

Food safety

Uncompromised sensitivity in polychlorinated dibenzo-*p*-dioxins/furans analysis using triple quadrupole GC-MS with cost-effective helium-saving technology

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Goal

The goal of this application note is to demonstrate the performance of the Thermo Scientific™ HeSaver-H₂Safer™ technology for the iConnect™ split/splitless (SSL) injector on the Thermo Scientific™ TRACE™ 1600 Series GC systems for the analysis of polychlorinated dibenzo-*p*-dioxins. In this study, a Thermo Scientific™ TSQ™ 9610 triple quadrupole mass spectrometer was used for detection and quantitation.

Introduction

Polychlorinated dibenzo-*p*-dioxins/furans (PCDDs/PCDFs) and their toxicity at trace levels continue to be a concern for regulatory agencies. The Thermo Scientific™ DFS™ Magnetic Sector gas chromatography high-resolution mass spectrometer (GC-HRMS) is the gold standard in dioxin analysis, providing worldwide compliance in environmental samples as outlined in the U.S. Environmental Protection Agency (EPA) Method 1613¹ and in food and feed under EU Regulations 644/2017 and 771/2017 for the analysis of PCDDs/PCDFs.^{2,3} With maximum allowable intake limits set at sub pg/g levels, sensitivity is of utmost importance for the analysis.

Helium is an ideal carrier gas in GC-MS instrumentation due to its high purity, inert nature, and overall performance. However, with global supply shrinking, laboratories are

facing challenges in maintaining operations with increasing costs and delayed delivery. Use of an alternative carrier gas, such as hydrogen, is a possible solution. However, its reduced pumping efficiency and reactivity can detrimentally impact sensitivity of MS instrumentation several fold, leading to challenges to meet regulatory requirements for tightly regulated substances, such as PCDDs/PCDFs.

To overcome challenges with gas supply and reduced performance with alternative carrier gases in GC-MS analysis, Thermo Fisher Scientific has released the innovative HeSaver-H₂Safer technology, available on the TRACE 1600 Series GC as an option for the standard iConnect SSL injector. This consists of a modified SSL injector body, where an alternative/cheaper gas (i.e., nitrogen) is used to pressurize the inlet and perform the functions of split and purge flow instead of helium.³ Meanwhile, helium is introduced at the base of the modified SSL body, as a carrier gas with a limited maximum flow rate, slightly higher than what required for the chromatographic separation. The pressurizing gas is used during the injection phase to transfer the sample into the column, but it is prevented from entering the column during the separation process by an anti-diffusion tubing. The combination of both leads to drastically reduced helium consumption during GC operation, as most of the gas consumption occurs through the split and purge lines. For users who wish to use hydrogen as a carrier gas, the HeSaver-H₂Safer is also compatible with hydrogen, where its flow is limited to allow for safe operation without the need for an additional hydrogen sensor installed.

Reducing helium gas consumption is the only way to mitigate helium shortage issues without impacting the analytical performance of GC-MS systems and compromising results. In this study, the performance of the HeSaver-H₂Safer option, together with its helium consumption and cost savings, was evaluated for trace analysis of PCDDs/PCDFs. The HeSaver-H₂Safer technology is used in this note on the TSQ 9610 GC-MS/MS, however it is also compatible with other GC-MS systems used for dioxins analysis, such as the [Thermo Scientific DFS Magnetic Sector GC-HRMS](#) and the [Thermo Scientific™ Orbitrap Exploris™ GC Mass Spectrometer](#).

Experimental

Standard and sample preparation

Isotopically labeled calibration standards (EPA-1613) were purchased from Wellington Laboratories (Canada). Each standard was diluted by a factor of 2 to construct a 6-level calibration curve. The lowest calibration standard was diluted by a factor of 5 to produce a limit of quantification (LOQ) check standard (0.01–0.1 pg·µL⁻¹). LOQ and sensitivity check standards (LOQ/2 and LOQ/4) were prepared through dilution of the lowest calibration standard.

