Introducing the Agilent GC/MS/MS Dioxin Analyzer

Cutting-edge technology enables fast and reliable Dioxin analysis at low levels:
1. New standard for GC/MS/MS sensitivity with 70:10:70 Triple Quadrupole EI source
2. An instrument with variable GC Sector MS.
3. MultiMode inlet (SSI) for effective cold splitless injections and more.

Developed from successful collaborations with leading Dioxin Labs in Europe:
1. The Agilent platform already validated according to new regulations in Europe for both food and feed (EC 589/2014, 709/2014).
3. Custom reporting with complete calculations have been developed and automated in MassHunter.

Ready for analysis:
1. Pre-configured and pre-tested at our factory so installation in your lab is fast and efficient.
2. The RTL advantage: guarantees the exact matching of our reference method on a new instrument.
3. A service engineer runs a complete check out standard so validation can begin.
4. Method never needs altering even when column maintenance is performed.

Validation of GC/MS/MS confirmatory method for the European official control of levels of dioxins, furans, and dioxin-like PCBs in foodstuffs

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Dioxin EU Regulation for food-feed ‘started’ with the Belgian Dioxin Crisis in 1999…

EU Commission Documents
- Council Regulation 2001/102/EC
- Commission Directive 2006/13/EC
- Commission Recommendation 2006/1881/EC
- Commission Recommendation 2011/516/EU
- Commission Regulation (EC) No 25/2012
- Commission Regulation (EC) No 27/2012
- Commission Regulation (EC) No 27/2012
- Commission Recommendation 2006/88/EC
- Commission Regulation (EC) No 252/2012
- Commission Regulation (EC) No 2375/2001
- Commission Regulation (EC) No 2375/2001
- Commission Regulation (EC) No 589/2014
- Commission Regulation (EC) No 709/2014

EU Commission Strategy (food-feed)
- Continuous monitoring
- Maximum-Action-(Target) level strategy
- Screening-Confirmatory approach
- RASFF (high capacities)
  Based on state-of-the-art methods

Analytical Methods?
- Evolutive Guidelines
  - PBMS
  - GC-IDHRMS vs CALUX
  - Validation @ LOQs (@1.5th level of interest)
  - Z-scores @ PTs
  - Recovery rates…

Recent EN 16215
- EN 16215
  - Animal feeding stuffs - Determination of dioxins and dioxin-like PCBs by GC-HRMS and/or of indicator PCBs by GC-HRMS
  - www.dioxin20xx.org
**Confirmatory Tool**

- **HRMS sector or MS/MS instruments**

**ANALYTICAL CRITERIA FOR USE OF MS/MS FOR DETERMINATION OF DIOXINS AND DIOXIN-LIKE PCBs IN FEED AND FOOD**

- New EU Regulation
- PCDD/Fs and DL-PCBs
- Criteria GC-MS / MS (589/2014)
- Detachable quantity - PCDD/F upper femto gram ($10^{-15}$g)
- NDL-PCBs nano gram ($10^{-9}$g)
- MO-PCBs picogram ($10^{-12}$g)
- Selectivity - Chromatographic separation of Relative RT ± 0.25% vs analyze $1,2,3,4,7,8$-HxCDF and $1,2,3,6,7,8$-HxCDF < 25% valley to peak
- MRM transitions - Monitoring 2 specific precursors with Monitoring at least 1 precursor and each specific product transition for all 2 products labeled and unlabeled analytes
- Tolerance ratio ± 20% if rel. intensity > 50%
- Relative ion intensities max ± 15% Tolerance ratio ± 25% if rel. intensity 20-50%
- Resolution MS quadrupole = unit Used for LOQ calculation
- Blank value < 30% of maximum level ML
- LOQ calculated from average blank to average LOQ < 1/5 of maximum level ML
- Difference between labeled and unlabeled levels < 20% ML

**Validation for vegetable oil (feed)**

- Top-down approach
- Control chart
- Blanks subtraction - Average blank, Blanks control chart
- Recovery experiments, QCs
- Accuracy
- Reproducibility
- LOD/LOQ
- Selectivity, linearity

**PTV Injections**

- Dioxin fraction runtime = 42min
- MO-PCBs fraction runtime = 38min
Chromatographic Profile

MO and NDL PCBs

PCDDs, PCDFs, NO PCBs

Chromatographic Separation

Selectivity

25% valley separation HxCDF

Tandem in-space MS

7000 MS/MS System

optimized for gas chromatography

MRM transition Ratio

Quant/Qual transition ratio

EU Reg 709/2014 says:

PCDD/Fs, DL-PCBs: 2 specific precursors with each specific product ions

- 2378 TCDD: 319.9 > 256.9  Average ratio = 0.964
- 2378 TCDD: 321.9 > 258.9

NDL-PCBs: at least 1 precursor and 2 product ions

- PCB 189: 393.8 > 323.8
- PCB 189: 395.8 > 325.8

Average ratio = 0.627

Tolerance:

± 15% R(quad)=unit

± 20% R(quad)=unit

Proper Estimation of LOQs

LOD/LOQ

Tolerance: 
95.1 ± 15%

- LOQ = blank mean + 6*stdev (12 injections – blank)

In MassHunter

Organic halogen compounds (2012), 156-159

Proper Estimation of LOQs

- LOD/LOQ

Tolerance: 
95.1 ± 15%

- LOQ = blank mean + 6*stdev (12 injections – blank)

- LOD = blank mean + 10*stdev (8 replicate injections – call point)
**Validation for vegetable oil (feed)**

- **iLOD/iLOQ**
  - Acceptable and consistent deviation to the average RRF
  - \( \text{Av RRF}_{1...6} = 0.9622 \)
  - \( \text{R}^2 \) (linear fit) = 0.996
  - \( \text{Av RRF} = 0.9435 \)
  - Deviation = -1.9%

- **LOQ matrix**
  - Procedure blanks
  - 10 independent injections
  - Average + 6*stdev.

