Prague, 29th -30th April 2015

New and emerging techniques in POPs analysis

Esteban Abad

Laboratory of Dioxins, Environmental Chemistry Department, IDÆA (CSIC)

Jordi Girona 18-26, 08034 Barcelona, Spain

10th International Symposium on Recent Developments in POPs Analysis

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M. Ábalos, D. Pemberthy, J. Sauló, E. Abad

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Previous Presentations

19th International Mass Spectrometry Conference

IMSC2012 KYOTO

Kyoto, 15th -23th September 2012

They were shown the following objectives:

- To show some new perspectives in the mass spectroscopy determination of dioxin-like substances in environmental and food samples
- To evaluate suitability of GC-QqQ(MS/MS) for the analysis of dl-POPs
- Comparison with reference techniques (GC-HRMS)

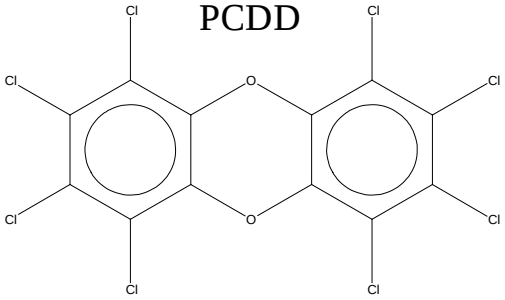
New Perspectives in the mass spectroscopy determination of dioxin-like substances in environmental and food samples

A. García-Bermejo, M. Ábalos, L. Mattioli, M.J. González, B. Gómara, E. Abad

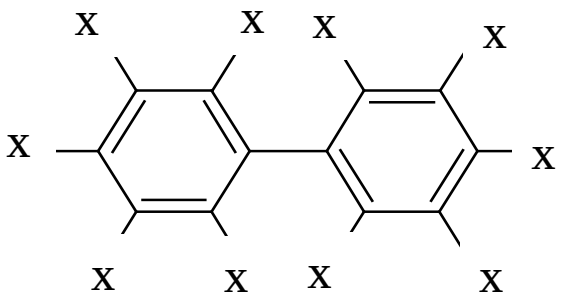
What are POPs?

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

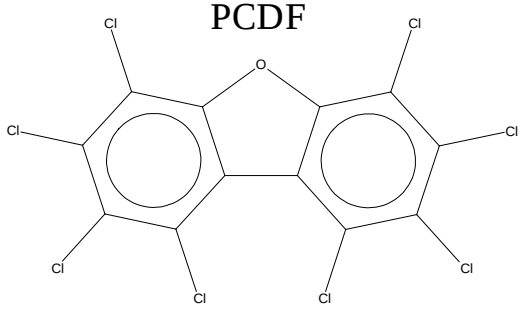
PCDD



PCB



PCDF



“Incidents” – Serious Economic Impacts

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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

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Page last updated at 17:11 GMT, Friday, 28 March 2008

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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

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Irish pork products recalled after dioxin is found

Published: Sunday, December 7, 2008

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From The Times

December 8, 2008

Contaminated pork from Ireland: your questions answered

Serious Impact – Global Treaty and Regulation

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

Serious Impact – Global Treaty and Regulation

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.

COMMISSION REGULATION (EU) No 589/2014 (2 June 2014)

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).

The POPs Analysis Workflow in the Lab

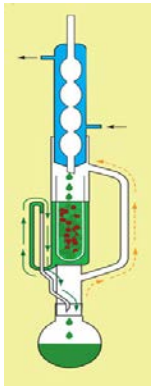
Confirmatory Scheme

Sample Preparation

Food, Feed for PCDD/Fs, PCBs, OCPs, BFRs, Toxaphene, etc.

Extraction

Soxhlet



ASE

Cleanup

FMS PowerPrep or manual clean up

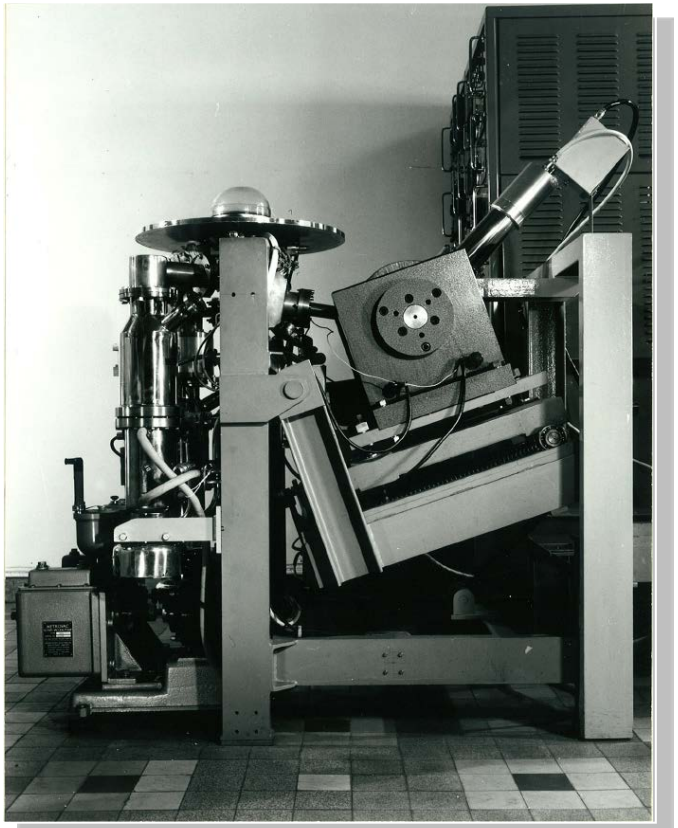
GC-HRMS

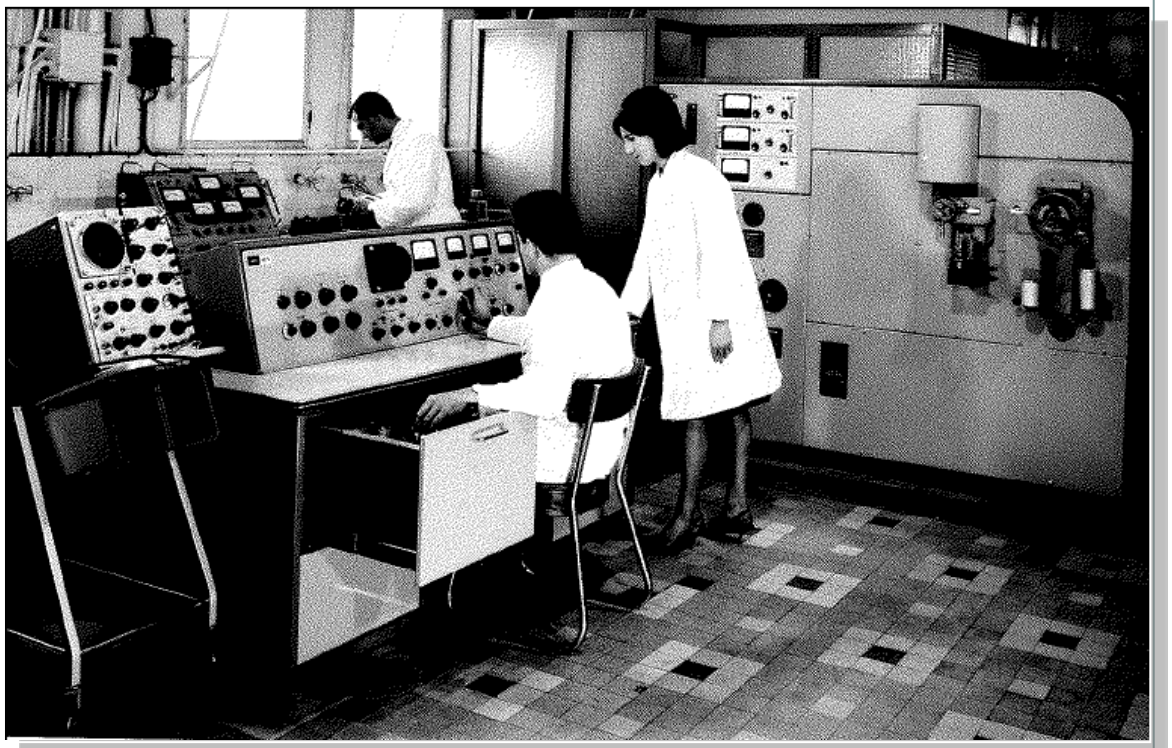
Analysis and final TEQ Calculation

GC-MS/MS

HRMS-Magnetic Sector Analyzer

Confirmation technique





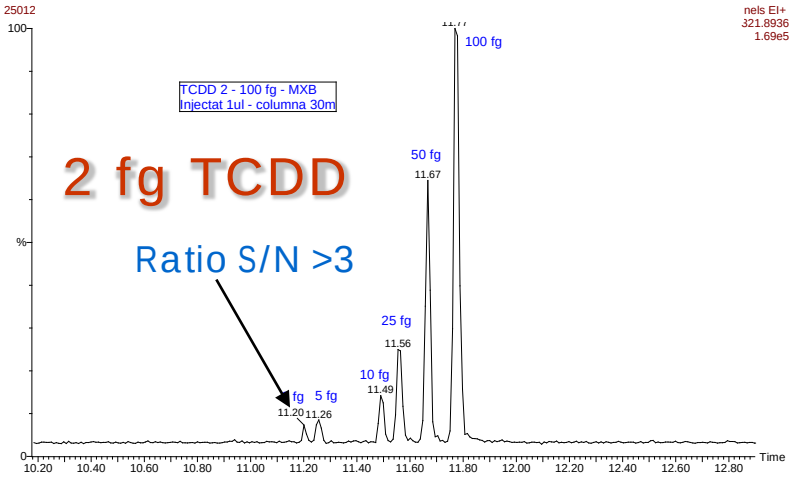
París, 1964 -1969

Two patents:
- Housing source on the top position
- Magnet Radius < 8cm
- Special magnetic sector design

HRMS-Magnetic Sector Analyzer

Thermo Scientific™ DFS™ HR MS - Gold Standard Sensitivity

- Includes a **D**ouble **F**ocusing magnetic **S**ector (DFS) MS, latest technology in magnetic sector high resolution GC/MS.
- DFS offers excellent analytical performance for POPs analysis: Sensitivity, Robustness, Throughput, Flexibility.
- In use for routine analysis in leading dioxin expert labs all over the world.