A 2 g soil sample was extracted in a bi-phasic mixture of acetonitrile/hexane (4 mL:4 mL) followed by centrifugation at 3,000 rpm. 100 µL of the hexane layer was evaporated to dryness and spiked with 15 µL of the lowest calibration standard and reconstituted in nonane to 100 µL total volume. The spiked soil extract was injected repeatedly in two separate analyses over a 4-day period with calibration and LOQ/sensitivity check standards to assess instrument precision and sensitivity robustness over the entire duration of the analysis.

Instrument and method setup

The instrumental configuration of the TSQ 9610 GC-MS/MS has been previously described,⁴ with details summarized in Tables 1 and 2. Automated liquid injection was carried out by a Thermo Scientific™ TriPlus™ RSH autosampler. Chromatographic separation was performed on a TRACE 1610 GC equipped with a Thermo Scientific™ TraceGOLD™ TG-Dioxin 60 m × 0.25 mm i.d. × 0.25 µm film capillary column (P/N 26266-1540). Timed selected reaction monitoring (SRM) was performed for data acquisition, while quantification was performed using isotopic dilution. All required calculation steps are included in the Dioxin analyzer workflow available within the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software.

Table 1. GC injection and column conditions

Trace 1610 GC system parameters	
Injector	iConnect SSL with HeSaver-H ₂ Safer
Injection volume (µL)	1.5
Liner	Thermo Scientific™ Single taper with quartz wool (P/N 453A1925-UI)
Injection mode	Splitless (split flow 120 mL·min ⁻¹ after 2 min)
Split flow (mL·min ⁻¹)	120 (nitrogen)
Injector temperature (°C)	280
Pressurizing gas	Nitrogen
Carrier gas, (mL·min ⁻¹)	He, 1.2 (constant flow)
Oven temperature Program	
Initial temperature (°C)	120
Hold time (min)	2
Rate 1 (°C·min ⁻¹)	25
Temperature 1 (°C)	250
Hold time 1 (min)	0
Rate 2 (°C·min ⁻¹)	2.5
Temperature 2 (°C)	285
Hold time 2 (min)	0
Rate 3 (°C·min ⁻¹)	10
Temperature 3 (°C)	320
Hold time 3 (min)	15
Total run time (min)	44.7

Table 2. Ion source and mass spectrometer conditions

TSQ 9610 Triple Quadrupole GC-MS parameters	
Transfer line temperature (°C)	300
Advanced Electron Ionization (AEI) ion source temperature (°C)	350
Electron energy (eV)	50
Acquisition mode	Timed-SRM with dwell time prioritization (10 × (native (High), labeled (Low)))
Emission current (µA)	50
Collision gas and pressure (psi)	Argon, 70 psi

Results and discussion

Robust injection performance

The performance and durability of the HeSaver-H₂Safer SSL was evaluated in two separate injection sequences of a soil extract (no cleanup) spiked with PCDD/F with concentrations ranging from 15 to 150 fg·µL⁻¹ for the various dioxin and furan congeners. The relative standard deviation (RSD) of the absolute peak area for each PCDD/F congener measured within the spiked soil extract over the entire sequences is shown in Figure 1. After 69 injections in sequence 1, variation in peak area response was less than 9% RSD for all congeners. An additional sequence of 31 injections was carried out over two additional days with a new soil extract spiked at the same concentration level, without any injector or column maintenance carried out in between. Peak area response %RSD over the sequence 2 was less than 8%, showing excellent inter and intra-day repeatability injection performance.

