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The use of GC-MS/MS technologies for the determination of pesticides and other contaminants in food

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Overview

- Introduction
- Tandem mass spectrometry using
  - Atmospheric Pressure GC (APGC)
  - Electron Ionisation (EI)
- Case studies
  - Dioxins analysis
  - Pesticides by GC- and LC-MS/MS on a single system
  - Fast GC-MS/MS
  - Pesticide residue analysis
- Summary

Introduction

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- There is a growing need for analytical testing associated with food and water
- In food national authorities control and enforce regulatory limits by testing samples for levels of residues and contaminants using analytical surveillance programs
- The food industry also carries out its own analyses:
  - Due diligence
  - Positive release
  - Brand protection
- Authorities and water companies have the obligation to ensure that regular monitoring of the quality of water is carried out in order to check that drinking water available to consumers meets regulatory requirements but also environmental quality is improved

Gas chromatography has always, and will continue to be, an essential tool for testing

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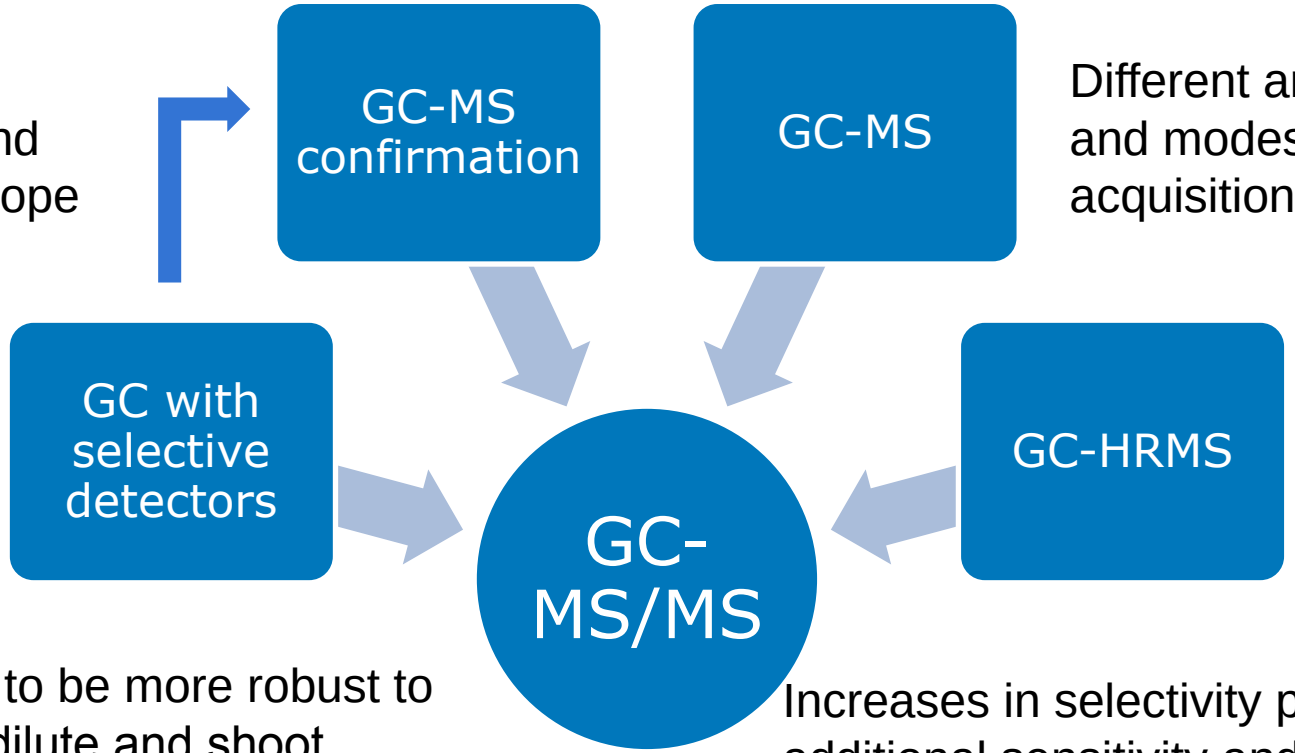
Insufficient specificity and analytical scope



Different analyzers and modes of acquisition

Also needs to be more robust to cope with “dilute and shoot approaches to sample prep…

Increases in selectivity provide additional sensitivity and improved scan speeds facilities more analytes

A reminder of the benefits of MS/MS using Multiple Reaction Monitoring (MRM) over SIM

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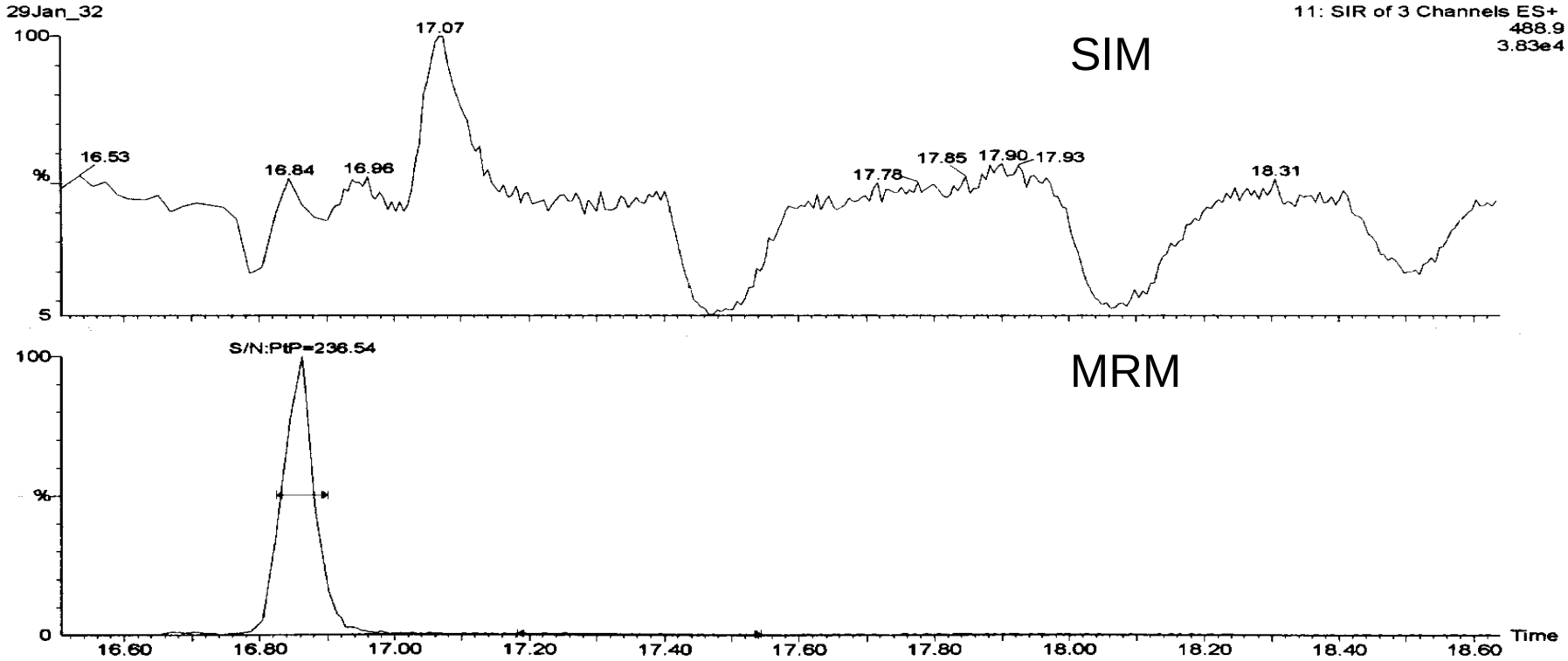

Different ionisation modes available for GC-MS/MS

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- Electron Ionization (EI) results in classical 70eV spectra
  - Mature and highly optimised technology
    - Robust and easy to use
  - The lack of $M^{+\cdot}$ in some EI spectra forces the user to take the risk of using fragment ions of unknown identity
    - Issue solved by using pre-optimised transitions and conditions supplied in a database
  - If ion current is distributed over many ions with poor intensity, overall sensitivity can be compromised

Different ionisation modes available for GC-MS/MS

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- Chemical Ionisation (CI) results in much less fragmentation so spectra usually show high abundance of charged pseudo-molecular ions
  - Positive and negative ions are formed so the MS can be set up in either polarity modes
  - Negative CI or electron capture ionisation gives better selectivity than EI for “electron-trapping” compounds
  - CI is not a universal technique
- Atmospheric Pressure GC (APGC)
  - Produces radical cations and/or protonated molecules using nitrogen and corona discharge
  - Can run under optimum conditions or compromise
  - Relatively high gas consumption compared with EI systems
  - Need for good control of moisture content in gases

Mass spectra of endosulfan

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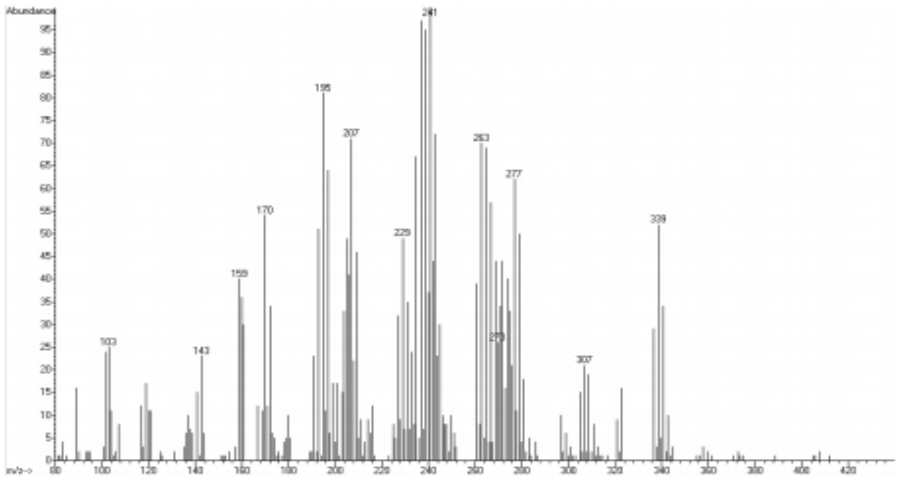
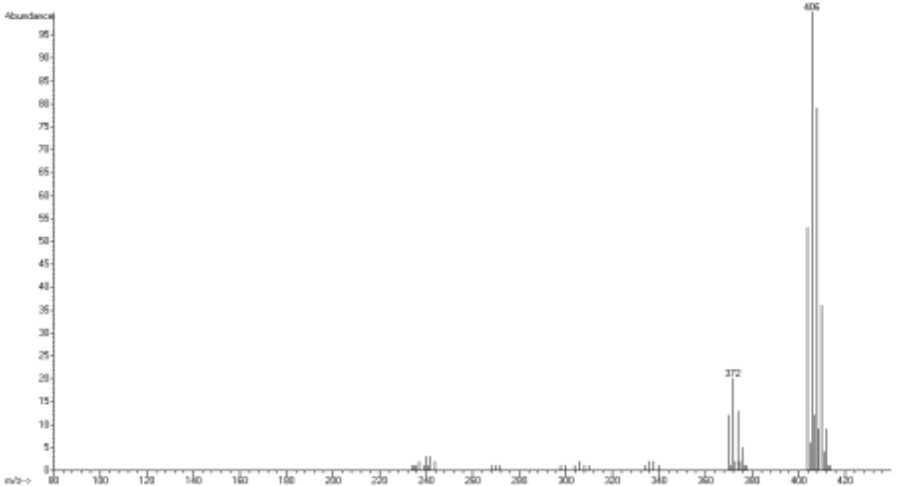
EI @ 70eV



CI with methane



Benefits of Atmospheric Pressure Gas Chromatography (APGC) for GC-MS/MS

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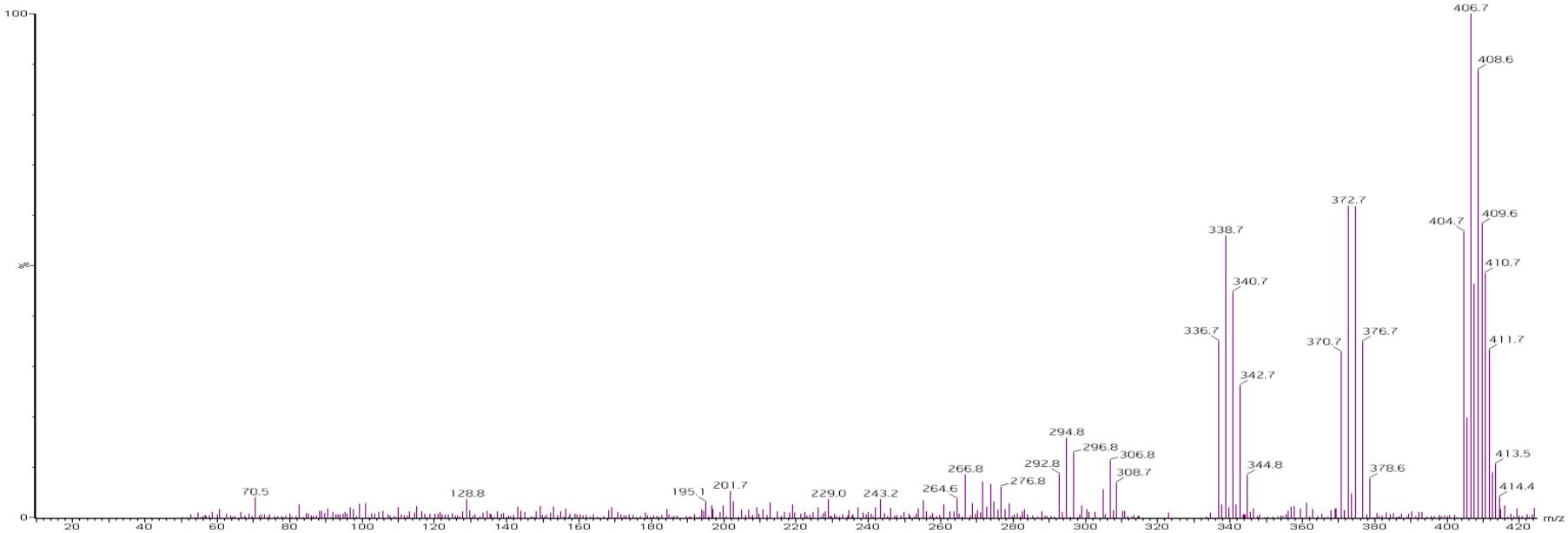
- Provides excellent sensitivity and selectivity
  - Less fragmentation observed in mass spectra
  - Spectra typically dominated by molecular or protonated molecular ions
  - Where fragmentation is observed the number of fragment ions is limited and they are of relatively high mass
- There is no need for extensive tuning or specific reagent gases
- Able to run fast GC as ion source not constrained by vacuum
- Easy maintenance
- Can switch between GC and LC inlets