HRMS-Magnetic Sector Analyzer

Screening methods may comprise bioassays and GC/MS methods; confirmatory methods are high-resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) methods. Following criteria have to be complied with on total TEQ value:

- High gas chromatography coupled to resolution mass spectrometry (HRGC-HRMS)
- Isotopic dilution method for quantification

Requirements
•Investment
•Expertise
•……

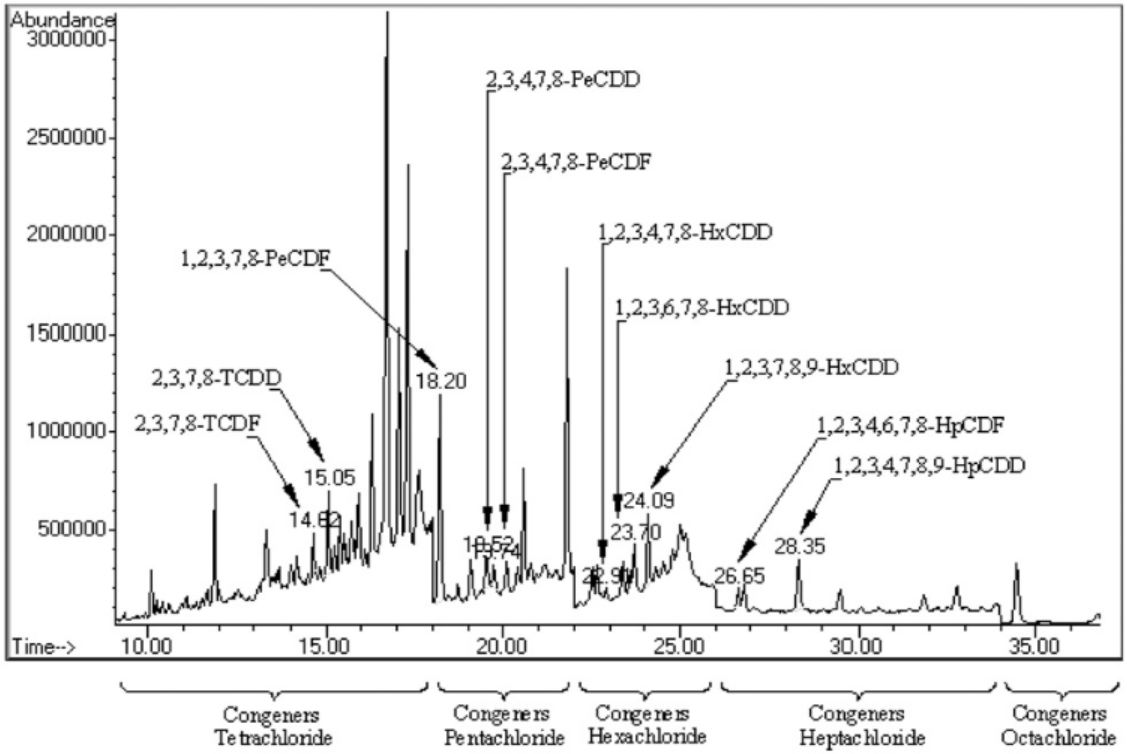
- ✓ GC-ECD
- ✓ The 90's: GC-MS (quadrupole)
- ✓ Early 2000's: GC-ITD(MS/MS)
- ✓ Other…..
- ✓ 2010 – nowadays: GC-QqQ(MS/MS)

Low Resolution Mass Spectrometry

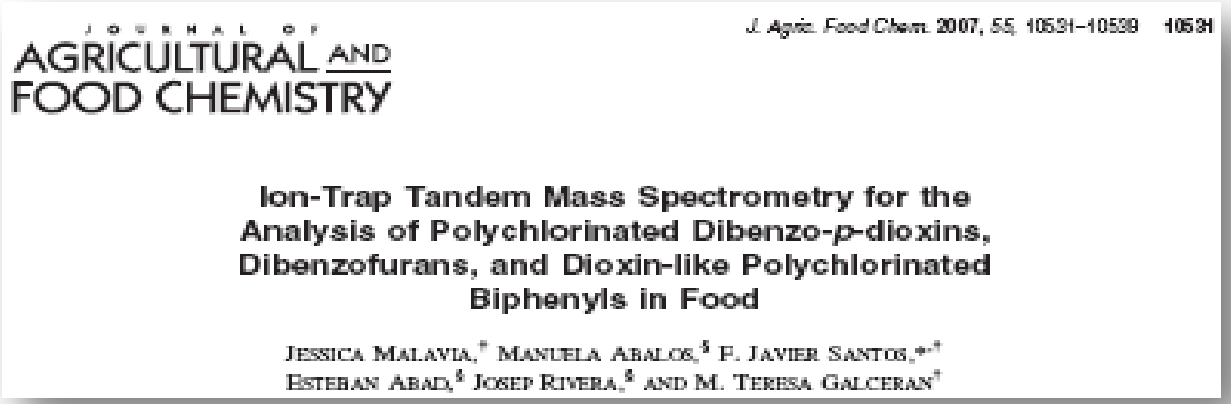
Dioxins in beef samples from Mexico using a low resolution GC/MS screening method

Lidia Naccha[ab], Guadalupe Alanis[b], Anabel Torres[a], Esteban Abad[c], Manuela Ábalos[c], Josep Rivera[c], Lorenzo Heyer[d], Alberto Morales[e] and Noemí Waksman[a*]

Food Additives and Contaminants: Part B
Vol. 3, No. 1, March 2010, 64–72



Ion-trap Mass Spectrometry

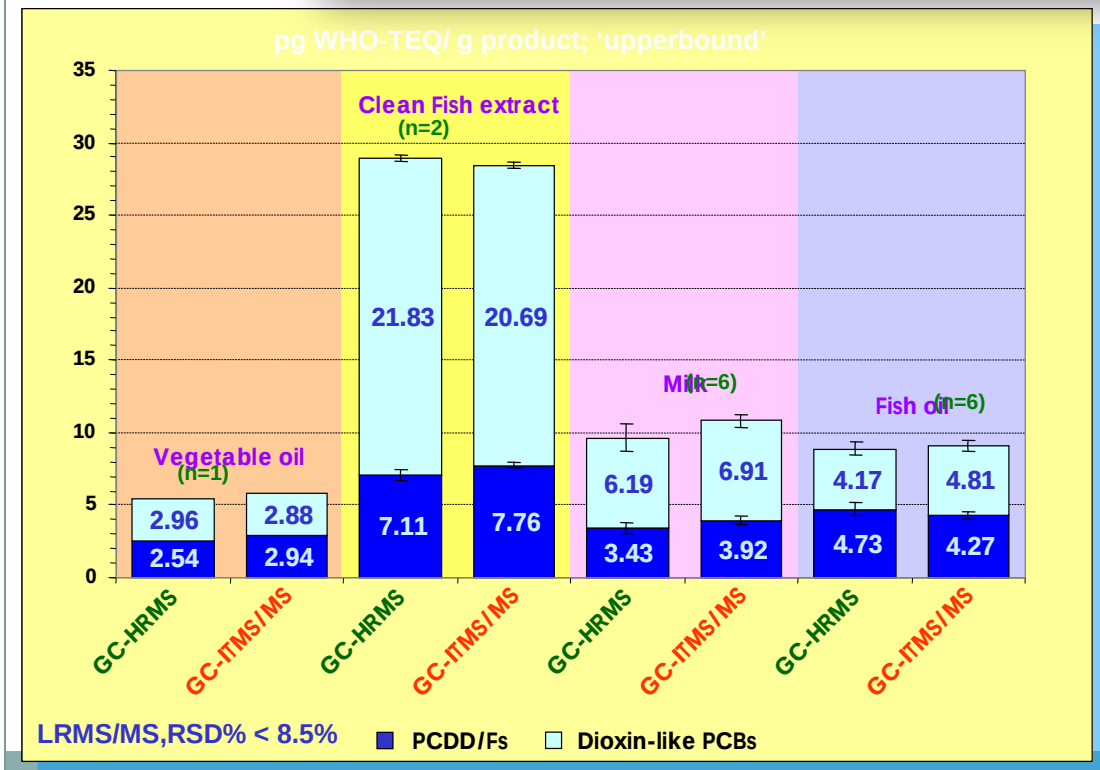


JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. 2007, 55, 10531–10539 10531

Ion-Trap Tandem Mass Spectrometry for the Analysis of Polychlorinated Dibenzo-*p*-dioxins, Dibenzofurans, and Dioxin-like Polychlorinated Biphenyls in Food

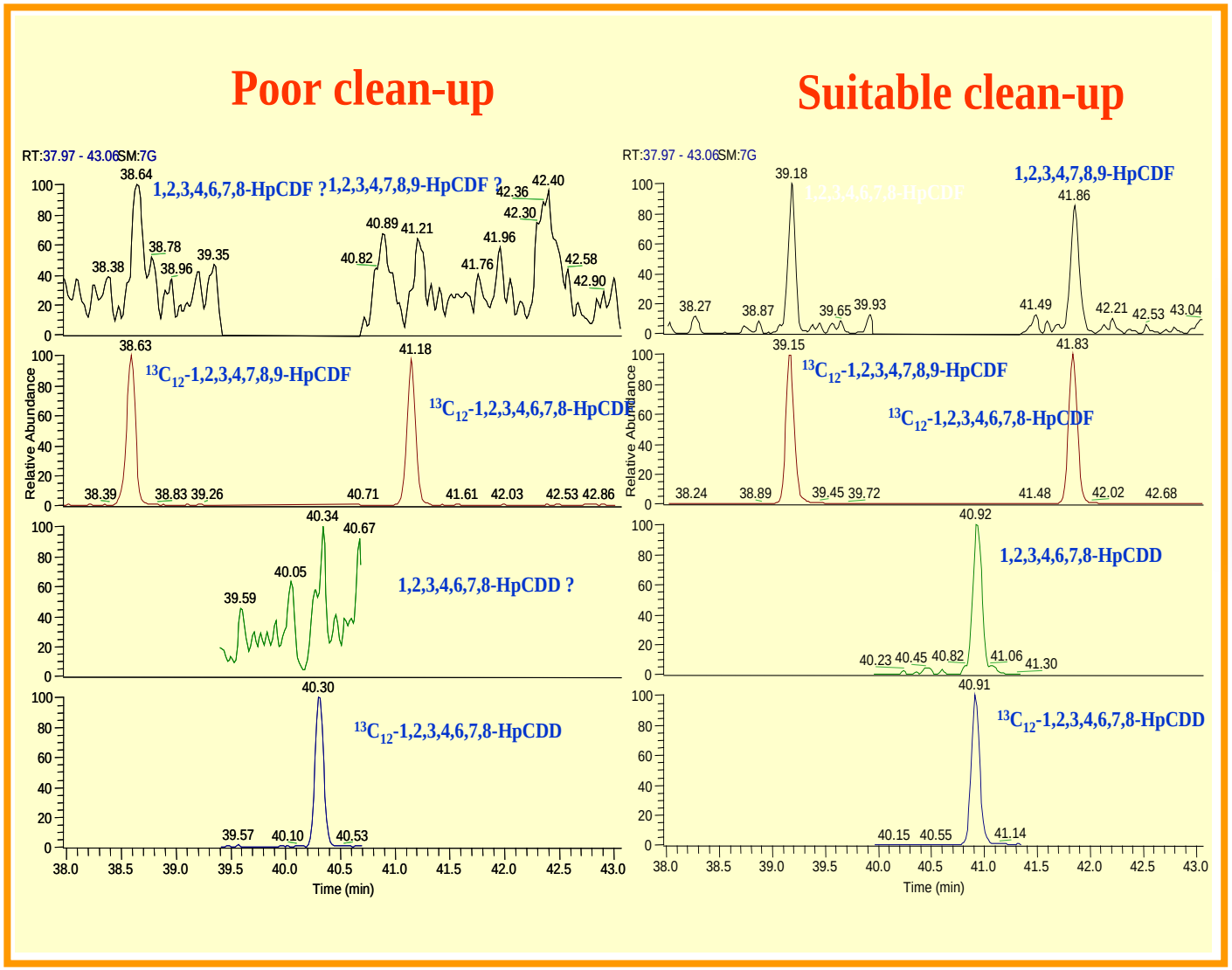
JESSICA MALAVIA,† MANUELA ABALOS,§ F. JAVIER SANTOS,*,† ESTEBAN ABAD,§ JOSEP RIVERA,§ AND M. TERESA GALCERAN†