Uncompromised sensitivity

Due to the inherent toxicity of PCDD/F, sensitivity is of upmost importance for instrumentation to meet the strict regulatory requirements of maximum allowable levels within food and feed. Sensitivity and LOQ check standards were analyzed during the sample analysis for LOQ determination. To ensure accurate quantification at trace levels, method performance criteria described under both EPA Method 1613¹ and current EU regulations^{2,3} were applied to LOQ check standards:

- Quantification and confirmation ions must be detected within the specified retention window within a ±0.01 min retention time tolerance
- Ion confirmation ratio between quantification and confirmation ions must be within 15% of the theoretical value
- Average relative response factor (RRF) value can deviate no more than 20% (at the start and end of analysis sequence) across all calibration curve points

At an injection volume of 1.5 µL, the LOQ/2 check solution passed all these criteria at fg levels for PCDD/F homologue groups (Figure 2). This demonstrates that MS performance remains uncompromised using nitrogen as a pressuring gas with the HeSaver-H₂Safer SSL.

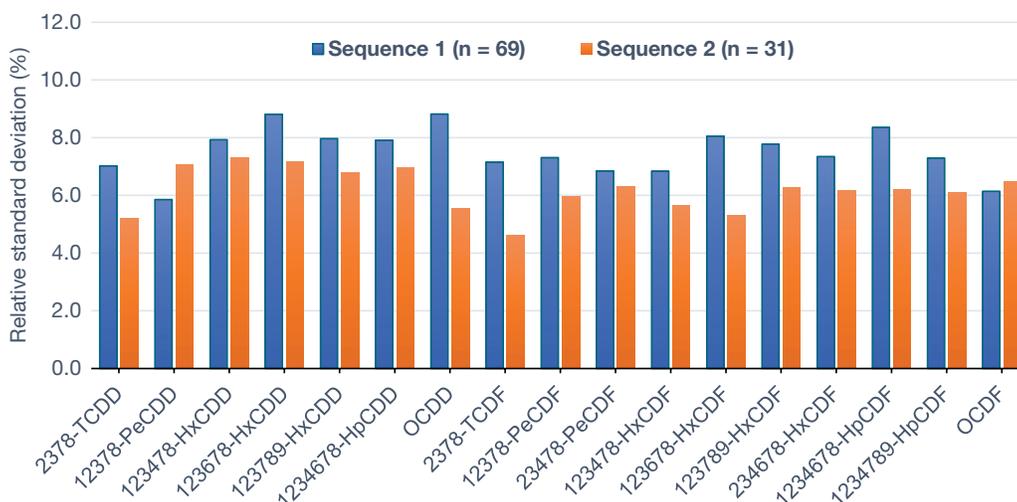


Figure 1. Relative standard deviation (%) of peak area response calculated for repeated injections of a soil extract spiked with PCDD/F (15–150 fg·µL⁻¹) in two separate analyses. The number of injections for separate analyses is represented by n value.

Selectivity and precision at ultra-trace concentrations

Sensitive performance is not only needed with solvent standards, but in real sample matrices over large analysis batches. Robust injection performance was demonstrated in Figure 1 using a raw soil extract (no cleanup). In terms of detector selectivity, Figure 3A shows full scan acquisition (50–500 m/z) together with the extracted SRM channels (Figure 3B) of the PCDD/F spiked into the raw soil extract. Defined amounts of PCDD/F were spiked into the soil extract to obtain a concentration slightly above the level previously tested with the LOQ/2 solution. Despite no cleanup being employed, selective detection of PCDD/F was easily obtained at fg levels. Quantification of PCDD/F through isotopic dilution using a dedicated Dioxin processing method within Chromeleon CDS software showed excellent precision-to-target values

spiked within the raw soil extract (Figure 4). The variation in the quantified concentration was ≤ 7.2 fg across 100 injections from both analysis sequences 1 and 2. Calculated concentrations were in good agreement with spiked concentrations, except for 1,2,3,4,6,7,8-HpCDD, OCDD, and OCDF. These congeners were present in the soil sample prior to spiking, resulting in higher calculated concentrations compared to the spike concentrations.

Helium consumption/savings

A key advantage of using the HeSaver-H₂Safer technology is that no modification of existing methods is needed, as would be the case when changing to a different carrier gas. The above results show that injection, chromatography, and detector performance remain uncompromised, while providing significant savings in terms of helium consumption and cost.

