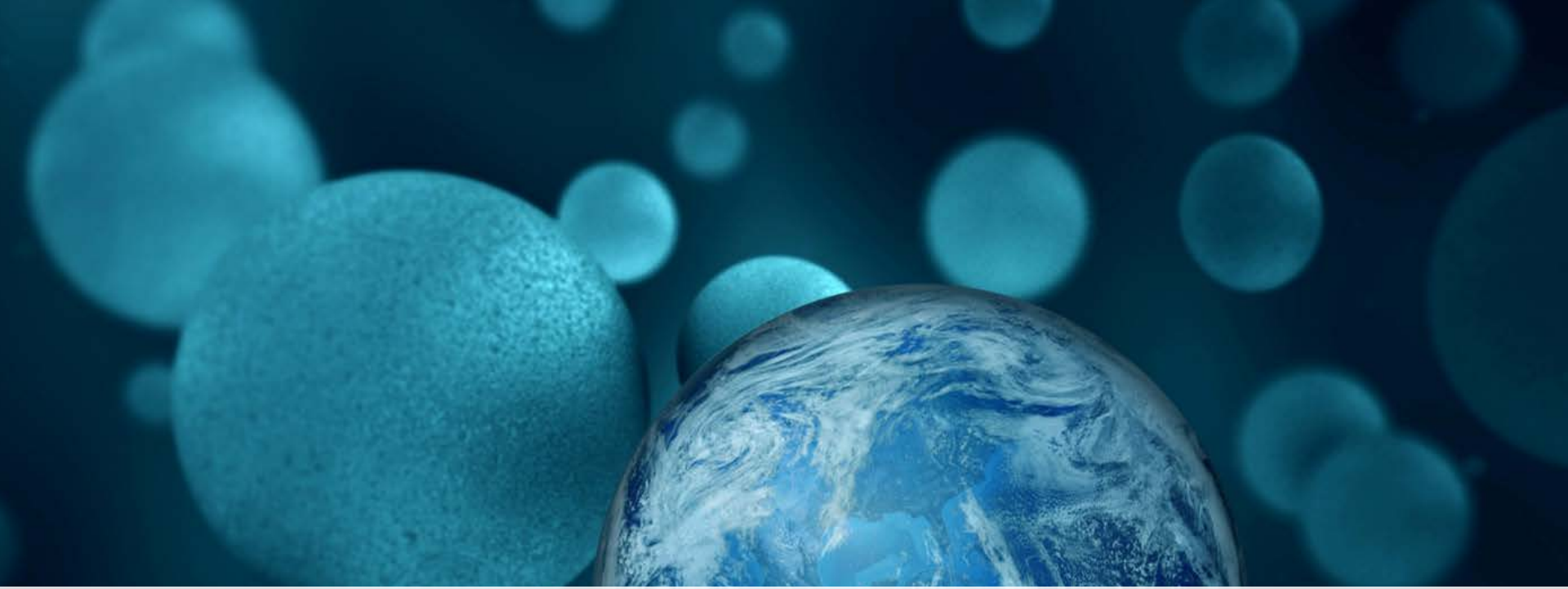


ThermoFisher SCIENTIFIC

GC-MS Evolutionary Technology & the Art of POPs Analysis

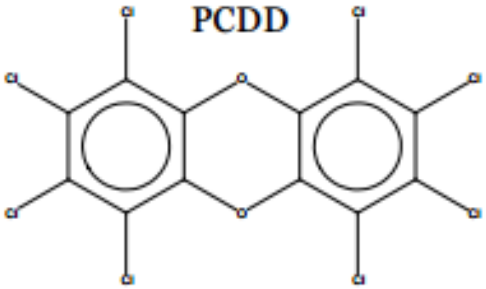
Chris Cheah

Senior Laboratory Manager - SEA

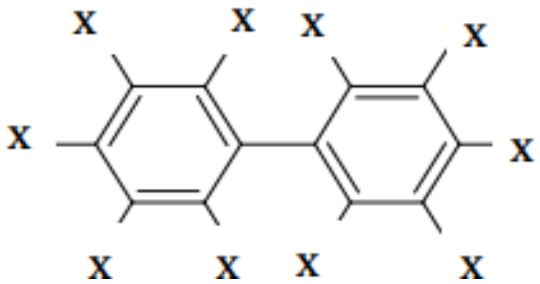
Persistent Organic Pollutants

Organochlorine pesticides	Aldrin, Chlordane, DDT, Dieldrin, Endrin, Heptachlor, Hexachlorobenzene, Mirex and Toxaphene
Polychlorinated biphenyls	They are used as dielectric oil in transformers and accumulators and other uses
Dioxins	Anthropogenic substances coming from different sources, despite a minor natural contribution

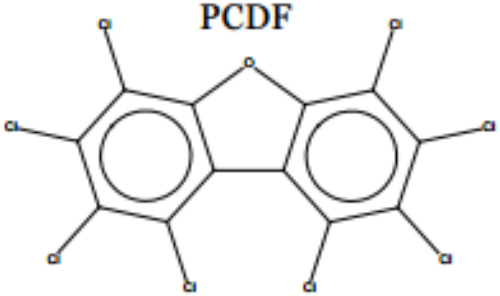
PCDD



PCB



PCDF



ThermoFisher SCIENTIFIC

Dioxin Incidents” – Huge Economical Impact

BBC ONLINE NETWORK

BBC NEWS

Front Page
World
UK
UK Politics
Business
Sci/Tech
Health
Education
Sport
Entertainment
Talking Point
In Depth
On Air
Archive

Wednesday, June 2, 1999 Published at 12:10 GMT 13:10 UK

World: Europe

Belgian chickens banned in Europe

Belgium exports about half its egg and chicken produce

BBC

NEWS

ONE-MINUTE WORLD NEWS

News Front Page
Africa
Americas
Asia-Pacific
Europe
Middle East
South Asia
UK
Business
Health
Science & Environment

Italy recalls tainted mozzarella

The Italian government has recalled from sale the mozzarella cheese linked to dioxin contamination.

Italy's health ministry said the affected cheese came from 25 producers in the Campania region near Naples, where buffalo mozzarella is made.

France has now lifted a ban on sales

Mozzarella production is big business in Italy

HOME PAGE | TODAY'S PAPER | VIDEO | MOST POPULAR | TIMES TOPICS

The New York Times

Europe

WORLD | U.S. | N.Y. / REGION | BUSINESS | TECHNOLOGY | SCIENCE | HEALTH | SPORTS | OPINION

AFRICA | AMERICAS | ASIA PACIFIC | EUROPE | MIDDLE EAST

Irish pork products recalled after dioxin is found

Published: Sunday, December 7, 2008

TIMESONLINE

NEWS | COMMENT | BUSINESS | MONEY | SPORT | LIFE & STYLE | TRAVEL | DR

UK NEWS | WORLD NEWS | POLITICS | SCIENCE | ENVIRONMENT | WEATHER | TECH &

Where am I? › Home › News › World News › Ireland News

From The Times

December 8, 2008

Contaminated pork from Ireland: your questions answered

“Dioxin Incidents” – Huge Economical Impact

- **Dioxin and PCB incidents**
- **… are expensive.**
  - Egg production enterprise with 5 million hens would cost in excess of $30 million.
  - Broiler enterprise producing 3 million broilers per week would exceed $85 million.
  - Times Beach, Missouri, road dust covered by oil spread. The cleanup cost with a total of $110 million.
- Sources to the Environment
  - Combustion processes
  - Landfill sites
  - Improper waste managment
  - Sewedge sludge
- Accumulation in Food Chain
  - Regular control required
  - In environment, feed, and food

Repeated “Dioxin” cases from only recent years:

- 1998 Milk from citrus pulp pellet feed
- 1999 Belgian PCB/dioxin in eggs, poultry
- 1999 Clay and zeolithes for feed
- 2000 Choline chloride
- 2002 “Carbosan Copper”
- 2004 Potato pulp
- 2005 Hydrochloric acid
- 2007 Indian Guar Gum thickener
- 2008 Irish Pork Meat
- 2008 Italian Mozarella Cheese …
- ... still on today around Naples.

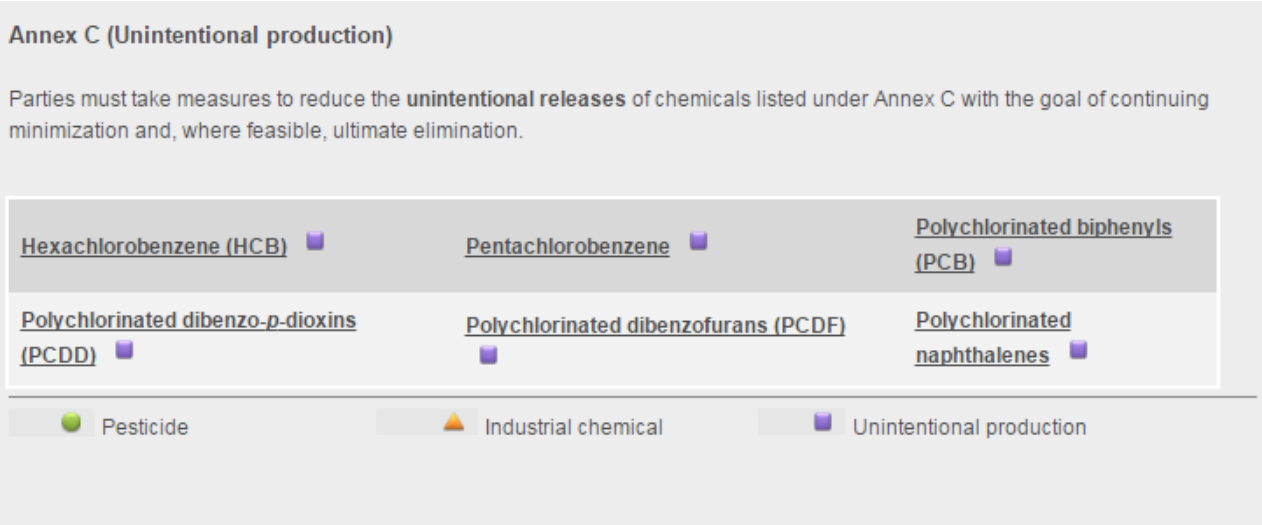


Global Regulation & Response

Different dynamic and abatement strategies:

- **Global Scale – Stockholm Convention on POPs**
- **EU Regulation**:
  - Setting limits in well-known sources
  - Setting limits in feed and food for dioxins and PCBs
  - Defining not only the limits but also developing methods for sampling and analysis for the official control of levels of dioxins and PCBs in food and feed: Confirmatory and Screening methods

Global Response - Stockholm Convention



EU Commission Regulation – Past/Present

Past

Commission Regulation (EU) No 252/2012

- Specifies use of GC-HRMS for confirmatory dioxin analysis in food and feed.
- **GC-MS/MS** allowed as screening technique.

Present

Commission Regulation (EU) No 589/2014

- Specifies use of **GC-HRMS** or **GC-MS/MS** for confirmatory dioxin analysis in food and feed.

Commission Regulation (EU) No 589/2014 of 2 June 2014

- Specifies use of **GC-HRMS** **or** **GC-MS/MS** for confirmatory dioxin analysis.
- **GC-MS/MS** is "an appropriate confirmatory method for checking compliance with the maximum", only.
- **GC-HRMS** remains the recommended technique for "determination of low background levels in food monitoring, following of time trends, exposure assessment of the population".

HRMS is recognized to deliver superior sensitivity, as required for **low level background** studies

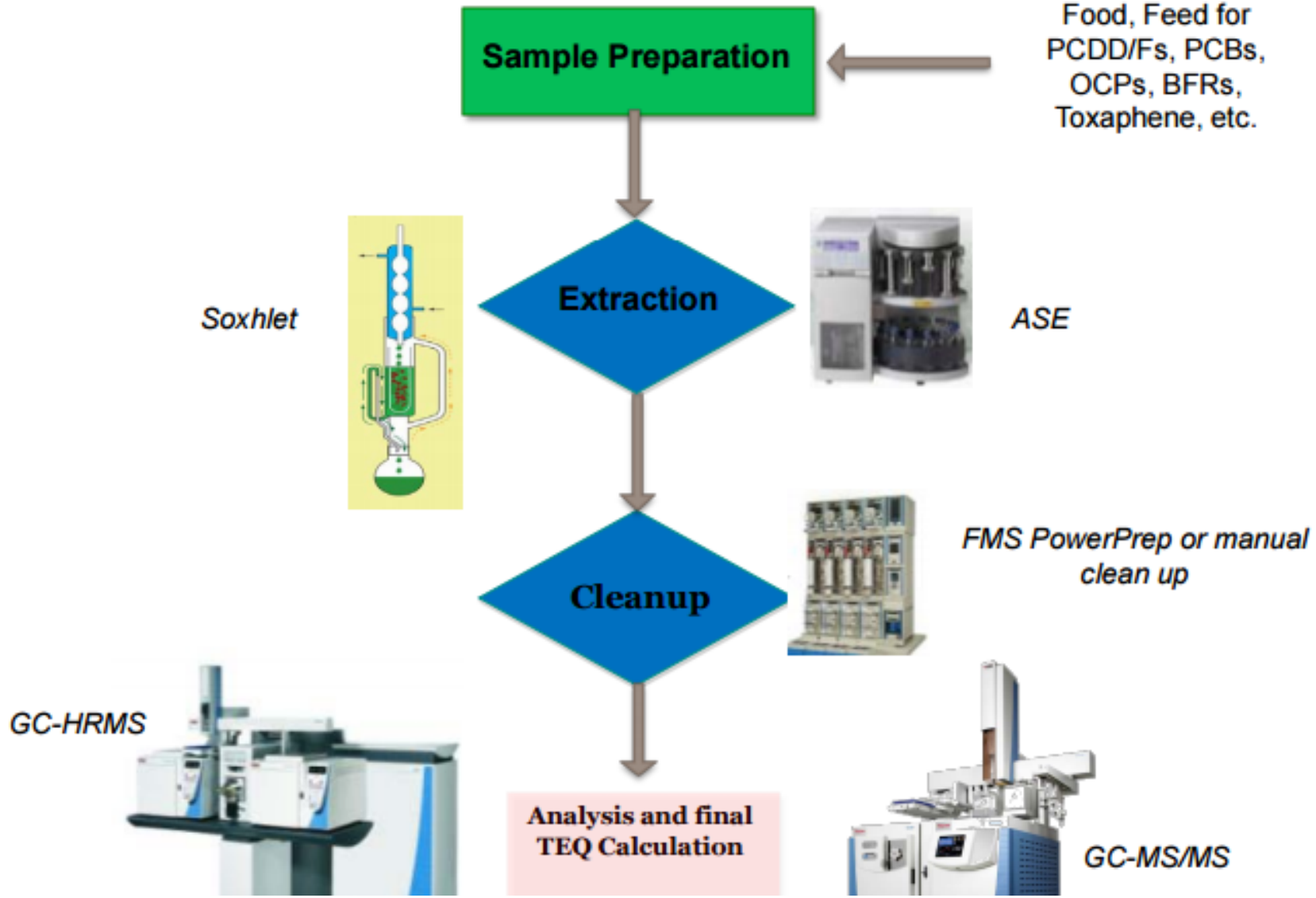
HRMS fulfills all requirements for all types of dioxin applications, is considered the ***Reference*** standard for dioxin analysis.