DIFFERENCE PROJECT

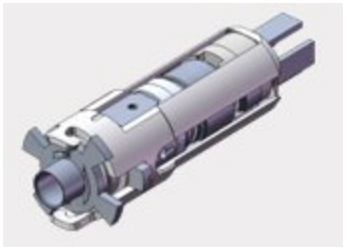
V EU Framework
Coord.. Prof. Jacob de Boer

Ion-trap Mass Spectrometry



Tiple Quadrupole Mass Spectrometry

- 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

Experimental: General conditions

- For all experiments a **GC-MS/MS** coupled with a Thermo Scientific™ TRACE™ 1310 GC was used.
- Sample introduction was performed with a Thermo Scientific™ TriPlus RSH autosampler.
- Data processing was achieved using TargetQuan 3.1 software.

Experimental: Injector & Autosampler

TRACE 1310 GC Parameters	
Injection Volume (mL):	2
Liner	SSL single taper
Inlet (°C):	260
Inlet Module and Mode:	Splitless
Carrier Gas, (mL/min):	He, 1.2
Oven Temperature Program:	
Temperature 1 (°C):	100
Hold Time (min):	2
Temperature 2 (°C):	250
Rate (°C/min)	25
Temperature 3 (°C):	285
Rate (°C/min)	2.5
Temperature 4 (°C):	330
Rate (°C/min)	10
Hold Time (min):	5

Experimental: MS/MS

TSQ 8000 Evo Mass Spectrometer Parameters	
Transfer line (°C):	280
Ionization type:	EI
Ion source(°C):	300
Electron energy (eV):	40
Acquisition Mode:	timed-SRM
Q2 Gas Pressure(argon)(psi):	60
Q1 Peak Width (Da):	0.7
Q3 Peak Width (Da):	0.7

Overview

- To evaluate suitability of GC-QqQ(MS/MS) for the analysis of dl-POPs:
  - Optimizing instrumental conditions for the analysis of PCDD/Fs by GC-QqQ(MS/MS)
  - Analysis in real samples: The matrices were analyzed for their dioxin content:

Dry fish sample (previously used in inter-laboratory studies)

Fish sample (previously used in inter-laboratory studies)

Milk powder sample (reference material)

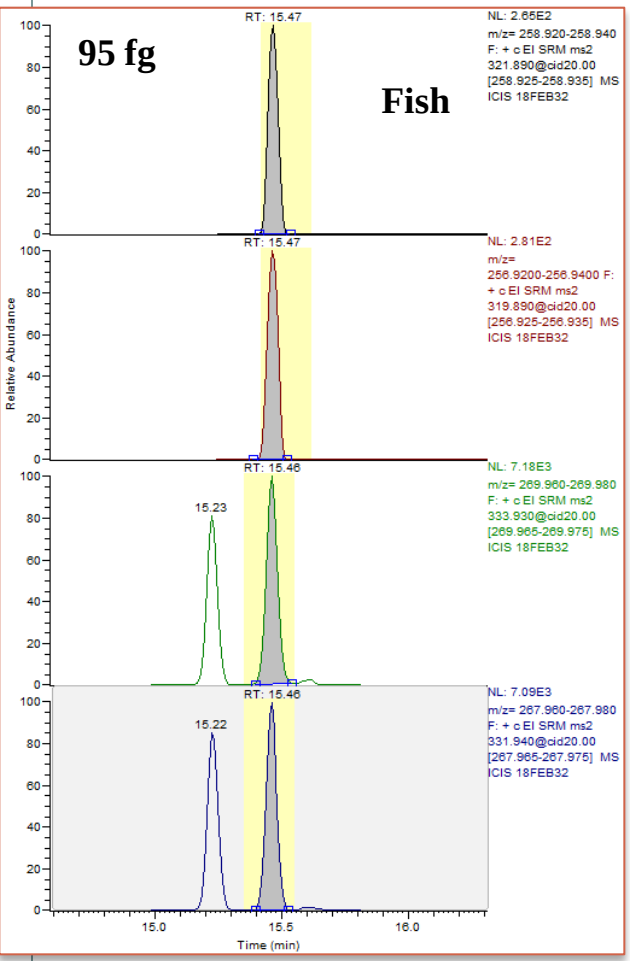
Feed sample (internal reference material)

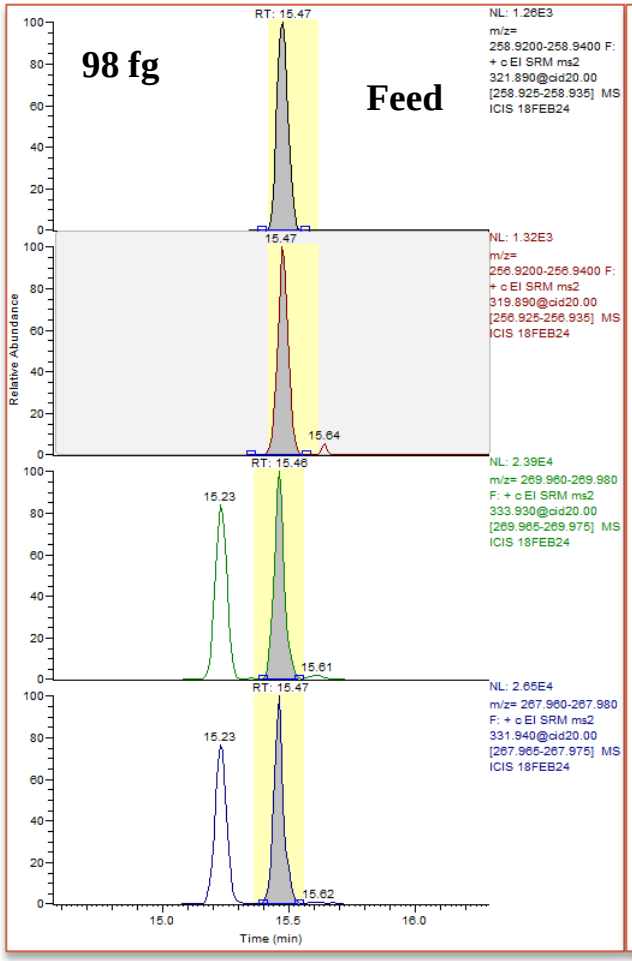
Fly ash sample (certified reference material)

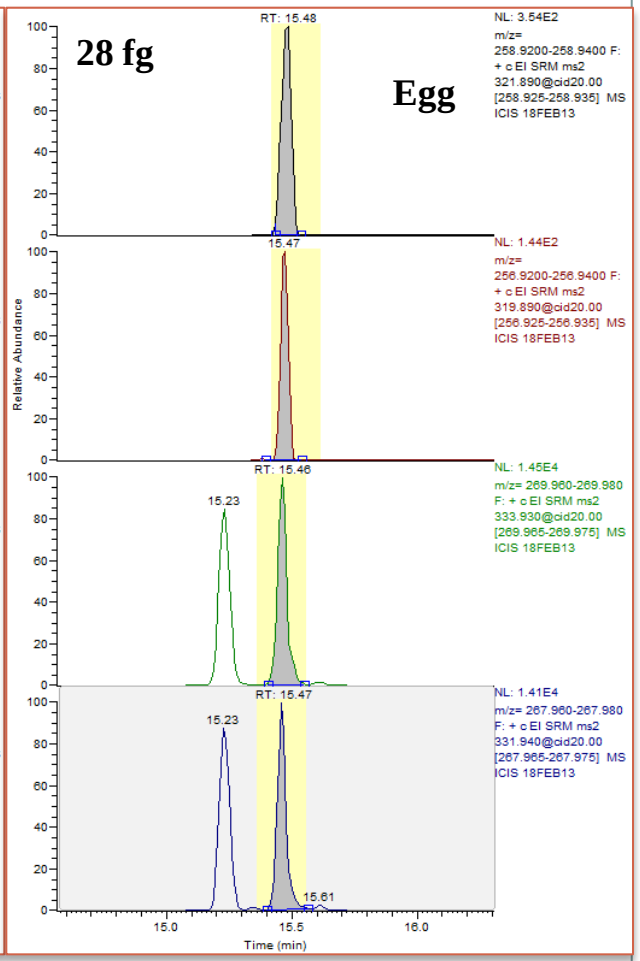
Sewage sludge (certified reference material)

