Optimized Sample to Result Workflow of Dioxin and PCBs Automated Sample Prep and GC-MS/MS Analysis in Environmental and Food

Reinvigorating Dioxins Analysis with GC/MS/MS

Joel Ferrer
Product Manager, Triple Quadrupole GC/MS

Agilent
Trusted Answers

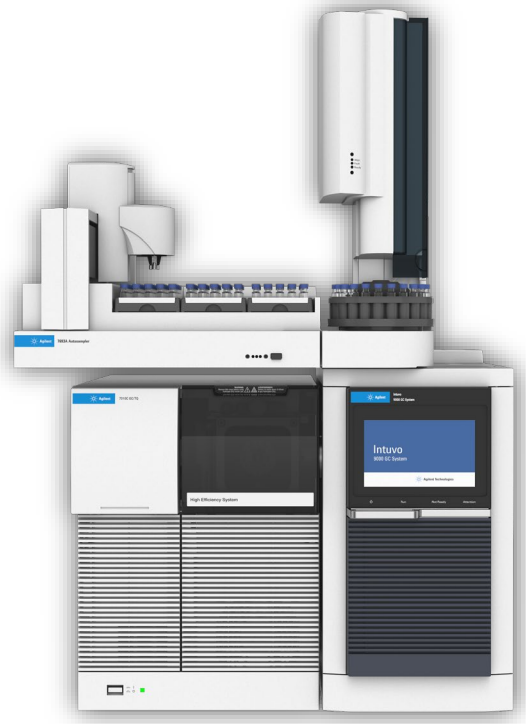
Introducing the 7000E GC/TQ and 7010C GC/TQ

7000E GC/TQ

Inert Plus EI Source | CI Source | HydroInert Source

8890 GC

Intuvo 9000 GC | Intuvo 9000 GC



7010C GC/TQ

HES EI Source | CI Source

8890 GC

Intuvo 9000 GC

Next-Generation Mass Spec Intelligence

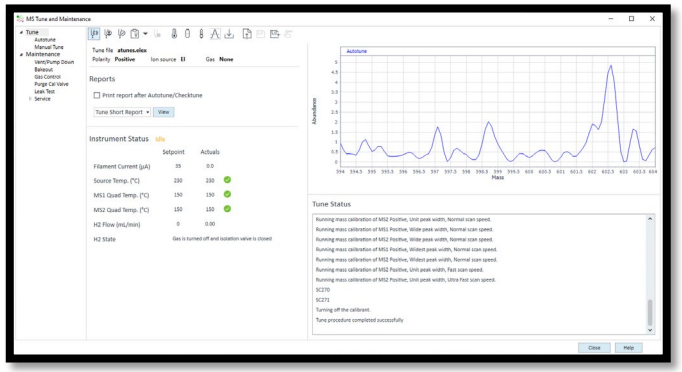
Intelligence Powered Advances on GC/TQ

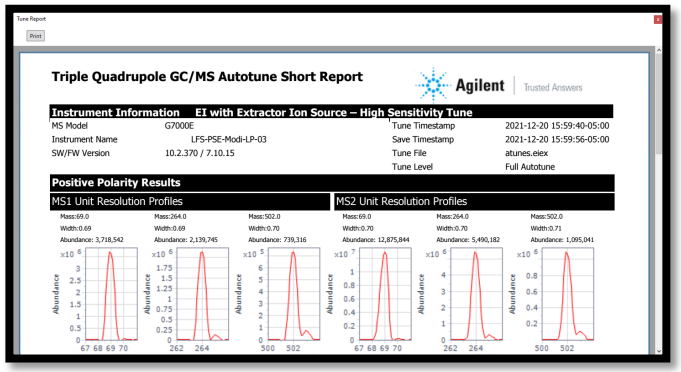
NEW SWARM Autotune completes 2X faster

System Screening & System Evaluation assess presence of ions and electronics status

Diagnostic Tune used for troubleshooting

A detailed system report (MSR) can be sent to a service engineer for speedy diagnostic efforts





Introduction

Persistent Organic Pollutants (POPs) in Stockholm Convention

- The Stockholm Convention on POPs (2001) is a global treaty to protect **human health** from chemicals that remain in the environment and are **persistent**, **bioaccumulative** and transportable across the globe.

- Include:
  - Industrial chemicals ex. PCBs, hexachlorobenzene
  - Pesticides ex. Aldrin, DDT, endrin, toxaphene etc.
  - Pharmaceuticals
  - Solvents
  - By-products ex. **Dioxins & Furans**

- Initially ‘dirty dozen’ but new POPs include Perfluoro alkyl substances (**PFAS**), short chain chlorinated paraffins (**SCCPs**), pesticides, Deca-BDE etc.

- Provisions include eliminating production and intentional uses, managing and disposing wastes in a suitable environmentally safe manner

A History of Dioxin Analysis and USEPA 1613B

Most Widely Used Dioxin Method Globally

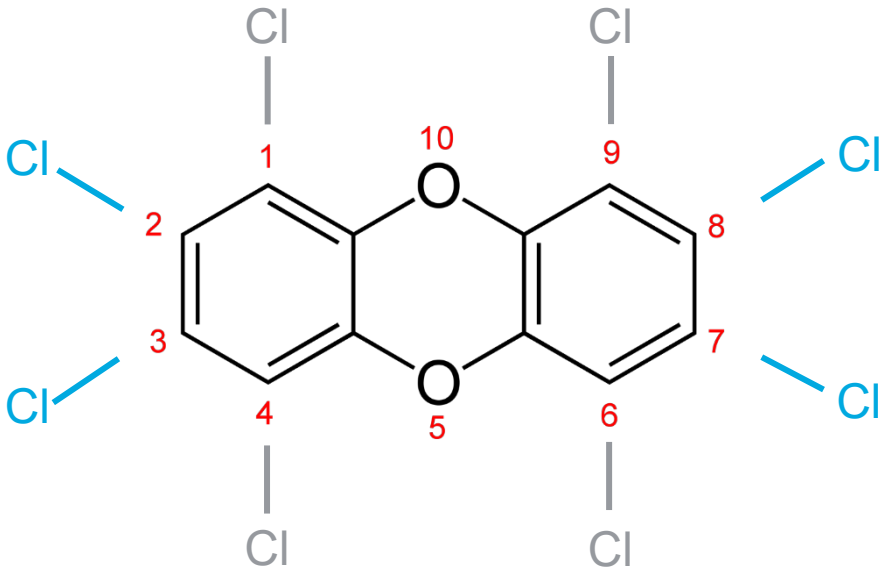
- A **HRGC/HRMS** method that is used to analyze 2,3,7,8-tetrachloro dibenzo-p-dioxin (TCDD) in municipal and industrial discharges
- Used to determine **PCDDs and PCDFs** in water, soils, sludges and other matrices
- Specificity is provided for determination of the **seventeen 2,3,7,8-substituted PCDDs and PCDFs**
- This method is also used for the analysis of **drinking water, food products, and human/animal tissue** samples
- **Many regions** outside the U.S. also use the method as guidance or strictly follow it

