Application Note

Food and Feed Analysis

Hydrogen as Carrier Gas for the Analysis of Dibenzo-*p*-dioxins and Dibenzofurans (PCDD/Fs) in Food and Feed Samples Using an Agilent 7010 Series Triple Quadrupole GC/MS

Authors

Joerg Riener and Susann Lang
Agilent Technologies
Hewlett-Packard-Straße 8
76337 Waldbronn
Germany

Abstract

Until recently, the use of hydrogen as carrier gas for GC/MS/MS analysis of hydrophobic compounds at ultra-trace levels has been impractical. Drawbacks such as enhanced background, reduced signal-to-noise ratio (S/N), spectral changes, and most importantly, reduced sensitivity, were the limiting factors associated with the use of hydrogen. The Agilent 7010 series triple quadrupole GC/MS equipped with the high-efficiency electron ionization source (HES) enables much higher sensitivity by creating 20 times more ions, offering the possibility to compensate for method performance loss when using hydrogen. This is especially relevant in the field of ultra-trace analysis with GC/TQ, since limits of detection (LODs) and limits of quantification (LOQs) could not be reached otherwise.

This application note presents a validated GC/MS/MS method using hydrogen as carrier gas for the analysis of dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) in food and feed samples with the 7010 series GC/TQ. The aim was to reduce run times while maintaining method performance when transferring method settings from helium to hydrogen as carrier gas. Analysis time was reduced by a factor of approximately one third without undermining performance. Routine analysis of various food/feed matrices over six months verified robust system performance under high sample throughput conditions. The results clearly indicate that it is straightforward to transfer GC/TQ MS methods for stable hydrophobic organic compounds (HOCs) to hydrogen as carrier gas; PCDD/Fs are an excellent example due to challenges inherent in their analysis.

Introduction

The application of hydrogen as a carrier gas for chromatographic analyses has been discussed in the literature for various analytes, chromatographic systems, and detection techniques, in particular related to fast gas chromatography.[1–3] Due to some drawbacks of hydrogen, especially related to MS detection as well as safety considerations, hydrogen has not been widely adopted, even though it has several advantages compared to other conventionally used carrier gases, most commonly helium. Generally, when using hydrogen as carrier gas, improvements in resolution and separation and a reduction in runtime are observed.[4] Faster analysis is related to distinct properties of hydrogen, i.e., higher average linear velocity at a minimum of theoretical plates, related to density, diffusion coefficient, and viscosity (the van Deemter relationship). In contrast, potential disadvantages include reduced sensitivity, reflected by a 2–5-fold decrease in signal-to-noise ratio; differences in EI mass spectra, target-to-qualifier ion ratios, and abundance ratios; and possible reactivity of H_2 with analytes. Additionally, safety accommodations need to be considered when working with hydrogen due to its wide flammability range, which spans from 4% to 75% in air. Nevertheless, hydrogen is inexpensive and can be the best carrier gas of choice in certain applications.

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), together with dioxin-like (DL, including non-ortho (NO) and mono-ortho (MO) substituted) and non-dioxin-like (NDL) PCBs, are persistent organic pollutants of major toxicological concern that can bioaccumulate in the food chain and are regulated in the European Union through maximum levels in food under Regulation (EU) 2023/915, supported by official sampling and analytical requirements under Regulation (EU) 2017/644, with corresponding control provisions for feed under Regulation (EU) 709/2014. Based on legislation, the confirmation and quantitation of dioxins, furans, and dioxin-like polychlorinated biphenyls (dl-PCB) in food/feed samples had to be accomplished by isotope dilution capillary gas chromatography/high-resolution mass spectrometry (GC/HRMS). Since triple quadrupole GC/MS shows similar performance to high-resolution mass spectrometry (GC/HRMS) revisions have been initiated and accepted to allow GC/MS/MS as an adequate confirmatory method in an amendment to EU legislation 589/2014 and 709/2014 for checking compliance with maximum levels (ML) of PCDD/Fs in food and feed stuff.[5] On this basis, and with novel state-of-the-art technology, going one step further—converting the carrier gas from helium to hydrogen in ultra-trace level analysis—appears to be the next logical step.