Mass spectra of endosulfan using APGC



APGC on Xevo TQ-XS

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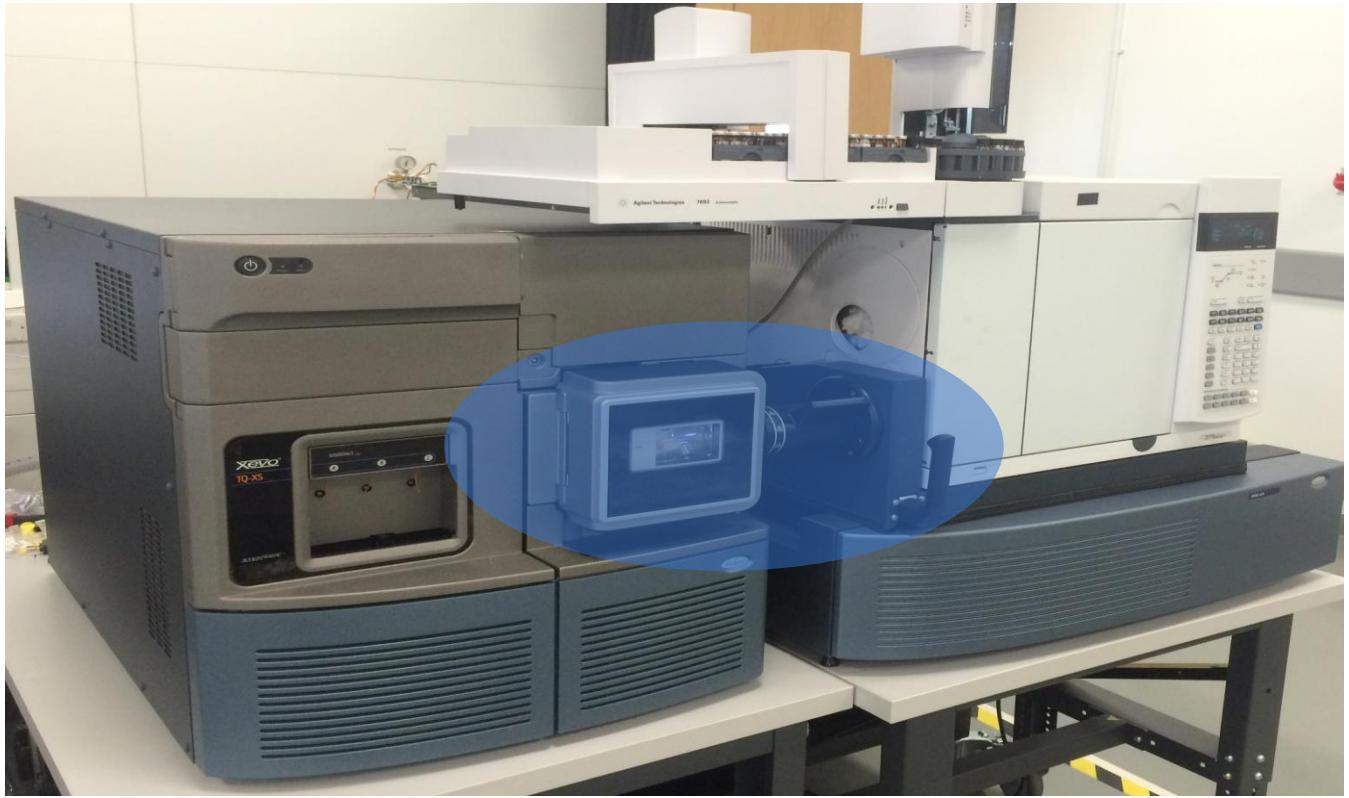

APGC on Xevo TQ-XS

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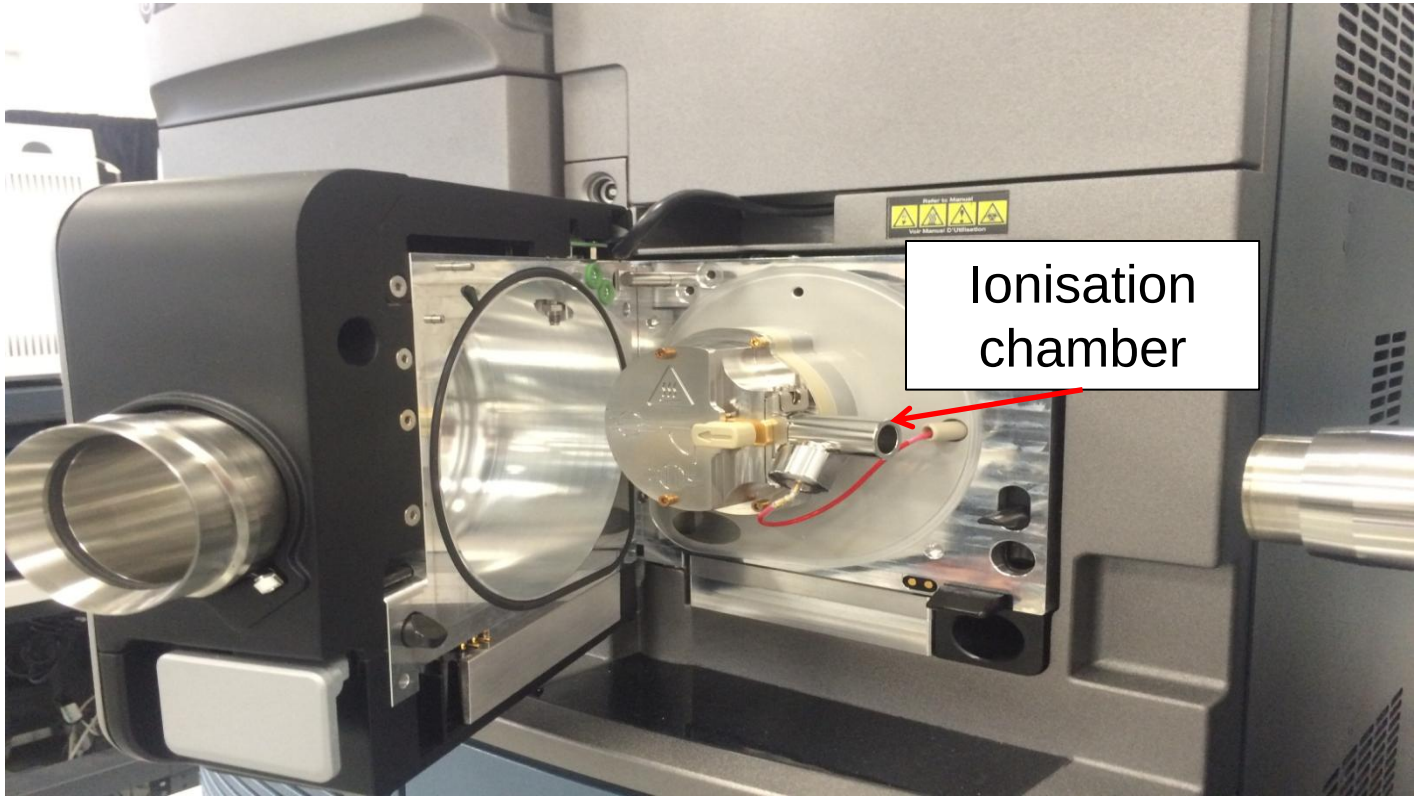

Atmospheric Pressure Gas Chromatography (APGC)

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- There are two mechanisms for generating ions by APGC
  - Charge transfer
  - Proton transfer
- Ionisation via the charge transfer route has been shown to be more effective for the determination of dioxins and furans and so source conditions are optimised to promote this mechanism
  - Cone gas, auxiliary gas, cone voltage and corona current
- As the presence of water vapour in the source can lead to ionisation from the competing proton transfer mechanism, reducing the intensity of the radical molecular ion, it is important to keep the source and the nitrogen gas dry (filter)
- Most pesticides ionise via proton transfer so the presence of some water is preferred (via a vial located in the source region)

Determination of dioxins and furans

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- APGC on a high performance tandem quadrupole (Xevo TQ-XS) can achieve extremely low limits of detection compared with EI-based GC-MS/MS or GC-HRMS systems
- Benefits:
  - Able to use less reference standard by diluting CSL/CS solutions
  - No need for large volume injection via PTV
  - There is potential for reducing the complexity of the sample preparation steps
  - Technique has been shown to be robust (ten Dam *et al.* (2016). *J Chromatogr. A* **1477:**76–90)
- Implementation
  - Excellent tool for research proposes including investigation into mixed halogened dioxins/furans
  - APGC-MS/MS is compliant with the EU requirements for analysis of food and feed
  - US EPA and European methods provide for the analysis and reporting of specific dioxin and furan isomers in environmental samples but specifies the use of GC-HRMS for confirmation
  - EPA Method 1613B in use for analysis of food and feed is still restricted to GC-HRMS

Performance for dioxins/furans using APGC on Xevo TQ-XS

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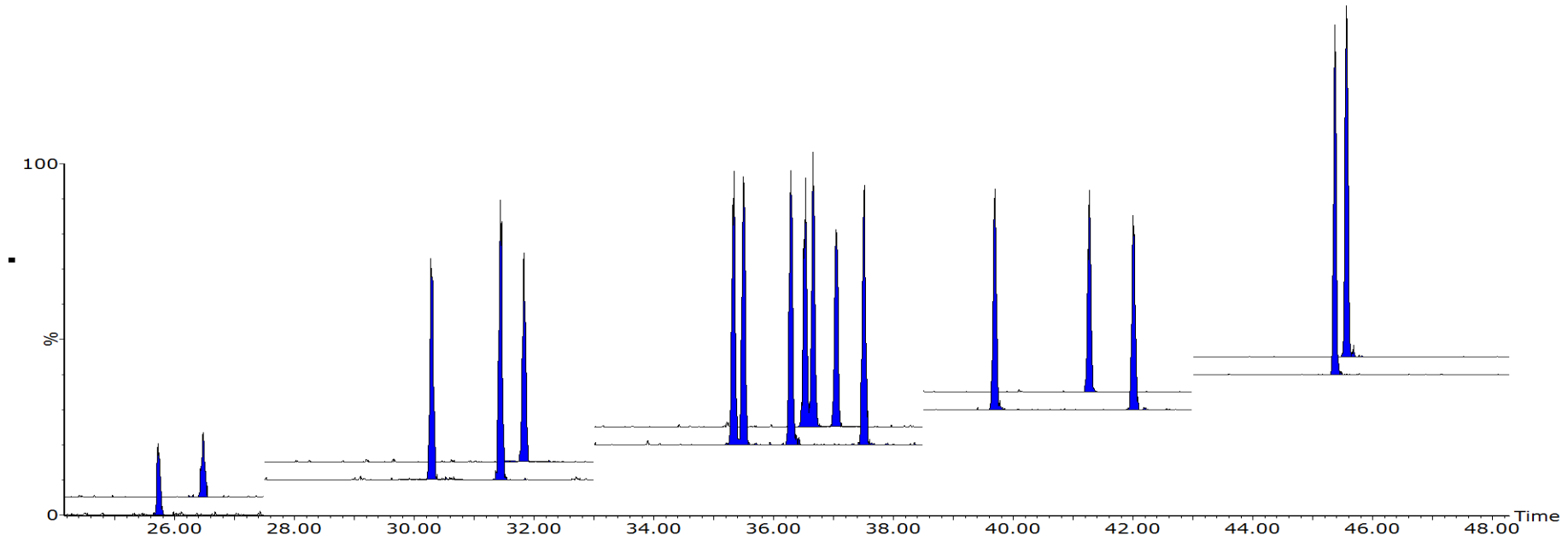
1/10 dilution of CSL standard - 10 fg on-column