PCDD and PCDF Nomenclature and Isomers

EPA 1613B Analytes

Dioxins

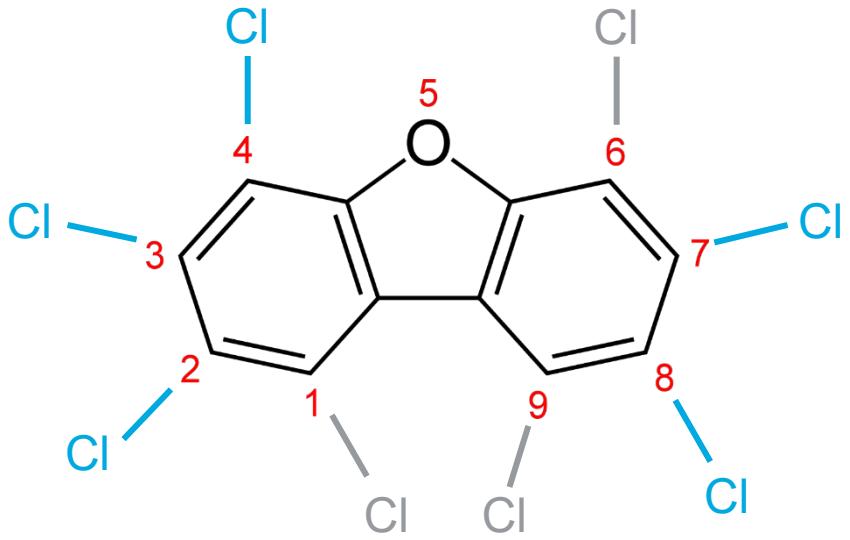
Polychlorinated dibenzodioxin (PCDD)



2,3,7,8 – TCDD
(2,3,7,8-Tetrachlorinated dibenzodioxin)

Furans

Polychlorinated dibenzofurans (PCDF)



2,3,4,7,8 – PCDF
(2,3,4,7,8-Pentachlorinated dibenzofurans)

Chlorine Atoms	PCDD Isomers	PCDF Isomers
1	2	4
2	10	16
3	14	28
4	22	38
5	14	28
6	10	16
7	2	4
8	1	1
Total	**75**	**135**

75 total PCDD isomers

135 total PCDF isomers

17 toxic isomer

Toxic Equivalent Factors (TEF)

17 Toxic Dioxin Compounds

Toxic PCDDs	I-TEF	WHO_{2005}-TEF
2,3,7,8-TetraCDD	1	1
1,2,3,7,8-PentaCDD	0.5	**1**
1,2,3,4,7,8-HexaCDD	0.1	0.1
1,2,3,6,7,8-HexaCDD	0.1	0.1
1,2,3,7,8,9-HexaCDD	0.1	0.1
1,2,3,4,6,7,8-HeptaCDD	0.01	0.01
1,2,3,4,6,7,8,9-OctaCDD	0.001	**0.0003**

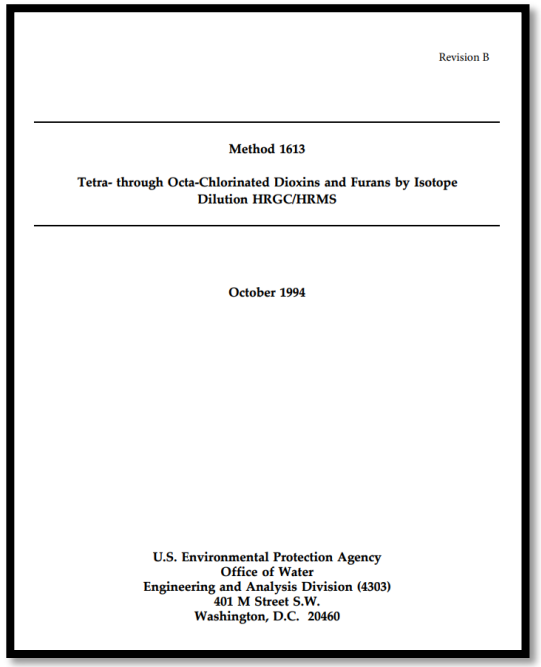
Toxic PCDFs	I-TEF	WHO_{2005}-TEF
2,3,7,8-TetraCDF	0.1	0.1
1,2,3,7,8-PentaCDF	0.05	**0.03**
2,3,4,7,8-PentaCDF	0.5	**0.3**
1,2,3,4,7,8-HexaCDF	0.1	0.1
1,2,3,6,7,8-HexaCDF	0.1	0.1
1,2,3,7,8,9-HexaCDF	0.1	0.1
2,3,4,6,7,8-HexaCDF	0.1	0.1
1,2,3,4,6,7,8-HeptaCDF	0.01	0.01
1,2,3,4,7,8,9-HeptaCDF	0.01	0.01
1,2,3,4,6,7,8,9-OctaCDF	0.001	**0.0003**

$$TEQ = \sum_{n=1}^{17}([PCDD/F]_i(^{ng}/_{L}) \times TEF_i)\ (^{ng\ TEQ}/_{L})$$

Environmental Dioxins Testing Today

Agilent 7010C Triple Quadrupole GC/MS

- The U.S. EPA has approved an alternate testing protocol for 1613B – Analysis of Dioxins
- The new alternate testing protocol accepts GC/TQ as an **equivalent technology** to analyze dioxins in Environmental Samples



Revision B

Method 1613

Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS

October 1994

U.S. Environmental Protection Agency
Office of Water
Engineering and Analysis Division (4303)
401 M Street S.W.
Washington, D.C. 20460

U.S. EPA 1613B

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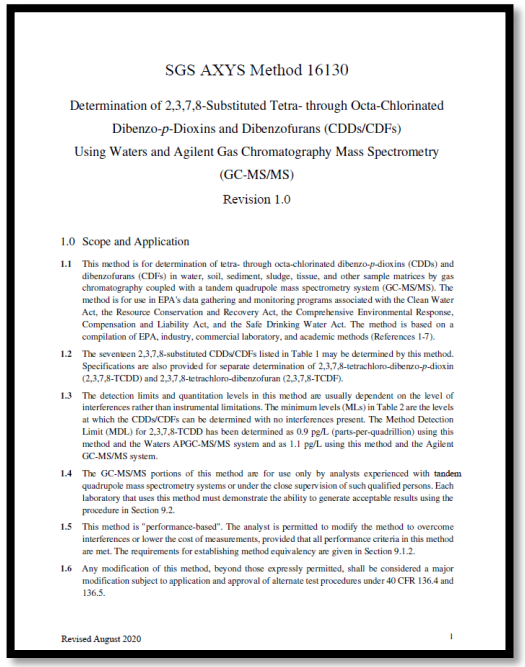


SGS AXYS Method 16130

Determination of 2,3,7,8-Substituted Tetra- through Octa-Chlorinated Dibenzo-*p*-Dioxins and Dibenzofurans (CDDs/CDFs) Using Waters and Agilent Gas Chromatography Mass Spectrometry (GC-MS/MS)

Revision 1.0

1.0 Scope and Application

1.1 This method is for determination of tetra- through octa-chlorinated dibenzo-*p*-dioxins (CDDs) and dibenzofurans (CDFs) in water, soil, sediment, sludge, tissue, and other sample matrices by gas chromatography coupled with a tandem quadrupole mass spectrometry system (GC-MS/MS). The method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act. The method is based on a compilation of EPA, industry, commercial laboratory, and academic methods (References 1-7).