The main objective of this study was to convert a PCDD/F analysis method for food and feed samples from helium to hydrogen as carrier gas without compromising analytical performance and shortening the analysis time. Therefore, in this study: (i) the 7000 series and 7010 series triple quadrupole systems were compared under helium conditions as a baseline study to specifically investigate the benefits for PCDD/F analysis generated by the 7010 technique, (ii) the PCDD/F method was converted to hydrogen as carrier gas, and system performance was observed by assessing initial stability, sensitivity, separation efficiency, mass spectra, and LOQs, (iii) the PCDD/F method was optimized for hydrogen application by developing novel chromatographic conditions (column and flow) and evaluating and verifying the system performance, (iv) method performance criteria were applied to food/feed samples as part of the validation (matrix effects), and (v) the method was applied in routine analysis for six months to assess robustness and stability of the system.

Experimental

Standards

Standards were purchased from Wellington Laboratories Inc. and Cambridge Isotope Laboratories. Calibration solutions, a test mix solution (TMS), and a window defining mix solution (WDM) were prepared in toluene. A 13-point calibration curve with varying levels of native and constant levels of isotope-labeled dioxin and furan analytes (Table 1) was analyzed. Each calibration solution contained all 17 native compounds as well as isotope-labeled analogs. The test mix solution contained the same analytes in a medium concentration of the calibration solutions and was used for method development. The WDM solution consisted mainly of critical-to-separate native dioxin and furan compounds in order to assess the separation efficiency of the GC columns and to define first and last eluters of the mass spectrometric time segments.

Samples, blanks, and reference material

Samples, blanks, and reference material from routine analysis were randomly selected from the laboratory's regular analytical throughput. Nevertheless, it was verified that the extracts covered varying types of matrices and PCDD/F contamination levels to be included in the study. Matrices included food and feed stuff such as eggs, egg powder, spices, milk, yogurt, infant milk powder, fat (powder and oils from plant or animal), feed (oils and powders, plant or animal, urea), mineralic feed (copper sulphate, trace element powder), meat meal, different types of fish (tuna, sardines, salmon, shrimp, cod, herring), mussels, poultry (fat, meat, muscle), whey powder, and feather meal. Concentrations for all PCDD/Fs ranged between approximately 10 fg and 100 pg per sample across all matrices evaluated.

Table 1. Concentrations of the calibration solutions and the test mix solution (TMS).

Abbreviation	Analyte	Calibration Range (pg/µL)	TMS (pg/µL)
Native Compounds			
2378-etraCDD	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	0.0017–9.32	0.68
12378-PentaCDD	1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin	0.0027–15.10	0.68
123478-HexaCDD	1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin	0.0031–17.00	0.68
123678-HexaCDD	1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin	0.0033–16.70	0.68
123789-HexaCDD	1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin	0.0033–17.90	2.73
1234678-HeptaCDD	1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin	0.0034–18.70	1.36
OctaCDD	1,2,3,4,6,7,8,9-Octachlorodibenzo-*p*-dioxin	0.0065–35.50	1.36
2378-TetraCDF	2,3,7,8-Tetrachlorodibenzofuran	0.0017–9.28	0.68
12378-PentaCDF	1,2,3,7,8-Pentachlorodibenzofuran	0.0032–17.90	0.68
23478-PentaCDF	2,3,4,7,8-Pentachlorodibenzofuran	0.0035–19.00	0.68
123478-HexaCDF	1,2,3,4,7,8-Hexachlorodibenzofuran	0.0036–20.20	0.68
123678-HexaCDF	1,2,3,6,7,8-Hexachlorodibenzofuran	0.0031–16.00	0.68
123789-HexaCDF	1,2,3,7,8,9-Hexachlorodibenzofuran	0.0034–26.40	0.68
234678-HexaCDF	2,3,4,6,7,8-Hexachlorodibenzofuran	0.0034–18.60	0.68
1234678-HeptaCDF	1,2,3,4,6,7,8-Heptachlorodibenzofuran	0.0031–16.80	1.36
1234789-HeptaCDF	1,2,3,4,7,8,9-Heptachlorodibenzofuran	0.0036–19.90	1.36
OctaCDF	1,2,3,4,5,6,7,8-Octachlorodibenzofuran	0.0071–38.80	1.36
Isotope-Labeled Compounds			
$^{13}C_{12}$-1368-TetraCDD	$^{13}C_{12}$-1,3,6,8-Tetrachlorodibenzo-*p*-dioxin	10.00	0.57
$^{13}C_{12}$-2378-TetraCDD	$^{13}C_{12}$-2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	08.50	0.49
$^{13}C_{12}$-12378-PentaCDD	$^{13}C_{12}$-1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin	20.00	1.14
$^{13}C_{12}$-123478-HexaCDD	$^{13}C_{12}$-1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin	23.00	1.30
$^{13}C_{12}$-123678-HexaCDD	$^{13}C_{12}$-1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin	18.00	1.01
$^{13}C_{12}$-1234678-HeptaCDD	$^{13}C_{12}$-1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin	18.50	1.02
$^{13}C_{12}$-OctaCDD	$^{13}C_{12}$-1,2,3,4,6,7,8,9-Octachlorodibenzo-*p*-dioxin	40.50	2.26
$^{13}C_{12}$-2378-TetraCDF	$^{13}C_{12}$-2,3,7,8-Tetrachlorodibenzofuran	11.00	0.62
$^{13}C_{12}$-12378-PentaCDF	$^{13}C_{12}$-1,2,3,7,8-Pentachlorodibenzofuran	15.50	0.87
$^{13}C_{12}$-23478-PentaCDF	$^{13}C_{12}$-2,3,4,7,8-Pentachlorodibenzofuran	19.50	1.08
$^{13}C_{12}$-123478-HexaCDF	$^{13}C_{12}$-1,2,3,4,7,8-Hexachlorodibenzofuran	20.50	1.13
$^{13}C_{12}$-123678-HexaCDF	$^{13}C_{12}$-1,2,3,6,7,8-Hexachlorodibenzofuran	20.50	1.13
$^{13}C_{12}$-123789-HexaCDF	$^{13}C_{12}$-1,2,3,7,8,9-Hexachlorodibenzofuran	27.00	1.50
$^{13}C_{12}$-234678-HexaCDF	$^{13}C_{12}$-2,3,4,6,7,8-Hexachlorodibenzofuran	22.50	1.26
$^{13}C_{12}$-1234678-HeptaCDF	$^{13}C_{12}$-1,2,3,4,6,7,8-Heptachlorodibenzofuran	17.50	0.96
$^{13}C_{12}$-1234789-HeptaCDF	$^{13}C_{12}$-1,2,3,4,7,8,9-Heptachlorodibenzofuran	26.00	1.45
$^{13}C_{12}$-OctaCDF	$^{13}C_{12}$-1,2,3,4,5,6,7,8-Octachlorodibenzofuran	46.00	2.53