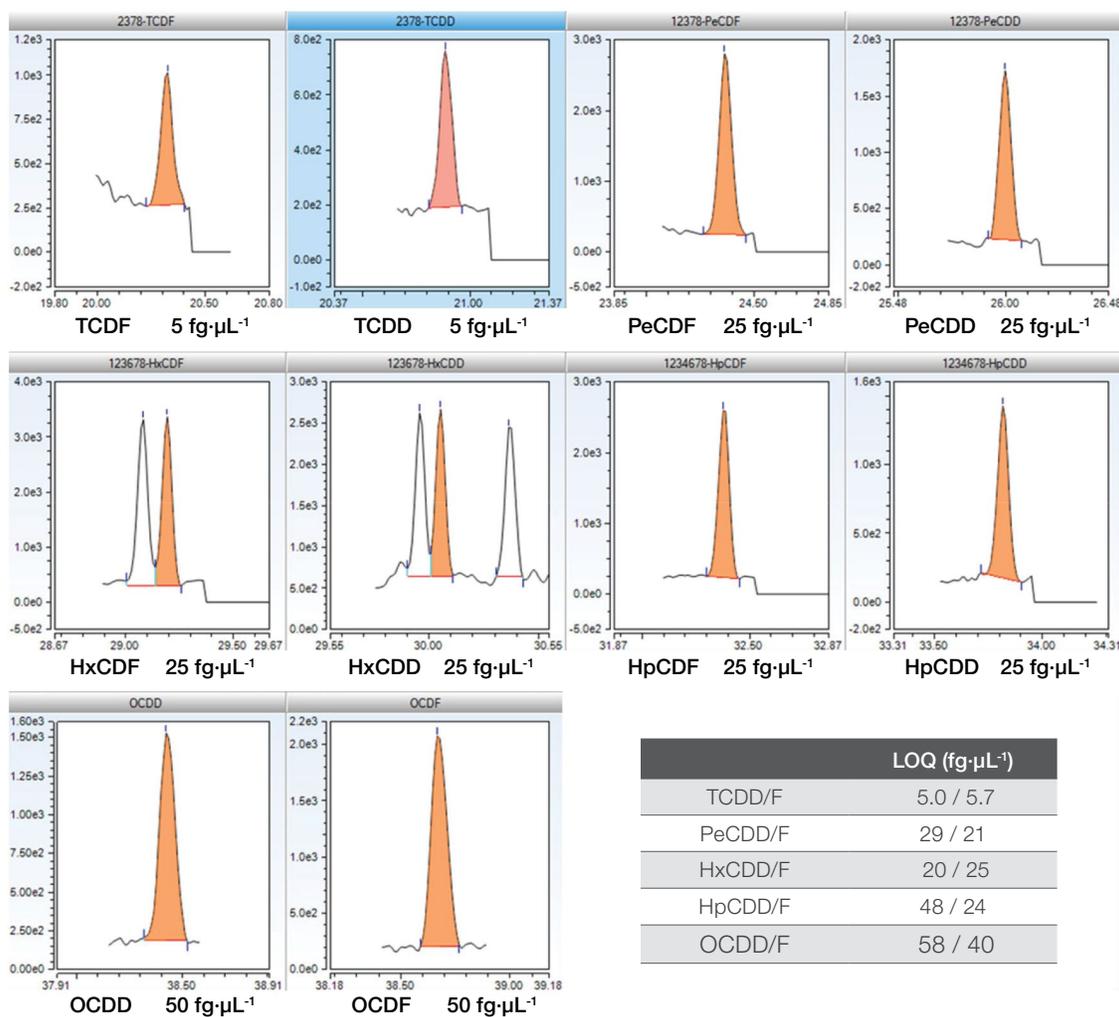


Figure 2. Extracted SRM channel of PCDD/F homologue groups in LOQ/2 check standard (5–50 fg·µL⁻¹) and LOQ calculated from analytical variation measured in LOQ/2 check standard