1.2 The seventeen 2,3,7,8-substituted CDDs/CDFs listed in Table 1 may be determined by this method. Specifications are also provided for separate determination of 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (2,3,7,8-TCDD) and 2,3,7,8-tetrachloro-dibenzofuran (2,3,7,8-TCDF).

1.3 The detection limits and quantitation levels in this method are usually dependent on the level of interferences rather than instrumental limitations. The minimum levels (ML's) in Table 2 are the levels at which the CDDs/CDFs can be determined with no interferences present. The Method Detection Limit (MDL) for 2,3,7,8-TCDD has been determined as 0.9 pg/L (parts-per-quadrillion) using this method and the Waters APGC-MS/MS system and as 1.1 pg/L using this method and the Agilent GC-MS/MS system.

1.4 The GC-MS/MS portions of this method are for use only by analysts experienced with tandem quadrupole mass spectrometry systems or under the close supervision of such qualified persons. Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedure in Section 9.2.

1.5 This method is "performance-based". The analyst is permitted to modify the method to overcome interferences or lower the cost of measurements, provided that all performance criteria in this method are met. The requirements for establishing method equivalency are given in Section 9.1.2.

1.6 Any modification of this method, beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

Revised August 2020 1

SGS AXYS 16130

Comparison of U.S. EPA 1613B vs SGS AXYS 16130

	Requirement	U.S. EPA 1613B	SGS AXYS 16130
No Change	**Valid for EPA compliance reporting**	Yes	Yes
	Analyte List	Tetra through Octa Dioxins and Furans (210congeners – 17 toxics)	Same as 1613B
	Matrices validated	Non-potable water, soil, sediment, fish tissue and other solids	Same as 1613B
	Sample Prep.	Soxhlet extraction/SPE + optional carbon clean-up	Same as 1613B
	Reporting units	Toxicity equivalents (TEQs)	Same as 1613B
Updated	**Method Detection Levels (MDLs)**	4.4 pg/L for 2,3,7,8-TCDD	1.1 pg/L for 2,3,7,8-TCDD (all MDLs calculated and in method)
	Detector	GC/HRMS (R>10,000 at 10% valley ex. Magnetic Sector)	GC/TQ (Unit resolution)
	Instrument tuning requirements	PFK tune to meet resolution and m/z deviation of <5 ppm	Standard Autotune to meet manufacturer's specifications of mass accuracy
	Lock mass reference requirement	Infusion of PFTBA	No lock mass; calibrant Infusion of PFTBA 'at manufacturer's specified limits' for matrix evaluation

Dioxins via SGS AXYS 16130

With this approval, some modification is required…

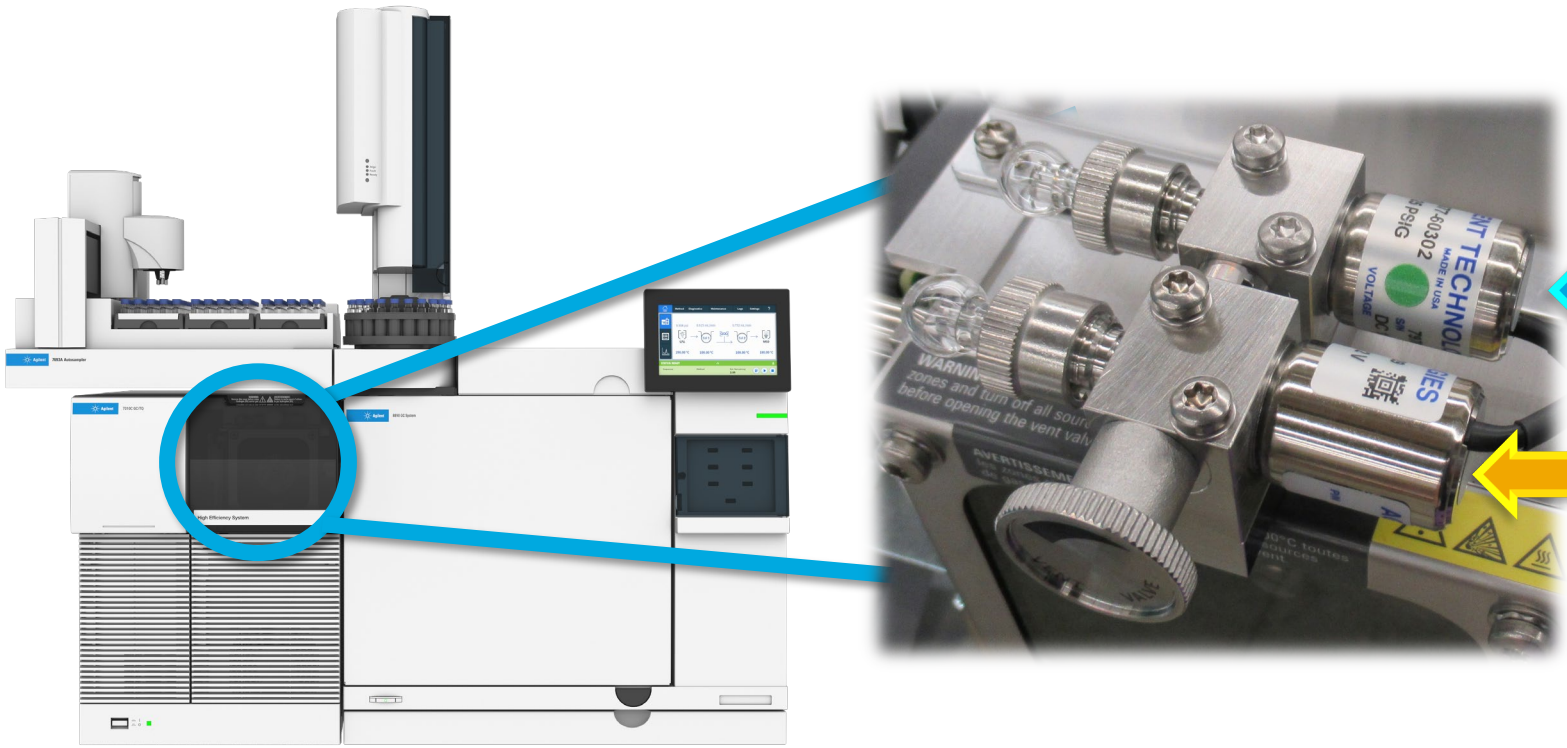
6.10 Mass spectrometer—Tandem quadrupole mass spectrometer equipped with either an atmospheric pressure (AP) ionization source or an electron ionization source (70 eV range) capable of repetitively selectively monitoring at least 32 transitions at unit resolution during a period of approximately 1 second, and shall meet all of the performance specifications in Section 10.

The MS/MS must have a mechanism for constantly bleeding PFTBA into the source during the analytical run, such as a needle valve or a capillary bleed directly into the source enclosure.