EU Commission Regulation – GC-MS/MS Requirements

SPECIFIC REQUIREMENTS FOR GC-MS/MS METHODS TO BE COMPLIED WITH FOR CONFIRMATORY PURPOSES (in food and feed samples):

- Monitoring of at least 2 specific precursor ions, each with one specific corresponding transition product ion, for all labelled and unlabelled analytes…
- Maximum permitted tolerance of relative ion intensities of ±15% for selected transition product ions in comparison to calculated or measured values (average from calibration standards), applying identical MS/MS conditions, in particular collision energy and gas pressure,…
- Resolution for each quadrupole to be set equal to or better than unit mass resolution (unit mass resolution: sufficient resolution to separate two peaks one mass unit apart)…
- LOQ: The method must demonstrate that it is able to distinguish between the blank and cut-off value. In reporting a value, a notification level should be established to decide what to do with the samples with a response below this level.
  - For PCDD/PCDF, LOD should be in the range of higher femtograms (10^{-15} g). For most congeners of PCBs, is sufficient LOQ in the nanogram (10^{-9} g). However, to measure congeners similar to dioxin-like PCBs (in particular non-ortho substituted congeners) the lower limit of the working range must reach the lowest picogram (10^{-12} g).

POPs Analysis Workflow



ThermoFisher SCIENTIFIC

POPs Analytical Tools

Magnetic Sector GC-HRMS

Ultimate sensitivity & Maximum robustness

The **Thermo Scientific™ DFS™ Magnetic Sector GC-HRMS**, the Gold Standard for Dioxins & POPs, is the only GC-MS specifically designed for Dioxin and POPs analysis. The DFS GC-HRMS offers worldwide full compliance with any official Dioxin, PCB or PBDE method (for example, EPA 1613, 1668, 1614). Exploit the benefits of highest available Dioxin sensitivity and robustness, delivered by our large-volume ion source.

Thermo Scientific DFS Magnetic Sector GC-HRMS

- World-Wide Method compliance (e.g. EPA 1613)
- Best Dioxin installation spec
- Highest available sensitivity
- Robust-by-size with large volume ion source

GC-MS Triple Quadrupole

High sensitivity & Ease-of-use

The **Thermo Scientific™ TSQ™ 8000 Evo GC-MS/MS** Analyzer brings a compelling productivity opportunity to your laboratory. It offers you fully compliant analysis of Dioxins in food and feed in respect to the latest EU regulations, meeting stringent European Union performance criteria.
High productivity within more efficient lab workflows accelerates sample turnaround times.

Thermo Scientific TSQ 8000 Evo GC-MS/MS Analyzer

- Ease-of-use
- Compliant with EU regulations for Dioxin food & feed analysis
- Great price/performance ratio

Orbitrap GC-MS

The ultimate tool for unknown identification

The **Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS** represents the first-ever combination of high-resolution gas chromatography and high-resolution, accurate-mass (HRAM) Orbitrap mass spectrometry. It is an easy-to-use, dedicated benchtop GC-MS system that provides highest confidence for emerging POPs research with unmatched performance in compound discovery, identification and quantitation for a comprehensive understanding of your samples.

Thermo Scientific Q Exactive GC Orbitrap GC-MS/MS

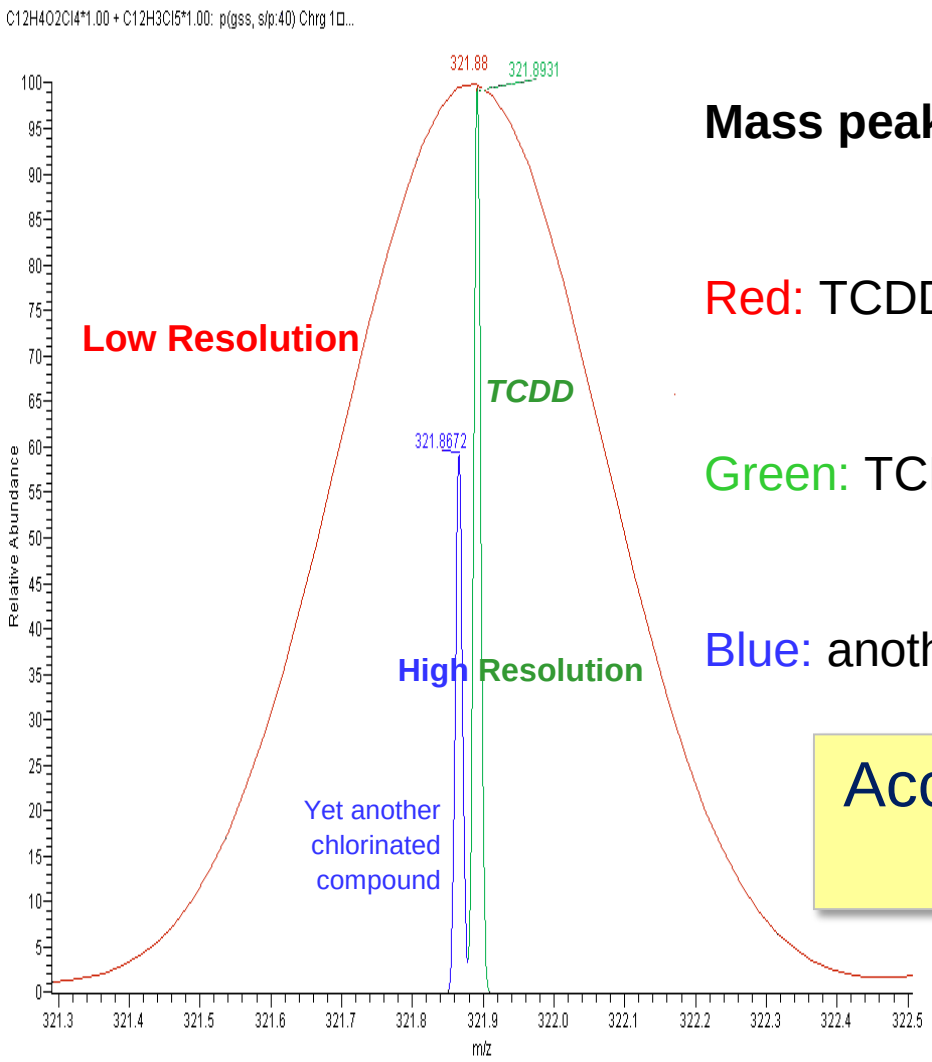
- The ultimate research tool
- Highest resolution and mass accuracy
- Unique unknown identification capabilities

DFS GC-HRMS: *Gold* Standard for Dioxins

T**or** GC-HRMS

- **Worldwide method compliance**
  - **EPA 1613**,1668,1614 & others
  - Use **PFK/PFTBA** , lock mass trace, etc.
- **Analytical Performance**
  - **best Dioxin spec** available
  - **large ion source**: ultimate sensitivity *combined with* highest ***robustness***
  - Unique **Dual GC** configuration
- **References (10 years DFS!)**
  - Worldwide use in **Dioxin expert labs**

Confirmation Analysis with *High Resolution*



Mass peaks at Different Resolution Settings:

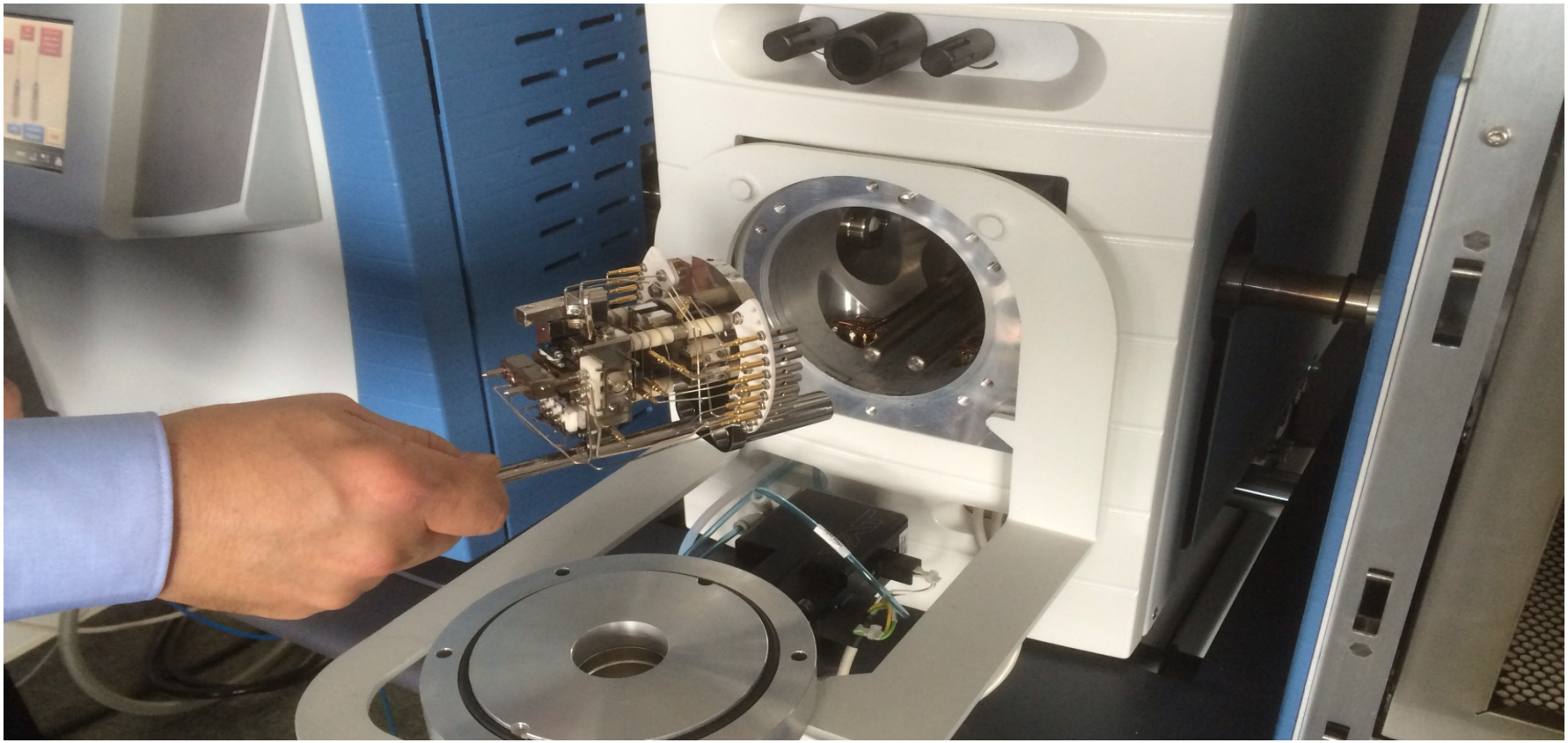
Red: TCDD mass trace at R 1.000 (GC-MS/MS)

Green: TCDD mass trace at R 10.000 (DFS)