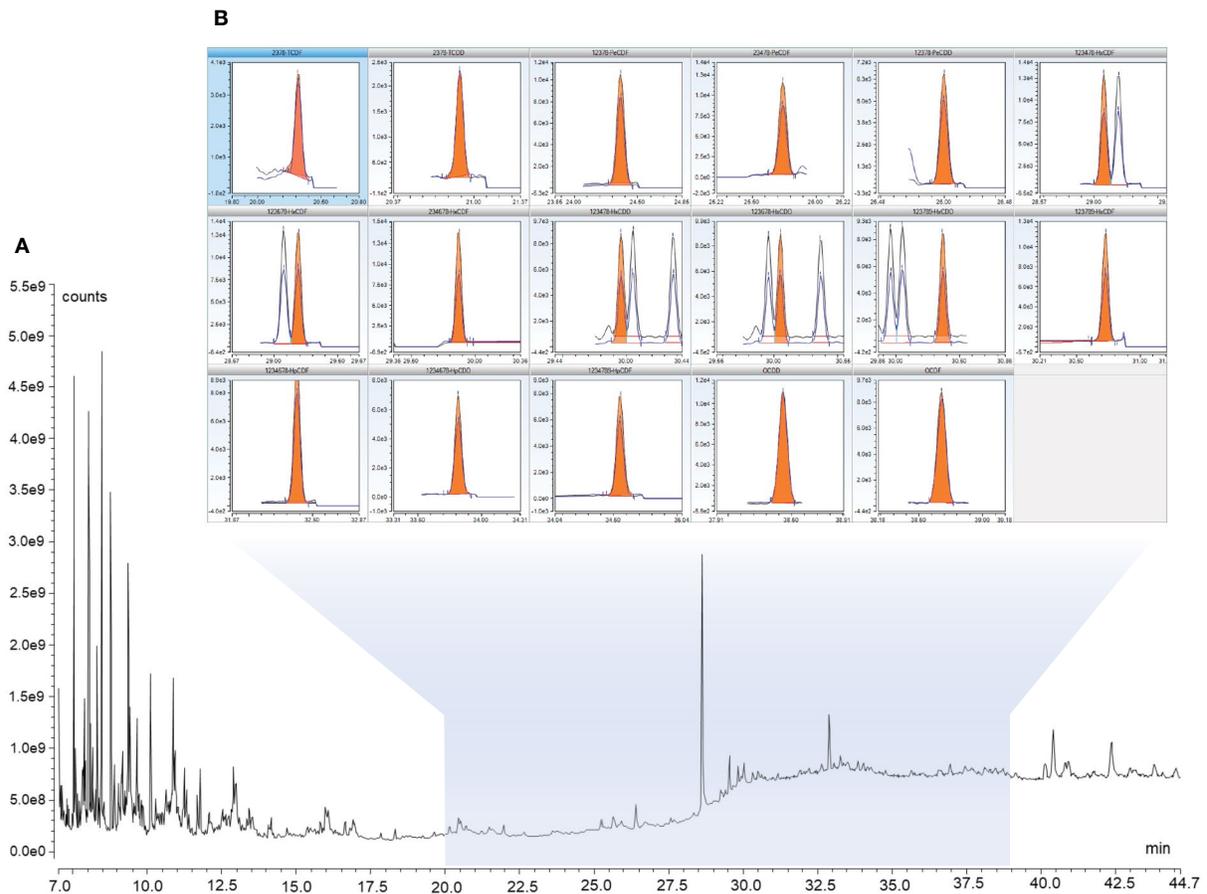


Figure 3. Full scan (50–500 m/z) total ion chromatogram (A) and extracted SRM (B) of PCDD/F spiked in a raw soil extract. Spike concentrations of homologue groups: TCDD/F: 15 $fg\cdot\mu L^{-1}$; PeCDD/F: 75 $fg\cdot\mu L^{-1}$; HxCDD/F: 75 $fg\cdot\mu L^{-1}$; HpCDD/F: 75 $fg\cdot\mu L^{-1}$; OCDD/F: 150 $fg\cdot\mu L^{-1}$