Sensitivity

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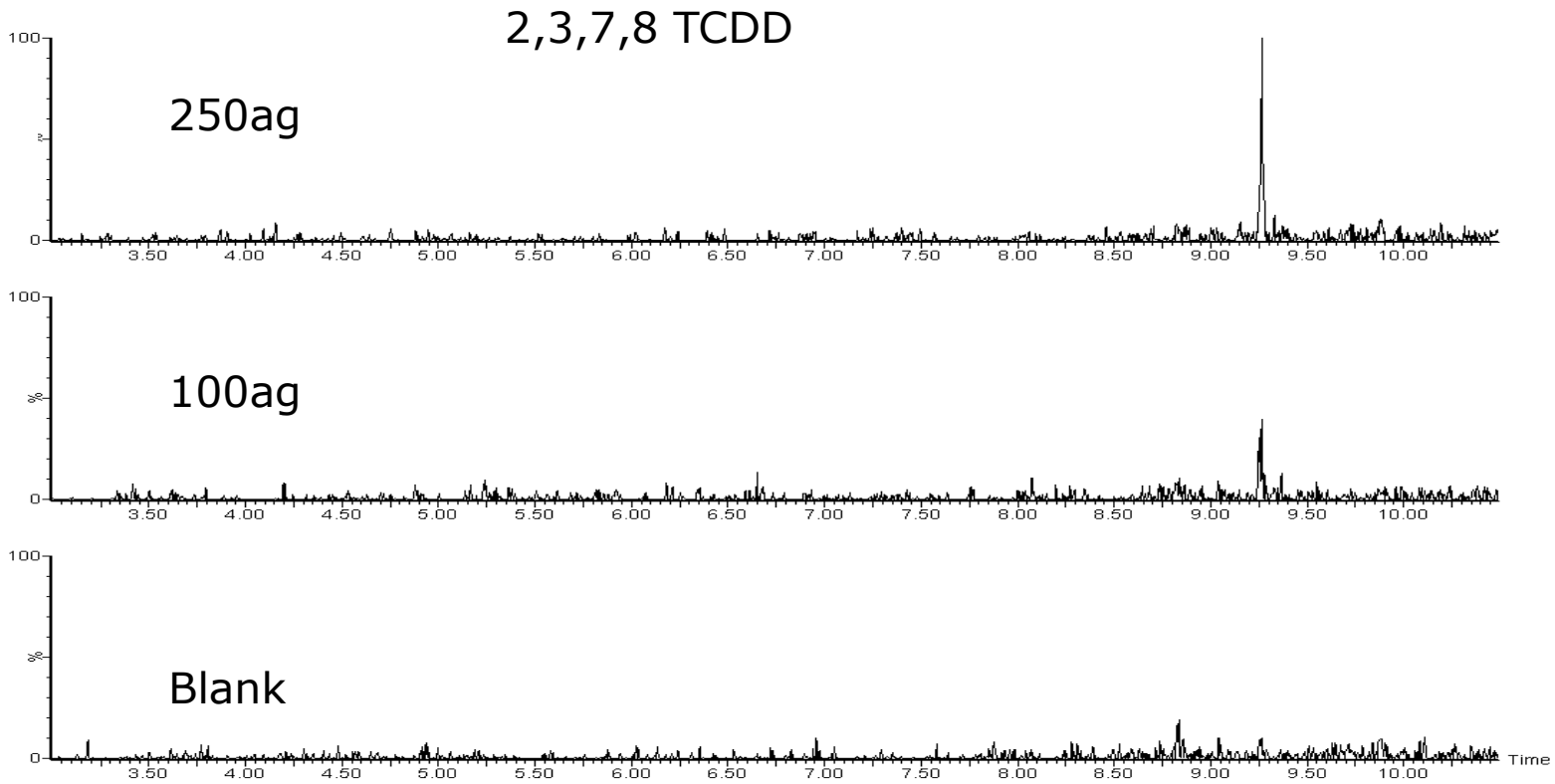

Comparison of data from Autospec and Xevo TQ-XS using APGC

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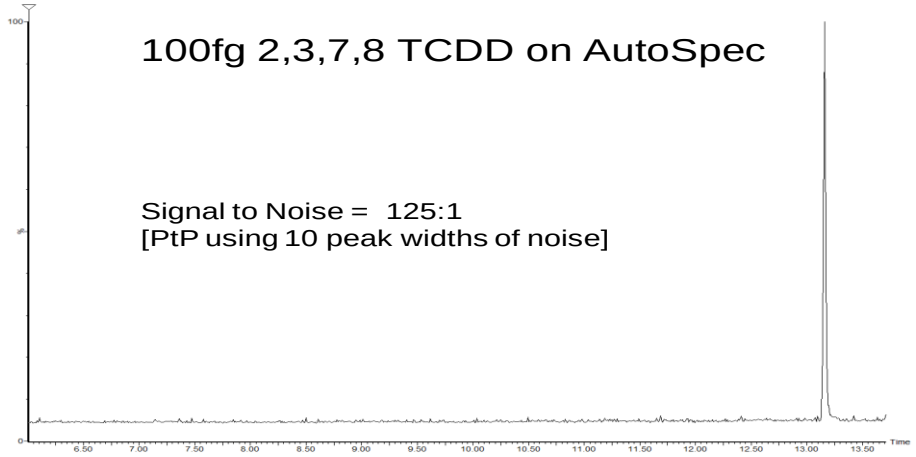
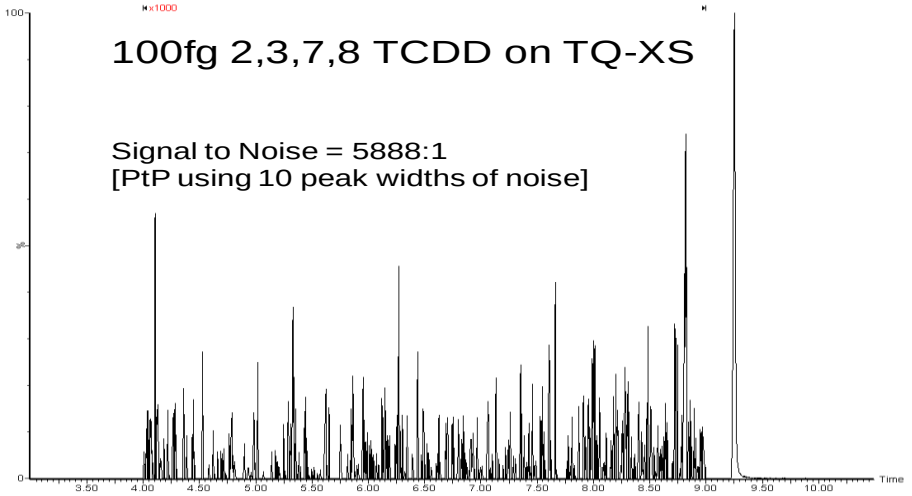
S/N 125:1



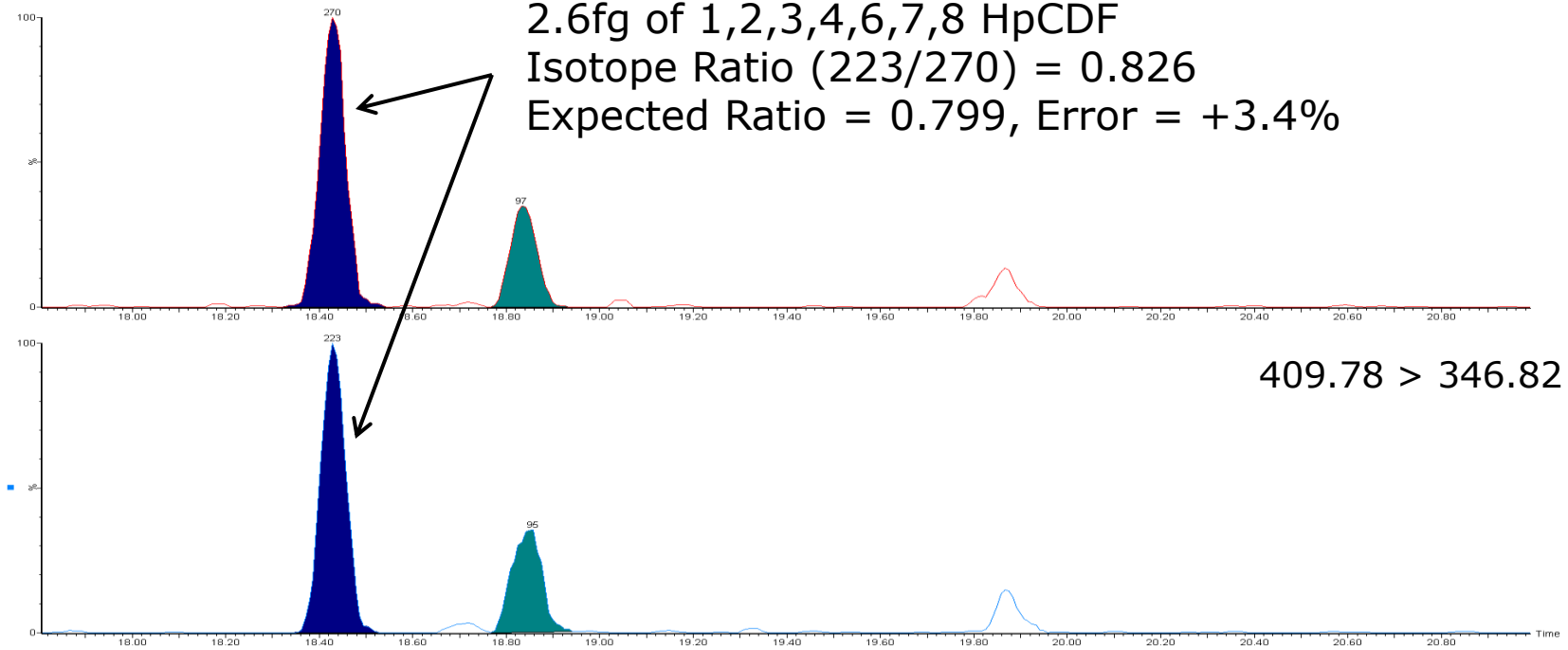
S/N almost 6000:1



Pork fat extract

- HpCDF trace of pork fat extract

407.78 > 344.82



Sample from LABERCA, France

Determination of pesticides in foodstuffs and the environment

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- APGC remains a popular option for pesticide residue analysis
  - Adds flexibility to the laboratory as can operate in combination with UPLC on a single platform; Xevo TQ micro or Xevo TQ-XS
  - Can generate extremely high sensitivity when employed with Xevo TQ-XS:
    - To meet regulatory requirements
    - To improve methodology such as reducing cleanup and/or moving to split injection
  - Can be used to verify results from EI-based GC-MS/MS systems
  - Can operate with short columns at high GC carrier gas flow rates
    - Decrease run time
    - Improve transmission of late elutors by reducing residence time
    - Increase S/N

APGC adds flexibility to existing LC-MS/MS system

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- Universal Source architecture provides access to both UPLC-MS/MS and GC-MS/MS on the same instrument, allows for an increase of laboratory efficiency, while maintaining required sensitivity and accuracy
  - <30 minutes needed to switch between chromatographic inlets
  - Analysis of same extracts
  - Easy generation of both methods using QuanPedia

Image courtesy of Nofalab, The Netherlands

Chromatograms for ~400 pesticides at 0.01 mg/kg in celery on Xevo TQ-S micro using APGC and UPLC

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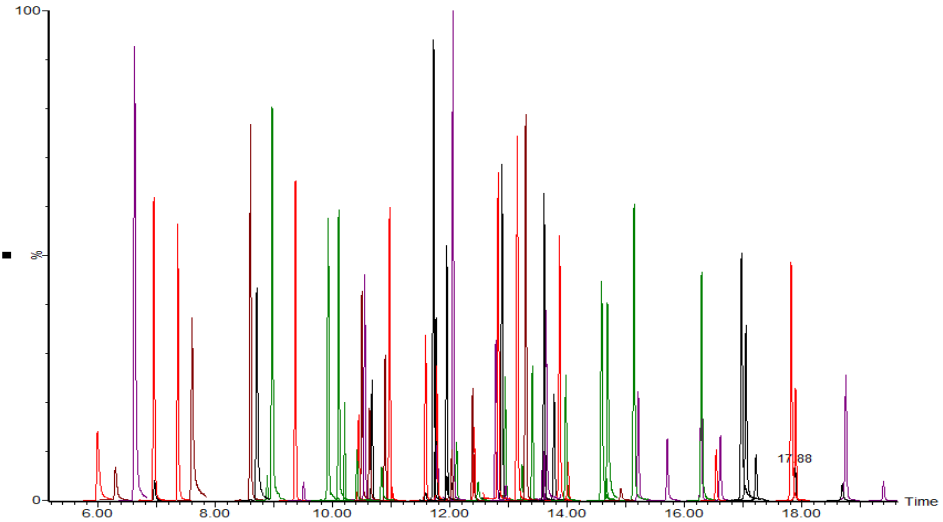
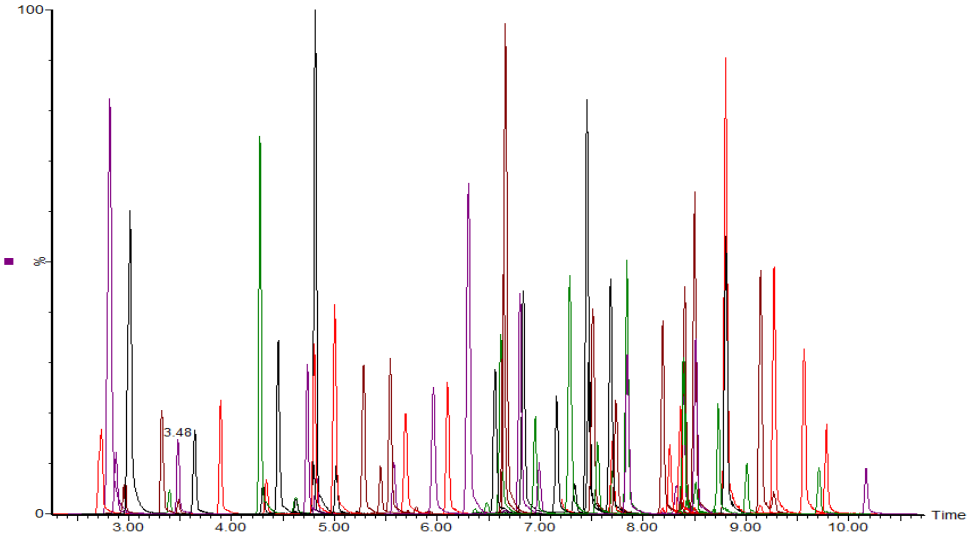
Sensitive with good precision (see Application Note 720006013 for more details and results)



APGC



UPLC

Typical calibration graphs and chromatograms for matrix-matched standards at 0.001 mg/kg