Blue: another chlorinated compound mass trace

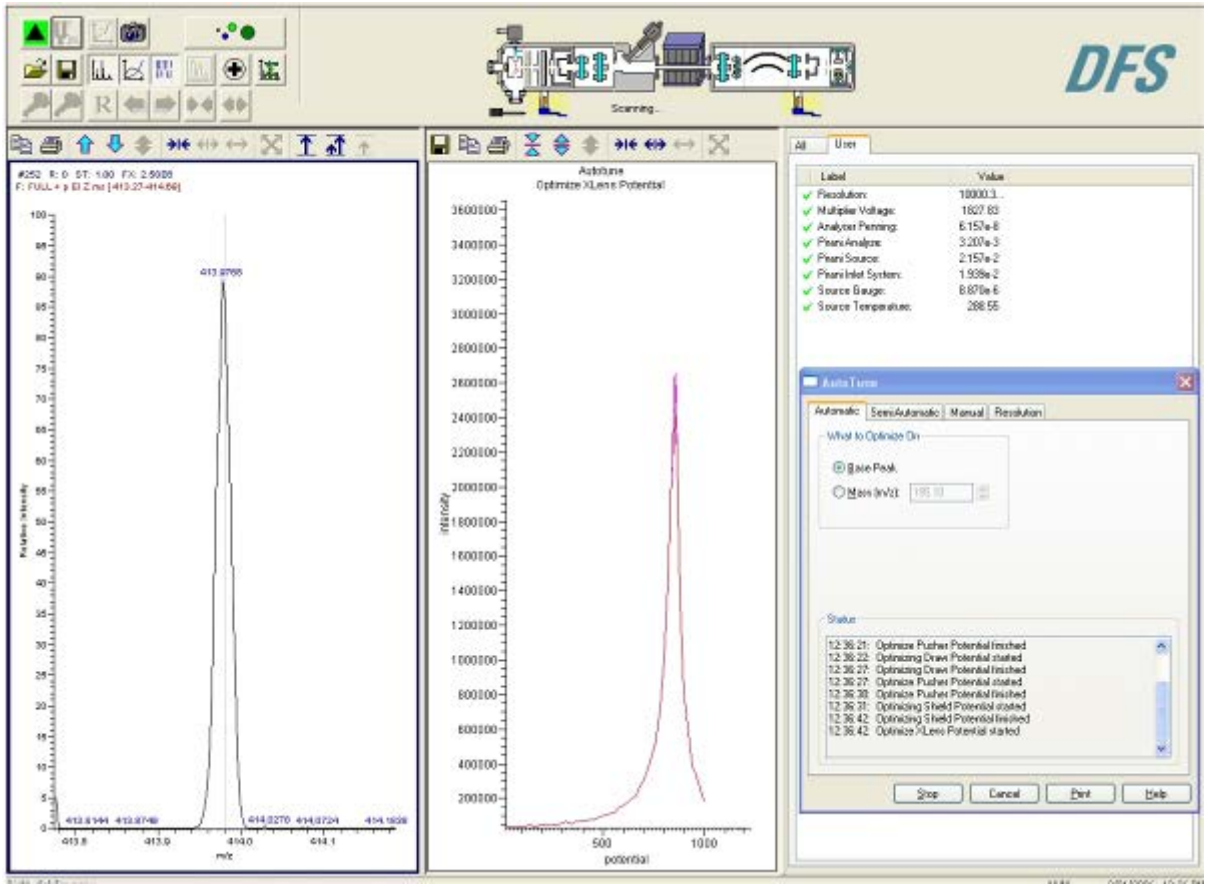
Accurate mass SIM is used for Confirmation Analysis

DFS Ion Source – Robustness by Size



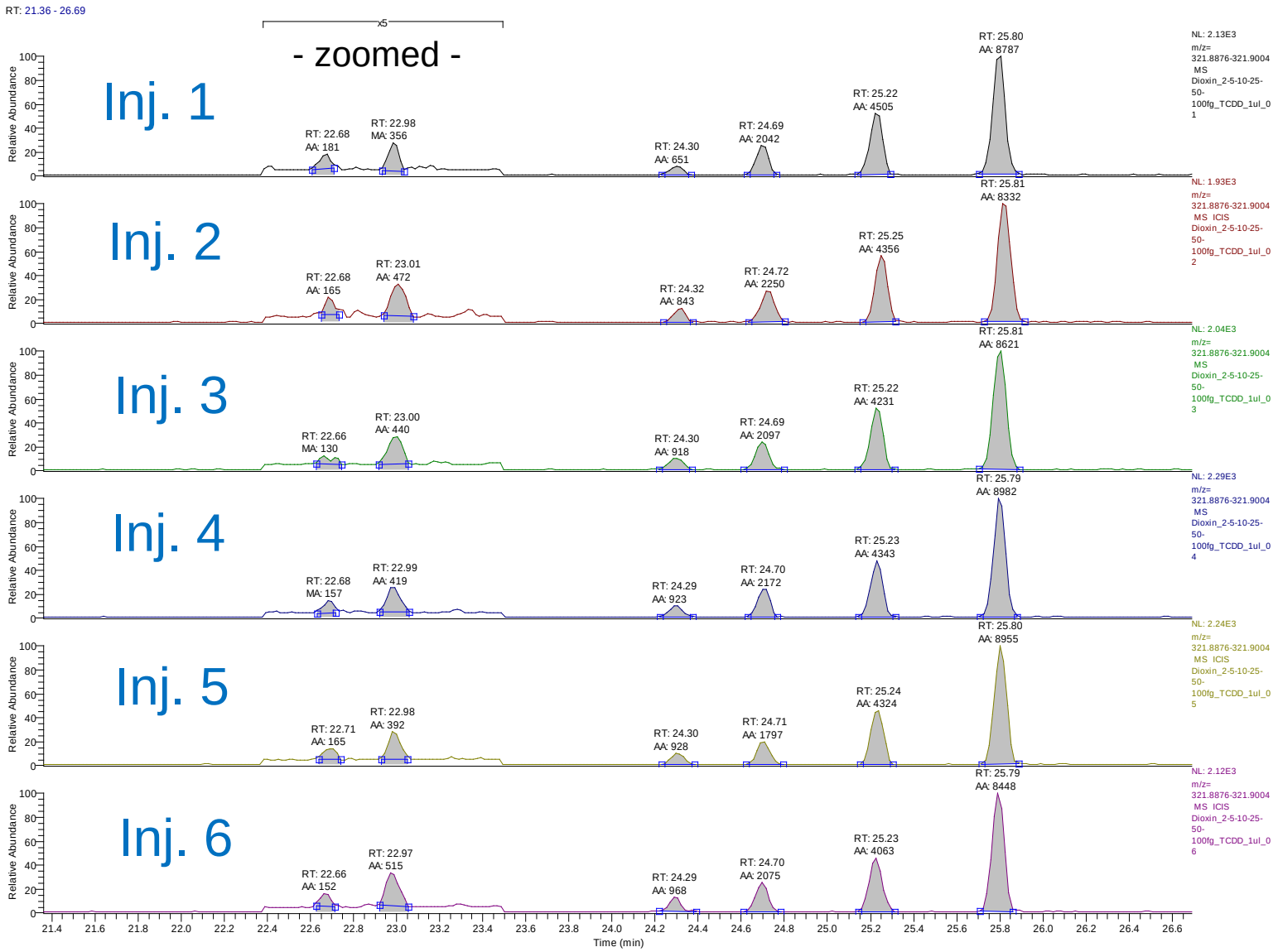
DFS ion source - designed for combining ultimate sensitivity with robustness, because sensitivity alone is not enough

Makes the DFS as easy to operate as a Benchtop MS



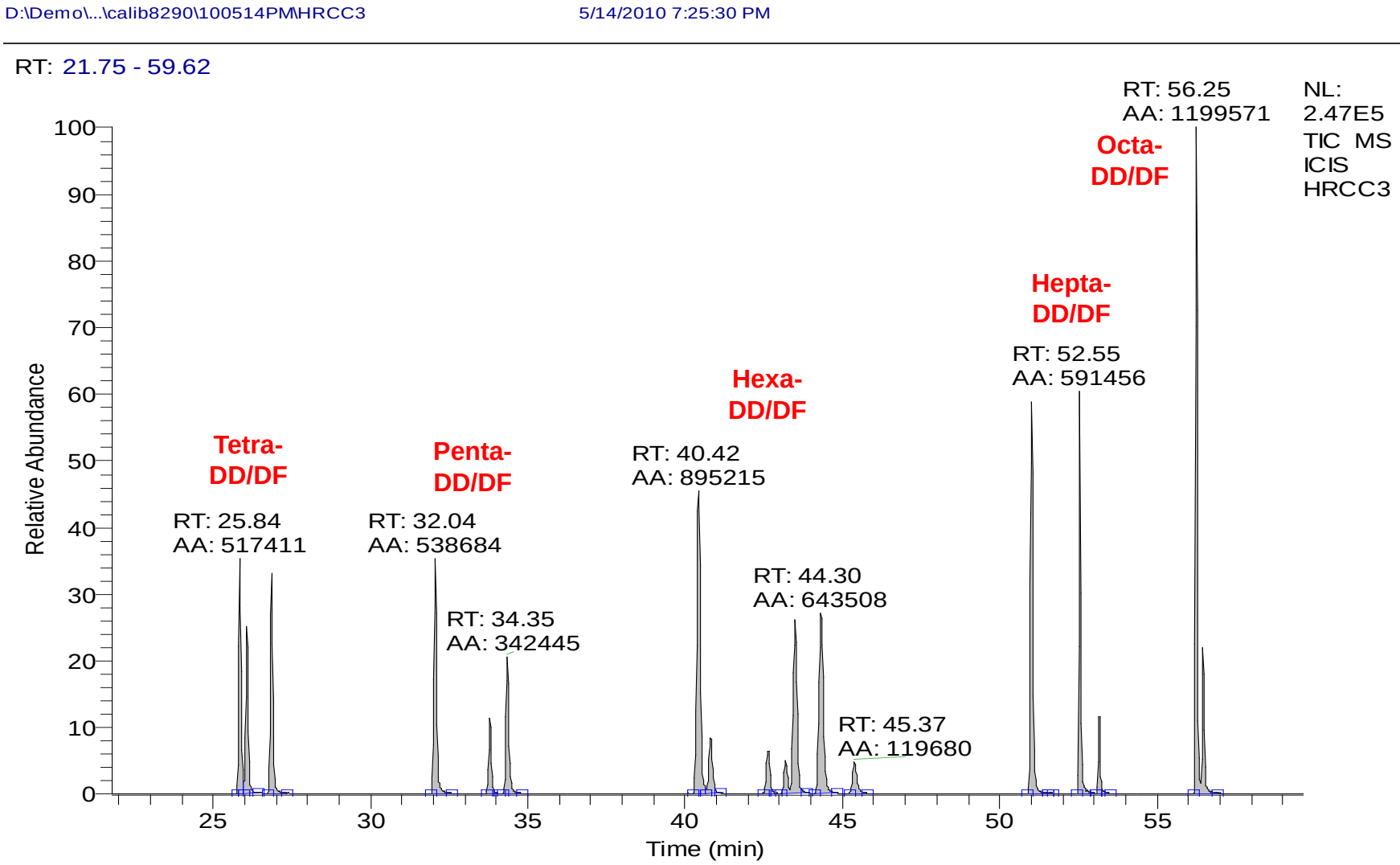
- Common User Interface known from DSQ, Polaris Q or LCQ/LTQ.
- **AutoTune**
- **Resolution** setting

2fg -100 fg TCDD on 60 m column



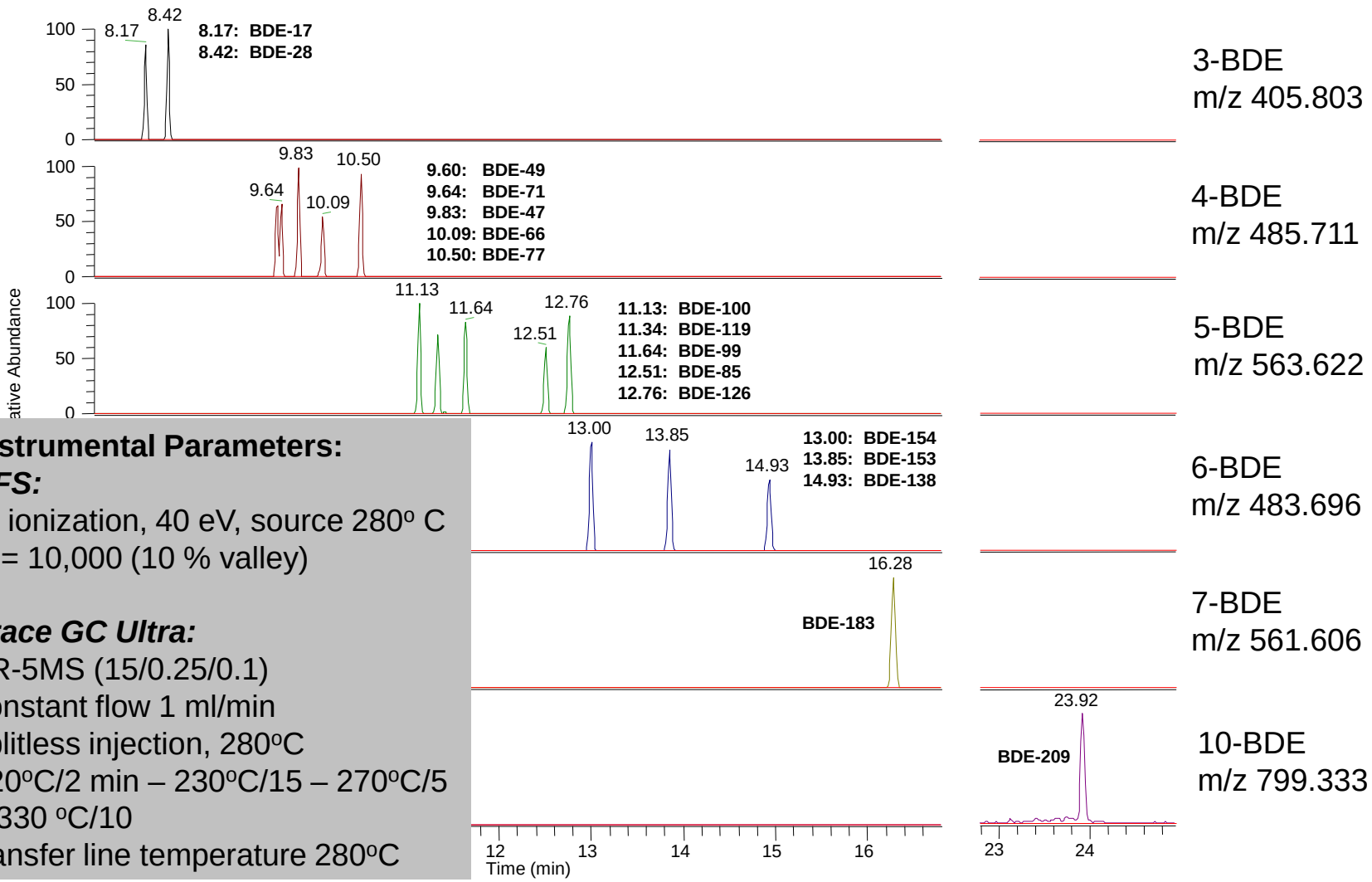
ThermoFisher SCIENTIFIC

Dioxin & Furan Analysis



ThermoFisher SCIENTIFIC

PBDE Brominated Flame Retardants (BFRs)



Instrumental Parameters:

DFS:

EI ionization, 40 eV, source 280° C

R = 10,000 (10 % valley)

Trace GC Ultra:

TR-5MS (15/0.25/0.1)

constant flow 1 ml/min

splitless injection, 280°C

120°C/2 min – 230°C/15 – 270°C/5 – 330 °C/10

transfer line temperature 280°C

TSQ 8000 EVO – State of Art GC-MS/MS

- Thermo Scientific™ TSQ™ 8000 Evo triple quadrupole GC-MS
- State-of-the-art triple quadrupole GC-MS system introduced at ASMS 2014
- Fast collision cell instrument with EvoCell technology for high SRM transition speeds, precision, and sensitivity

- Thermo Scientific™ ExtractaBrite™ ion source is heated throughout for high matrix tolerance
- Ion source is fully removable, hot, under vacuum when cleaning is necessary or swapping with a spare

EU Regulation 589/2014 Requirements

6.5. **Specific criteria for confirmatory methods**

— For GC-HRMS:

In HRMS, the resolution shall typically be greater than or equal to 10 000 for the entire mass range at 10 % valley.