Instruments and analysis

All investigations were carried out using an Agilent 7890B gas chromatograph coupled to an Agilent 7000C triple quadrupole MS and an Agilent 7010 series triple quadrupole MS, equipped with a Gerstel MPS auto sampler system and a cold injection system (CIS) with programmed temperature vaporization (PTV). The comparison of both MS/MS systems (7000C versus 7010) for PCDD/Fs was performed under helium conditions. It should be noted that although the same 7890 B GC was used throughout, the 7000C MS was converted (upgraded) to the 7010 series during the study. The 7010 series triple quadrupole system was used for measurements applying hydrogen as carrier gas. Measurements were carried out in Multiple Reaction Monitoring (MRM) mode. Two precursor ions and their respective product ions were monitored. Detailed hydrogen method conditions are given in Tables 2 and 3.

Table 2. GC and MS/MS conditions using hydrogen as carrier gas.

GC Conditions	
Column	VF-Xms, 40 m × 0.18 mm, 0.18 µm (CP9049)
Carrier Gas	Hydrogen
Flow	Constant, 0.9 mL/min
ALV	45.9 cm/sec
Oven Program	120 °C (0.5 min hold), 50 °C/min to 210 °C (0.22 min hold), 3 °C/min to 270 °C, 45 °C/min to 320 °C (4.00 min hold)
Inlet	Cold Injection system (CIS)
Inlet Program	80 °C (initial), 12 °C/sec to 320 °C, equilibration time 0.1 min, initial time 1.00 min
Injection	5 µL, Solvent vent
Purge Flow (Split Vent)	80 mL/min at 2 min
MS/MS Conditions	
Transfer Line Temperature	300 °C
MS Temperatures	150 °C
Source Temperature	280 °C
Gain	80
MS Resolutions	wide
Electron Energy	70 eV
Quench Gas	off
Collision Gas	1.5 mL/min
Solvent Delay	10.5 min

Table 3. MS/MS acquisition settings for PCDD/Fs using hydrogen as carrier gas.