10.2.1.2 The response of the MRM transition shown in Table 8 for the reference compound (PFTBA) is monitored throughout the run. The response of the MRM product ion shall not vary by more than ±20% throughout the run. Variations by more than 20% within the possible elution window of a CDD/CDF indicate the presence of coeluting interferences that may significantly alter the response of the mass spectrometer. Reinjection of another aliquot of the sample extract will not resolve the problem. Additional cleanup of the extract may be required to remove the interferences.

System Configuration

Reference Compound Introduction Valve (RCI Valve)



NEW
Reference Compound Introduction Valve (RCIV)

Calibration Valve

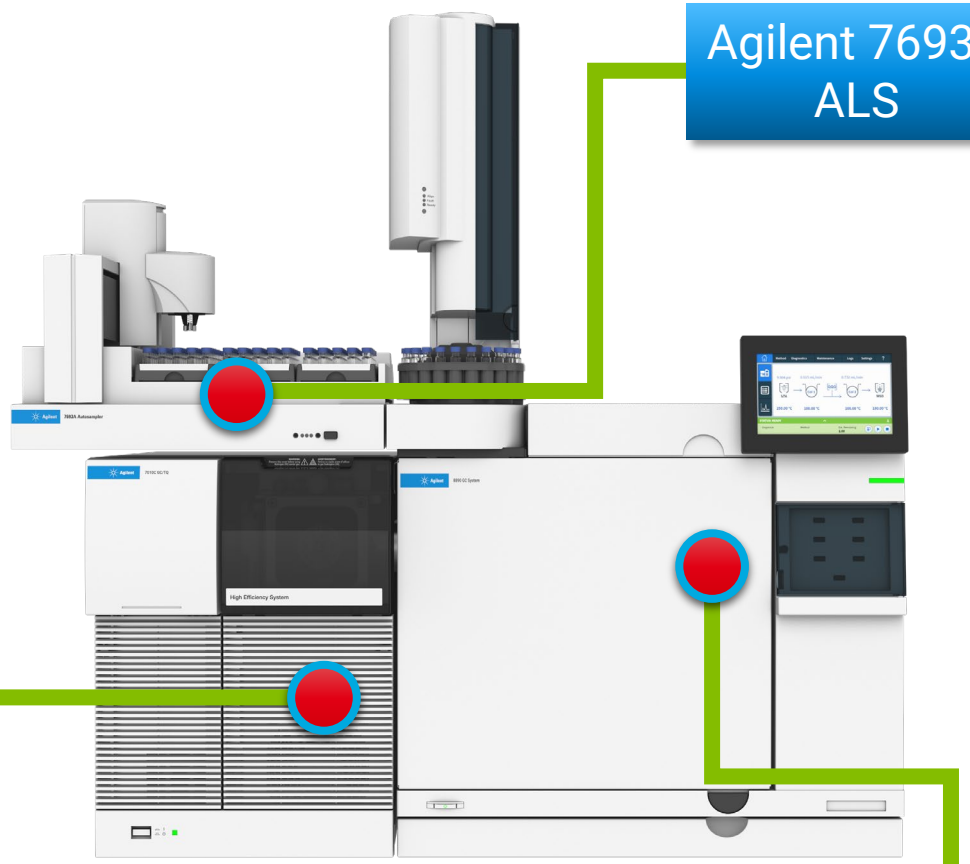
System Configuration

Also compatible with CTC PAL for sample introduction (1 mL vial)

Agilent 7693A ALS

Splitless
1 µL injection

Agilent 7010A/B/C GC/TQ



Equip with: Reference Compound Introduction Valve
Source: 290 °C
Quads: 150 °C
Transfer Line: 290 °C

He Quench Gas:
2.25 mL/min
N_2 Collision Gas:
1.5 mL/min

DB-5
60 m x 0.25 mm x 0.10 µm

Agilent 8890 GC

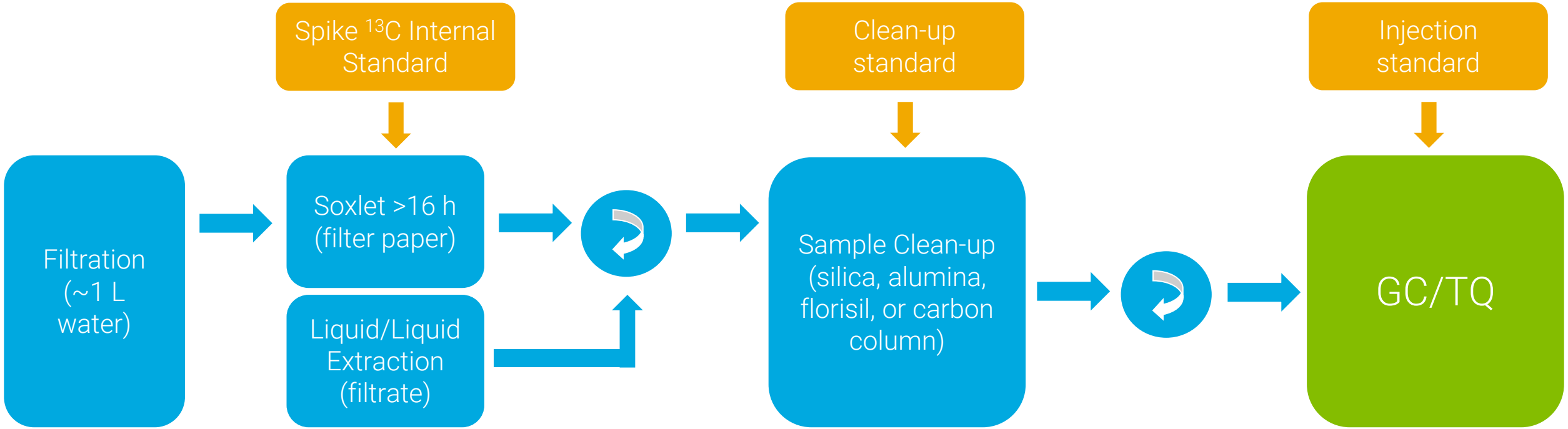
Inlet liner
Splitless, double taper, 4 mm ID

GC Parameters
S/SL Inlet → MSD
Constant Flow
Flow 1.1 mL/min

Oven program:
90 °C (2 min)
22 °C/min to 200 °C
1 °C/min to 215 °C (10 min)
5.2 °C/min to 300 °C (2.7 min)