**Calibration curve (lowest level)**
- 8 replicates
- 10*stdev

**Accuracy & Reproducibility**
- Spiked materials at 0.5 ML, ML, 2 ML (6 series, 3 days)
- Bias < 20%
- Within lab reproducibility < 15%

**Proficiency test (PT)**
- Z score = 0.80
- Z score = 0.59

**Within Lab Reproducibility**
- QC pork fat
**MassHunter Report Generator**

**Report Time** 2014-06-12 15:30
**Analysis Time** 2014-06-12 10:33
**Last Calib Update** 2014-05-12 16:56

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### Sample Name

14.4441 _dio x-

### Type

Sample, Vial

### Comments

Dio x-

### Compound

**RT [min]** | **Conc [ng/ml]** | **TEQ Conc [ng/ml]** | **LOQ** | **Upper Bound [ng/ml]** | **Medium Bound [ng/ml]** | **Lower Bound [ng/ml]** | **WHO-TEF 2005**
--- | --- | --- | --- | --- | --- | --- | ---
TCDD | 2378 | <LO Q | <LO Q | 0.0200 | 0.02000 | 0.01000 | 0.00000 | 1.0
PeCDF | 12378 | <LO Q | <LO Q | 0.0300 | 0.03000 | 0.01500 | 0.00000 | 1.0
HxCDD | 123478 | 24.27 | 0.0376 | 0.00376 | 0.0200 | 0.00376 | 0.00376 | 0.00376 | 0.1
HxCDD | 123678 | <LO Q | <LO Q | 0.1400 | 0.01400 | 0.00700 | 0.00000 | 0.1
HxCDD | 123789 | <LO Q | <LO Q | 0.0600 | 0.00600 | 0.00300 | 0.00000 | 0.1
HpCDD | 1234678 | 33.08 | <LO Q | <LO Q | 0.4400 | 0.00440 | 0.00220 | 0.00000 | 0.01
OCDD | 1234789 | 39.46 | 4.9895 | 0.00150 | 1.9900 | 0.00150 | 0.00150 | 0.00150 | 0.0003
TCDF | 2378 | <LO Q | <LO Q | 0.1000 | 0.01000 | 0.00500 | 0.00000 | 0.1
PeCDF | 12378 | <LO Q | <LO Q | 0.5600 | 0.01680 | 0.00840 | 0.00000 | 0.03
PeCDF | 123478 | 24.08 | <LO Q | <LO Q | 0.0800 | 0.02400 | 0.01200 | 0.00000 | 0.3
PeCDF | 123678 | <LO Q | <LO Q | 0.0800 | 0.00800 | 0.00400 | 0.00000 | 0.1
HxCDF | 123478 | 27.05 | <LO Q | <LO Q | 0.0700 | 0.00700 | 0.00350 | 0.00000 | 0.1
HxCDF | 123678 | 27.19 | <LO Q | <LO Q | 0.0600 | 0.00600 | 0.00300 | 0.00000 | 0.1
HxCDF | 123789 | 29.06 | <LO Q | <LO Q | 0.1800 | 0.01800 | 0.00900 | 0.00000 | 0.1
HxCDF | 234678 | 27.84 | <LO Q | <LO Q | 0.0800 | 0.00800 | 0.00400 | 0.00000 | 0.1
HpCDF | 1234678 | 31.29 | <LO Q | <LO Q | 0.5500 | 0.00550 | 0.00275 | 0.00000 | 0.01
HpCDF | 1234789 | 34.01 | <LO Q | <LO Q | 0.0200 | 0.00020 | 0.00010 | 0.00000 | 0.01
OCDF | 1234678 | 39.89 | <LO Q | <LO Q | 0.6000 | 0.00018 | 0.00009 | 0.00000 | 0.0003
PCB 77 | 1234678 | 18.01 | <LO Q | <LO Q | 49.6600 | 0.00497 | 0.00248 | 0.00000 | 0.0001
PCB 81 | 1234678 | 18.01 | 6.7691 | 0.00203 | 3.6700 | 0.00203 | 0.00203 | 0.00203 | 0.0003
PCB 126 | 1234678 | 21.04 | <LO Q | <LO Q | 1.3700 | 0.13700 | 0.06850 | 0.00000 | 0.1
PCB 169 | 1234678 | 24.22 | <LO Q | <LO Q | 0.0700 | 0.00210 | 0.00105 | 0.00000 | 0.03
**Total TEQ** | | | | | 0.32144 | 0.16436 | 0.00729 | 0.0
**Sum TEQ PCDD/F** | | | | | 0.17534 | 0.09030 | 0.00526 | 0.0
**Sum TEQ PCBs** | | | | | 0.14610 | 0.07406 | 0.00203 | 0.0

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**Take Home Message**

- PTV-GC/MS/MS accepted as a confirmatory tool under EU Regs
- Full validation on challenging matrix
- MS/MS, but still dioxin analyses…
- MS/MS & sectors to be properly perceived