Fulfilment of further identification and confirmation criteria as described in internationally recognised standards, for example, in standard EN 16215:2012 (Animal feed — Determination of dioxins and dioxin-like PCBs by GC/HRMS and of indicator PCBs by GC/HRMS) and/or in EPA methods 1613 and 1668 as revised.

— For GC-MS/MS:

✓ Monitoring of at least 2 specific precursor ions, each with one specific corresponding transition product ion, for all labelled and unlabelled analytes in the scope of analysis.

✓ Maximum permitted tolerance of relative ion intensities of ± 15 % for selected transition product ions in comparison to calculated or measured values (average from calibration standards), applying identical MS/MS conditions, in particular collision energy and collision gas pressure, for each transition of an analyte.

✓ Resolution for each quadrupole to be set equal to or better than unit mass resolution (unit mass resolution: sufficient resolution to separate two peaks one mass unit apart) in order to minimise possible interferences on the analytes of interest.

✓ Fulfilment of the further criteria as described in internationally recognised standards, for example, in standard EN 16215:2012 (Animal feed — Determination of dioxins and dioxin-like PCBs by GC/HRMS and of indicator PCBs by GC/HRMS) and/or in EPA methods 1613 and 1668 as revised, except the obligation to use GC-HRMS.

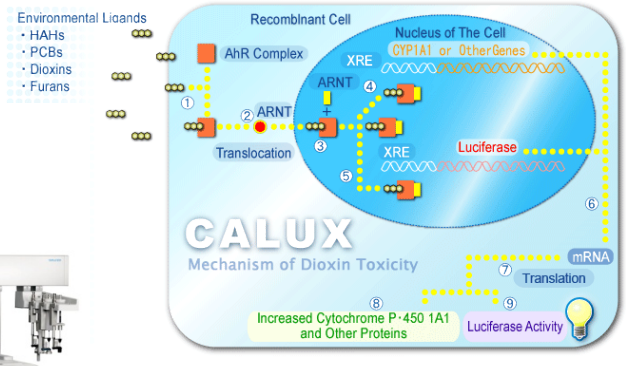
Methods for DioxinFurans & PCBs Measurement

SCREENING:

- GC-MS
- GC-MS/MS
- Magnetic Sector
- Bioassay (e.g. CALUX)

CONFIRMATION:

- Magnetic Sector – MLs and background
- GC-MS/MS – MLs as outlined in EU regulations 709/2014 and 589/2014



Products	Maximum levels for dioxins (PCDD + PCDF) [25] (pg WHO-PCDD/F-TEQ/g fat or product)
5.1.1 Meat and meat products [28] originating from	
- Ruminants (bovine animals, sheep)	3 pg WHO-PCDD/F-TEQ /g fat[26,27]
- Poultry and farmed game	2 pg WHO-PCDD/F-TEQ /g fat[26,27]
- Pigs	1 pg WHO-PCDD/F-TEQ /g fat[26,27]
5.1.2 Liver and derived products	6 pg WHO-PCDD/F-TEQ /g fat[26,27]
5.2. Muscle meat of fish and fishery products [29] and products thereof	4 pg WHO-PCDD/F-TEQ /g fresh weight[26]
5.3. Milk [30] and milk products, including butter fat	3 pg WHO-PCDD/F-TEQ /g fat[26,27]
5.4 Hen eggs and egg products [31,32]	3 pg WHO-PCDD/F-TEQ /g fat[26,27]
5.5.Oils and fats	
- Animal fat	
- from ruminants	3 pg WHO-PCDD/F-TEQ /g fat[26]
- from poultry and farmed game	2 pg WHO-PCDD/F-TEQ /g fat[26]
- from pigs	1 pg WHO-PCDD/F-TEQ /g fat[26]
- mixed animal fat	2 pg WHO-PCDD/F-TEQ /g fat[26]
- Vegetable oil	0.75 pg WHO-PCDD/F-TEQ /g fat[26]
- fish oil intended for human consumption	2 pg WHO-PCDD/F-TEQ /g fat[26]

ThermoFisher SCIENTIFIC

Experimental conditions (GC & MS)

TRACE 1310 GC Parameters	
Injection Volume (µL):	2.0
Liner	SSL Single tapered P/N: 453A2342
Inlet (°C):	280
Inlet Module and Mode:	SSL splitless
Carrier Gas, (mL/min):	He, 1.2
Oven Temperature Program:	
Temperature 1 (°C):	120
Hold Time (min):	2
Temperature 2 (°C):	250
Rate (°C/min)	25
Hold Time (min):	0
Temperature 3 (°C):	285
Rate (°C/min)	2.5
Hold Time (min):	0
Temperature 4 (°C):	320
Rate (°C/min)	10
Hold Time (min):	15

Column

Type	Length (m)	I.D.(mm)	Film (µm)	P/N
TG-5SilMS	60	0.25	0.25	26066-1540

TSQ 8000 Evo Mass Spectrometer Parameters	
Transfer line (°C):	280
Ionization type:	EI
Ion source(°C):	300
Electron energy (eV):	40
Acquisition Mode:	SRM (Timed Acquisition)
Collision gas:	Argon (60 psig)
Resolution Q1:	0.7 u (FWHM)
Resolution Q3:	0.7 u (FWHM)

All 17 PCDD/F and 12 dl-PCBs native and labeled congeners can be acquired in one method

Sample Preparation

- PCDD/Fs and dl-PCB standards containing the native and the ^{13}C-labelled compounds were obtained from ***Wellington Laboratories*** Inc. The following food and feed extracted samples were provided by the Institute of Environmental Assessment and Water Research, CSIC Barcelona, Spain:

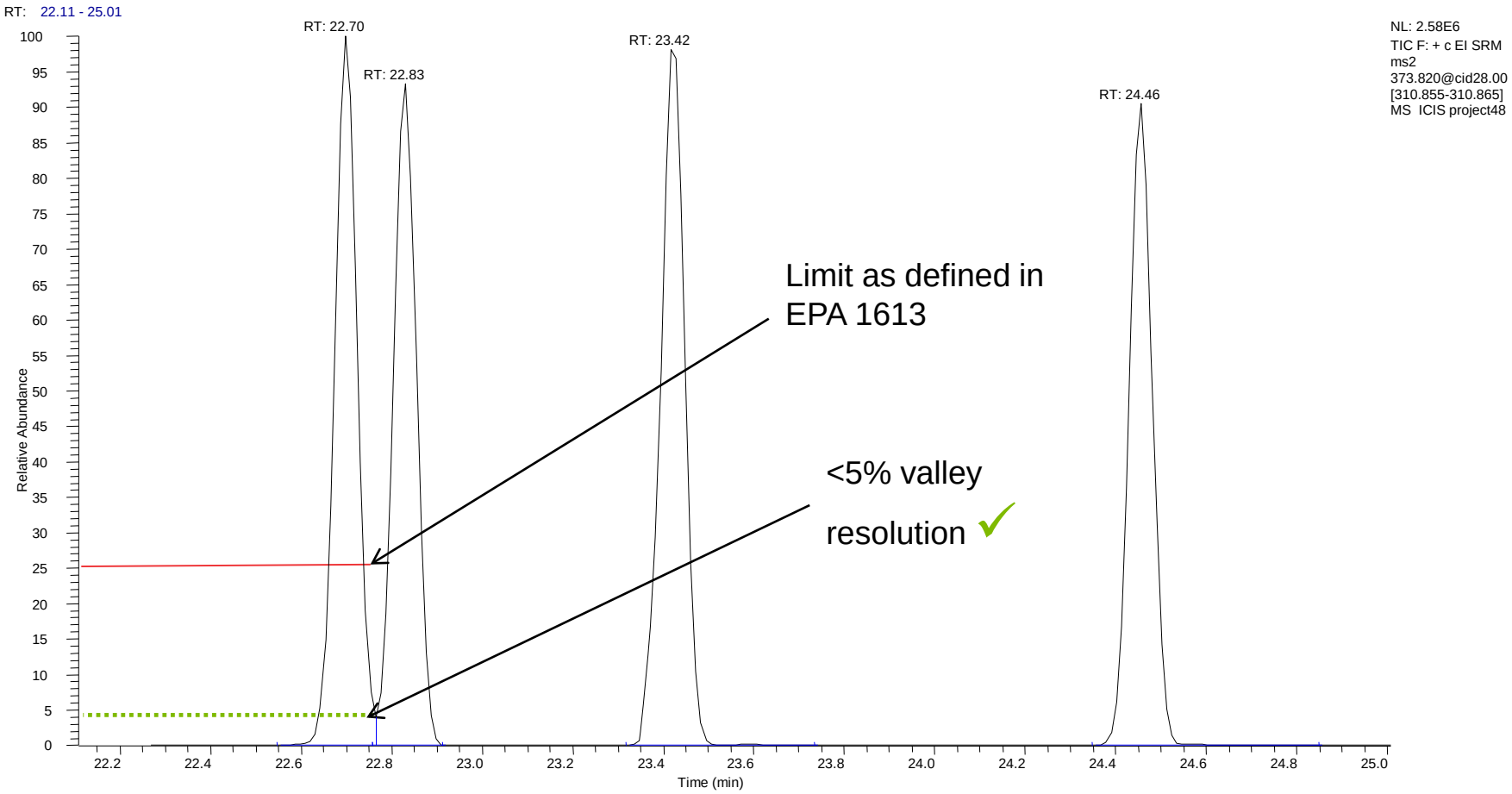
dl-PCBs
- Adipose tissue
- Fish liver
- Fish oil
- Dry fishspiked feed sample

PCDD/Fs
- milk powder sample (certified reference material).
- 3x dry fish samples (previously used in inter-laboratory studies)
- one feed sample (internal reference material).
- one milk powder sample (certified reference material).

- Extraction and clean-up of the matrix samples was performed either by PowerPrep™ SPE system (feed sample) or using a manual clean-up with multilayer silica, followed by basic alumina and a final carbon column (milk, tissue and fish samples).