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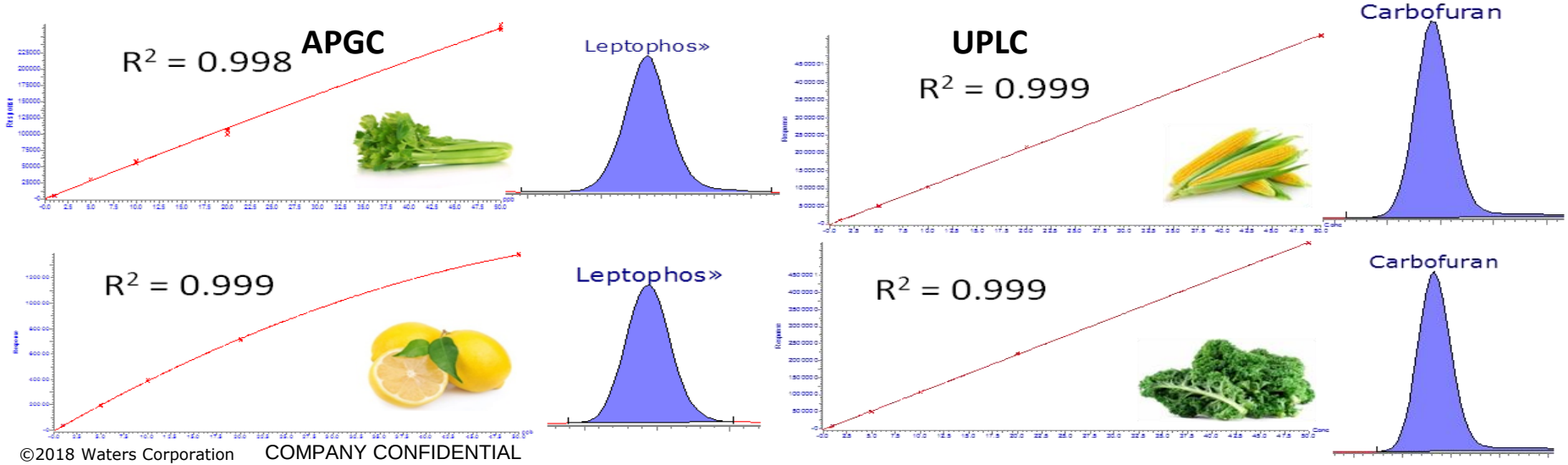
- Matrix matched standards were prepared from QuEChERS extracts of celery, lemon, corn and kale (0.001 to 0.050 mg/kg) and replicate injections made using the UPLC and APGC methods
- Detection at the EU default MRL of 0.01 mg/kg was easily achieved for >99% of pesticides with good precision (RSDs <10%) and RT and ion ratios within SANTE tolerance for most analytes
- A majority of the compounds in both analysis methods had values for R^2 ≥0.995 and residuals from triplicate injections at each calibration point were within ±20%



The sensitivity and repeatability from the APGC-MS/MS analysis of matrix-matched standards at 0.01 mg/kg

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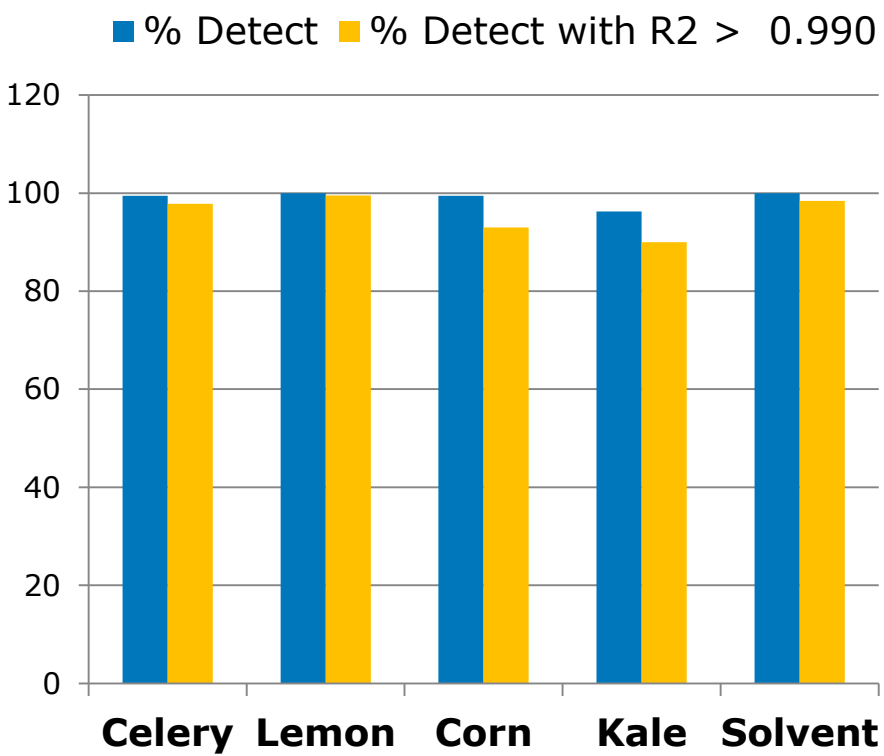
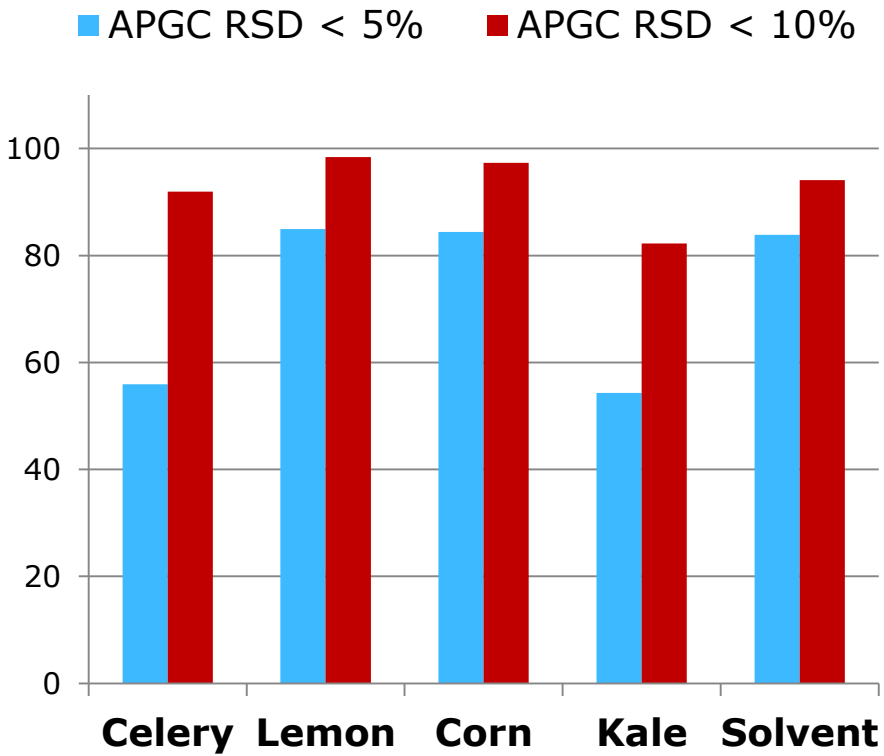
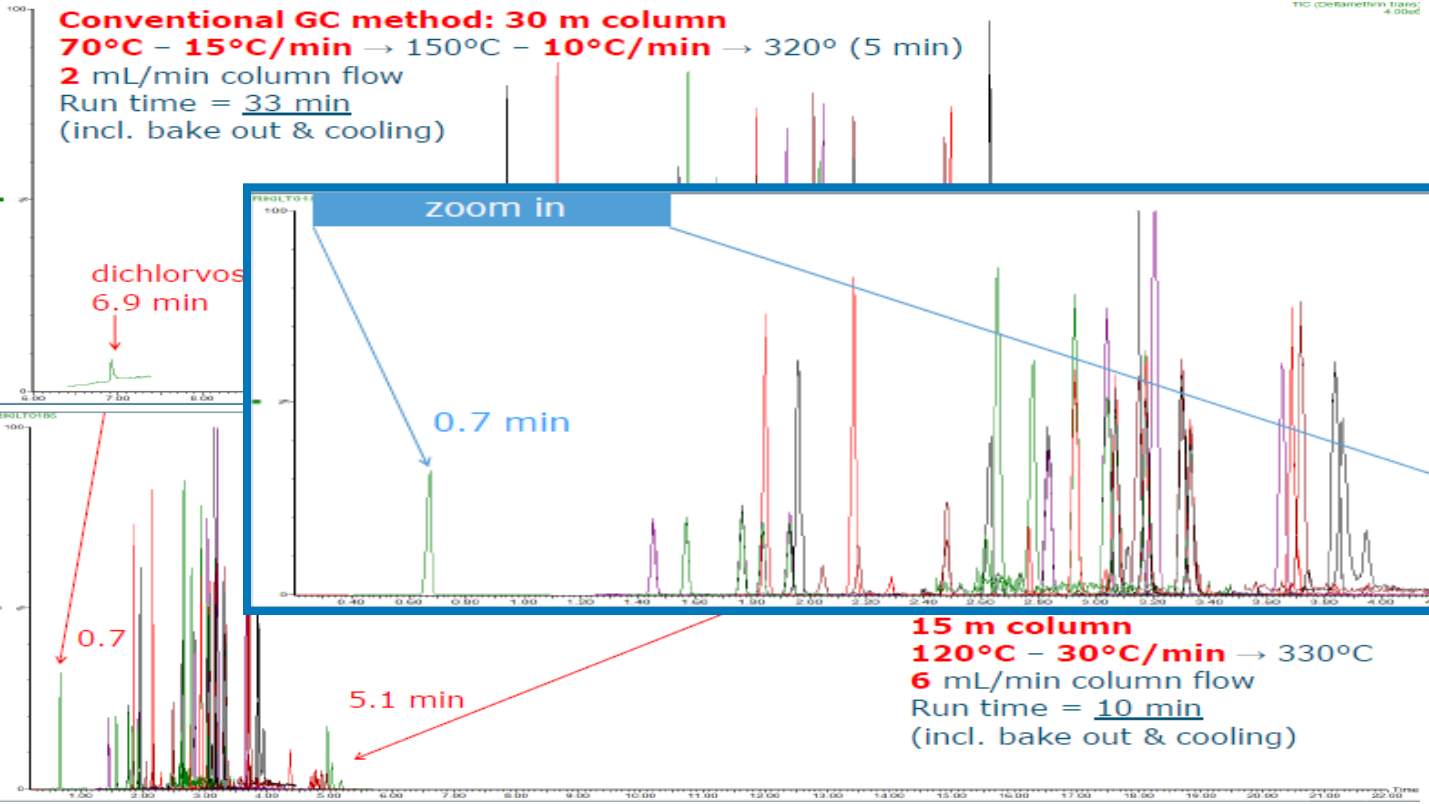




Fast GC using APGC



Tienstra, *et al.*, Fast GC method for pesticides and PCBS in feed matrices, EPRW 2014

APGC can generate extremely high sensitivity when employed with Xevo TQ-XS

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- Specific MRLs were set in Europe for food intended for infants and young children
  - A default MRL of 0.01 mg/kg is applicable for such food unless lower legal limits for the residue levels are defined in the Directives
  - Specific MRLs were set for selected substances (based upon toxicity)
    - *e.g.* Cadusafos @ 0.006 mg/kg
  - A banned list @ 0.003 mg/kg (based upon LOQ)
    - e.g. Endrin
- The food industry expect to work at even lower limits for such products
- The expected LOQ to comply with the WFD EQS for cypermethrin in surface waters is 0.024 ng/L!!!

Chromatograms from analysis of baby food extract spiked at 0.001 mg/kg for a selection of pesticides

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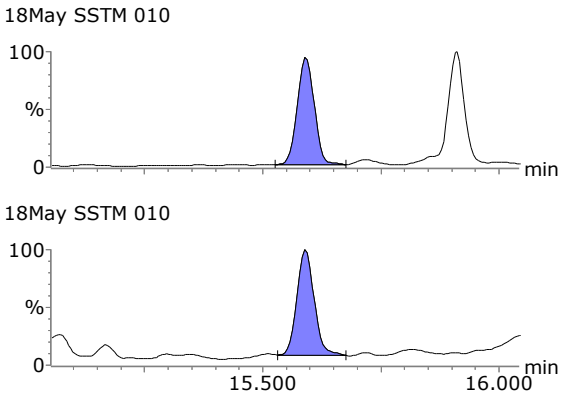
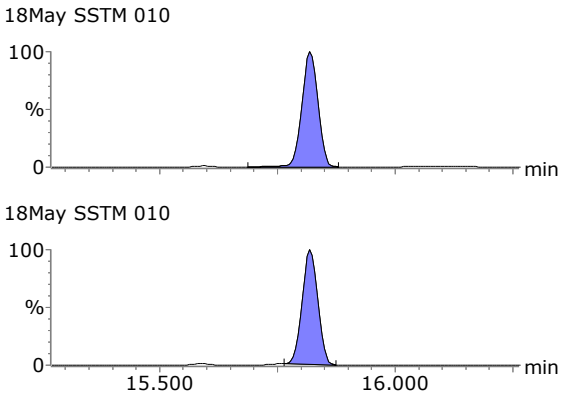
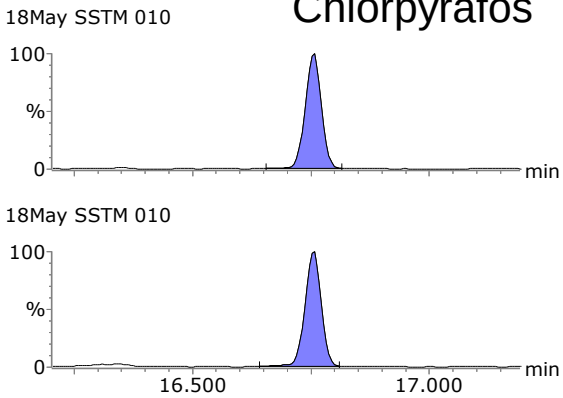
Acetochlor



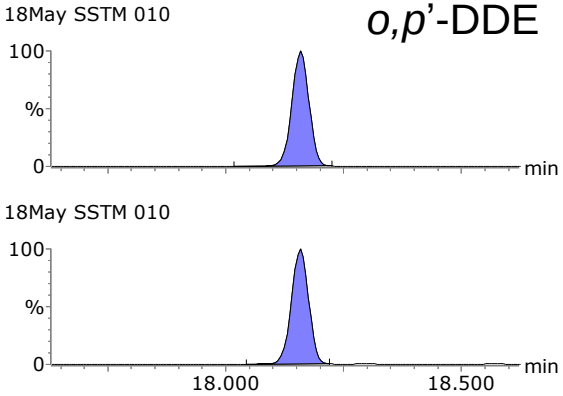
Alachlor



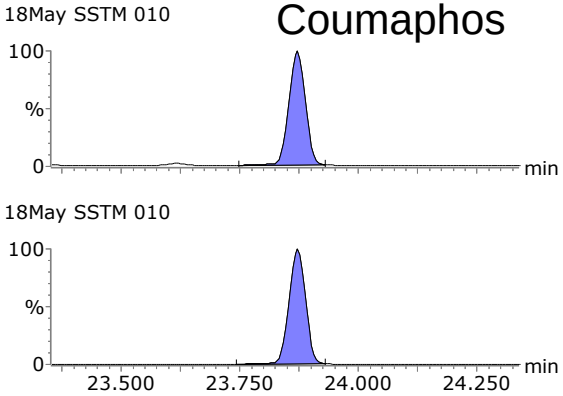
Chlorpyrafos



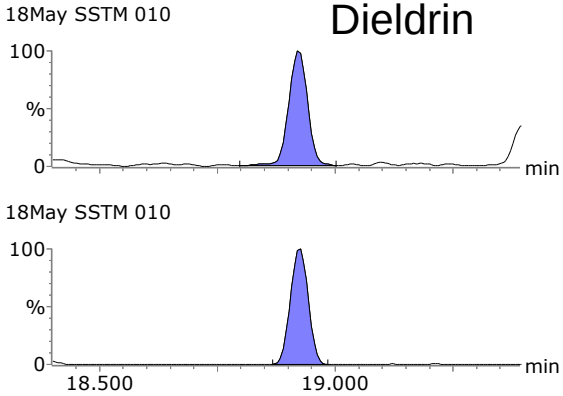
o,p'-DDE



Coumaphos



Dieldrin



Calibration graphs for a selection of pesticides from analysis of matrix matched standards (ppb) in baby food extract