EPA Method 1613B Sample Preparation

No changes to sample preparation or collection for SGS AXYS 16130



EPA 1613B is a performance-based method

Chromatography Performance

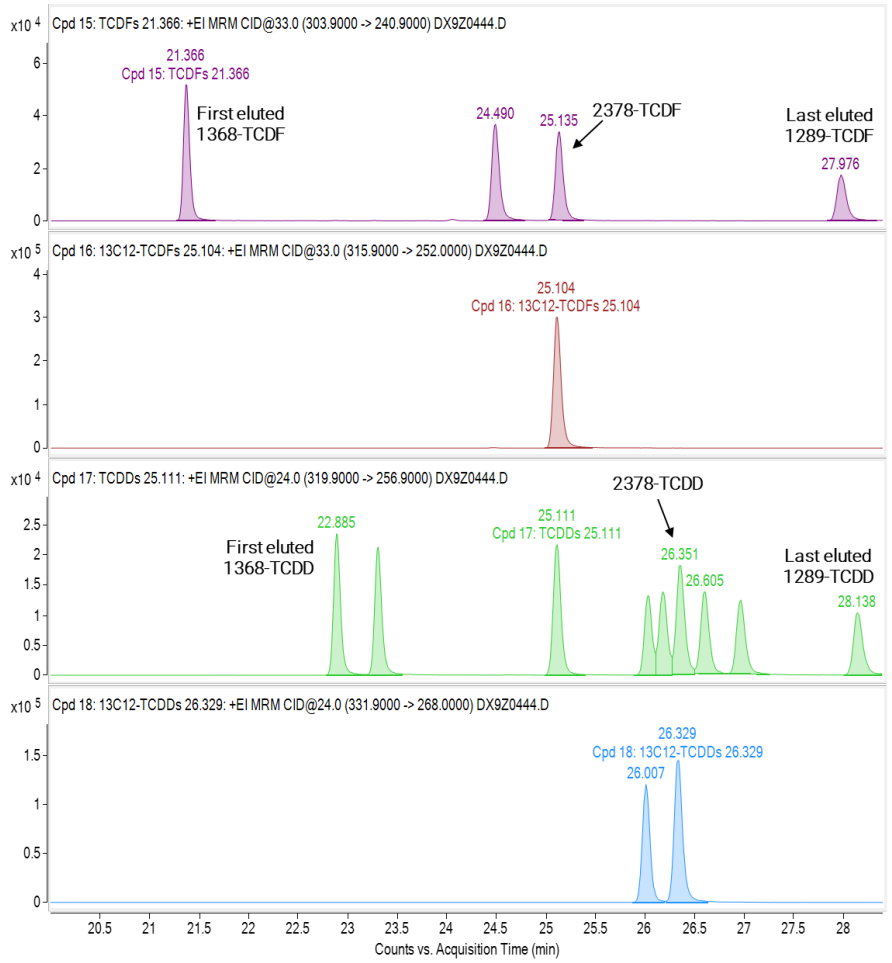


Figure 1. MRM chromatograms for TCDFs, labeled TCDF ISTD, TCDDs, and labeled TCDD ISTD.

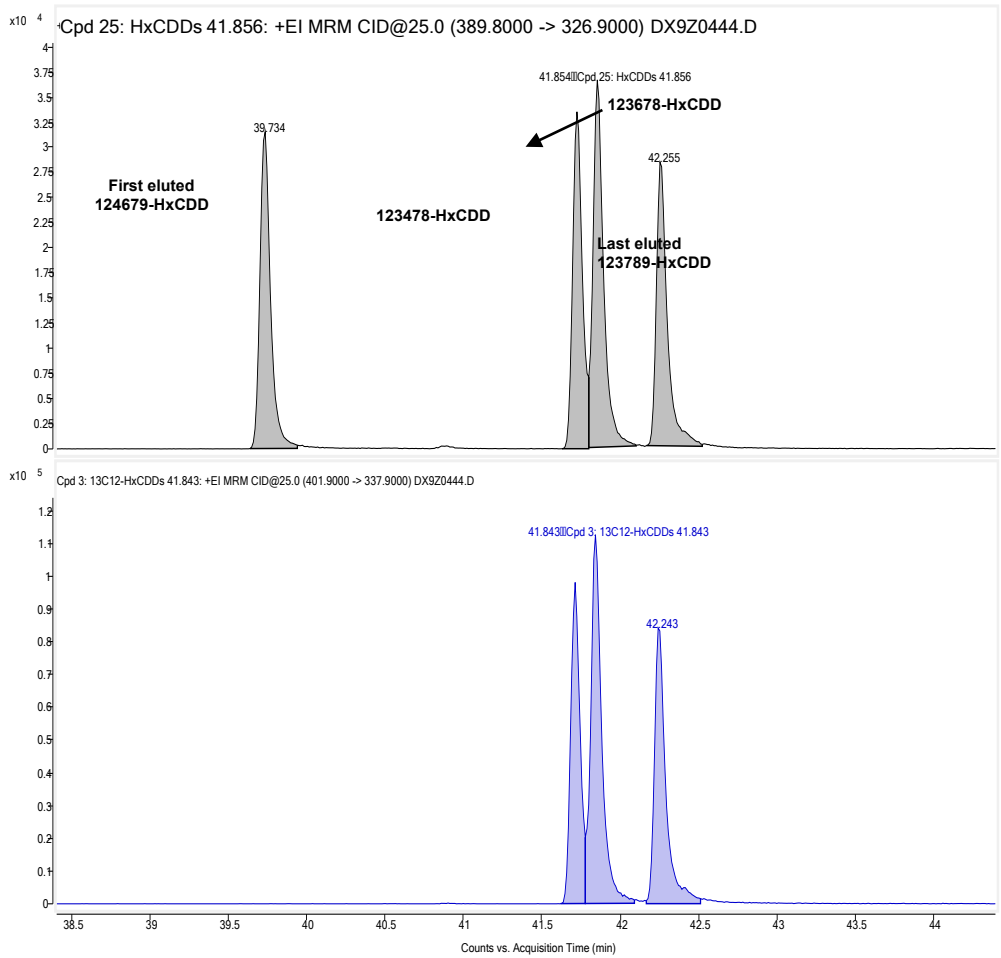
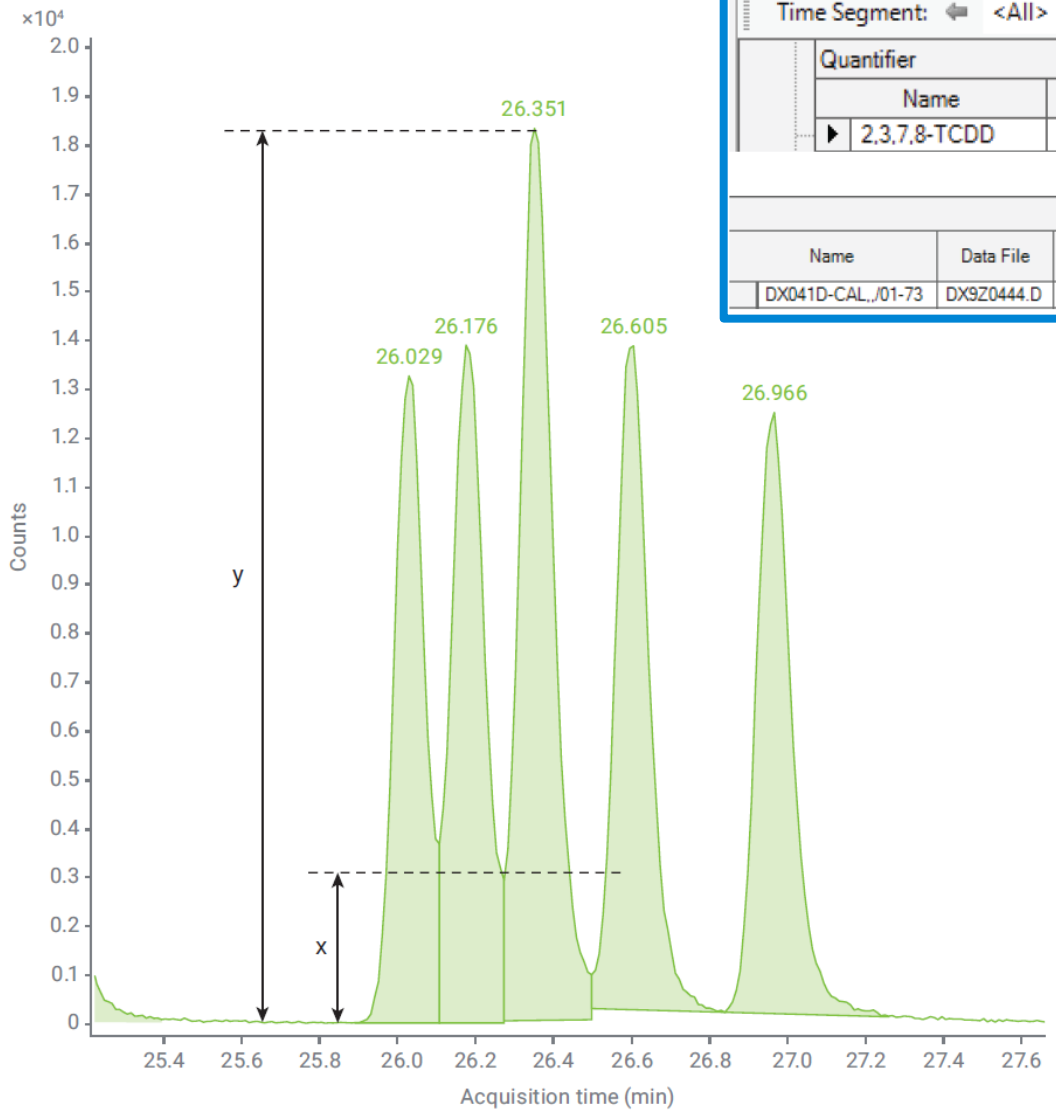