Results:Determination of Dioxins in Sample Extracts

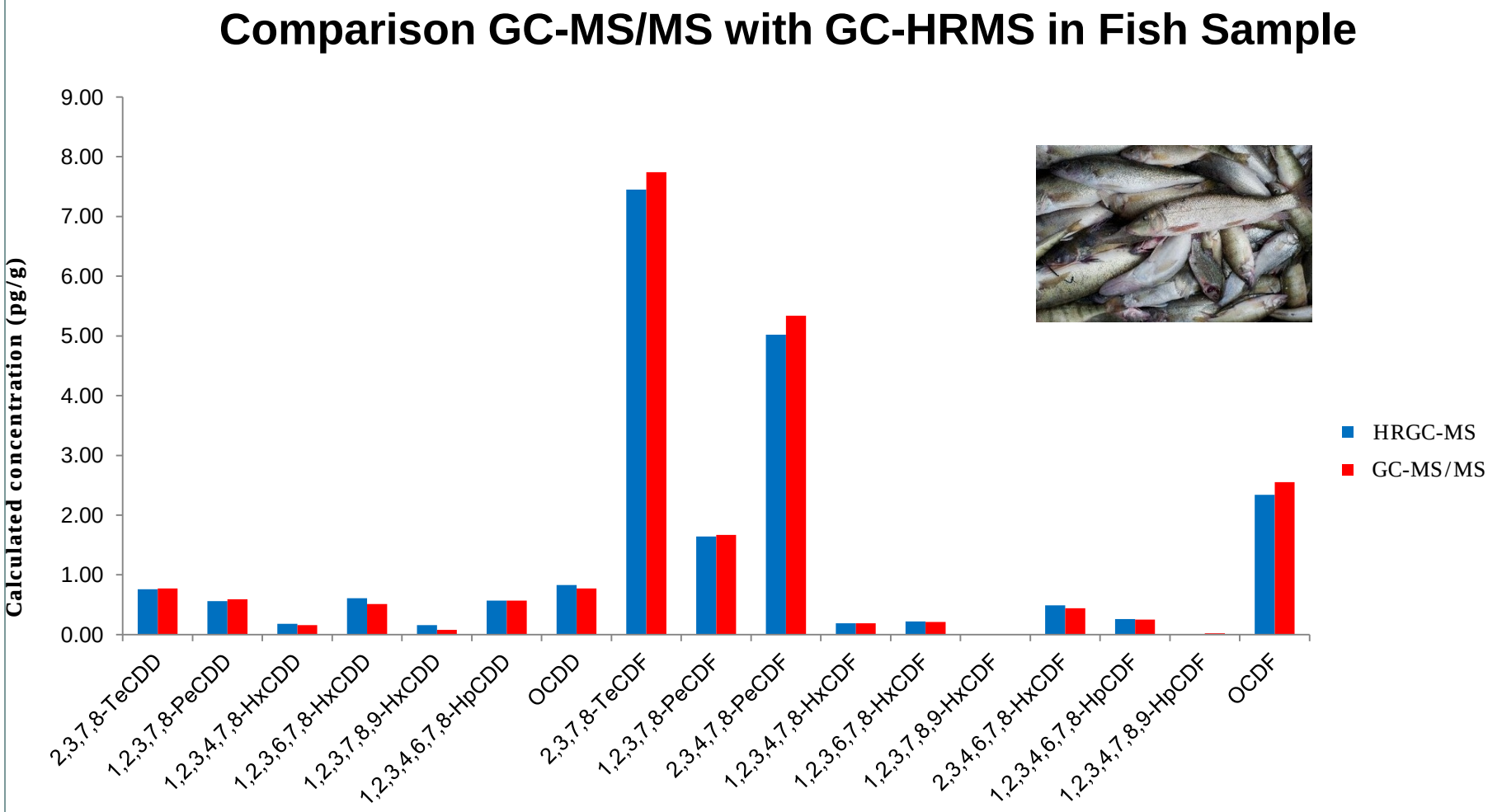
2378-TCDD in Sample Extracts







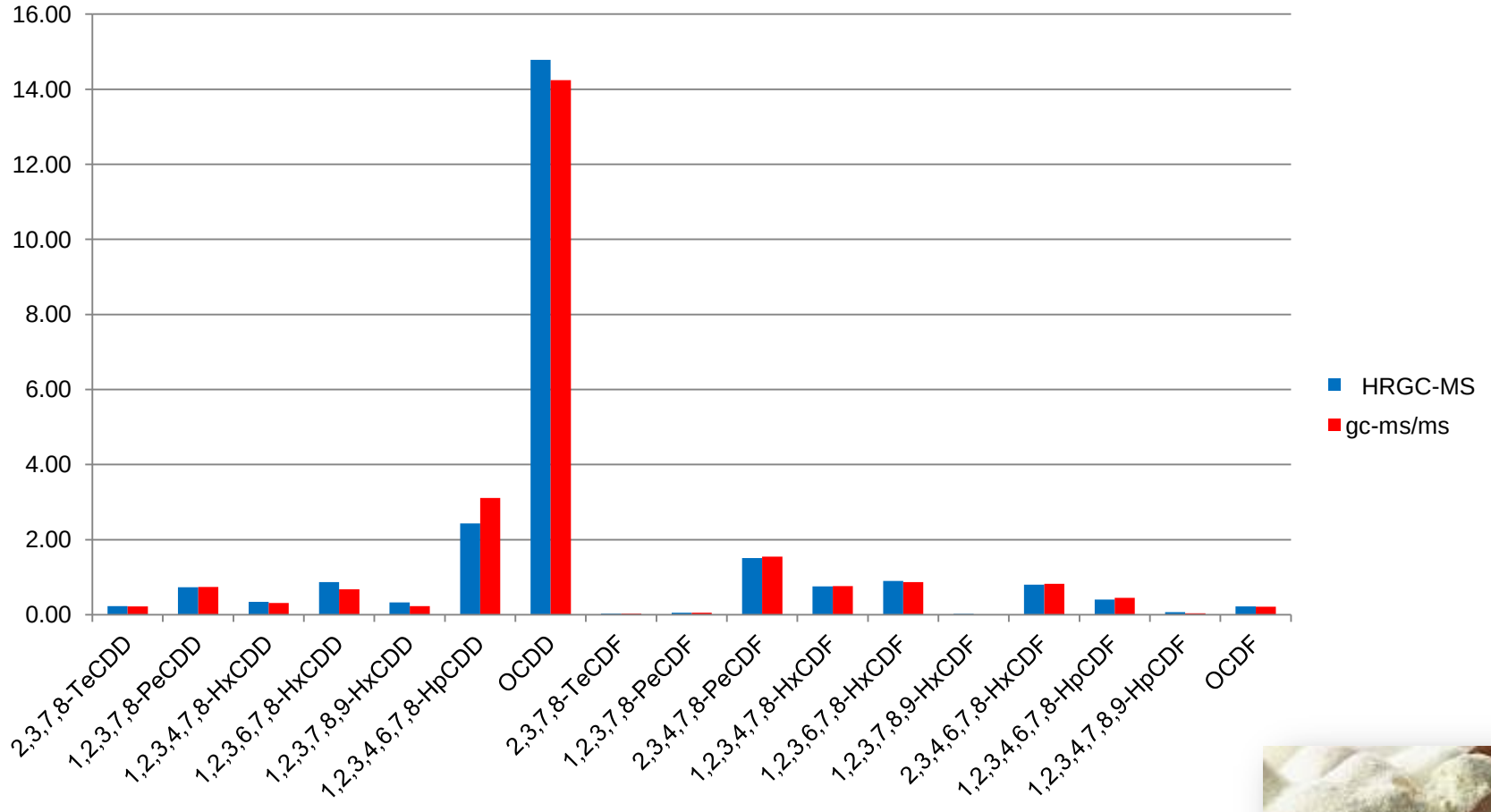
Results:Determination of Dioxins in Sample Extracts



Good correlation between GC-QqQ and GC-HRMS

Results:Determination of Dioxins in Sample Extracts

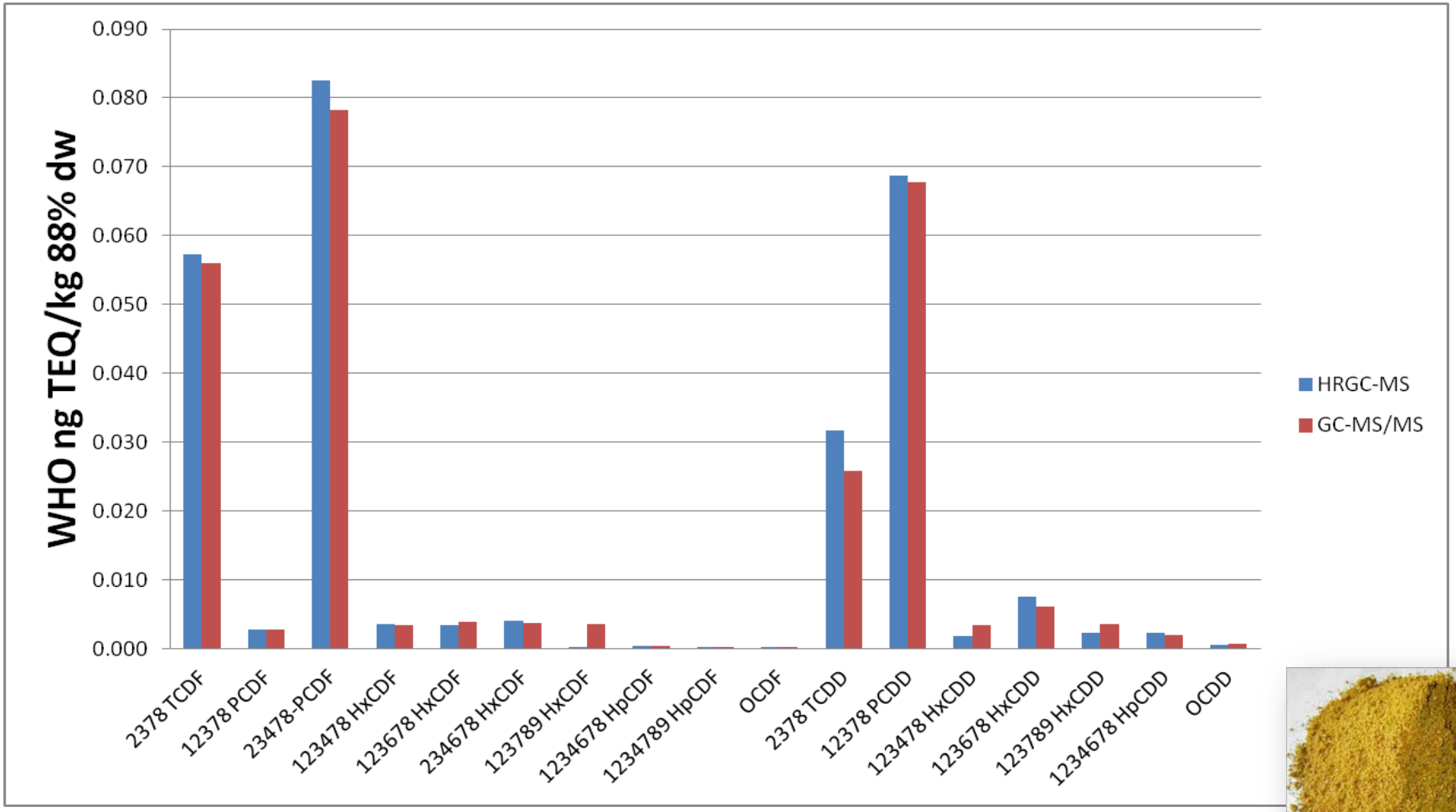
Comparison GC-MS/MS with GC-HRMS in Milk powder Sample