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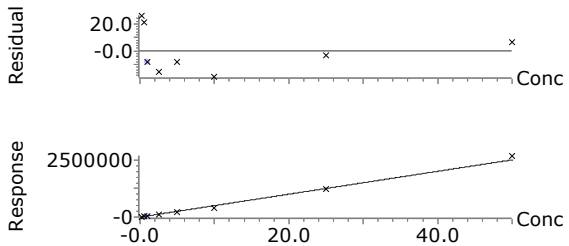
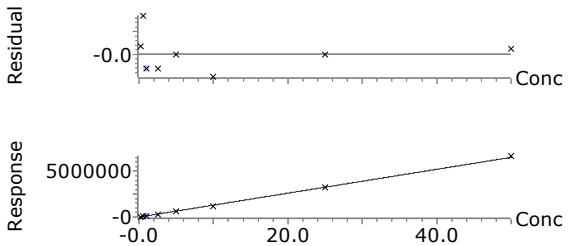
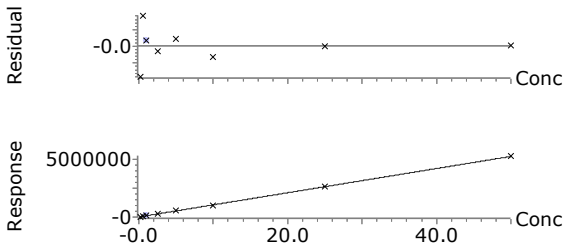
Compound name: Acetochlor
Correlation coefficient: r = 0.995637, r^2 = 0.991294
Calibration curve: 50421.7 * x + -7192.41
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis tra



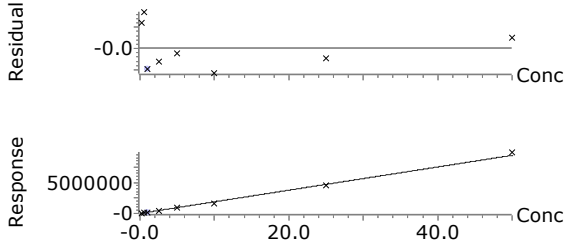
Compound name: Alachlor
Correlation coefficient: r = 0.999118, r^2 = 0.998236
Calibration curve: 130629 * x + -13166.1
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis tra



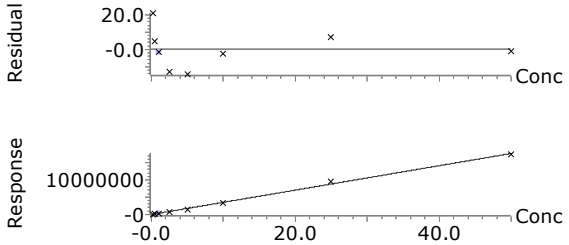
Compound name: Chlorpyrifos
Correlation coefficient: r = 0.999526, r^2 = 0.999052
Calibration curve: 104896 * x + 7651.52
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis tra



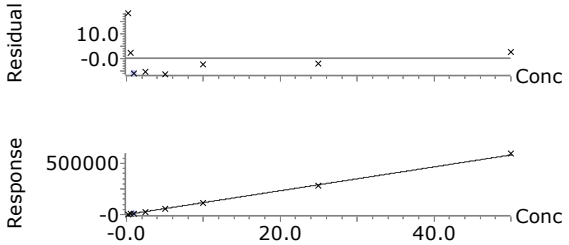
Compound name: DDE op
Correlation coefficient: r = 0.997919, r^2 = 0.995843
Calibration curve: 188822 * x + -21435.4
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis tra



Compound name: Coumaphos
Correlation coefficient: r = 0.998217, r^2 = 0.996438
Calibration curve: 353760 * x + -52005.4
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis tra



Compound name: Dieldrin
Correlation coefficient: r = 0.999476, r^2 = 0.998953
Calibration curve: 11674.5 * x + -587.961
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis tra



Preview of Waters' new EI-based GC-MS/MS system

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- Waters is introducing the Xevo TQ-GC GC-MS/MS system later this year
- Sensitive, robust and easy to use
  - Innovative user-centred hardware design
- Acquisition and data processing/review using software shared with LC-MS/MS systems
  - Minimal training for existing MassLynx users
  - IntelliStart for tuning and system readiness
  - Quanpedia includes GC method information
  - Pre-optimised methods can be simply loaded by the user

Software tools

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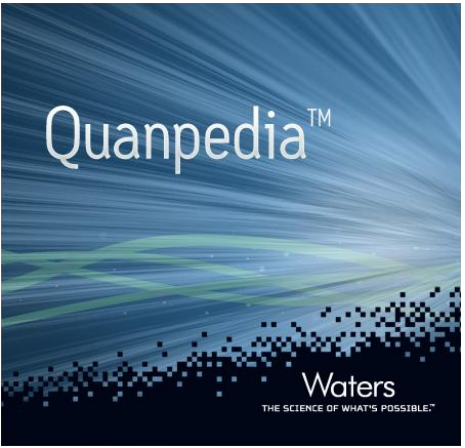
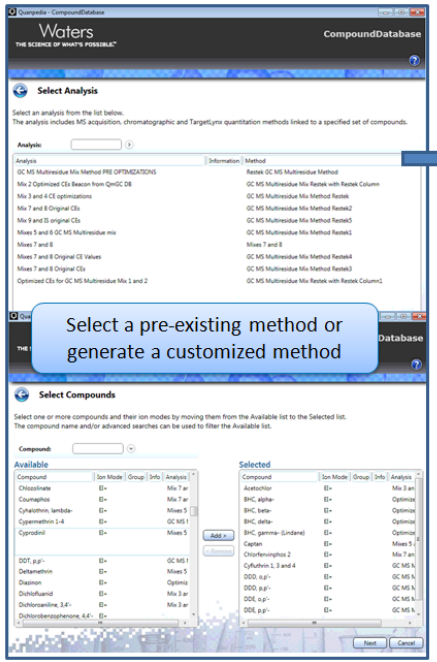
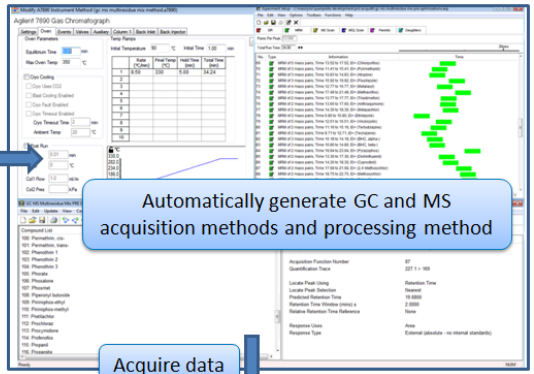


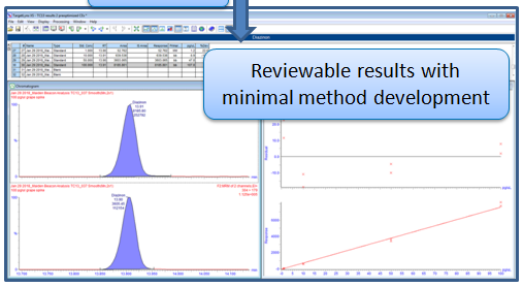


Select a pre-existing method or generate a customized method

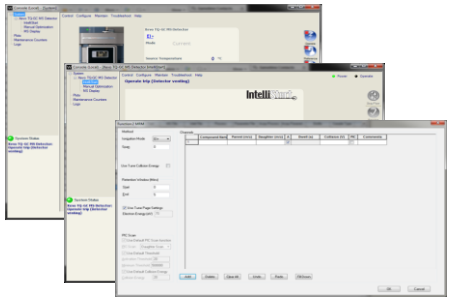


Automatically generate GC and MS acquisition methods and processing method

Acquire data

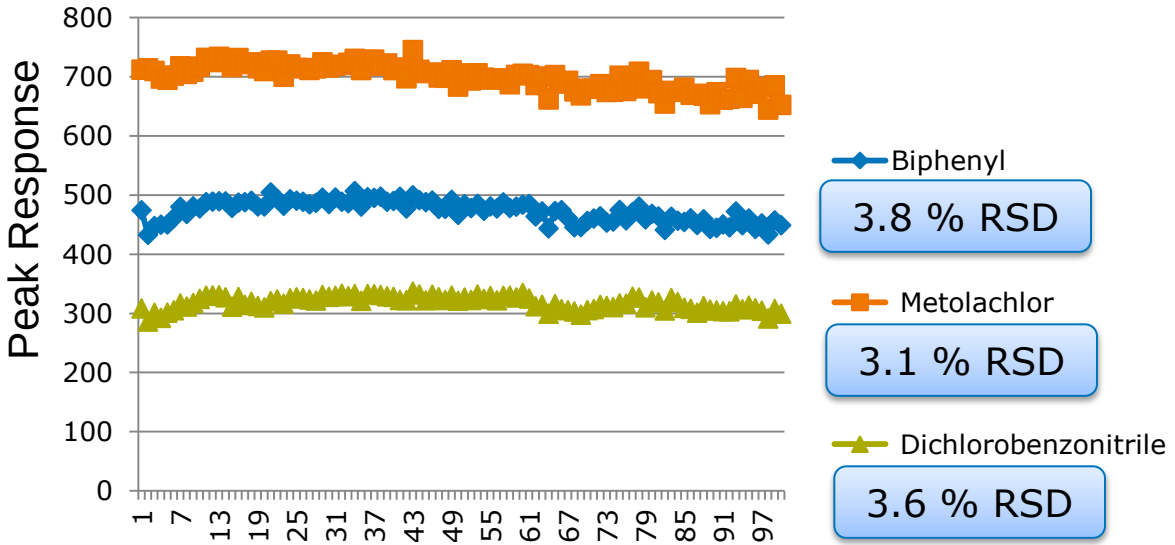


Reviewable results with minimal method development



Initial repeatability data

- Designed to minimized the need for user intervention
- Cleaning source is quick (under an hour), simple and intuitive



Repeatability over 100 injection of leek extract spiked at 50 ppb with liner change after 50 injections

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Initial results using Restek 203 pesticide mixture in QuEChERS extracts of various fruit and vegetables

Chromatograms for a selection of pesticides from analysis of carrot extract spiked at 0.005 mg/kg

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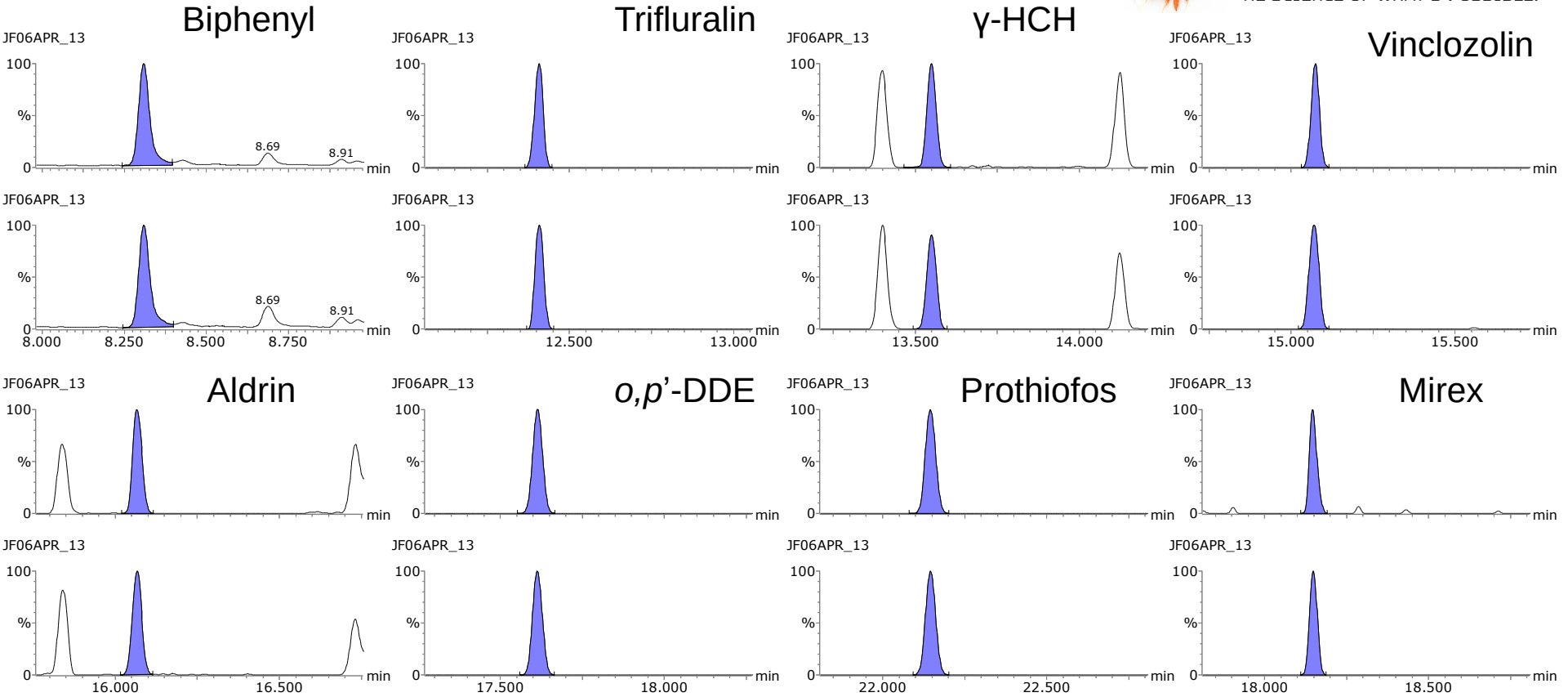