Chromatography - PCDD/Fs

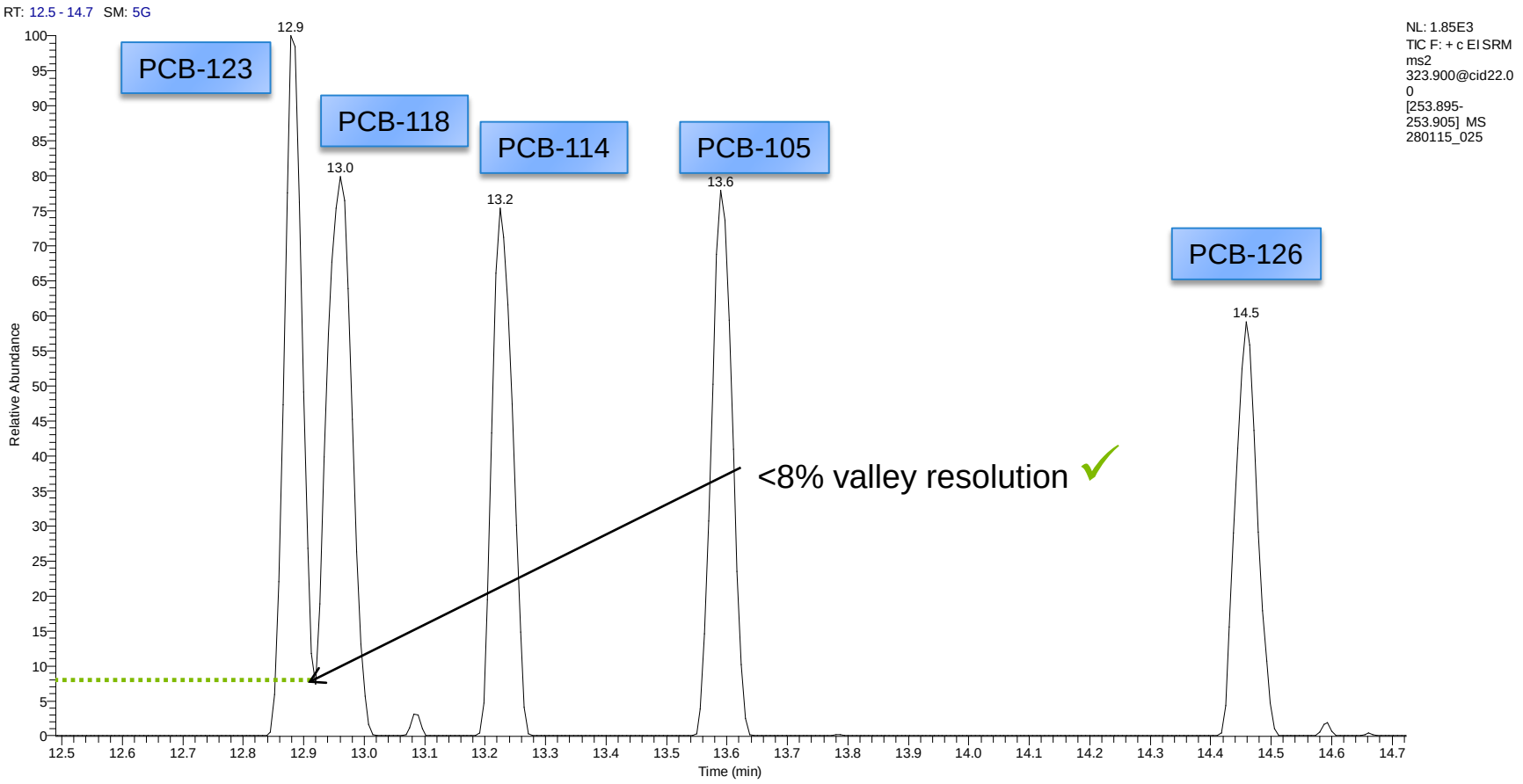
Excellent resolution of the isomeric pair 1,2,3,4,7,8 and 1,2,3,6,7,8 HxCDF



2µL EPA 1613 CS3 standard

ThermoFisher SCIENTIFIC

Chromatography - dl-PCBs



2µL 68C-CVS CS1 standard

Resolution of ‘Penta’ dl-PCBs – well below 25% valley

ThermoFisher SCIENTIFIC

The level of interest…

Unlike GC-HRMS, often in SRM data there is no noise and a signal to noise calculation based approached to LOD/LOQ can be meaningless. Therefore a statistical method was used.

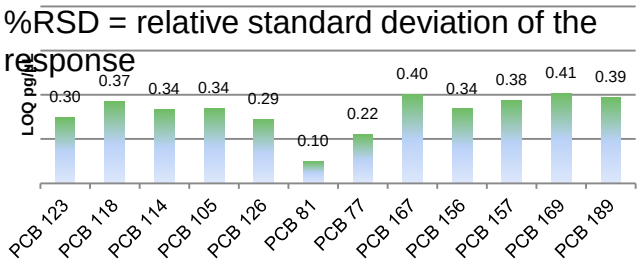
LOQ = t * Amount * %RSD

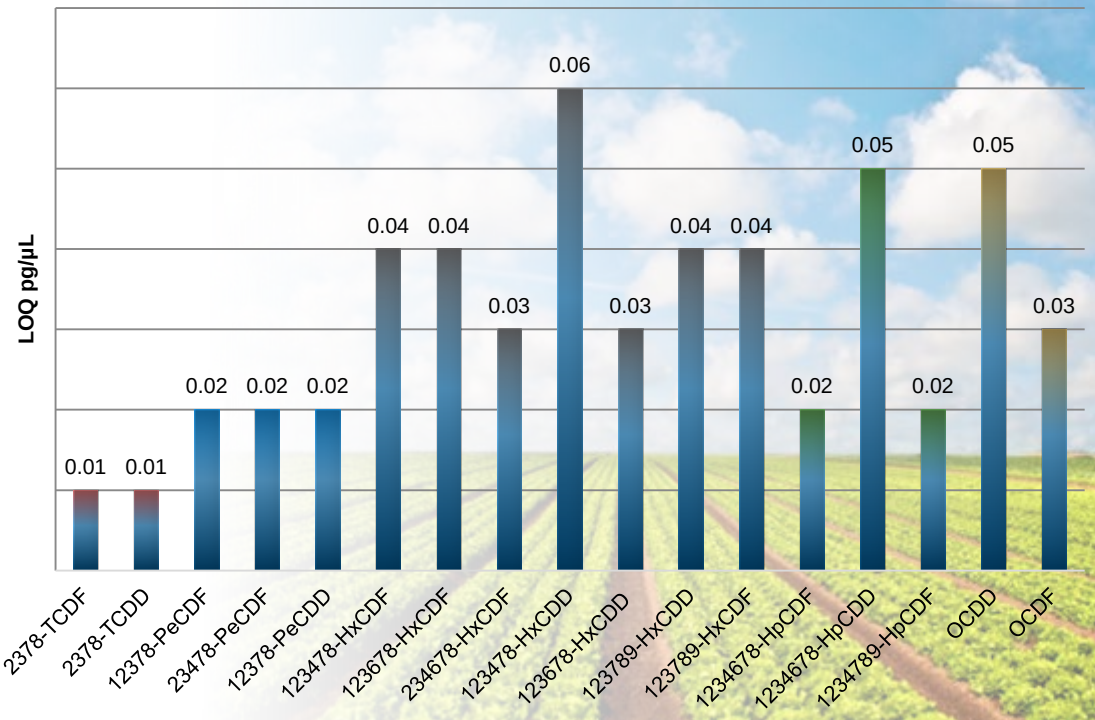
Where,

t = student *t*-value for **one-tailed** distribution: for n = 10 injections; *t* = 2.821

Amount = amount of analyte (on-column)

%RSD = relative standard deviation of the response





All ion ratios and response factor values met the required criteria at these limits

Linearity – Response factors (RF)

Linearity / Calibration (5 point)

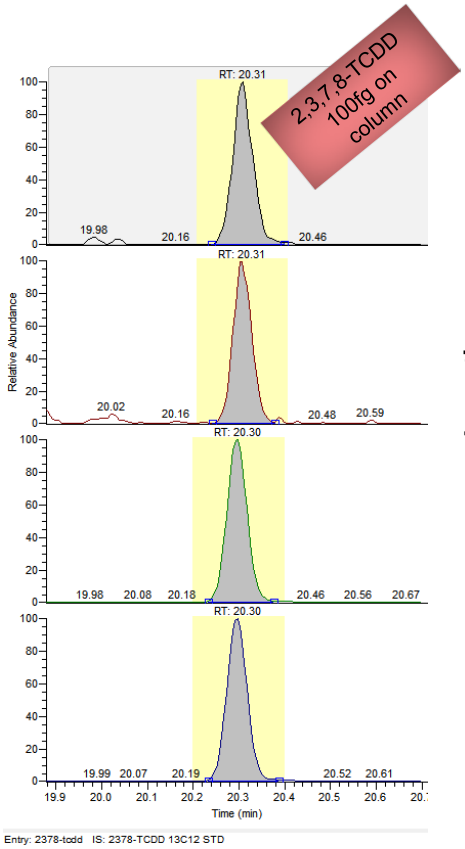
Compound	Concentration range (pg/µL)	Average RF	stdev	RF % RSD
PCB 77	1 - 2000	1.11	0.01	1.0
PCB 81	1 - 2000	1.10	0.01	1.0
PCB 105	1 - 2000	0.98	0.03	2.6
PCB 114	1 - 2000	1.08	0.04	3.7
PCB 118	1 - 2000	1.04	0.01	0.8
PCB 123	1 - 2000	1.01	0.02	1.7
PCB 126	1 - 2000	1.07	0.03	2.7
PCB 156	1 - 2000	1.20	0.02	1.4
PCB 157	1 - 2000	1.15	0.03	2.8
PCB 167	1 - 2000	1.22	0.07	5.6
PCB 169	1 - 2000	1.00	0.12	2.5
PCB 189	1 - 2000	1.01	0.04	4.3

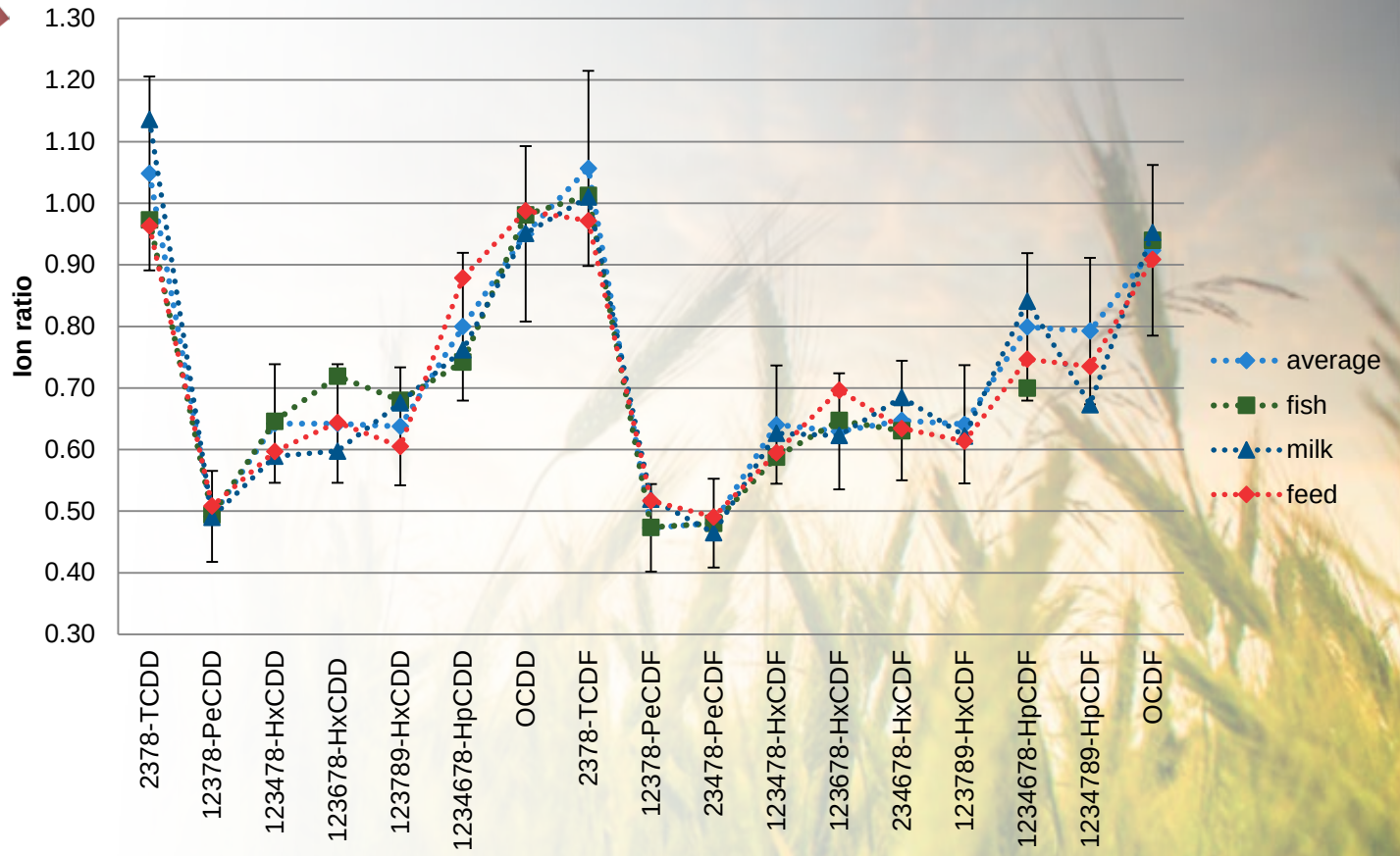
All RFs are well with the limits defined by US EPA 1613 (±15%)

Linearity / Calibration (6 point)

Compound	Concentration range (pg/µL)	Average RF	stdev	RF % RSD
2378-TCDF	0.1 - 40	1.04	0.02	1.9
2378-TCDD	0.1 - 40	1.12	0.02	2.2
12378-PeCDF	0.5 - 200	1.01	0.02	1.5
23478-PeCDF	0.5 - 200	1.03	0.02	1.6
12378-PeCDD	0.5 - 200	1.08	0.01	1.4
123478-HxCDF	0.5 - 200	1.03	0.01	1.2
123678-HxCDF	0.5 - 200	1.02	0.01	1.4
234678-HxCDF	0.5 - 200	1.06	0.03	3.2
123478-HxCDD	0.5 - 200	0.96	0.01	1.6
123678-HxCDD	0.5 - 200	1.21	0.04	3.6
123789-HxCDD	0.5 - 200	1.21	0.04	3.6
123789-HxCDF	0.5 - 200	1.36	0.11	0.8
1234678-HpCDF	0.5 - 200	1.06	0.02	1.7
1234678-HpCDD	0.5 - 200	1.07	0.02	2.1
1234789-HpCDF	0.5 - 200	1.12	0.02	2.2
OCDD	1.0 - 400	1.54	0.04	2.4
OCDF	1.0 - 400	1.09	0.03	3.2