Figure 2. MRM chromatograms for HxCDDs and the corresponding ISTD.

Chromatography Performance



Method Table

Time Segment: <All> Compound: 2,3,7,8-TCDD Reset Table View

Quantifier

Name	TS	Transition	Scan	Type	Resolution Calculation Type	Resolution Limit
2,3,7,8-TCDD	1	319.9 -> 256.9,...	MRM	Target	Valley Height/Peak Heig...	25.0

Sample							2,3,7,8-TCDD Results							
Name	Data File	Type	Level	Vial	Acq. Date-Time	Acq. Method File	RT	Resp.	MI	Calc. Conc.	Accuracy	S/N	Resolution F.	Resolution R.
DX041D-CAL,,/01-73	DX9Z0444.D	Cal	CS3	7	8/22/2019 2:31 AM	TQEI_DB5_DX_11	26.351	221151	☐	9.0183	90.2	2339.21	20.4	7.8

Figure 3. (Left)
2,3,7,8-TCDD and its close eluters.

Figure 4. (Above)

Top Row: Method setup for resolution check in MassHunter Quantitative Analysis.

Bottom Row: Front and rear valley height/peak height resolution calculated for 2,3,7,8-TCDD and its closest eluting isomers.

Reference Compound Stability

The need for lock mass monitoring of the GC/HRMS system for Method 1613B was replaced by use of a stability reference compound in the GC/TQ method.

The Agilent Reference Compound Introduction Valve (RCI Valve):

- Provides an **optimized flow** of PFTBA to observe any changes in the ionization efficiency and ion transmission
  - Seen as a change in the PFTBA signal intensity
- **No software changes or updates** are required– Controlled easily through Agilent MassHunter software

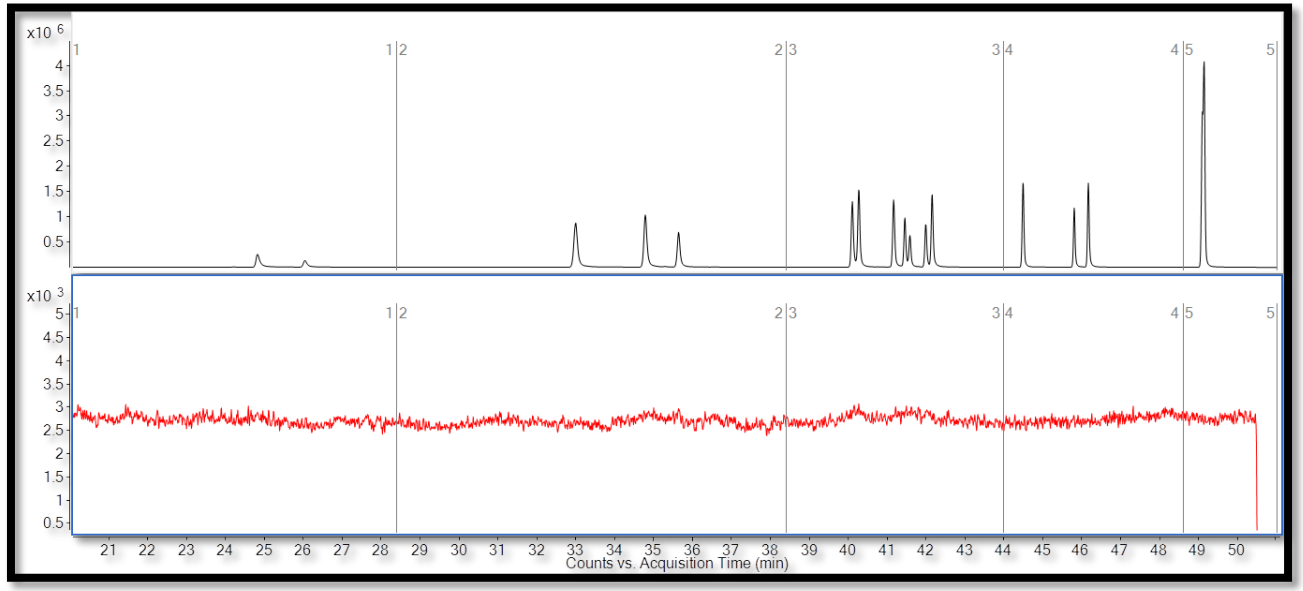


Figure 5. Stability of PFTBA response over the entire analytical run.