Good correlation between GC-QqQ and GC-HRMS

Results:Determination of Dioxins in Sample Extracts

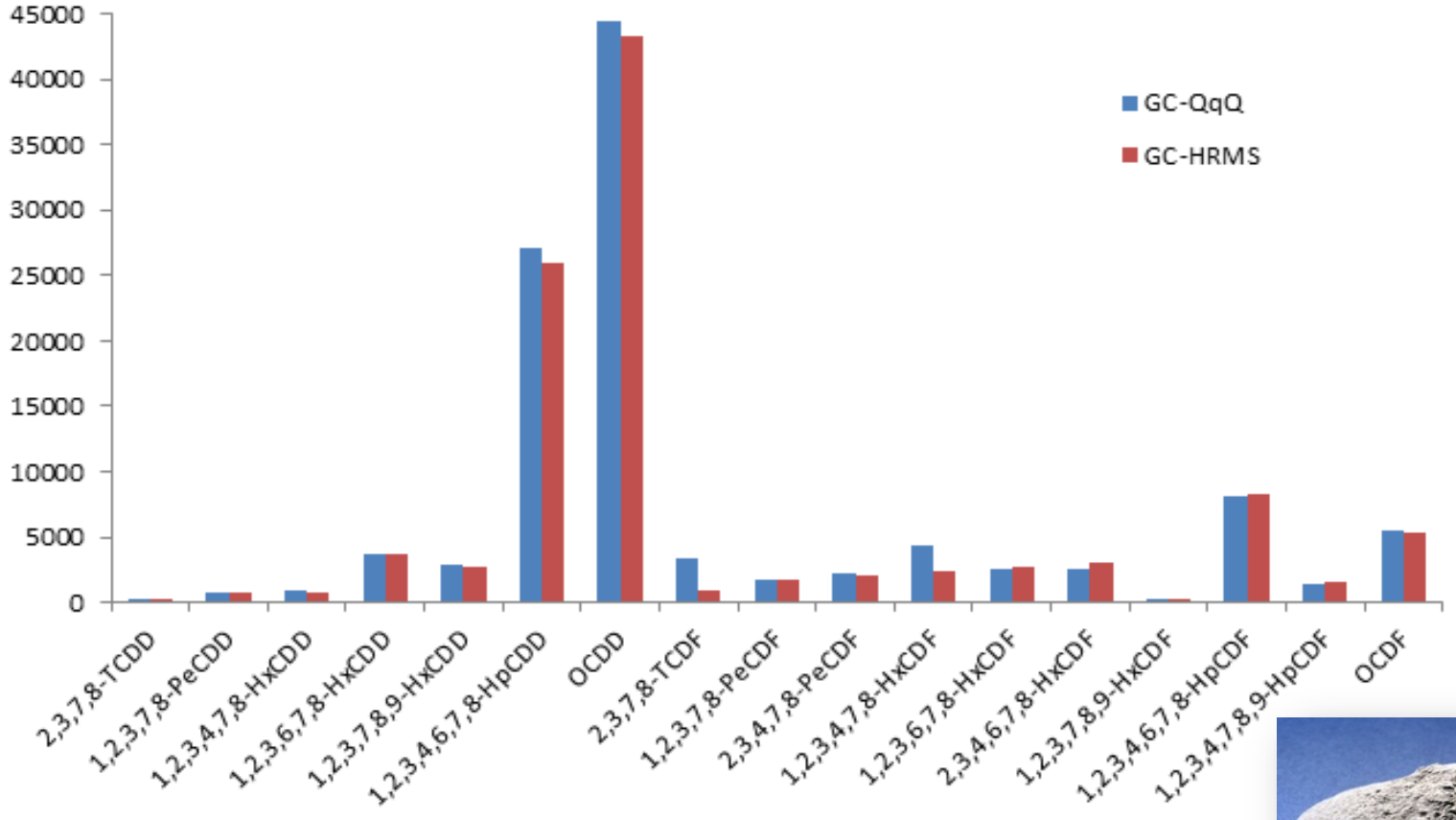
Comparison GC-MS/MS with GC-HRMS in Feed Sample



Good correlation between GC-QqQ and GC-HRMS

Results:Determination of Dioxins in Sample Extracts

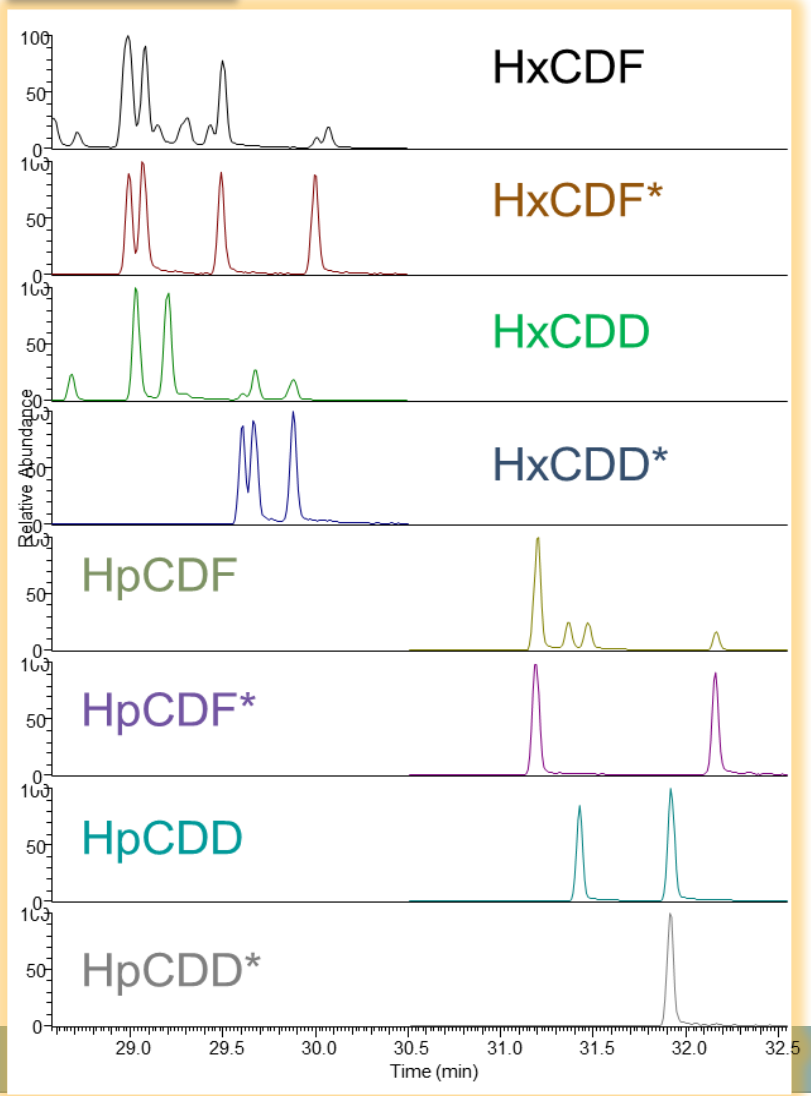
Comparison GC-MS/MS with GC-HRMS in Fly ash Sample (CRM 490)



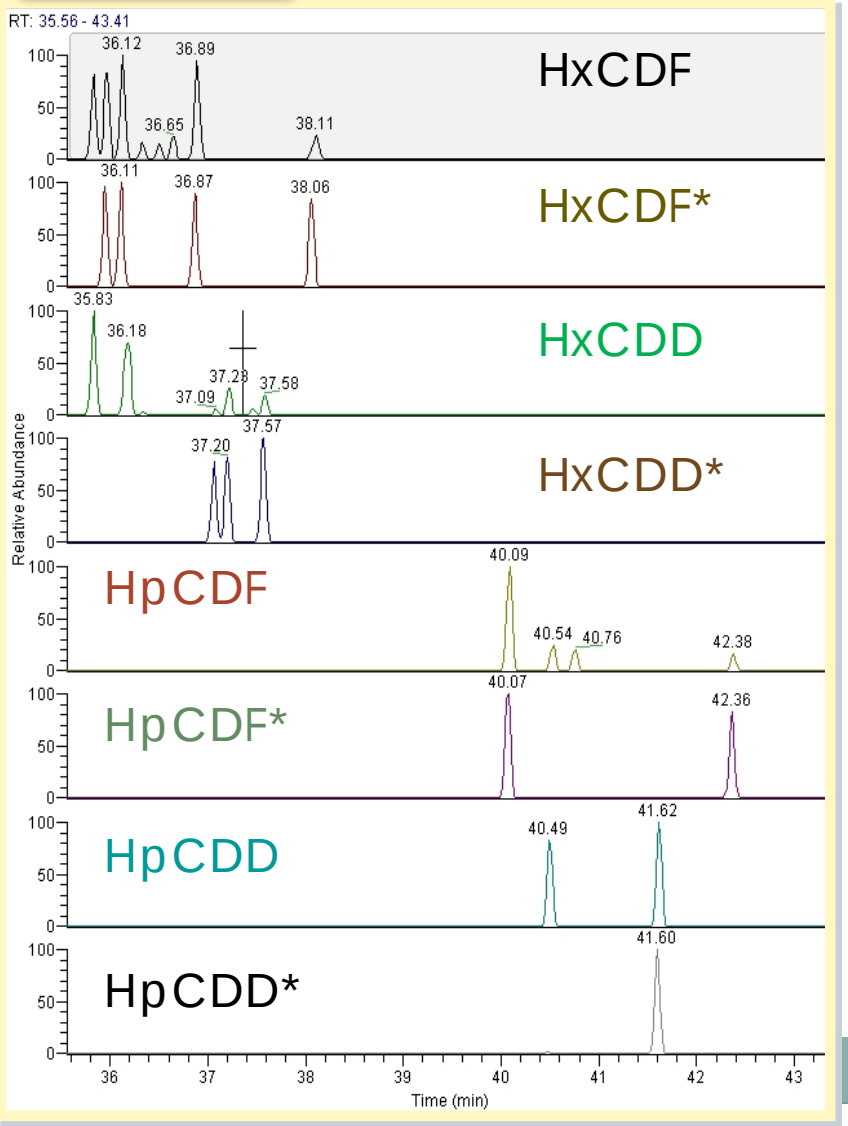
Results:Determination of Dioxins in Sample Extracts

Comparison GC-MS/MS with GC-HRMS in Fly ash Sample (CRM 490)