Ion Ratio Abundance – PCDD/Fs

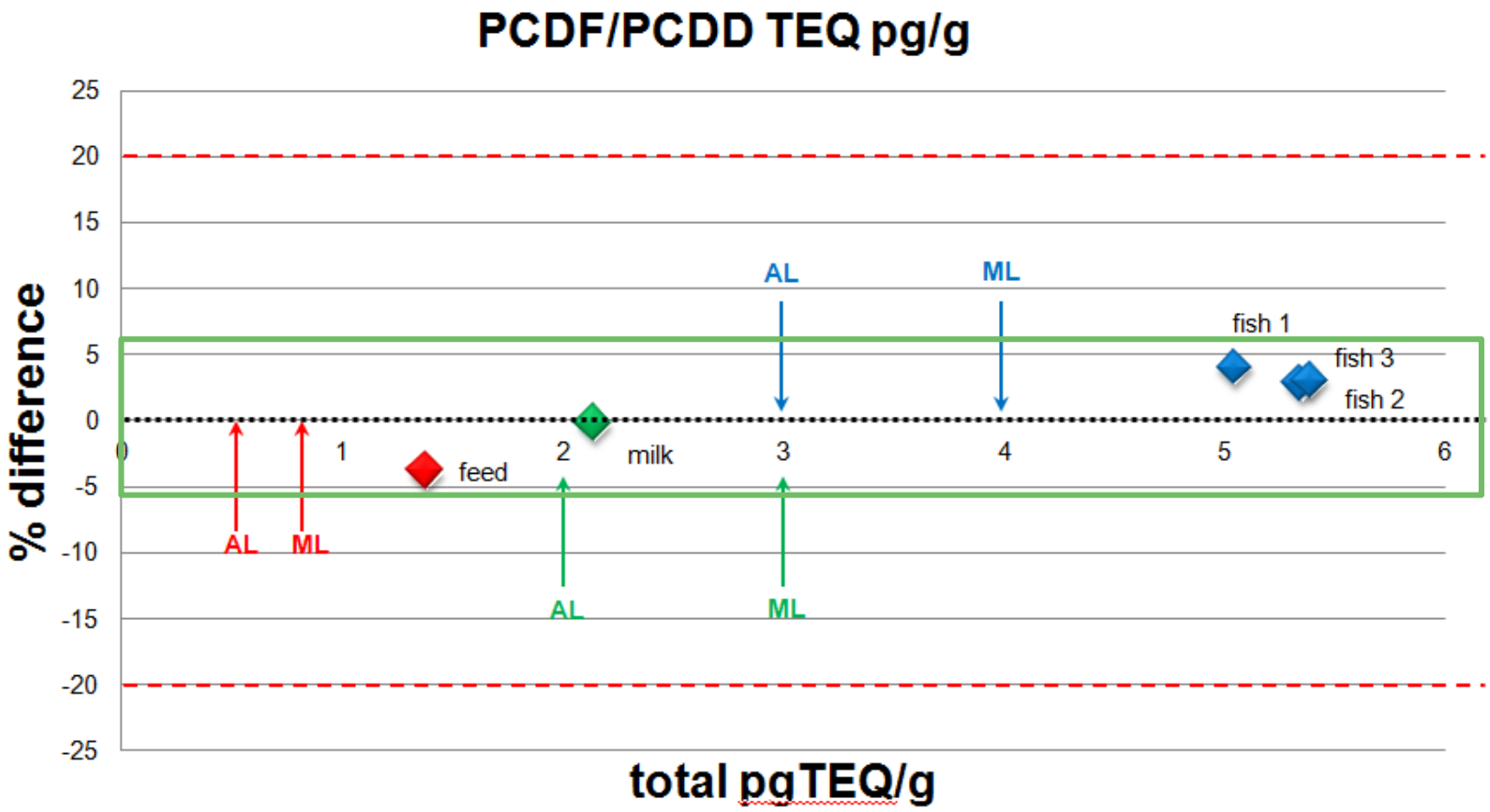




All ion ratios were within ±15% tolerance – meeting EU criteria

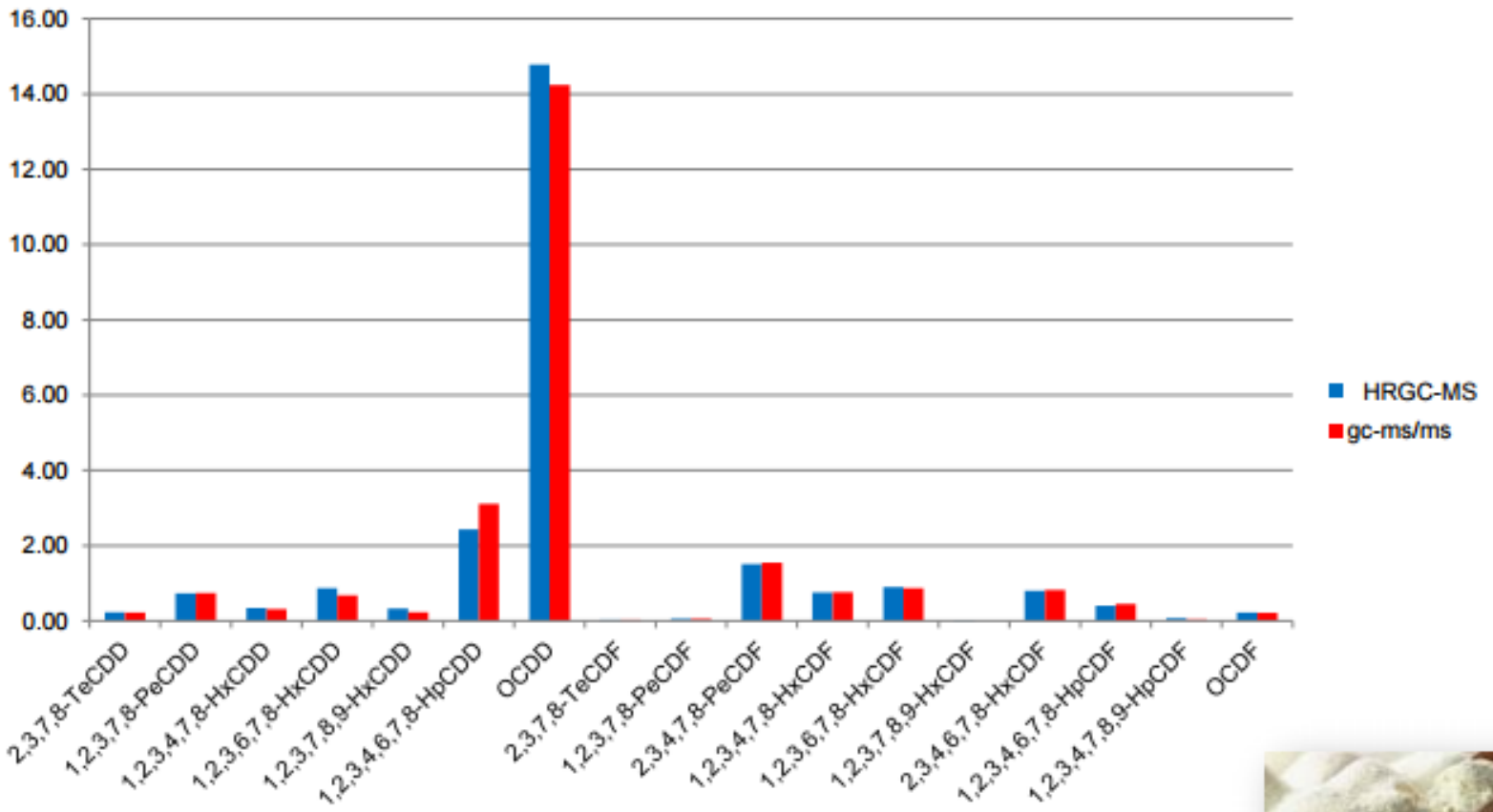
ThermoFisher SCIENTIFIC

Agreement with GC-HRMS (PCDD/Fs)



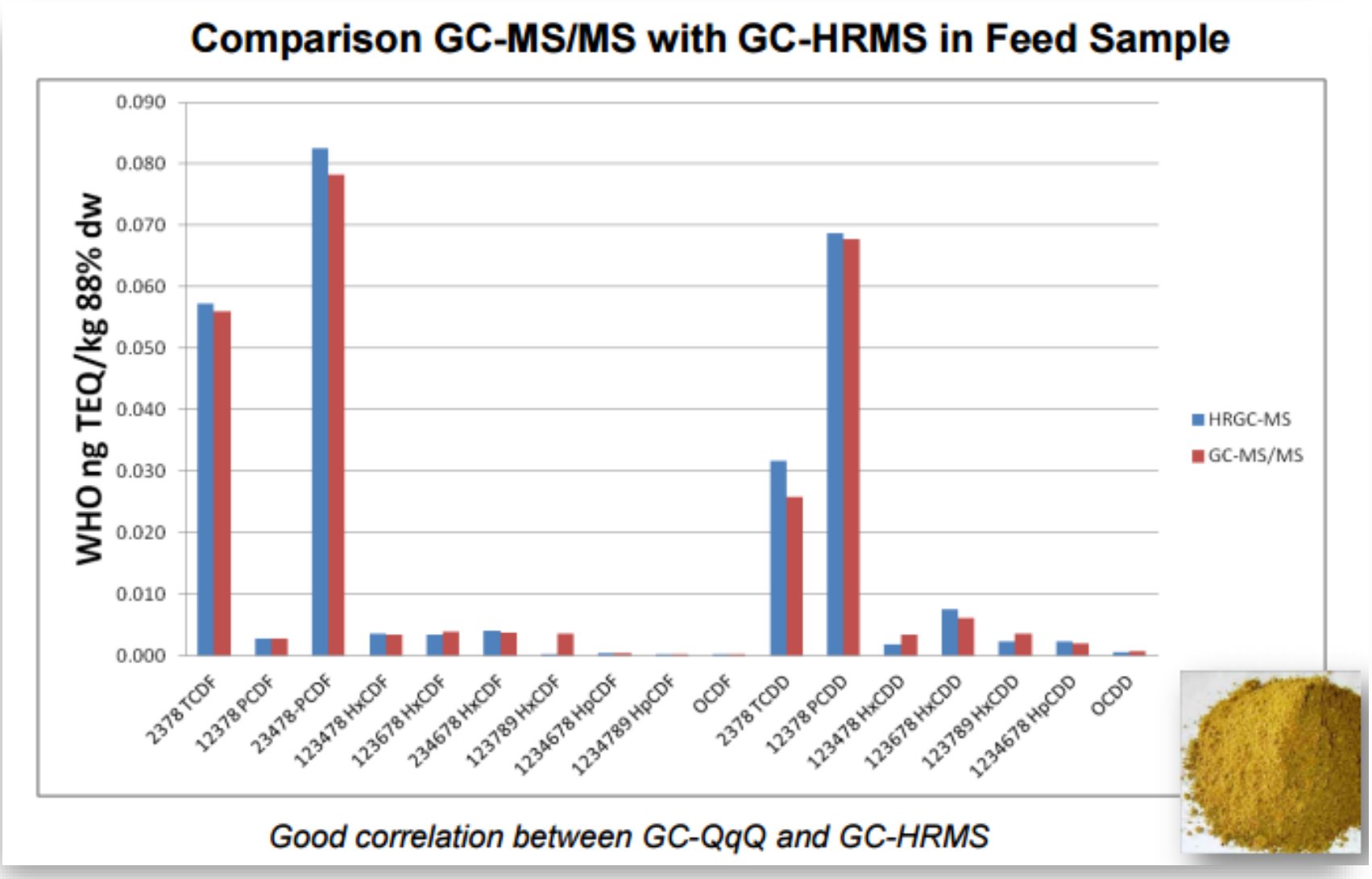
<5% difference between total dioxin concentration (WHO-PCDD/F-TEQ pg/g)