Compatible on 7010 Series GC/TQ

Method detection Limits (MDLs) with 7890/7010 GC/TQ Far Surpassed Method 1613B MDLs

The GC/TQ MDL results for the aqueous (1 L), solid (10 g), and tissue (10 g) samples:

Compound	Aqueous	Solid	Tissue
	MDL and (MRL) in pg/L	MDL and (MRL) in pg/g	MDL and (MRL) in pg/g
2,3,7,8-TCDD	1.1 (10)*	0.029 (1)	0.057 (0.5)
1,2,3,7,8-PeCDD	1.39 (50)	0.037 (5)	0.051 (2.5)
1,2,3,4,7,8-HxCDD	1.05 (50)	0.042 (5)	0.061 (2.5)
1,2,3,6,7,8-HxCDD	1.08 (50)	0.045 (5)	0.033 (2.5)
1,2,3,7,8,9-HxCDD	1.78 (50)	0.064 (5)	0.067 (2.5)
1,2,3,4,6,7,8-HpCDD	1.19 (50)	0.070 (5)	0.032 (2.5)
OCDD	9.4 (100)	0.311 (10)	0.085 (5)
2,3,7,8-TCDF	0.56 (10)	0.60 (1)	0.056 (0.5)
1,2,3,7,8-PeCDF	1.0 (50)	0.037 (5)	0.046 (2.5)
2,3,4,7,8-PeCDF	1.25 (50)	0.039 (5)	0.033 (2.5)
1,2,3,4,7,8-HxCDF	0.89 (50)	0.032 (5)	0.029 (2.5)
1,2,3,6,7,8-HxCDF	1.11 (50)	0.031 (5)	0.046 (2.5)
1,2,3,7,8,9-HxCDF	1.22 (50)	0.048 (5)	0.084 (2.5)
2,3,4,6,7,8-HxCDF	1.26 (50)	0.026 (5)	0.034 (2.5)
1,2,3,4,6,7,8-HpCDF	0.92 (50)	0.255 (5)	0.064 (2.5)
1,2,3,4,7,8,9-HpCDF	1.35 (50)	0.028 (5)	0.043 (2.5)
OCDF	2.81 (100)	0.365 (10)	0.113 (5)

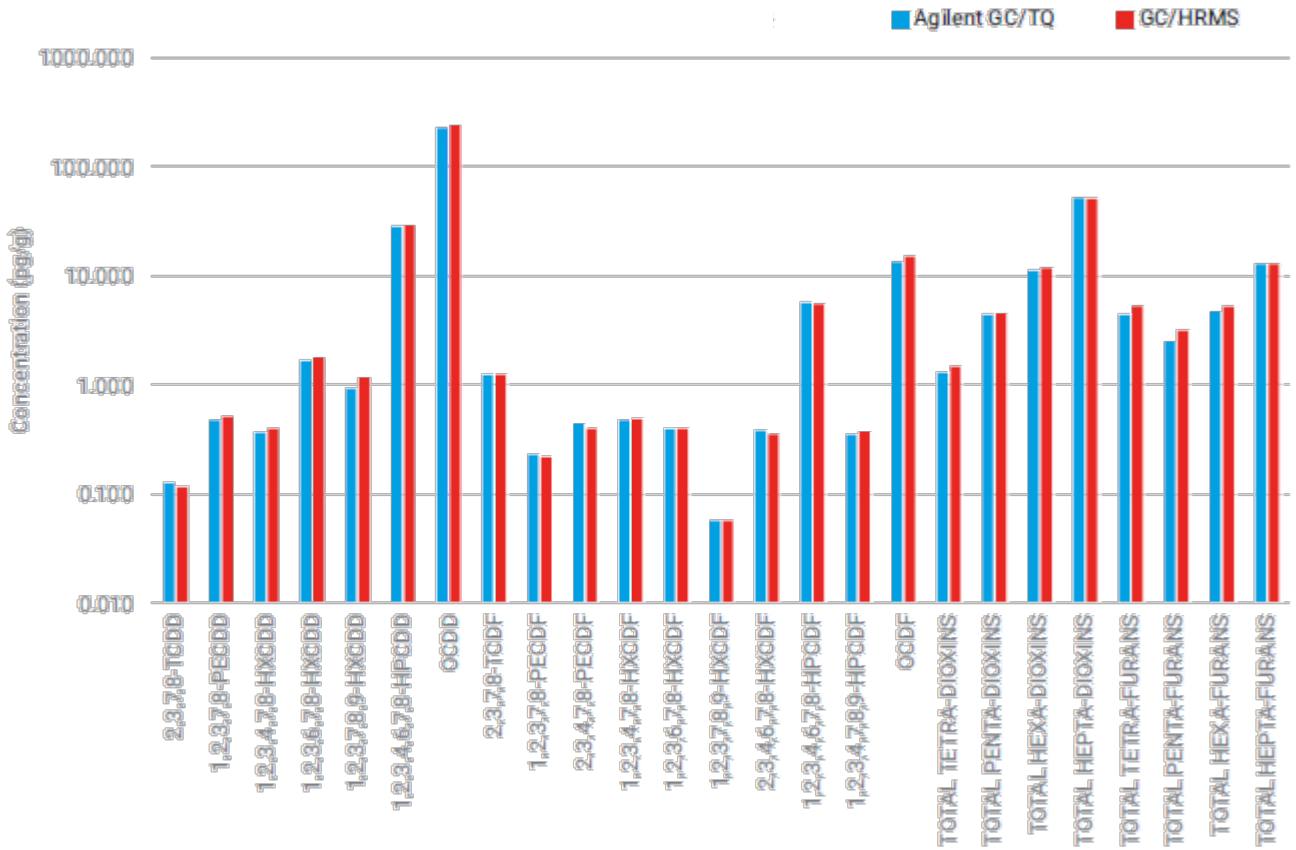
*The current MDL for 2,3,7,8-TCDD with the GC/HRMS 1613B is 4.4 pg/L

The MDL achieved with Agilent 7010B GC/TQ is **4 times** better!

Total PCDDs and PCDFs Reporting

Total PCDD and PCDF concentrations from the real-world sample extracts were reported by MassHunter software for each level of chlorination by **summing the concentration of the individual peaks meeting quantification criteria** (peak shape, S/N ≥ 2.5, and product ion ratio ±10%) in the appropriate retention time window.

Comparable total PCDDs and PCDFs reported using GC/HRMS and GC/TQ:



Conclusions

GC/TQ provides many of the specificity and sensitivity advantages of HRMS

Low Cost of Ownership

Lower instrument cost without the need for expensive consumables, maintenance, or specialized instrument operators