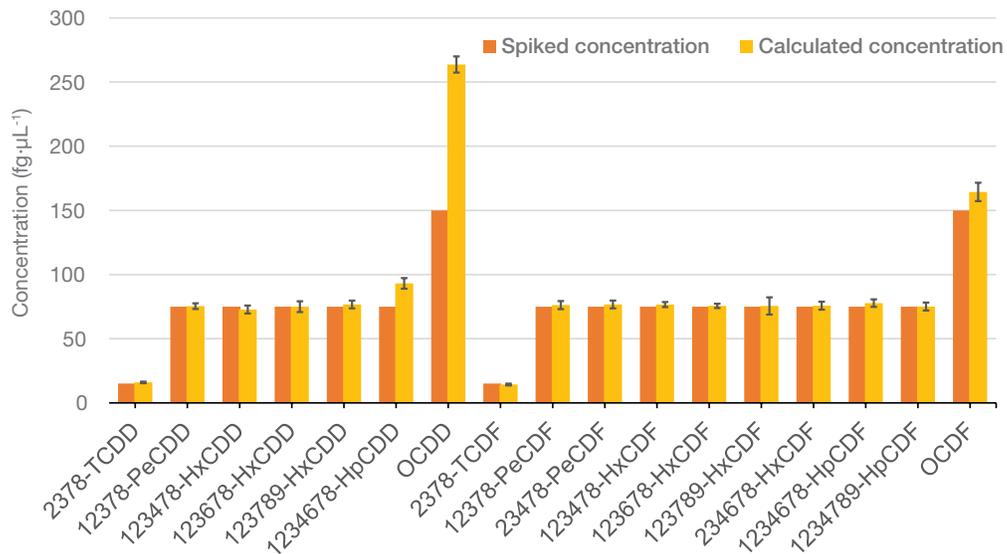


Figure 4. Spiked and calculated concentration of PCDD/F quantified from repetitive analysis of raw soil extract ($n=100$). Deviation between calculated and spiked concentrations for HpCDD, OCDD, and OCDF is attributed to their presence within the soil prior to spiking.

Helium consumption and cost were calculated with the online [Helium Saver Calculator](#) from the Thermo Fisher Scientific website and compared between using the HeSaver-H₂Safer SSL and standard SSL with the method parameters described above for PCDD/F analysis. Input parameters with helium consumption and cost savings are shown in Tables 3 and 4. The HeSaver-H₂Safer SSL consumed 7.6 times less helium compared to the standard SSL per sample analysis. If considering the situation where PCDD/F analysis was being carried out 24 hours a day for 365 days a year, a standard helium tank would last 1.8 years with

the HeSaver-H₂Safer SSL compared to 0.25 years (3 months) with a standard SSL. Under these conditions, a laboratory would consume up to 8 cylinders of helium using a standard SSL, whereas the same workload could be handled with 1 cylinder using the HeSaver-H₂Safer SSL (Figure 5). However, such conditions for dioxin analysis are not typically common, meaning a cylinder lifetime of up to almost 8 years could be achieved under more standard conditions (8 hours/day, 5 days a week, for 365 days) with cost savings over 10,000 USD within the 10-year lifespan of the instrument.