Determination of DioxinFurans in Milk Powder



ThermoFisher SCIENTIFIC

Determination of DioxinFurans in Animal Feed

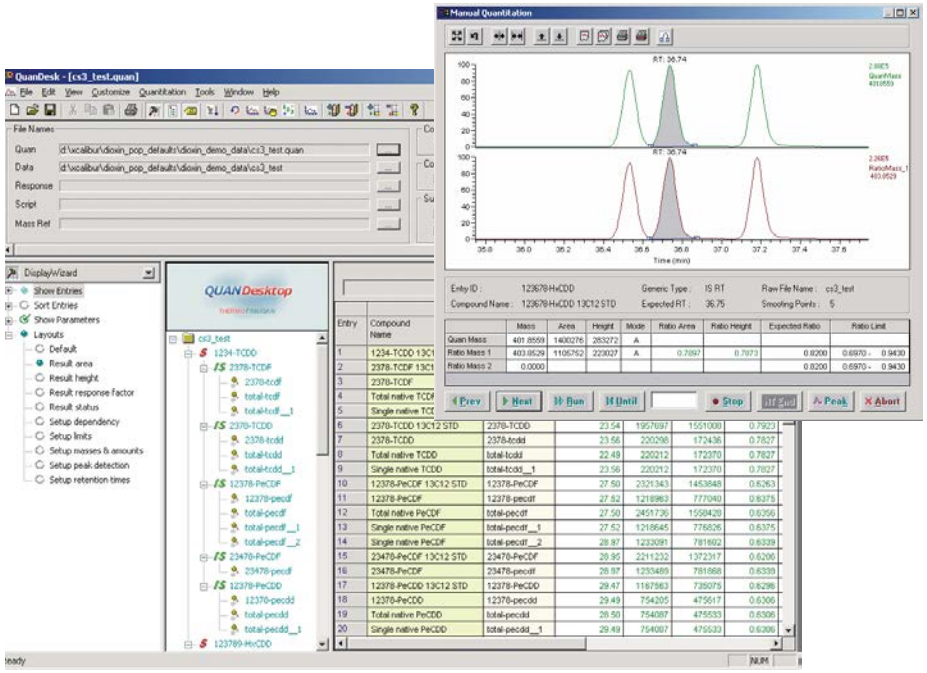


ThermoFisher SCIENTIFIC

TargetQuan – POPs Quantitation for TSQ and DFS

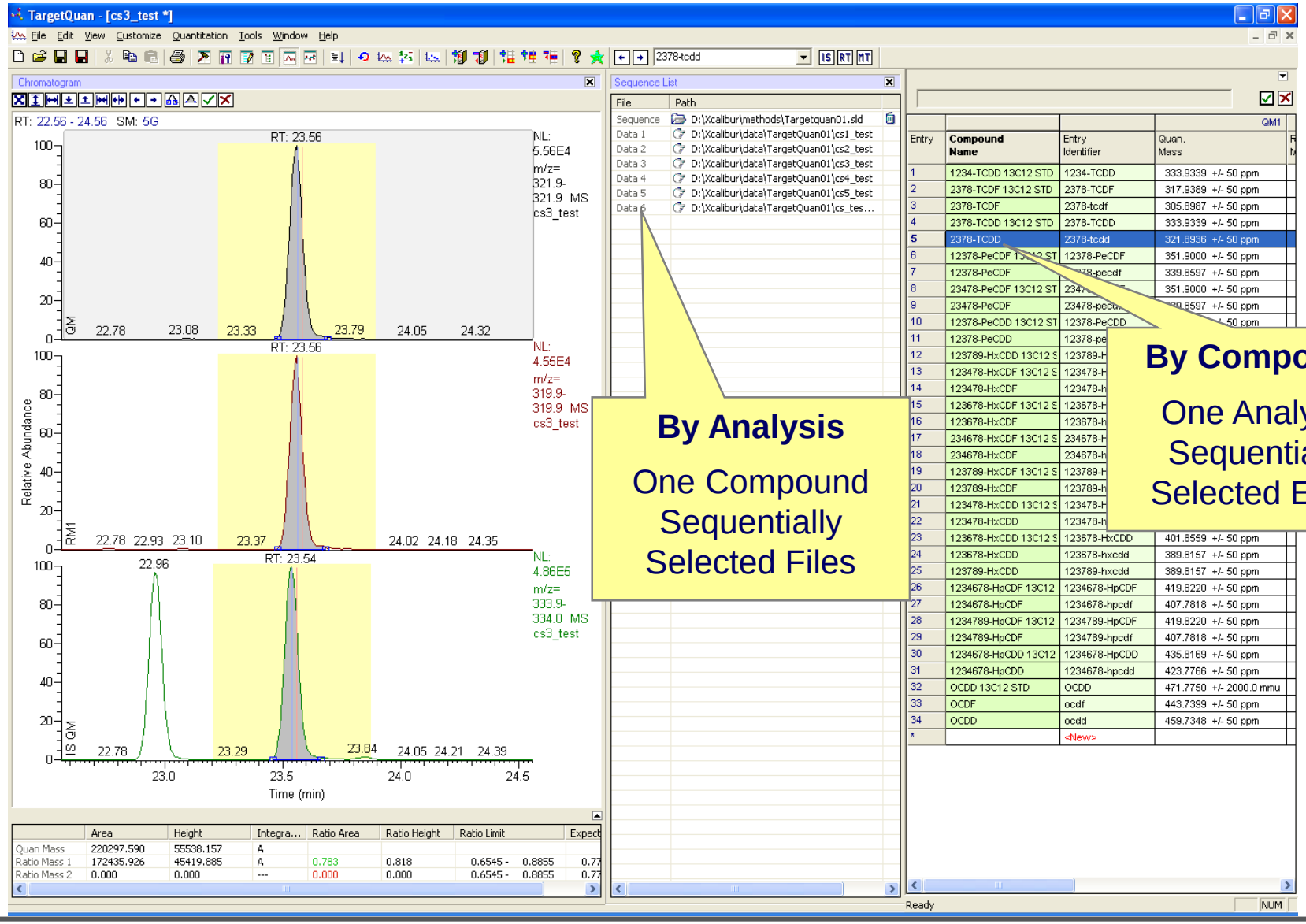
Features for dioxin applications:

- Toxicity equivalents (TEQ)
  - according to WHO definition
  - including lower, medium and upper boundary calculation
- User definable summation
  - Of calculated amounts or TEQs for reporting of sum TEQ values
- Ion ratio confirmation
  - One quantitation mass and up to two masses based on abundance
- EU and EPA 1613, 1668 compliant
  - Allows quantification based on average response of selected compound Retention time correction



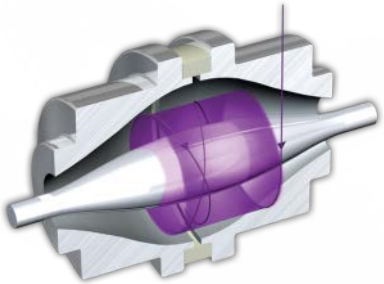
ThermoFisher SCIENTIFIC

TargetQuan – POPs Quantitation for TSQ and DFS



ThermoFisher SCIENTIFIC

Integration of 3 highly successful technologies



Orbitrap mass analyzer technology

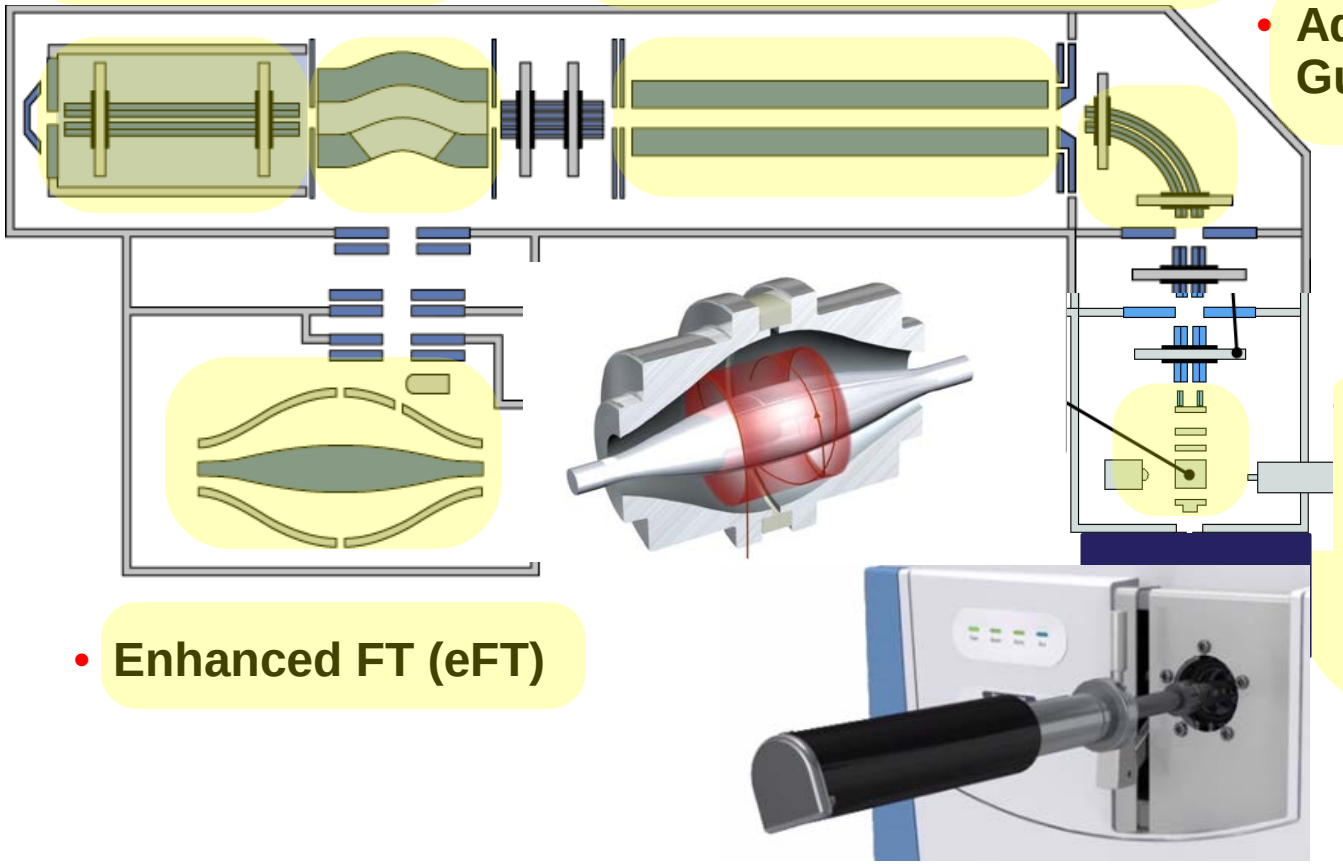
Incredible HR/AM performance

Highly regarded QExactive platform

ThermoFisher SCIENTIFIC

Q Exactive GC Mass Spectrometer: Hardware Innovations

- **HCD collision cell**
- **Quadrupole mass filter with Advance Quadrupole Technology (AQT)**
- **Advanced Active Beam Guide (AABG)**



- **Enhanced FT (eFT)**

ExtractaBrite EI/CI source

- Patented RF lens
- RF lens is removed with source cartridge without venting
- Replacement of GC Column without venting

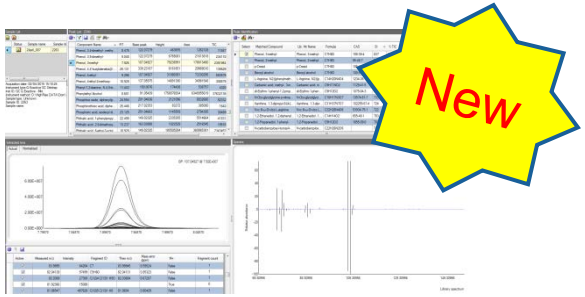
QExactive GC - Application Workflow Capability & Flexibility

Screening

Targeted + Untargeted

Multiple High Resolution Databases

High Resolution Mass Deconvolution



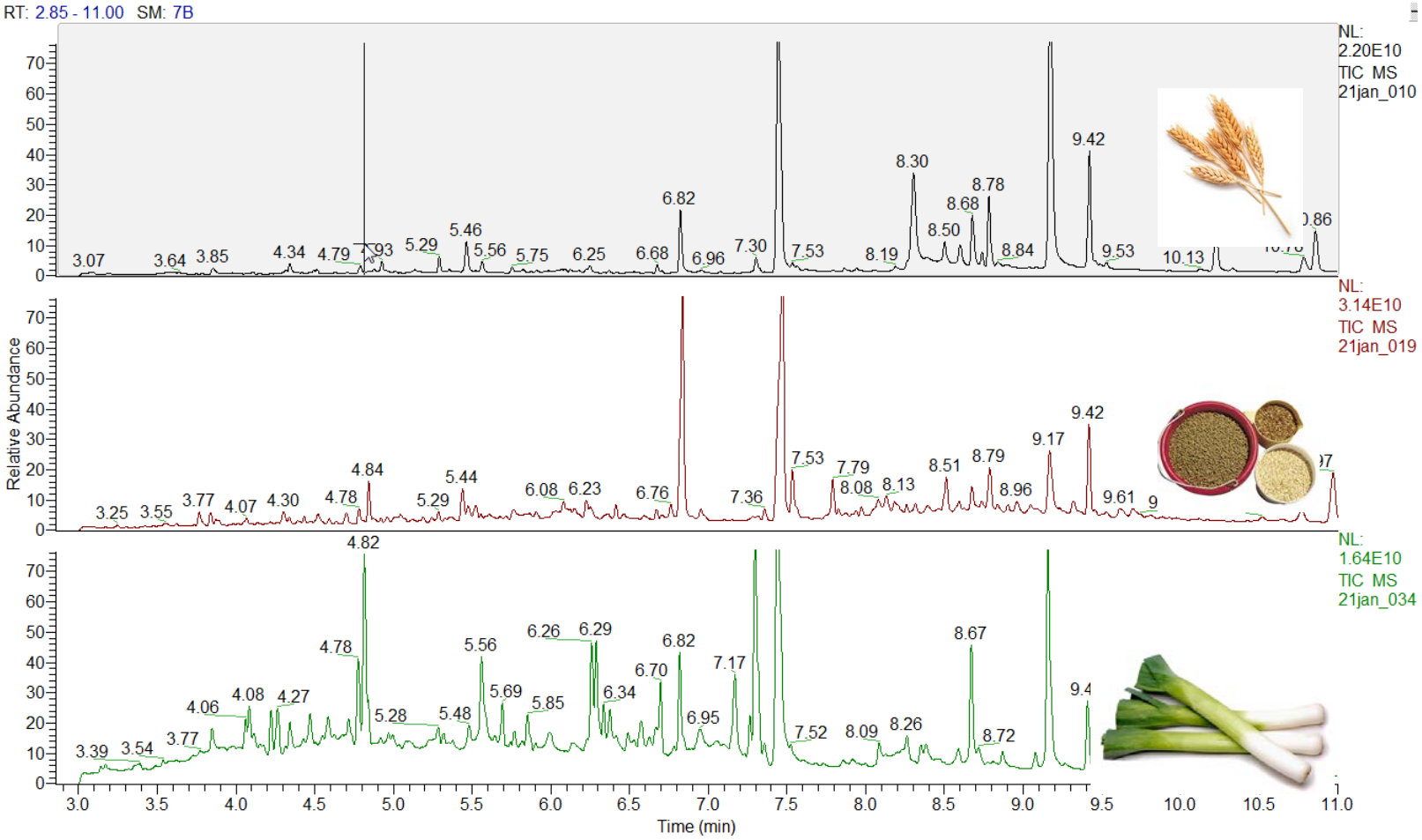
Multiple Libraries

(NIST has over 276,248 spectra
Wiley 719,000 spectra
Wiley MS Designer Drugs, 19,000 spectra
Wiley MS Of Drugs, Poisons, Pesticides
Wiley MS Steroids …)