GC-QqQ

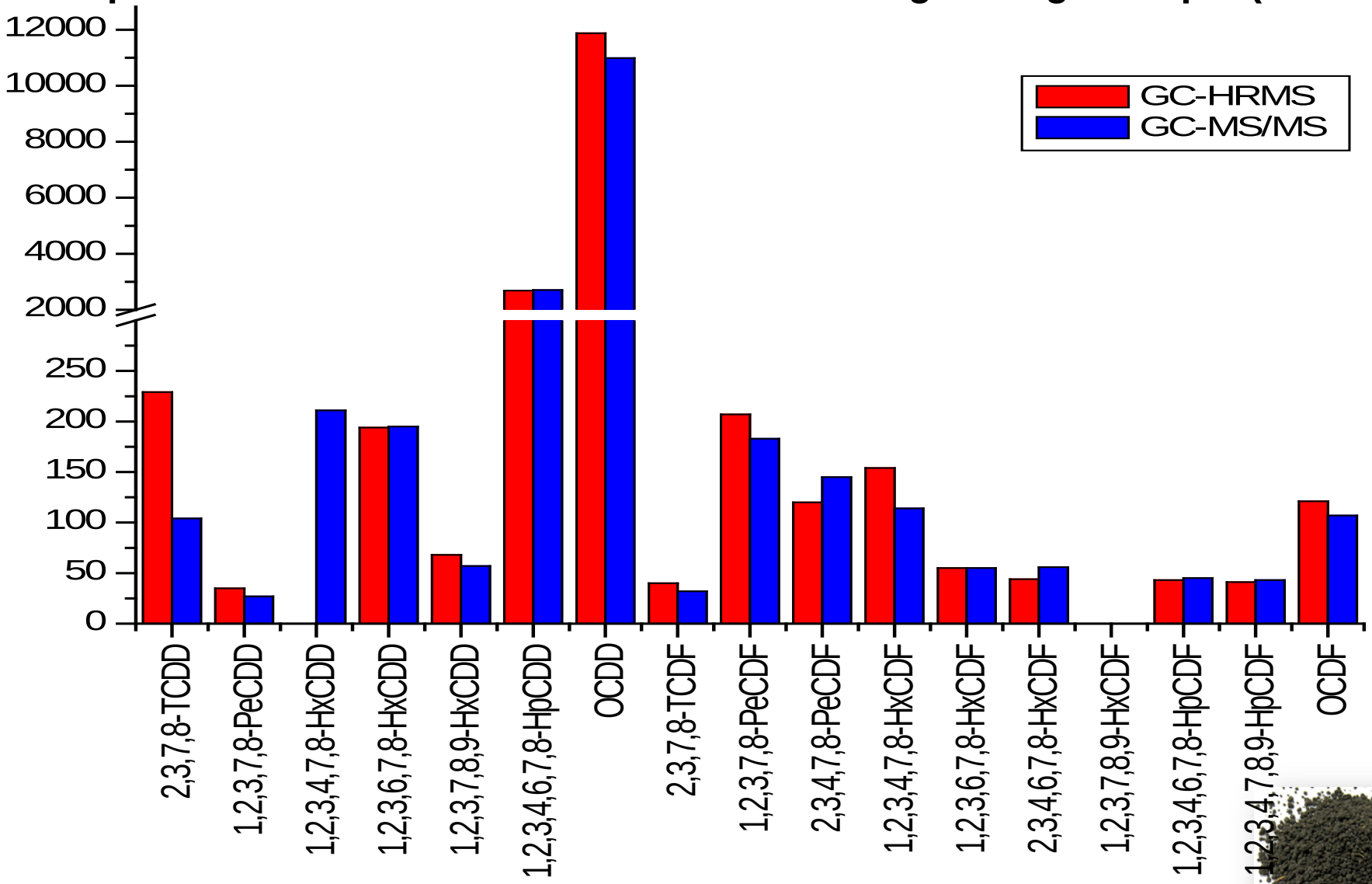


GC-HRMS



Results:Determination of Dioxins in Sample Extracts

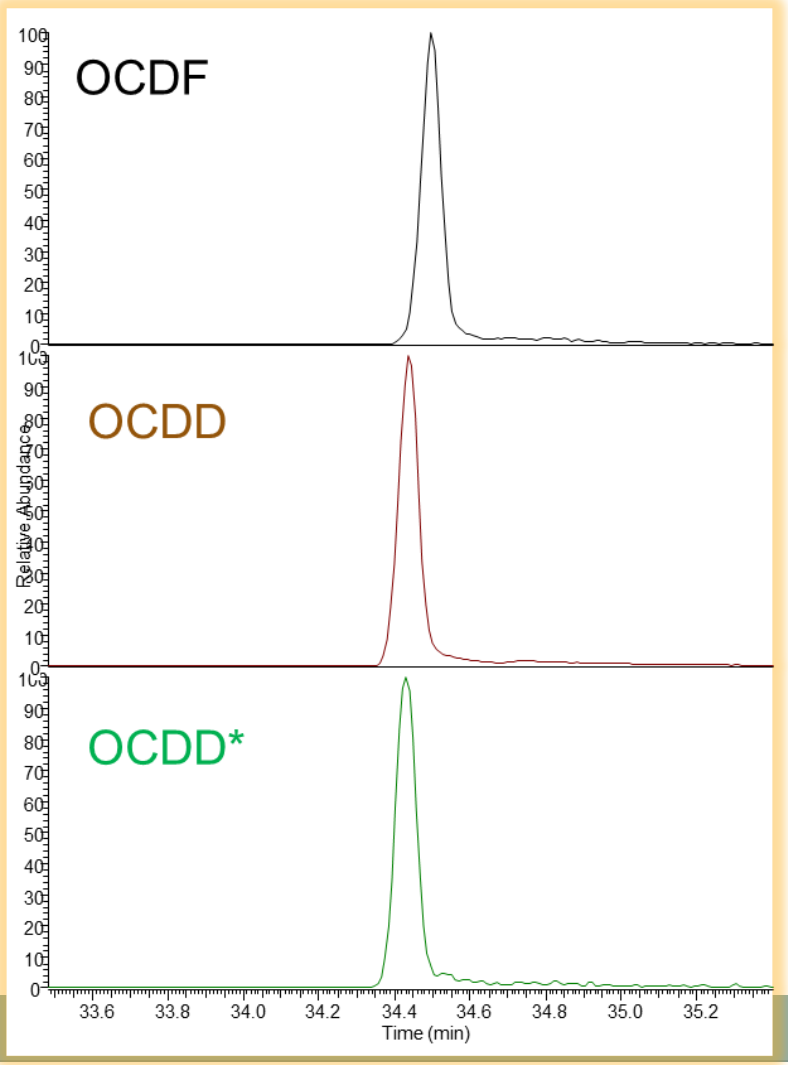
Comparison GC-MS/MS with GC-HRMS in Sewage sludge sample (CRM 677)



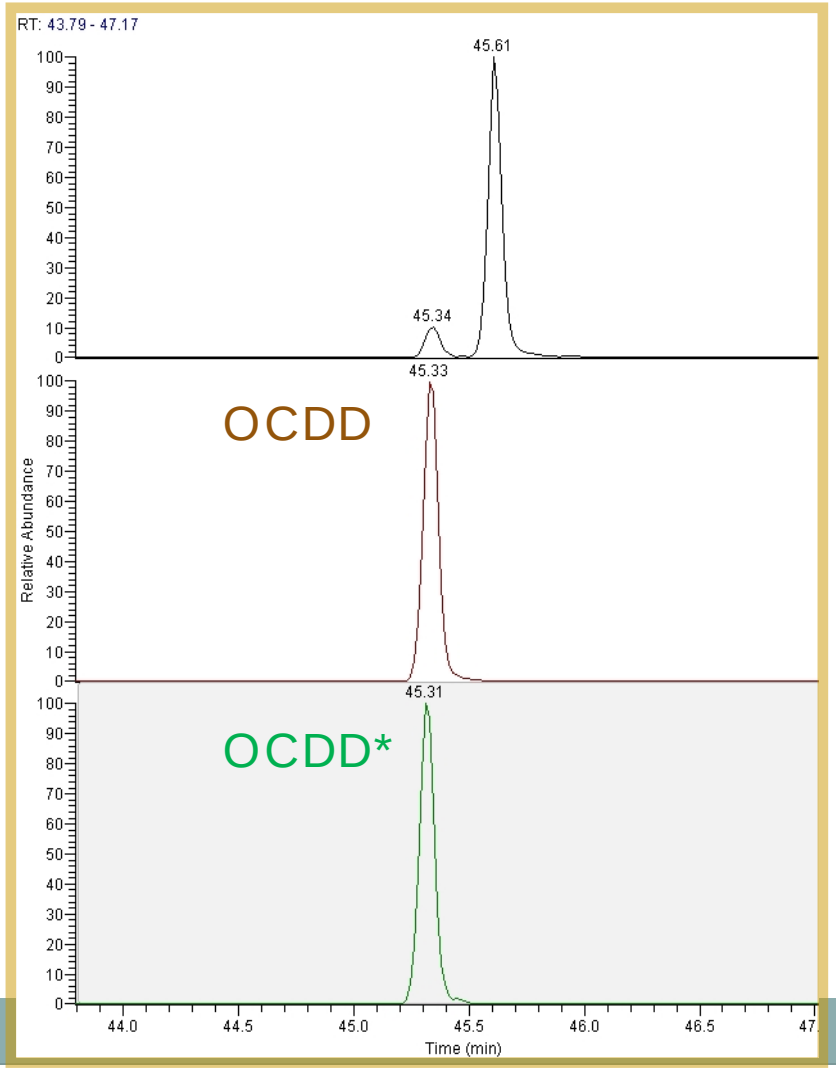
Results:Determination of Dioxins in Sample Extracts

Comparison GC-MS/MS with GC-HRMS in Sewage sludge (CRM 677)