Table 3. Column and injection parameters with estimated helium and nitrogen cylinder cost

Column length (m)*	60	Column flow (sccm)*	1.2
Column ID (mm)*	0.25	Total time per sample (mins)	44.7
Film thickness (µm)*	0.25	Cost of helium cylinder (UHP 5.0)	350
Split flow setting (sccm)*	120	Cost of nitrogen cylinder (UHP 5.0)	50

Table 4. Estimated helium consumption and costs for PCDD/F analysis for HeSaver-H₂Safer SSL and standard SSL

	Helium usage featuring Helium Saver Technology	Standard helium usage
He volume used per sample:	0.33 Liters	2.50 Liters
N ₂ volume used per sample:	2.17 Liters	0
Estimated lifetime of helium cylinder (if using 24/7/365):	1.8727 Years	0.2479 Years
Estimated lifetime of helium cylinder (if using 8 hrs x 5 days/wk for 365):	7.6847 Years	1.0411 Years
Annual cost savings (if using 24/7/365):	\$1,049.23	\$0.00
Lifetime cost savings (assuming 14 years of GC-MS instrument life time):	\$14,689.19	\$0.00

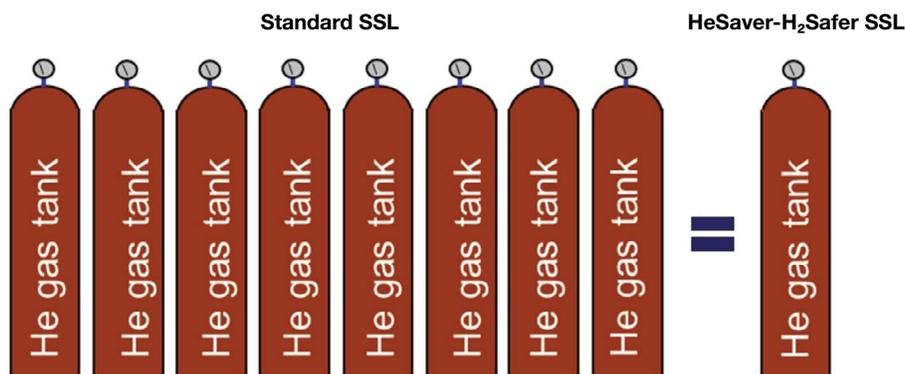


Figure 5. Visual diagram representing estimated helium consumption between standard and HeSaver-H₂Safer SSL inlets for PCDD/F analysis

Conclusion

This application demonstrates the performance of the HeSaver-H₂Safer technology used for trace analysis of PCDD/F in combination with the TSQ 9610 GC-MS/MS, which offers mitigation of helium shortage issues without impacting analytical results and without the need to re-optimize the methodology:

- Inter- and intra-day variation were less than 10% for absolute peak area for all PCDD/F congeners investigated within a sample matrix (no cleanup), demonstrating stable injection repeatability and robust performance of the HeSaver-H₂Safer technology.
- Mass spectrometric performance was uncompromised with precise quantification and fg-level sensitivity while maintaining retention time, ion ratio, and RRF method criteria.
- Helium consumption was reduced by a factor of 8 with the HeSaver-H₂Safer technology compared to the standard SSL, helping labs sustain both operation and performance.

References

1. United States Environmental Protection Agency (EPA) Method 1613 (1994). Tetra-through Octa-chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS.
2. Commission regulation (EU) 2017/644 (2017). Laying down method of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repeal Regulation (EU) No 589/2014.
3. Commission regulation (EU) 2017/771 (2017). Amending Regulation (EC) No. 152/2009 as regards the methods for the determination of the levels of dioxins and polychlorinated biphenyls.
4. Thermo Fisher Scientific, Technical Note 001218: Addressing gas conservation challenges when using helium or hydrogen as GC carrier gas. <https://assets.thermofisher.com/TFS-Assets/CMD/Technical-Notes/tn-001218-gc-hesaver-h2safer-trace1600-tn001218-na-en.pdf>
5. Thermo Fisher Scientific, Application Note 10590: Low level quantification of PCDD/Fs in animal feed using the Thermo Scientific TSQ 9000 triple quadrupole GC-MS/MS system with AEI source. <https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-10590-gc-ms-ms-pcdd-fs-animal-feed-an10590-en.pdf>

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