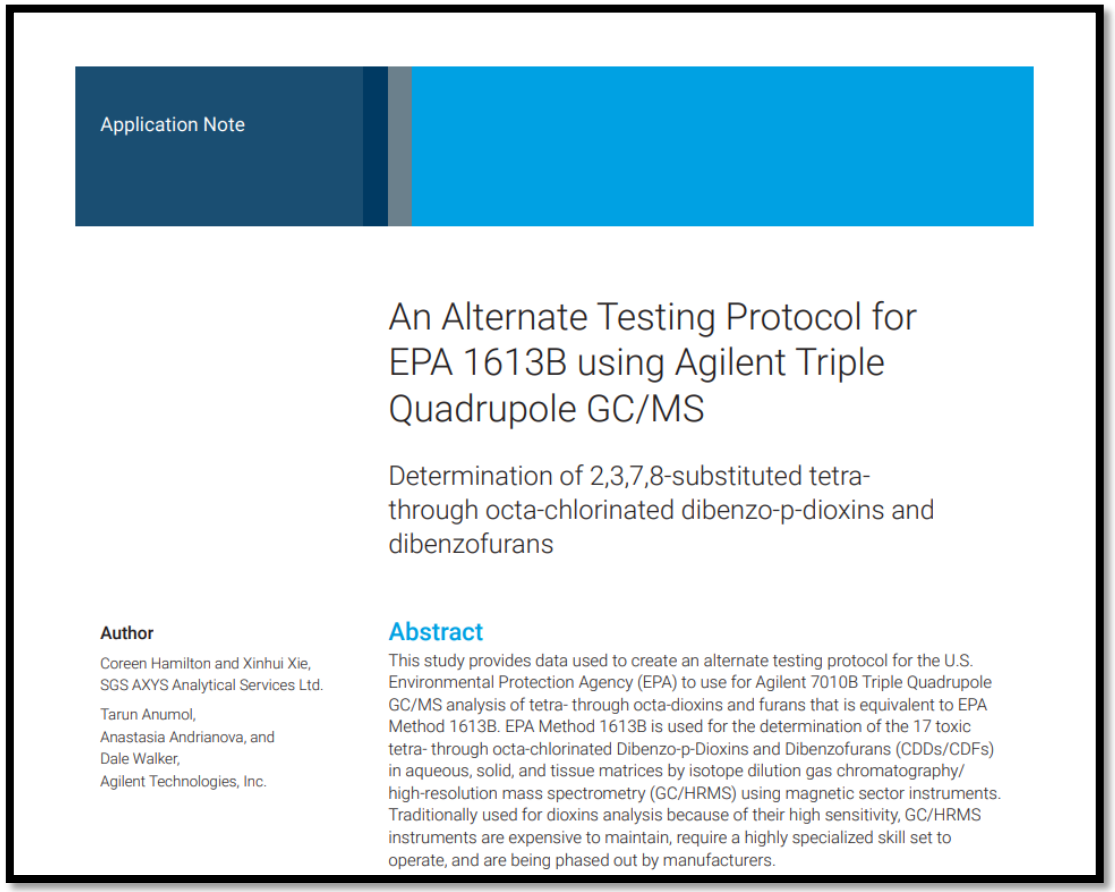
Versatility

7010C can be used strategically with other EPA methods leading to faster ROI

Flexibility

7010C utilizes electron ionization meaning it is compatible with several established libraries including the NIST GC Library

For More Information…



Application Note

An Alternate Testing Protocol for EPA 1613B using Agilent Triple Quadrupole GC/MS

Determination of 2,3,7,8-substituted tetra- through octa-chlorinated dibenzo-p-dioxins and dibenzofurans

Author

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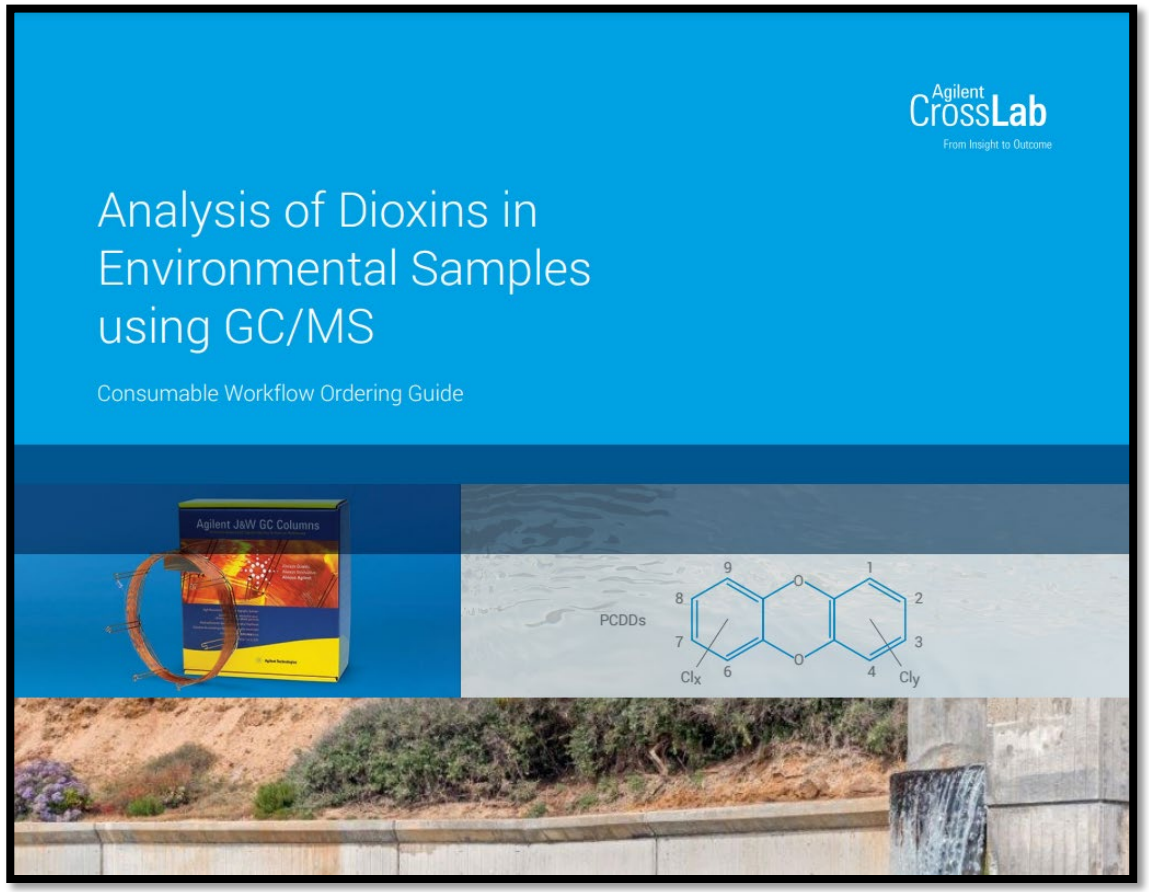
SGS AXYS Analytical Services Ltd.

Tarun Anumol,
Anastasia Andrianova, and
Dale Walker,
Agilent Technologies, Inc.

Abstract

This study provides data used to create an alternate testing protocol for the U.S. Environmental Protection Agency (EPA) to use for Agilent 7010B Triple Quadrupole GC/MS analysis of tetra- through octa-dioxins and furans that is equivalent to EPA Method 1613B. EPA Method 1613B is used for the determination of the 17 toxic tetra- through octa-chlorinated Dibenzo-p-Dioxins and Dibenzofurans (CDDs/CDFs) in aqueous, solid, and tissue matrices by isotope dilution gas chromatography/ high-resolution mass spectrometry (GC/HRMS) using magnetic sector instruments. Traditionally used for dioxins analysis because of their high sensitivity, GC/HRMS instruments are expensive to maintain, require a highly specialized skill set to operate, and are being phased out by manufacturers.



Agilent CrossLab
From Insight to Outcome

Analysis of Dioxins in Environmental Samples using GC/MS

Consumable Workflow Ordering Guide

Visit us at www.Agilent.com