GC-QqQ



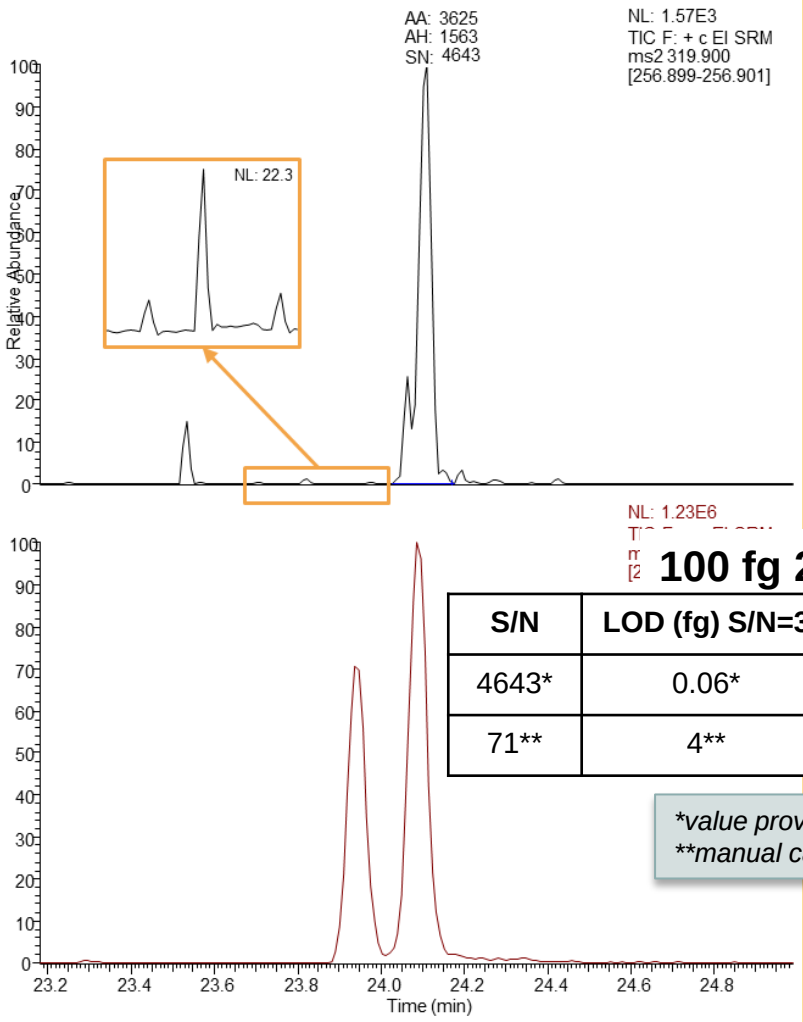
GC-HRMS



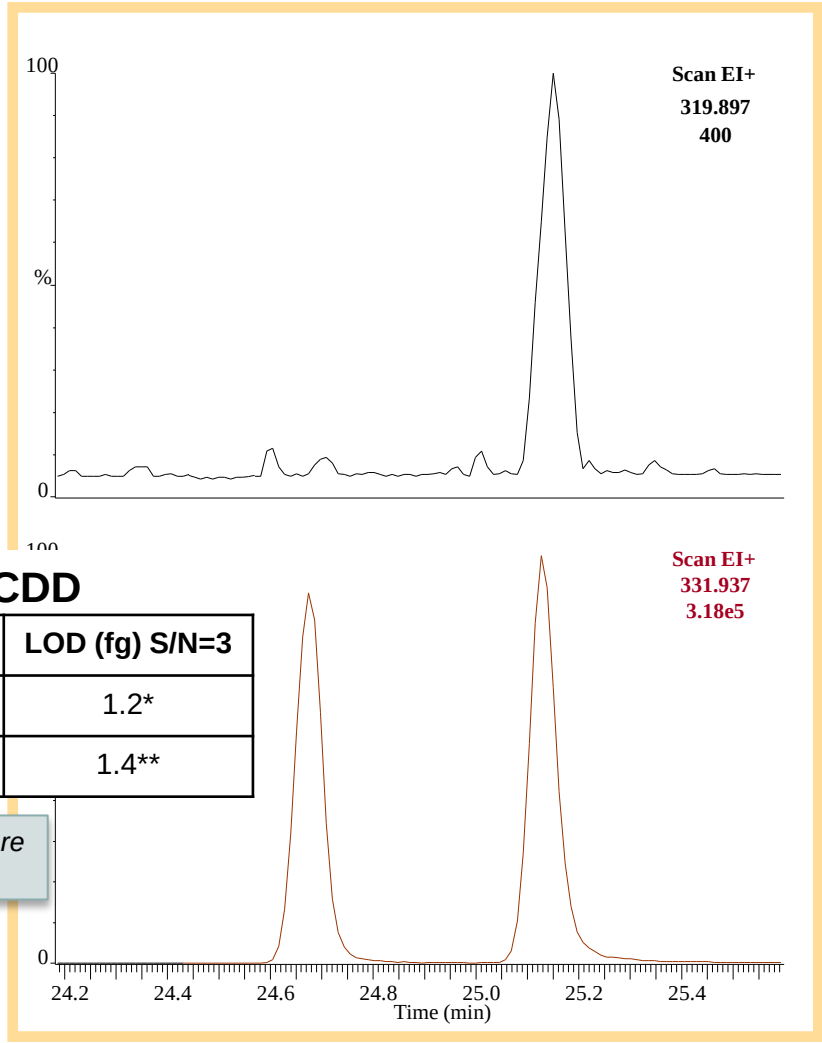
Results: LOD

S/N ratios for 2,3,7,8-TCDD
(EPA1613-CSL, 0.1 pg/µL)

GC-QqQ



GC-HRMS



100 fg 2,3,7,8-TCDD

S/N	LOD (fg) S/N=3	S/N	LOD (fg) S/N=3
4643*	0.06*	246*	1.2*
71**	4**	216**	1.4**

**value provided by software*
***manual calculation*

COMMISSION REGULATION (EU) No 589/2014 (2 June 2014)

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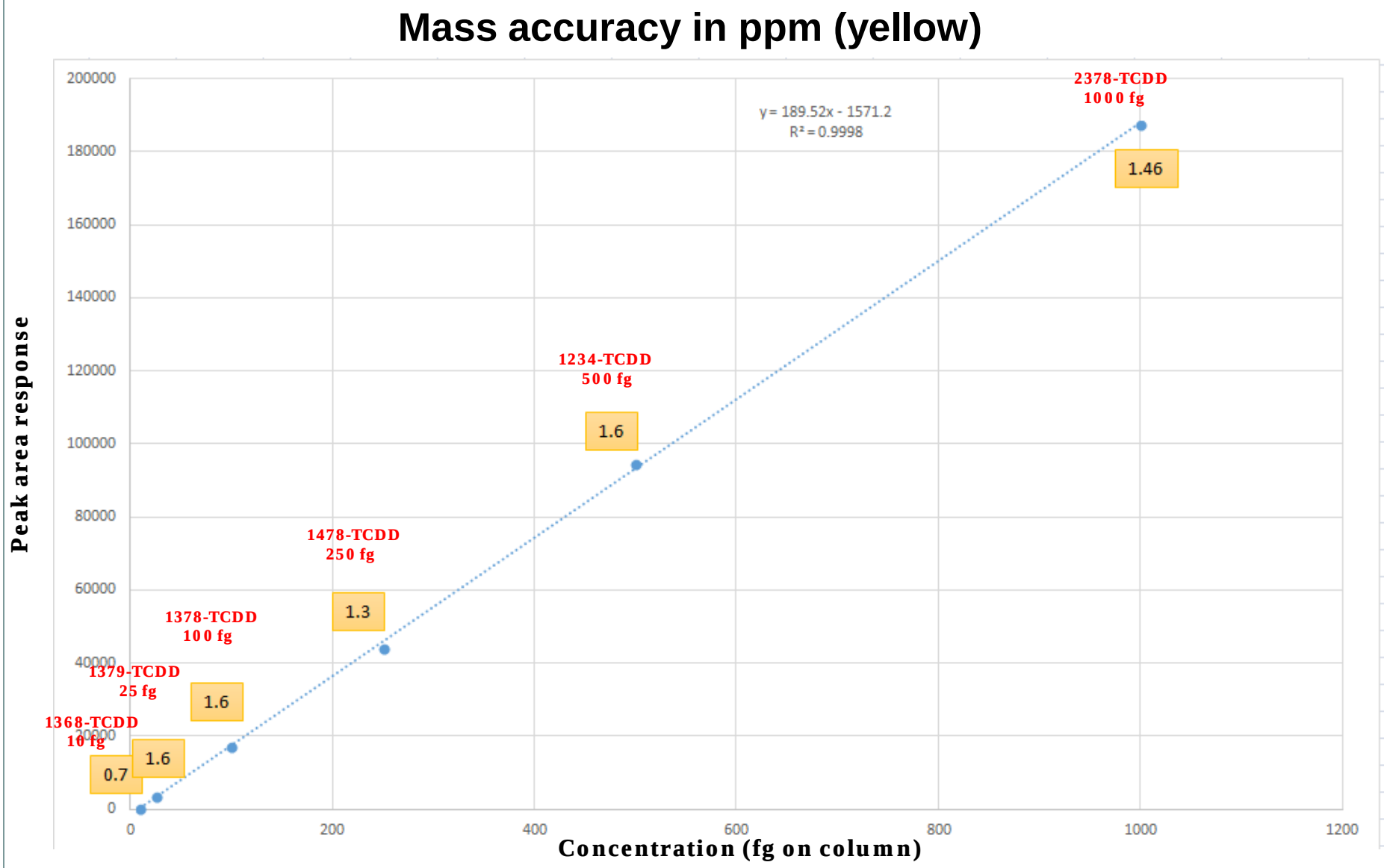
- LOQ: The method must demonstrate that it is able to distinguish between the blank and cut-off value. In reporting a value, a notification level should be established to decide what to do with the samples with a response below this level.
  - For PCDD/PCDF, LOD should be in the range of higher femtograms (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).