Calibration graphs for a selection of pesticides from analysis of matrix matched standards (ppb) in carrot extract

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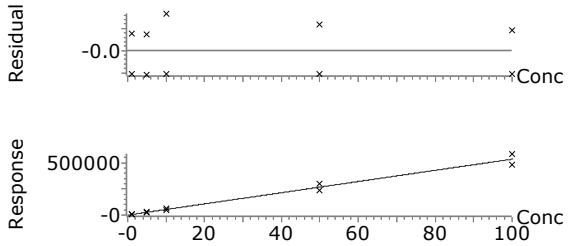
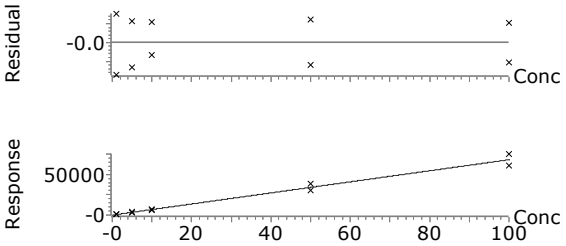
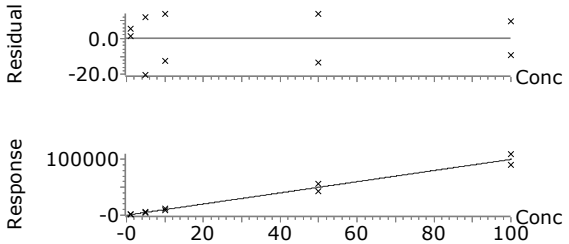
Compound name: DDE, o,p'-_
Correlation coefficient: r = 0.993807, r^2 = 0.987653
Calibration curve: 5359.83 * x + -36.4556
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis tra



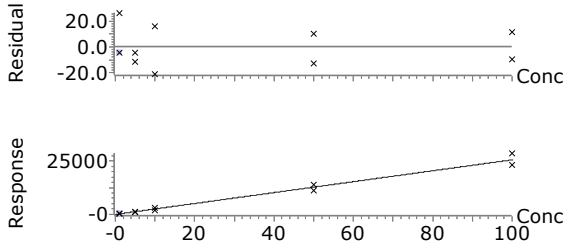
Compound name: Aldrin
Correlation coefficient: r = 0.993227, r^2 = 0.986500
Calibration curve: 682.033 * x + -103.083
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis tra



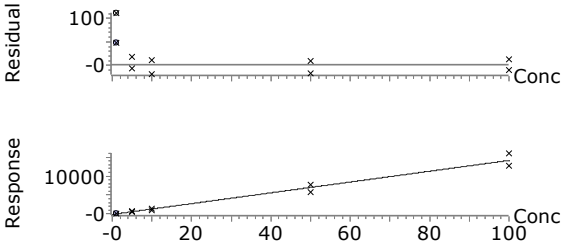
Compound name: BHC, gamma- (Lindane)
Correlation coefficient: r = 0.992908, r^2 = 0.985866
Calibration curve: 1001.97 * x + -219.57
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis tra



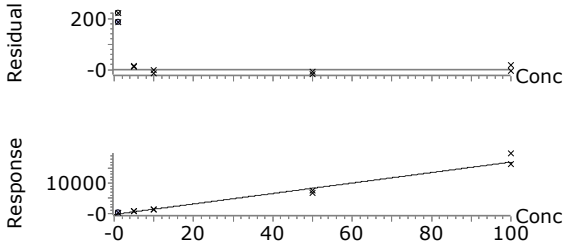
Compound name: Vinclozolin
Correlation coefficient: r = 0.992527, r^2 = 0.985109
Calibration curve: 257.416 * x + -115.819
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis tra



Compound name: Trifluralin
Correlation coefficient: r = 0.989352, r^2 = 0.978818
Calibration curve: 143.086 * x + -159.077
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis tra



Compound name: Prothiofos
Correlation coefficient: r = 0.989220, r^2 = 0.978556
Calibration curve: 173.995 * x + -397.355
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis tra



Repeatability for a selection of pesticides from replicate measurements (*n*=200) of matrix matched standards

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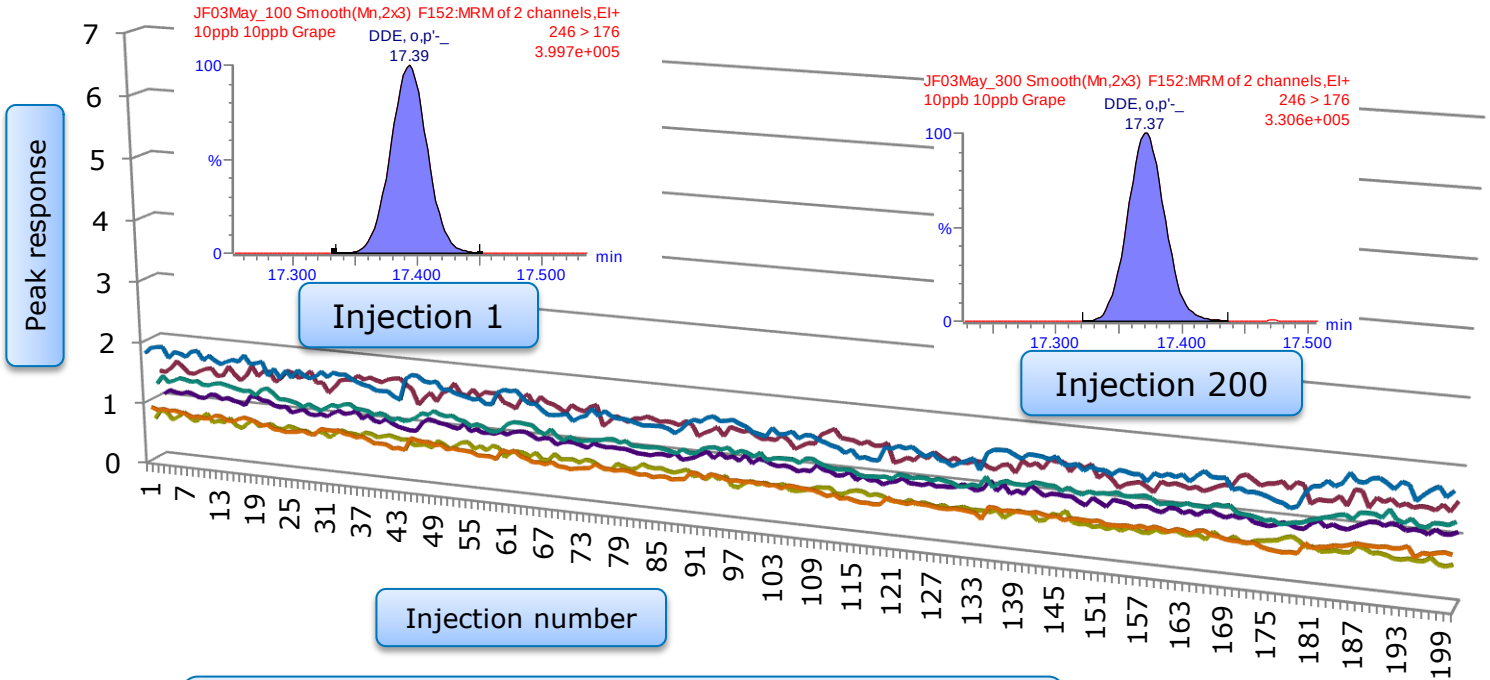

Repeatability from 200 injections of grape extract spiked at 0.01 mg/kg without any intervention

Accuracy for a selection of pesticides from replicate measurements (*n*=6) of matrix matched standards

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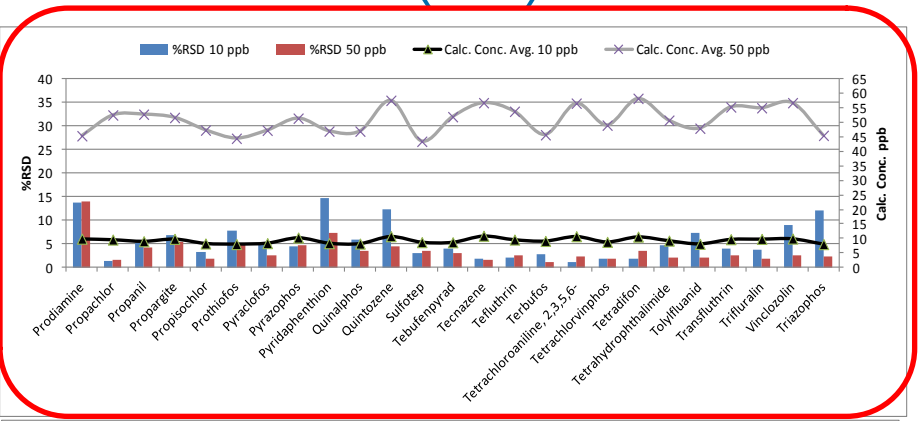

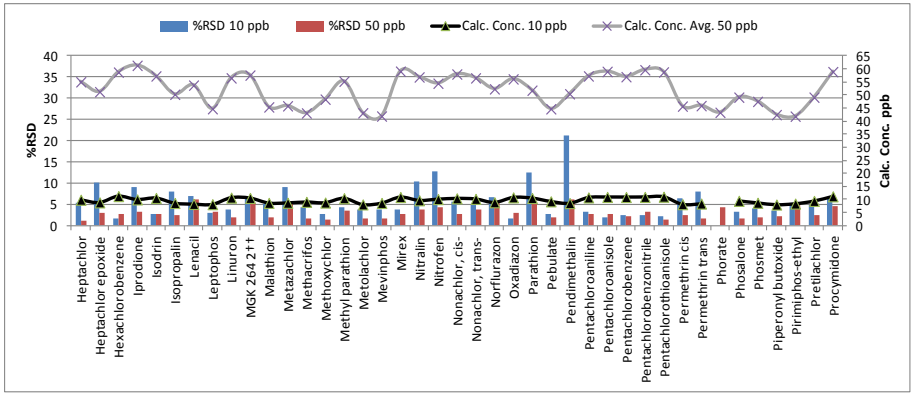

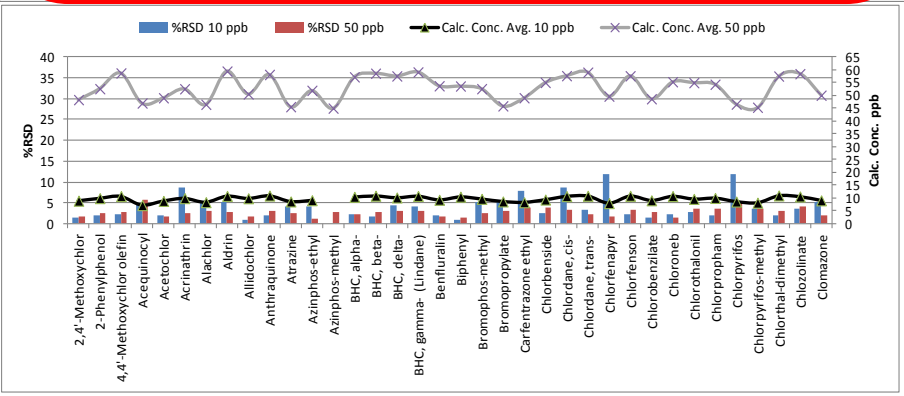

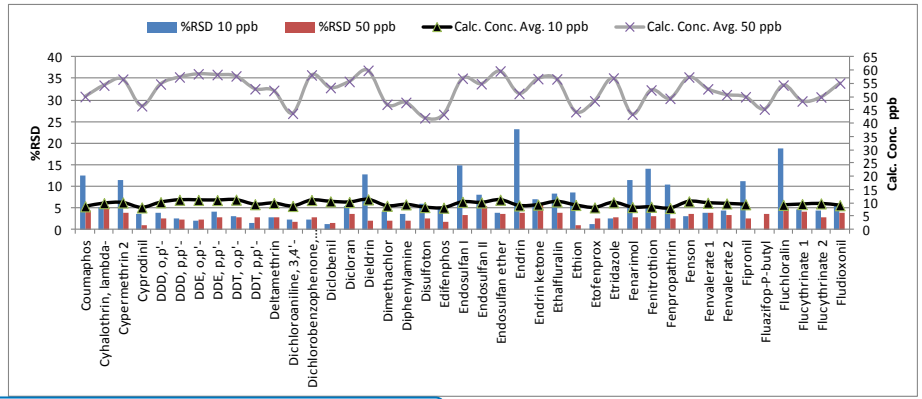

Carrot matrix samples at 50 and 10 ppb over 6 injections for 100 pesticides

Accuracy for a selection of pesticides from replicate measurements (*n*=6) of matrix matched standards