ThermoFisher SCIENTIFIC

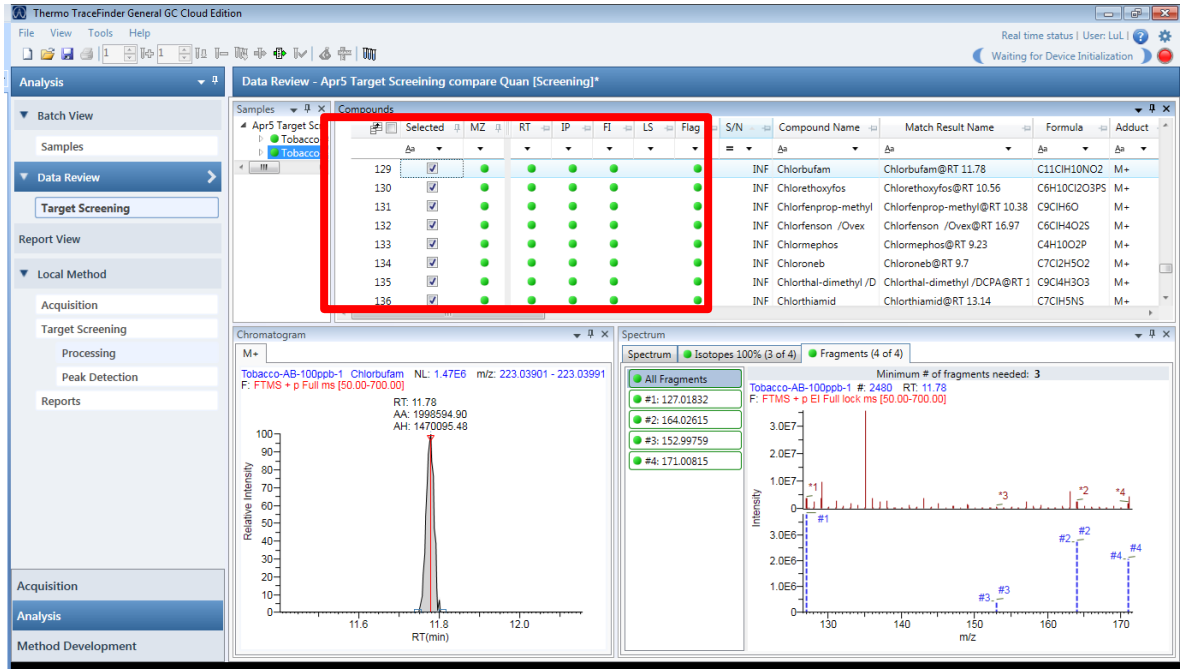
QExactive GC – Full Scan 60,000 RP Chromatography

- Full scan TIC for 55 pesticides spiked into wheat, horse feed and leek extracts.



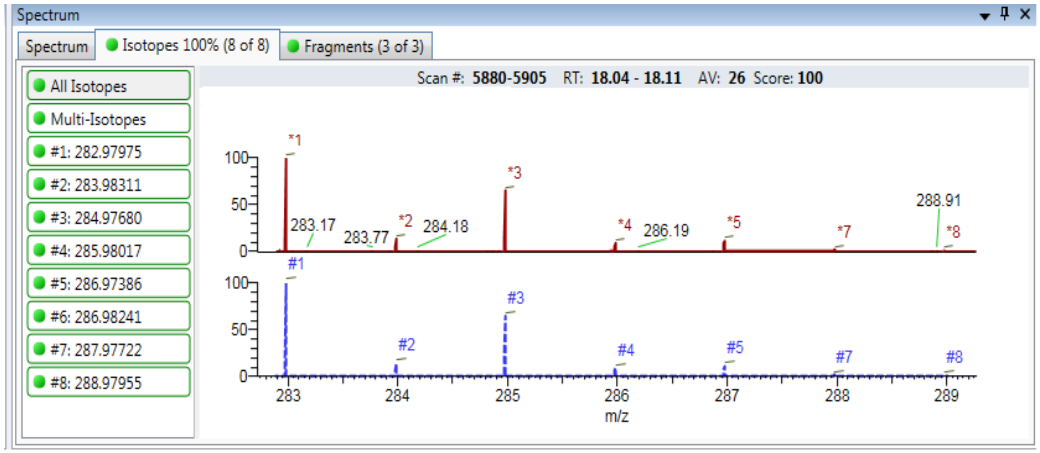
ThermoFisher SCIENTIFIC

Targeted Screening Review – Pesticides Identification



Confirmation Criteria

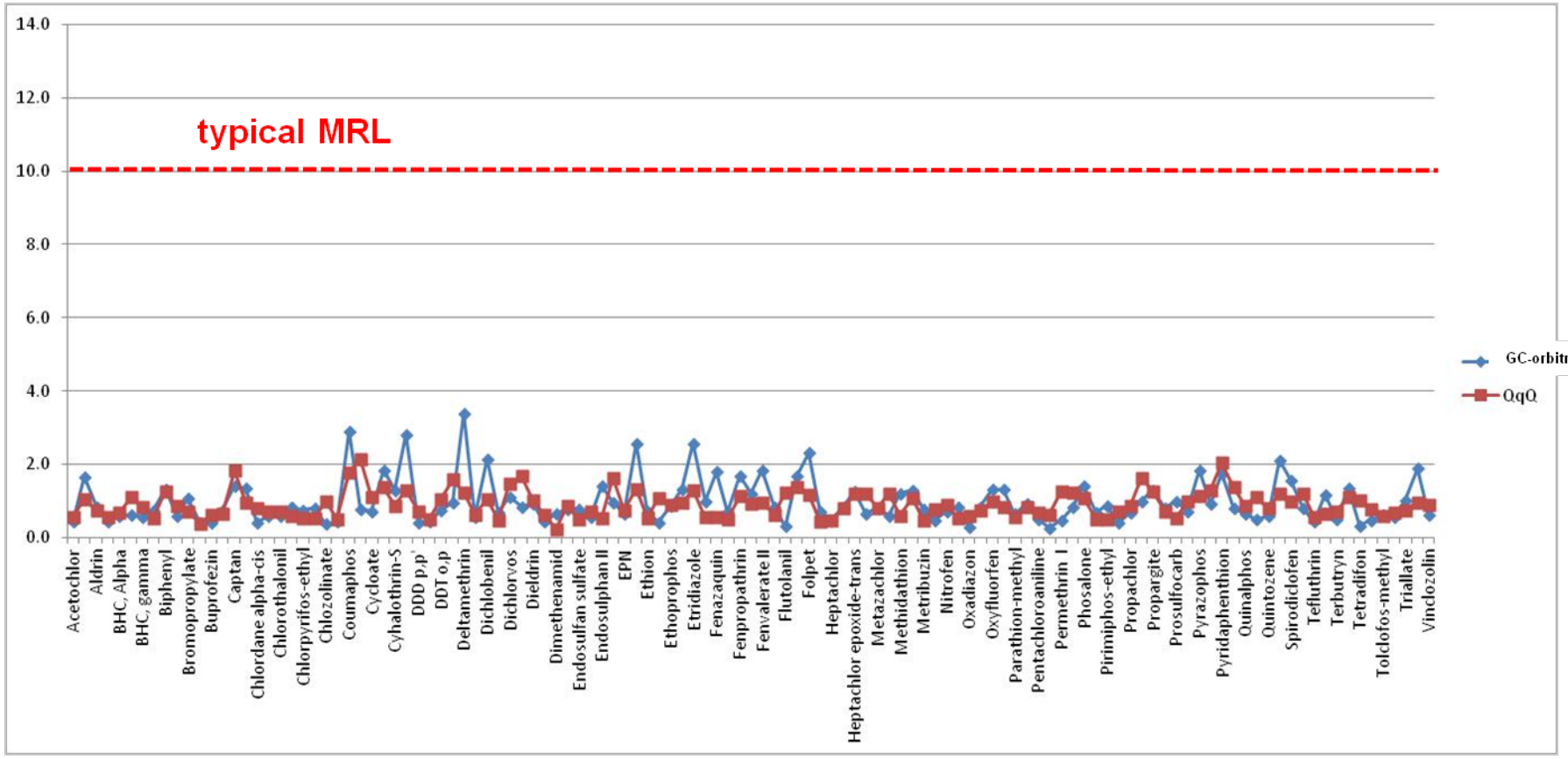
- 2-5 EI Fragment Ions
- Accurate Tolerance < 2ppm
- Retention Time
- Isotopic pattern



ThermoFisher SCIENTIFIC

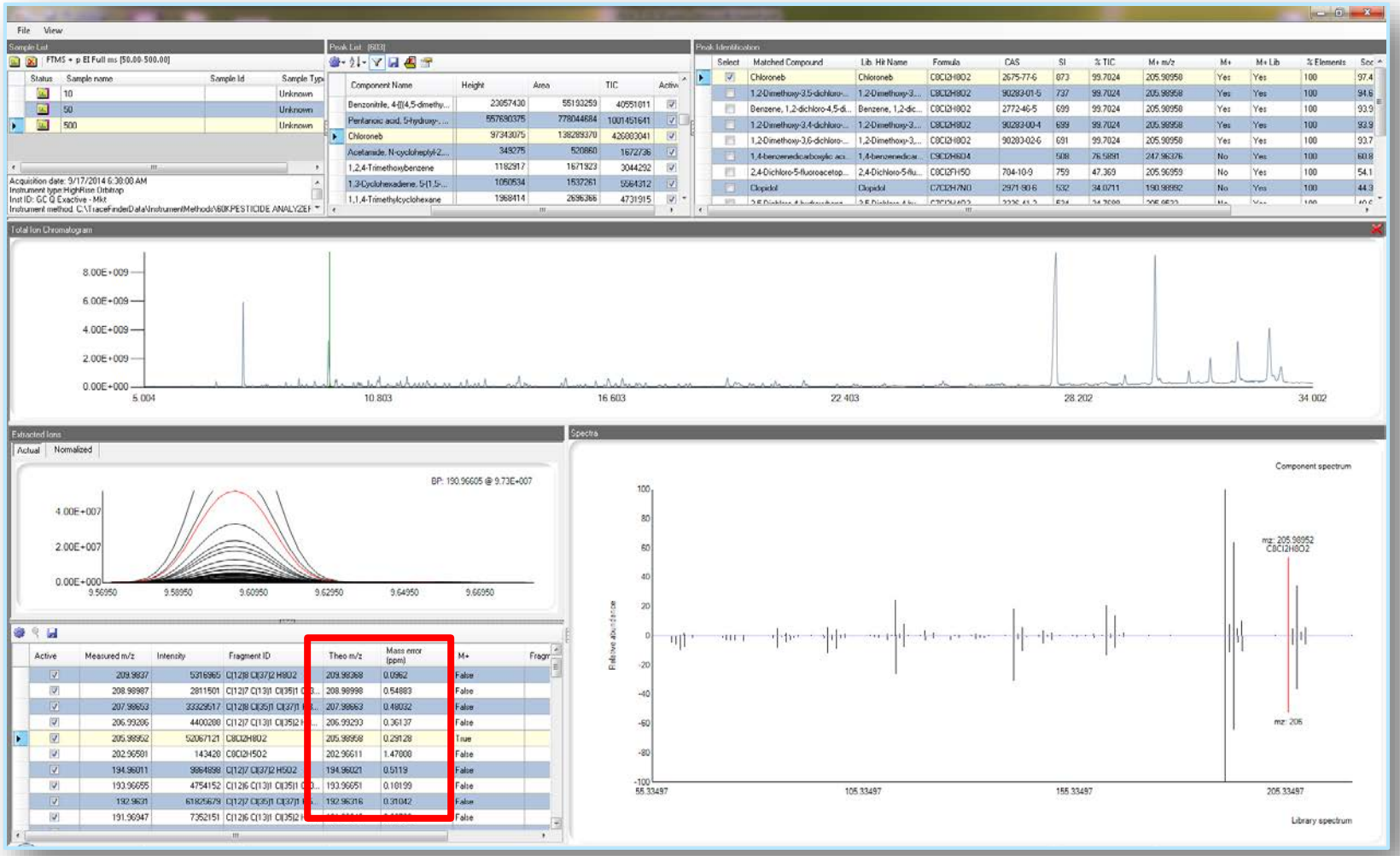
Achieving Pesticides MRL with Full Scan @ 60,000 RP

QE-GC: Full Scan at Resolution of 60,000 Resolving Power
QQQ: SRM



ThermoFisher SCIENTIFIC

EI Spectra Deconvolution & Library Search



Library search compatible with existing unit resolution libraries

ThermoFisher SCIENTIFIC

Summary – Take Away Message

- GC-MS/MS – Cost Efficient Alternative
  - EvoCell technology – High Speed SRM with Precision & Sensitivity
  - Removable ExtractaBrite™ Ion Source - easy maintenance
  - Viable and cost efficient alternative for food & feed analysis
  - Fast - with high throughput capability, automated runs

- GC-HRMS – The GOLD Standard Confirmation Solution
  - DFS Magnetic Sector - Compliant with international regulations
  - QExactive GC - New benchmark for powerful unknown compound identification for GC-MS, including POPs analysis
  - Highest sensitivity and precsion for low level analyses

- GC-MS/MS + GC-HRMS – Work Seamlessly Together
  - High precision data using labelled internal standards
  - Common TargetQuan software platform + LIMS sample manager