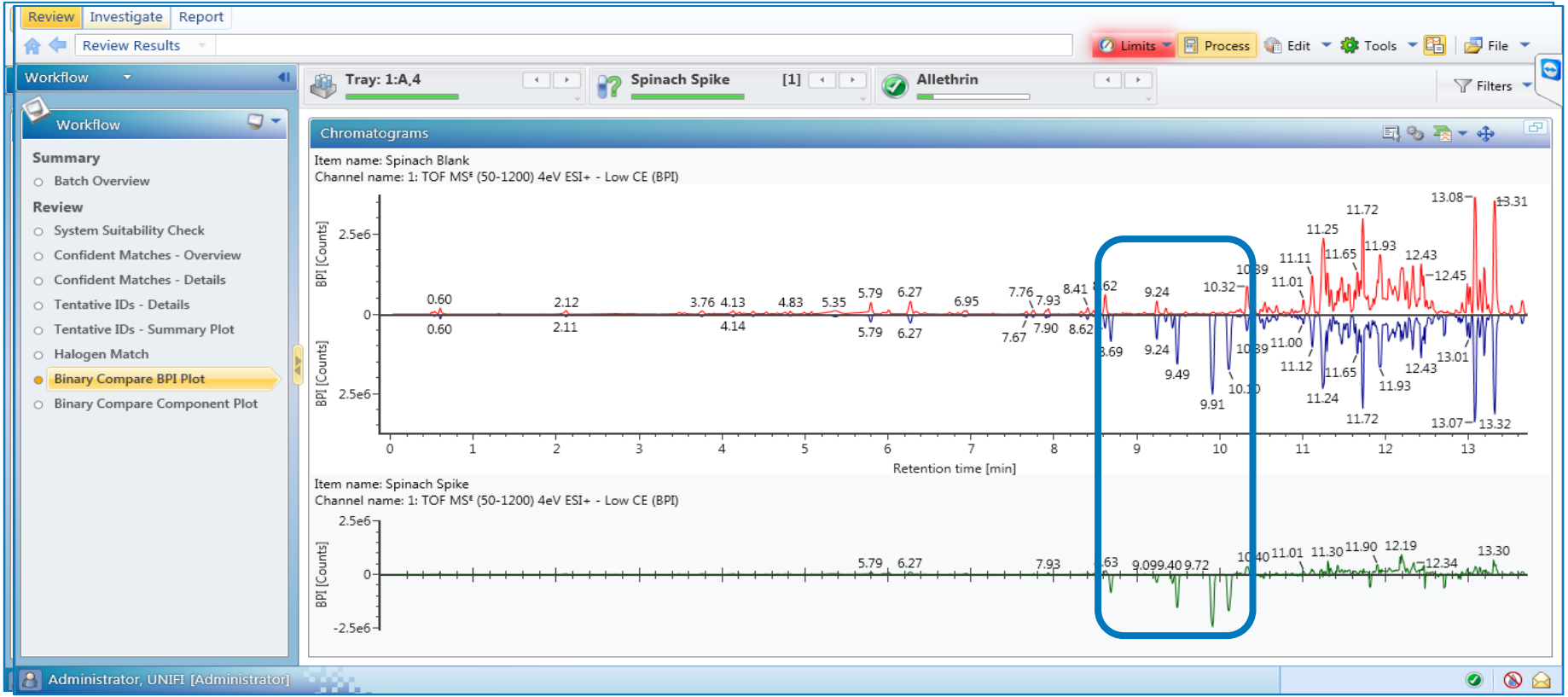
Qualitative Screen of Spinach:
Binary Compare



Unknown Screening: Halogen Match Spinach

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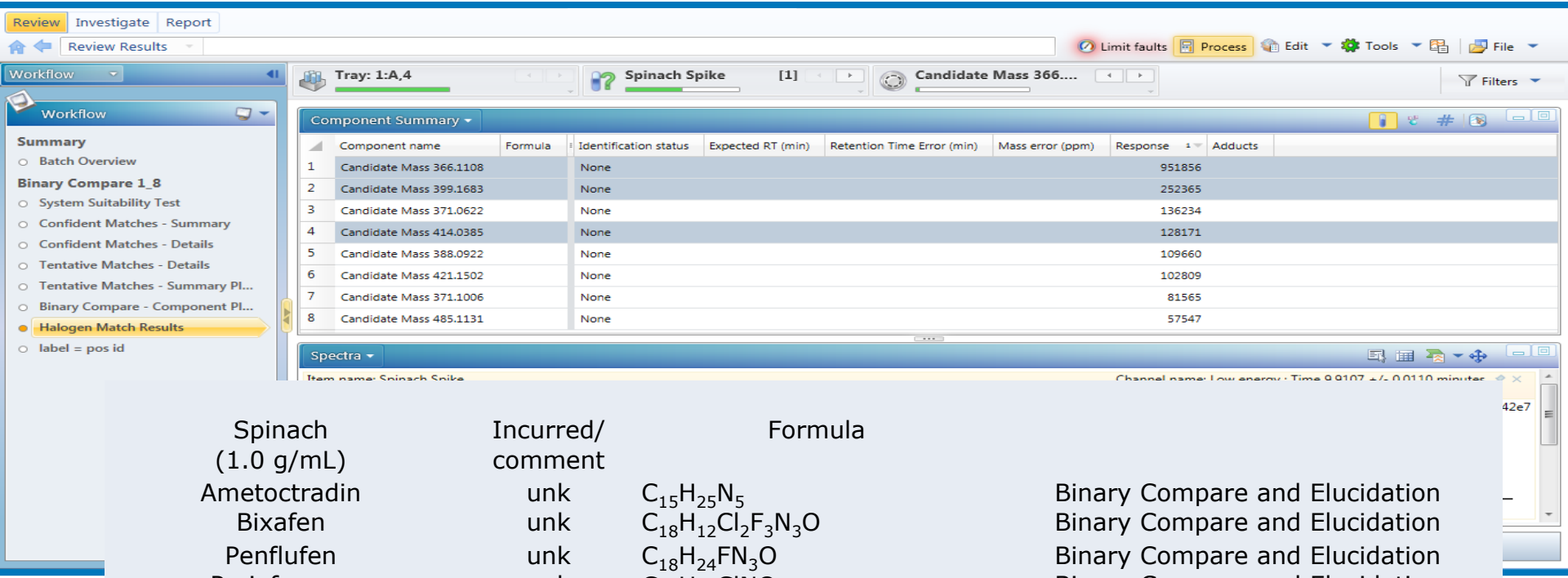

Spinach (1.0 g/mL)	Incurred/ comment	Formula	
Ametoctradin	unk	$C_{15}H_{25}N_5$	Binary Compare and Elucidation
Bixafen	unk	$C_{18}H_{12}Cl_2F_3N_3O$	Binary Compare and Elucidation
Penflufen	unk	$C_{18}H_{24}FN_3O$	Binary Compare and Elucidation
Pyriofenone	unk	$C_{18}H_{20}ClNO_5$	Binary Compare and Elucidation
Valifenalate	unk	$C_{19}H_{27}ClN_2O_5$	Binary Compare and Elucidation

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thank you!

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