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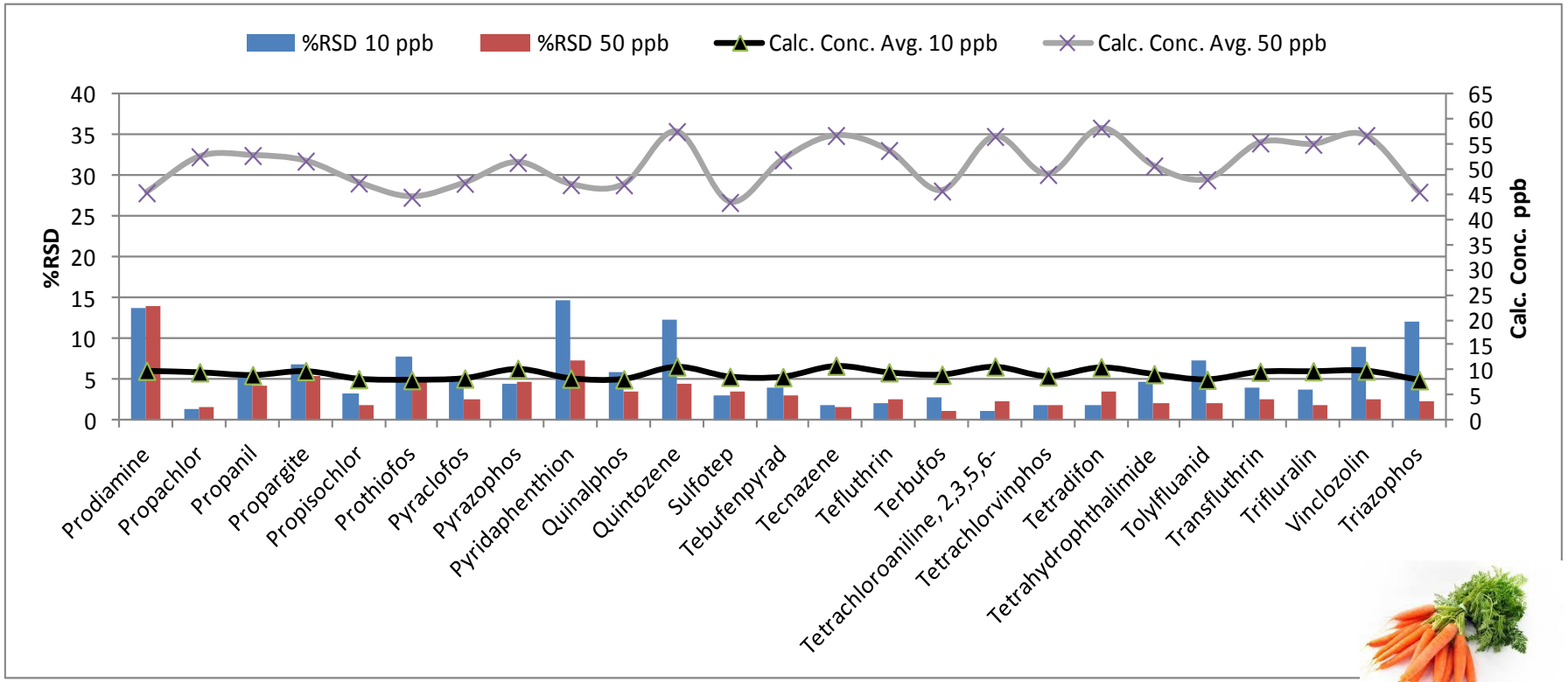

Xevo TQ-GC

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- Please come and have a chat and see the instrument shell in the hospitality suite
- Poster (288) on the determination of pesticides in green tea will be presented on Thursday in the GC-MS instrument applications session 2

Summary

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- GC-MS/MS is essential adding to LC-MS/MS to cover the required scope of analysis for food and environmental applications
- GC conditions for the determination of contaminants, such as pesticide residues in food, are well established
  - Care needs to be given to choice of liner to avoid breakdown in injector and routine preventative maintenance
  - Matrix effects are common and impact on quantification must be considered
- Optimising GC-MS/MS conditions can be tedious and lengthy so supply of pre-optimised methods is essential

Summary

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- The new Xevo TQ-GC is an EI-based GC-MS/MS system
  - Robust and easy to use with enough sensitivity to be successfully applied to most food and environmental contaminant applications
  - Sharing software with existing LC-MS/MS systems reduces the training burden for laboratories familar with Waters instruments
- Xevo TQ-S micro with APGC (and ACQUITY UPLC) offers to opportunity to run GC-MS/MS and LC-MS/MS on the same tandem mass spectrometer
  - Excellent performance with added operational benefits
- Xevo TQ-XS with APGC is an attractive solution for those challenging applications that require extremely high sensitivity (*e.g.* dioxins)
  - Also provides the opportunity to simplify cleanup and to switch to split injection technique

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Typical workflow for pesticide residue analysis using GC-MS/MS: QuEChERS CEN 15662

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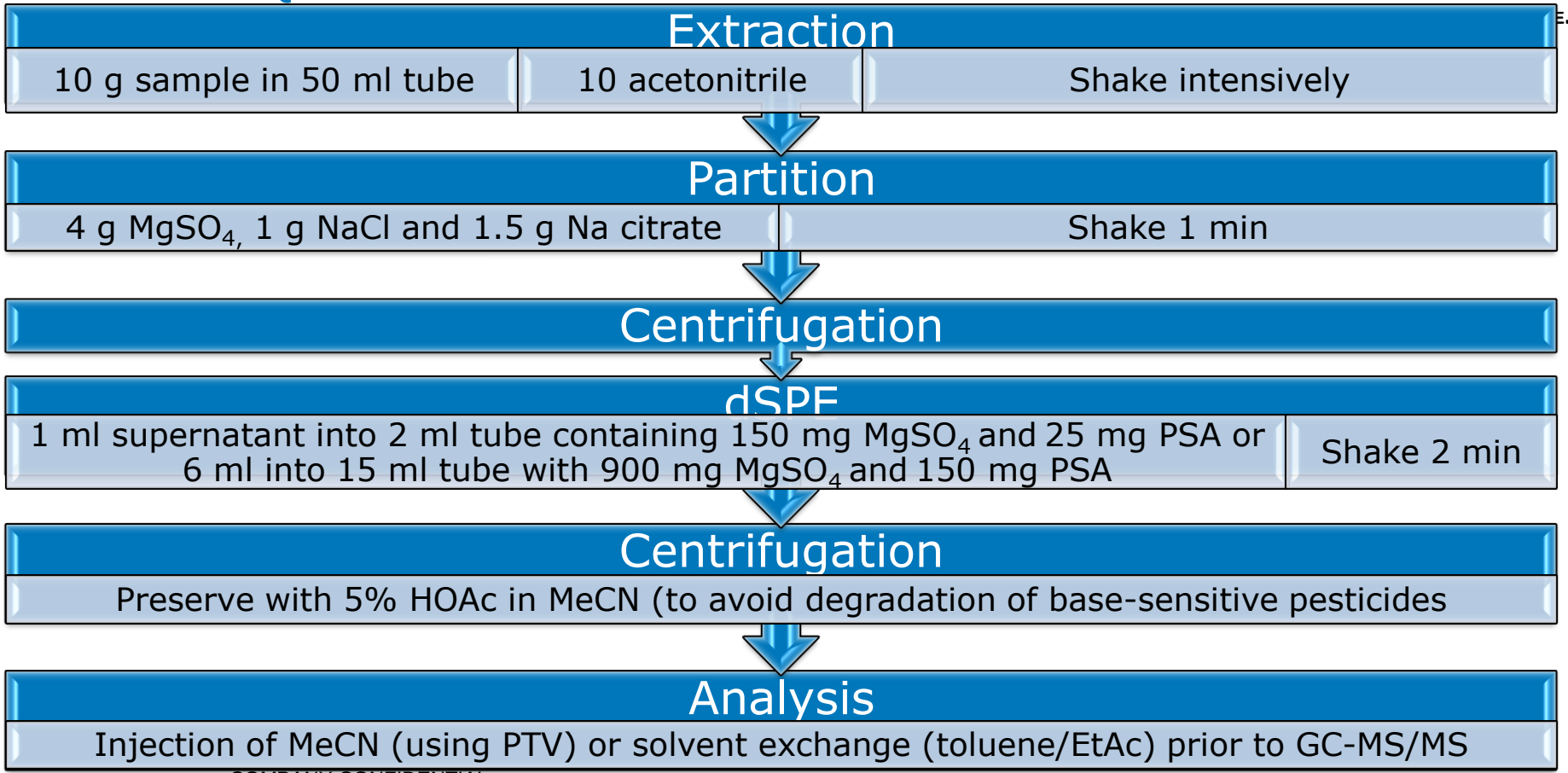
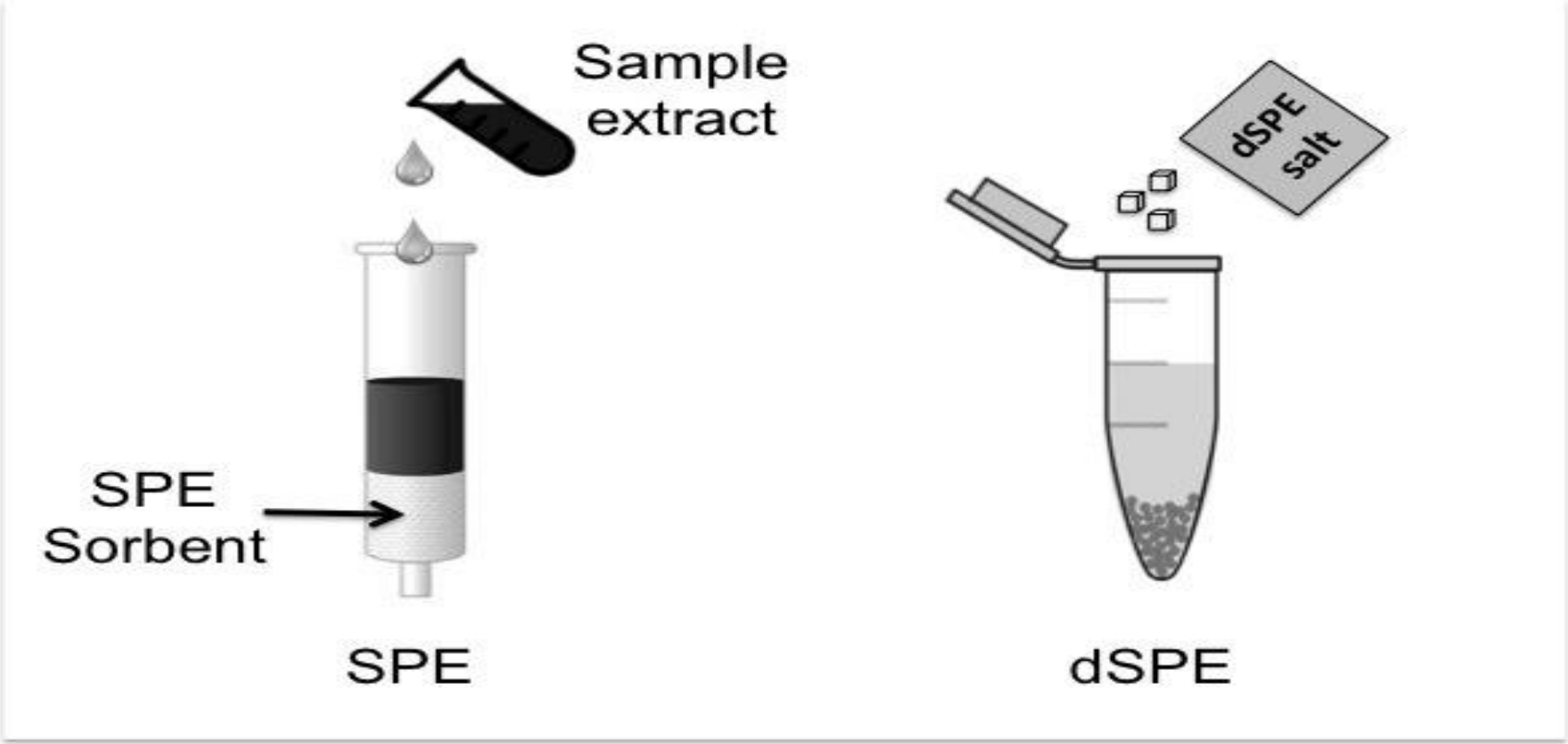


Different types of SPE



Selectivity of dSPE cleanup

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Amino-Sorbents, Alumina:

- Acids (including fatty acids)
- Sugars
- Pigments (Anthocyanes, some Chlorophyll)

Risk: Losses of acidic pesticides

Carbon-based Sorbents:

- Carotinoids, Chlorophyll, Sterols

Risk: Losses of planar pesticides

Reversed-Phase Sorbents:

- Lipids and Waxes

Risk: Losses of lipophilic pesticides

Alternative to dSPE: pass-through SPE

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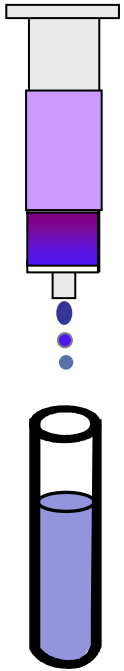
- Ideal for high organic (ACN) samples, like QuEChERS extracts
- Analytes not retained by the sorbent
- Retention of matrix components such as:
  - Fats
  - Phospholipids
  - Pigmentation
- Cartridge or syringe (Plus) formats

Pass-Thru Protocol

Load – Collect
Interferences Retained
Analytes Pass Through

Rinse – Collect
Analytes Pass Through



Effective removal of chlorophyll instead of using d-SPE with GCB

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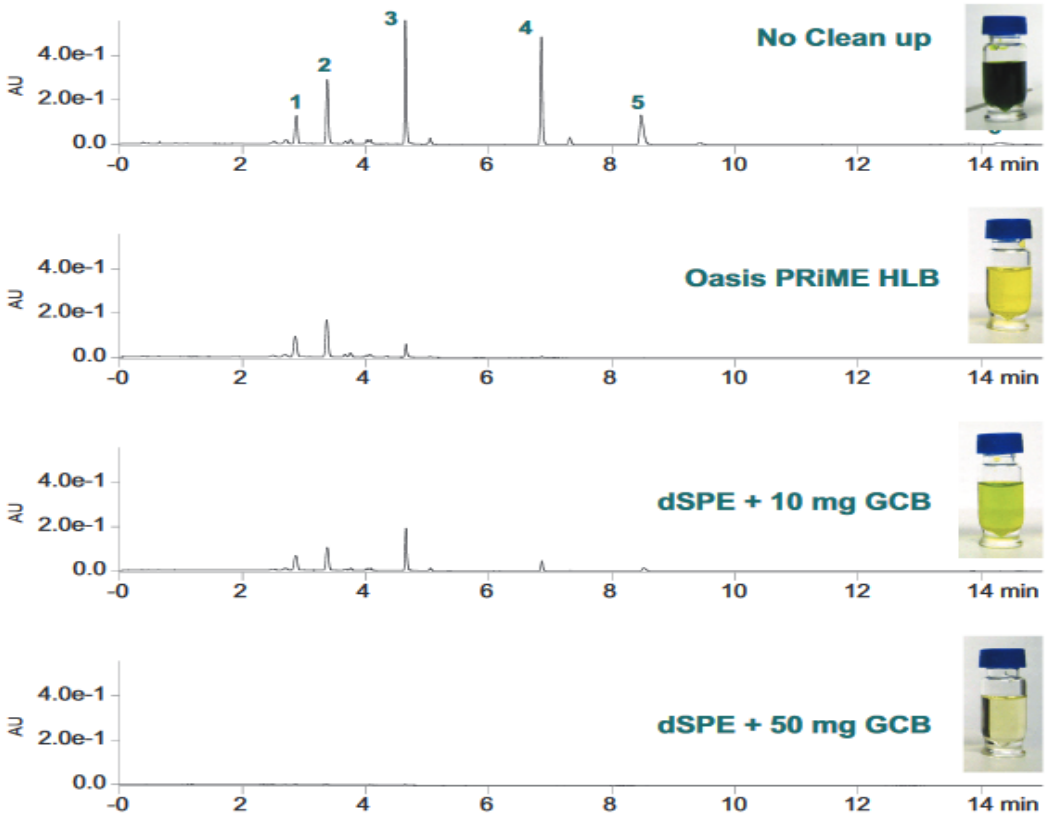
Compounds:
1. Violaxanthin
2. Antheraxanthin
3. Lutein
4. Chlorophyll b
5. Chlorophyll a
6. Carotene