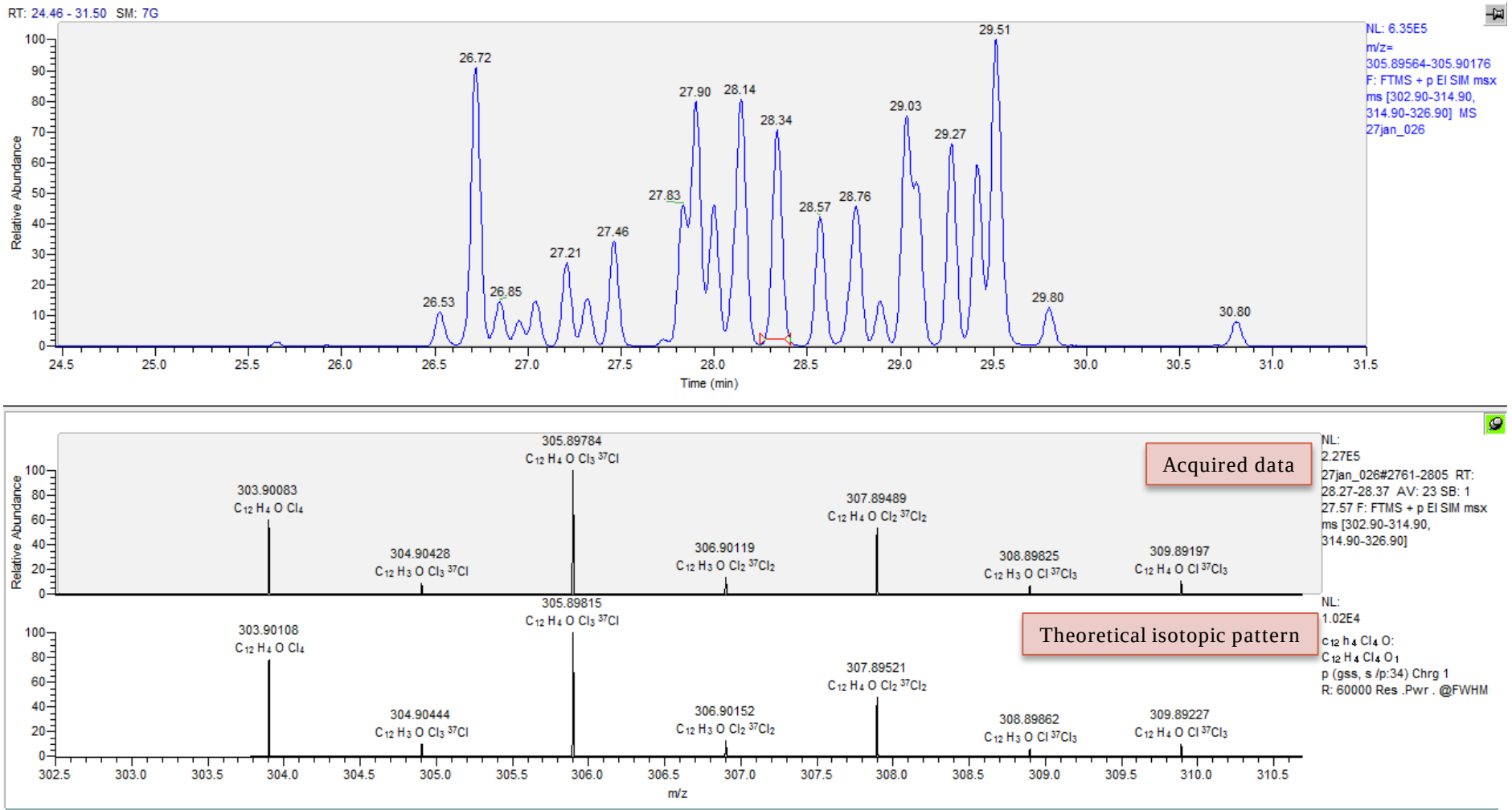
GC-Orbitrap technology for POPs analysis

Results: TCDD response across 10-1000 fg on column

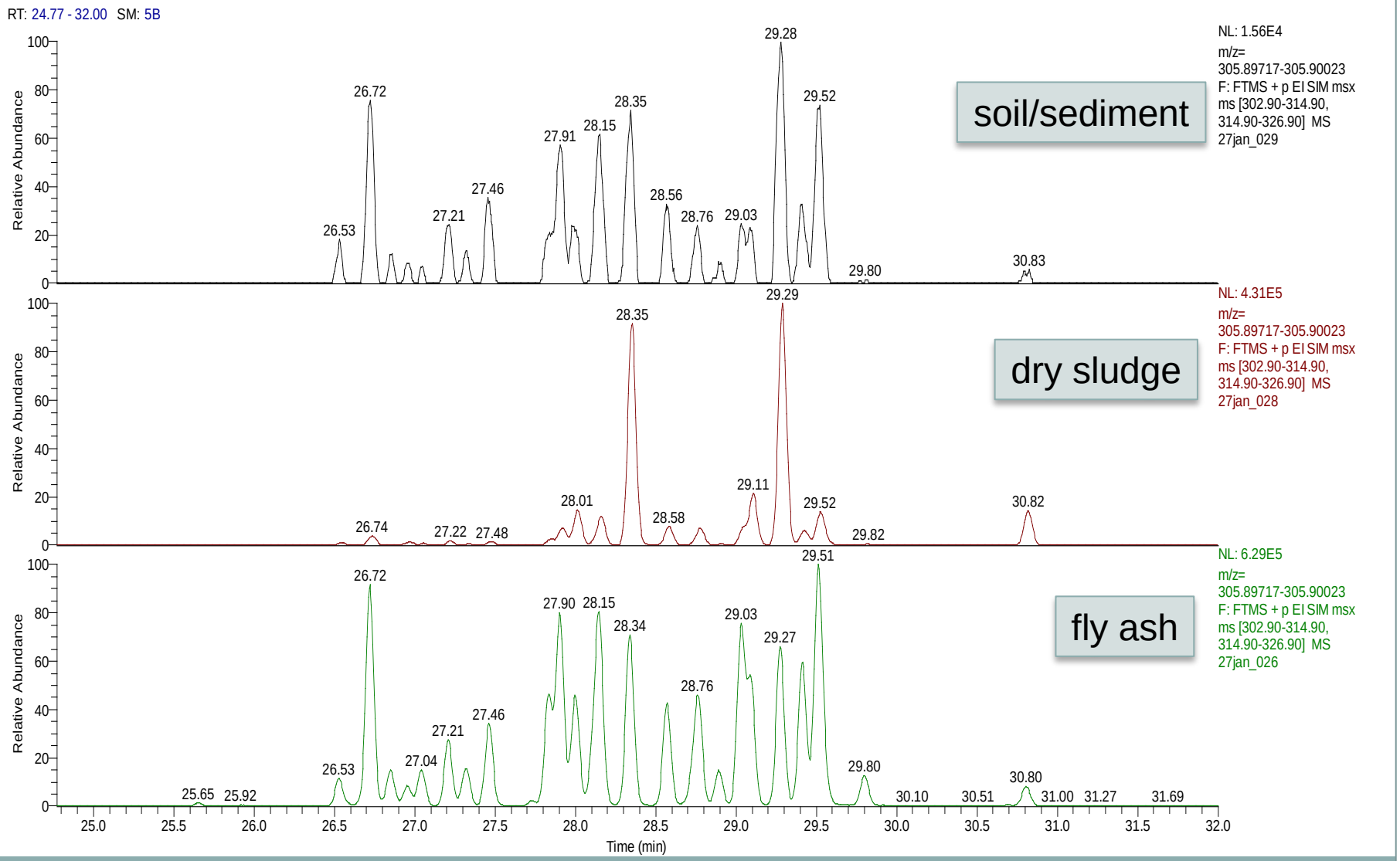


Results: TCDF native, fly ash

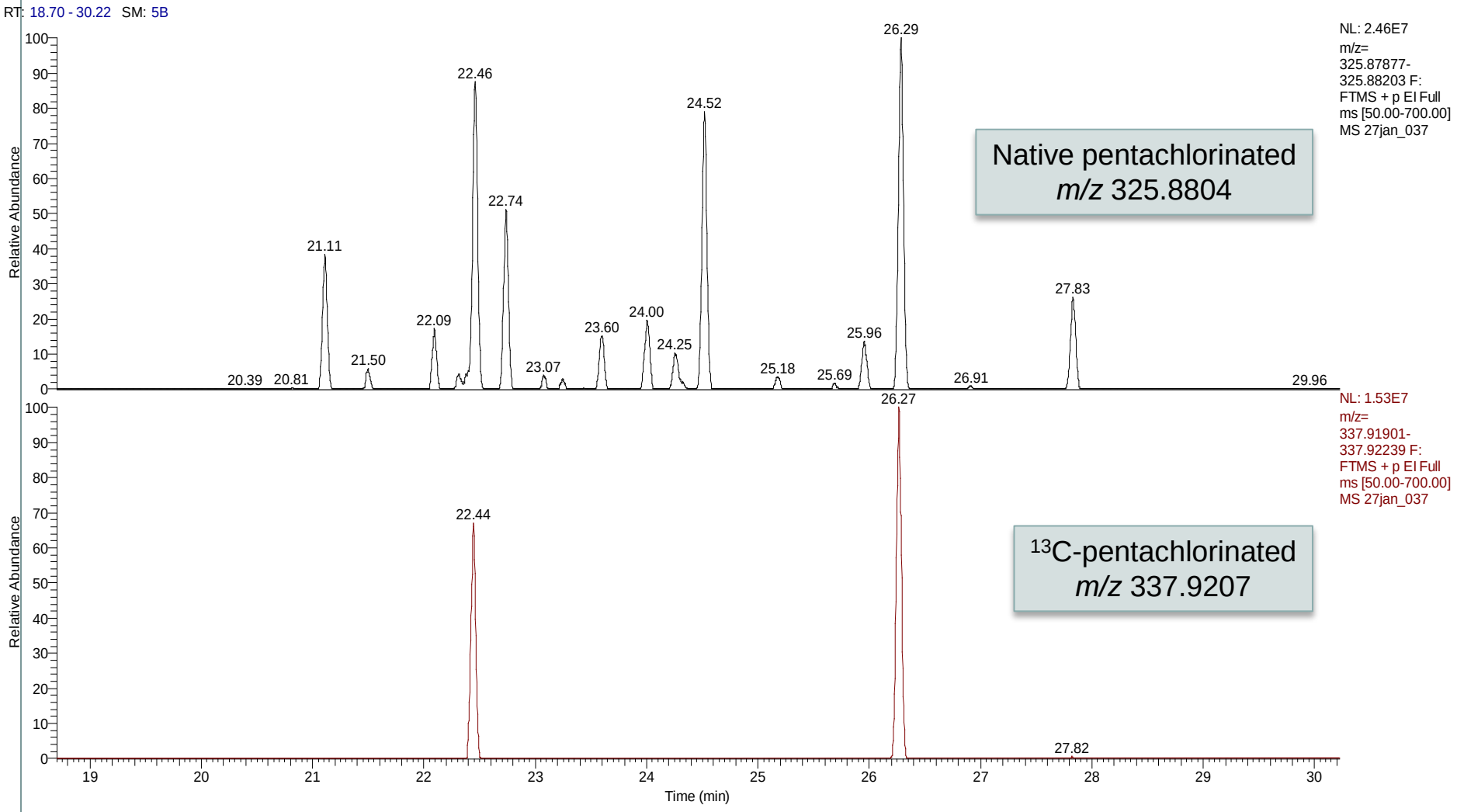
Isotopic pattern fit and accurate mass measurements for TCDD congener eluting at RT = 28.33 min



Results: Native TCDF profile in environmental samples



Pentachlorinated non- dioxin like PCBs profile in fish (CRM)



ACKNOWLEDGEMENTS

- Authors want to thank M.G Martrat, M. Adrados, J. Parera for their participation and contribution.

- A. García-Bermejo, M. J. González and B. Gómora from IQOG-CSIC.

- Many thanks to Paul Silcock, Cristian Cojocariu, Dominic Roberts and Sergio Guazzotti from Thermo Fisher Scientific for their encouragement in this study.

idaea

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Brock G. Chittim

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ThermoFisher SCIENTIFIC

Thank you for your attention!!!!

Prague, 29th -30th April 2015

New and emerging techniques in POPs analysis

Esteban Abad

Laboratory of Dioxins, Environmental Chemistry Department, IDÆA (CSIC)

Jordi Girona 18-26, 08034 Barcelona, Spain

10th International Symposium on Recent Developments in POPs Analysis

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