UPLC-PDA conditions:

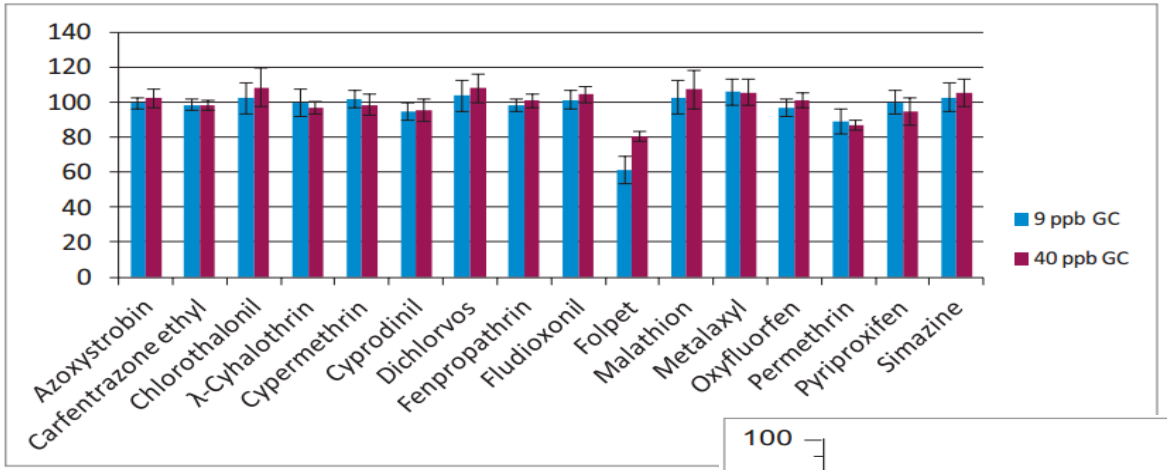
Detector:	ACQUITY® PDA
Wavelength:	450 nm
UPLC® System:	ACQUITY I-Class FTN
Column:	Cortecs® UPLC T3 (2.1 x 100 mm)
Temp.:	30 deg
Injection:	5 µL (QuEChERS extract diluted 1:4 with water)
Mobile phase A:	5 mM ammon. formate in water
Mobile phase B:	acetonitrile/methanol 75:25

Gradient:

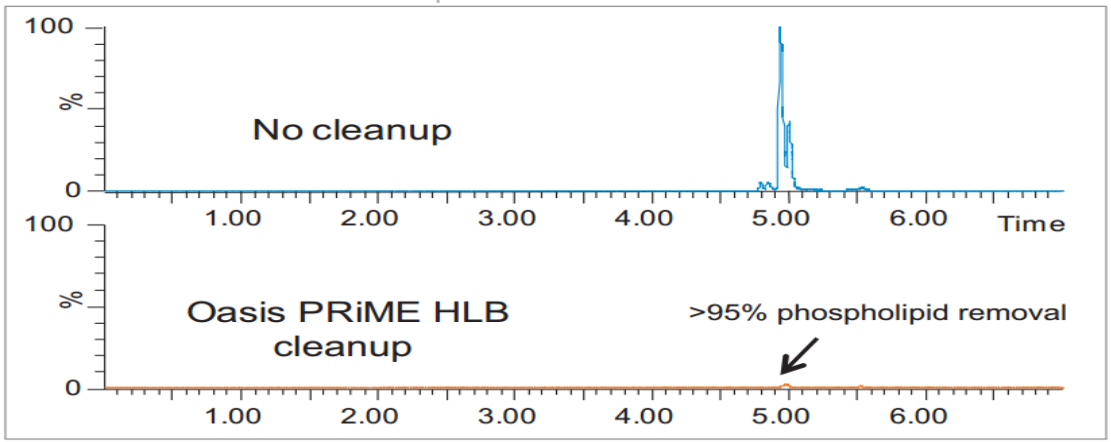
Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.5	25.0	75.0	Initial
5.0	0.5	1.0	99.0	6.0
20.0	0.5	1.0	99.0	6.0
20.2	0.5	25.0	75.0	6.0
21.0	0.5	25.0	75.0	6.0



Recovery and phospholipid removal during analysis of acocado



- 80 % of the fat was removed
- 95 % of phospholipids where removed
- 95% of chlorophyll was removed



QuEChERS CEN Extraction
Organic Banana/Pear/Mango Baby Food

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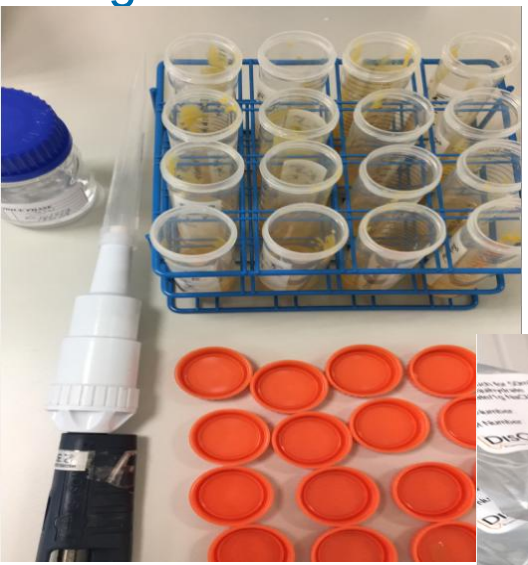

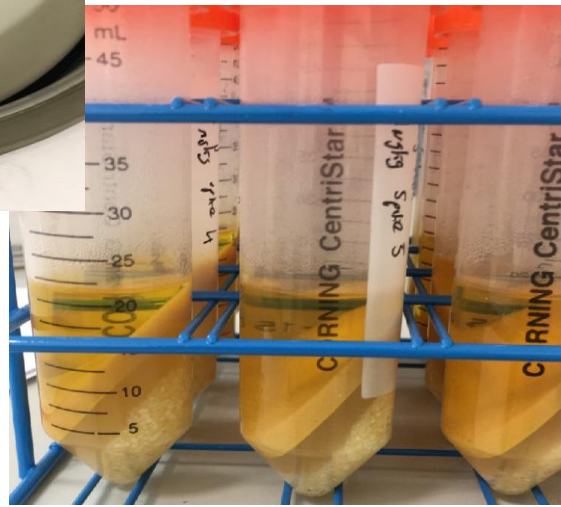

Oasis PRiME HLB Test Protocol

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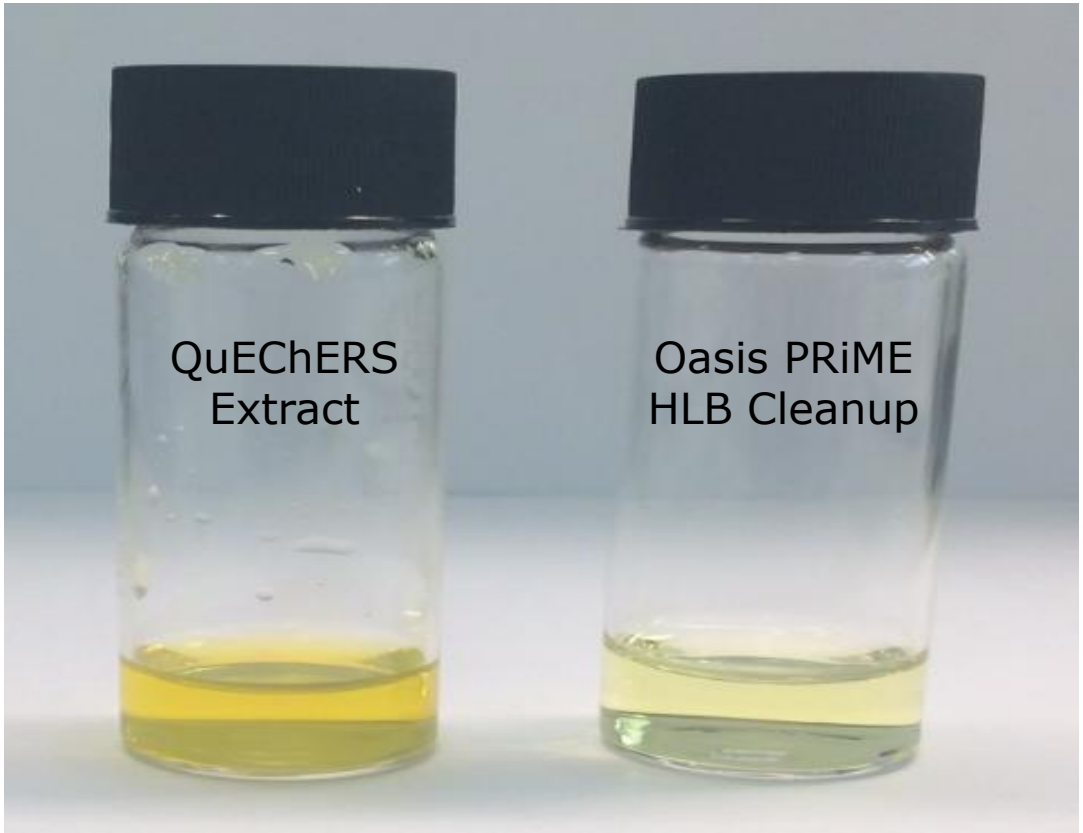
Oasis PRiME HLB

1. Pass 0.6ml of supernatant through Oasis PRiME HLB, plus light [186008886] (**discard**)
2. Pass 1.0ml of supernatant through Oasis PRiME HLB, plus light [186008886] (**collect**)
3. *Acidify a fixed volume of supernatant with 5% FA (50ul)



Cleanup on Oasis PRiME HLB; recovery at 0.001mg/kg

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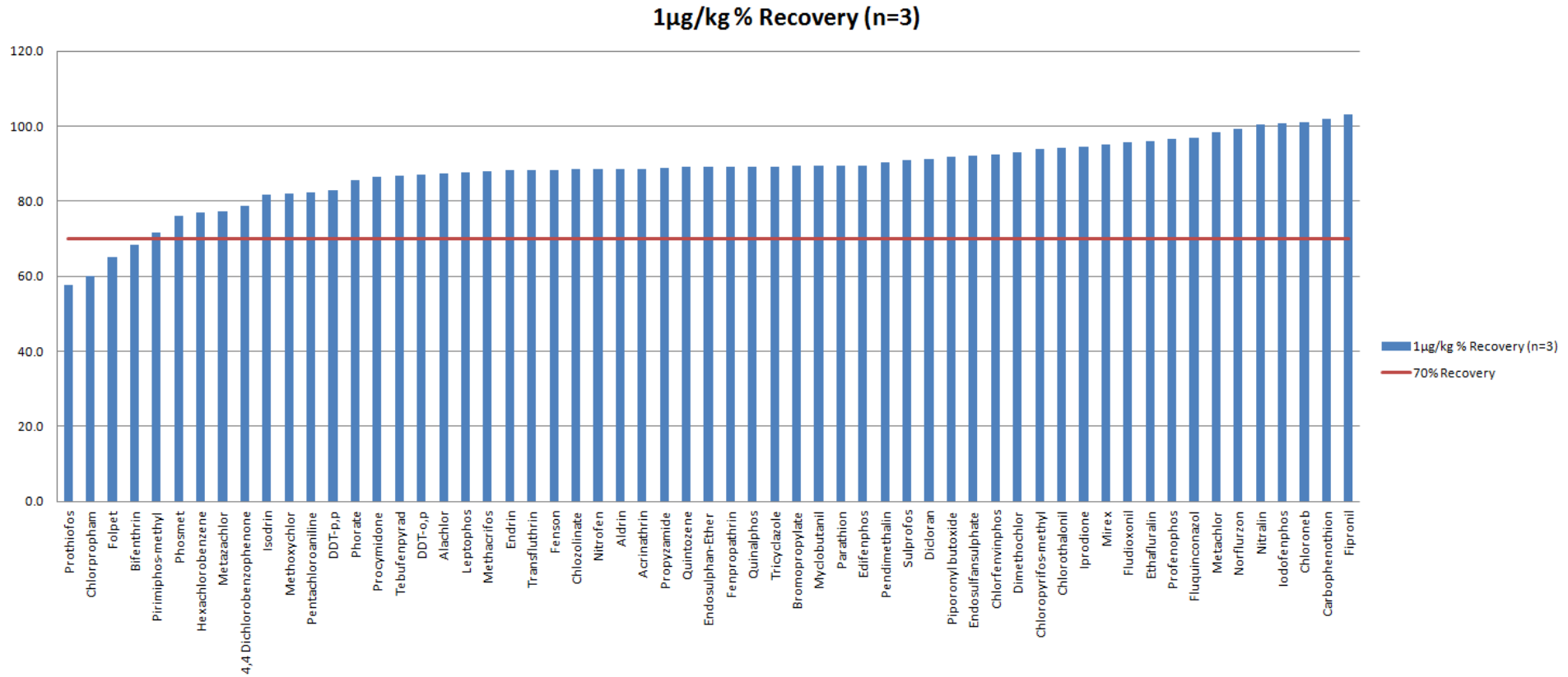

Cleanup on Oasis PRiME HLB; repeatability at 0.001mg/kg

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