Time Segment	TS Start Time (min)	Analyte	RT (min)	Precursor Ion	Product Ion	Dwell (ms)	CE (V)
1	10.00	$^{13}C_{12}$-1234-TetraCDD	13.18	333.9	269.9	25	24
				331.9	267.9	25	24
		$^{13}C_{12}$-2378-TetraCDF	13.22	317.9	253.9	25	33
				315.9	251.9	25	33
		2378-TetraCDF	13.24	305.9	242.9	75	33
				303.9	240.9	75	33
		$^{13}C_{12}$-2378-TetraCDD	13.58	333.9	269.9	25	24
				331.9	267.9	25	24
		2378-TetraCDD	13.59	321.9	258.9	75	24
				319.9	256.9	75	24
2	14.20	$^{13}C_{12}$-12378-PentaCDF	16.19	351.9	287.9	25	35
				349.9	285.9	25	35
		12378-PentaCDF	16.21	339.9	276.9	75	25
				337.9	274.9	75	25
		$^{13}C_{12}$-23478-PentaCDF	17.17	351.9	287.9	25	35
				349.9	285.9	25	35
		23478-PentaCDF	17.18	339.9	276.9	75	25
				337.9	274.9	75	25
		$^{13}C_{12}$-12378-PentaCDD	17.30	367.9	303.9	25	25
				365.9	301.9	25	25
		12378-PentaCDD	17.31	355.9	292.9	75	25
				353.9	290.9	75	25

Time Segment	TS Start Time (min)	Analyte	RT (min)	Precursor Ion	Product Ion	Dwell (ms)	CE (V)
3	17.80	$^{13}C_{12}$-123478-HexaCDF	19.98	387.8	323.9	25	35
				385.8	321.9	25	35
		123478-HexaCDF	19.99	375.8	312.9	75	35
				373.8	310.9	75	35
		$^{13}C_{12}$-123678-HexaCDF	20.13	387.8	323.9	25	35
				385.8	321.9	25	35
		123678-HexaCDF	20.14	375.8	312.9	75	35
				373.8	310.9	75	35
		$^{13}C_{12}$-234678-HexaCDF	20.87	387.8	323.9	25	35
				385.8	321.9	25	35
		234678-HexaCDF	20.88	375.8	312.9	75	35
				373.8	310.9	75	35
		$^{13}C_{12}$-123478-HexaCDD	20.96	403.8	339.8	25	25
				401.8	337.8	25	25
		123478-HexaCDD	20.97	391.8	328.9	75	25
				389.8	326.9	75	25
		$^{13}C_{12}$-123678-HexaCDD	21.09	403.8	339.8	25	25
				401.8	337.8	25	25
		123678-HexaCDD	21.11	391.8	328.9	75	25
				389.8	326.9	75	25
		$^{13}C_{12}$-123789-HexaCDD	21.39	403.8	339.8	25	25
				401.8	337.8	25	25
		123789-HexaCDD	21.40	391.8	328.9	75	25
				389.8	326.9	75	25
		$^{13}C_{12}$-123789-HexaCDF	22.00	387.8	323.9	25	35
				385.8	321.9	25	35
		123789-HexaCDF	22.01	375.8	312.9	75	35
				373.8	310.9	75	35
4	22.60	$^{13}C_{12}$-1234678-HeptaCDF	23.22	421.8	357.8	25	36
				419.8	355.8	25	36
		1234678-HeptaCDF	23.23	409.8	346.8	75	36
				407.8	344.8	75	36
		$^{13}C_{12}$-1234678-HeptaCDD	23.86	437.8	373.8	25	25
				435.8	371.8	25	25
		1234678-HeptaCDD	23.87	425.8	362.8	75	25
				423.8	360.8	75	25
		$^{13}C_{12}$-1234789-HeptaCDF	24.21	421.8	357.8	25	36
				419.8	355.8	25	36
		1234789-HeptaCDF	24.22	409.8	346.8	75	36
				407.8	344.8	75	36
5	24.60	$^{13}C_{12}$-OctaCDD	25.35	471.7	407.8	25	26
				469.7	405.8	25	26
		OctaCDD	25.36	459.7	396.8	75	26
				457.7	394.8	75	26
		$^{13}C_{12}$-OctaCDF	25.54	455.7	391.8	25	35
				453.7	389.8	25	35
		OctaCDF	25.54	443.7	380.8	75	35
				441.7	378.8	75	35

Before measurements with hydrogen as carrier gas were performed, certain technical changes on the GC/TQ system were initiated to address safety considerations. These included installation of a hydrogen sensor, removal of all loose covers, and connection of vent lines (split vent, septum purge) to the exhaust. Changing the draw out plate to a larger diameter should also be considered, because ions are more scattered in the MS source when using H_2; however, this was not applicable for the PCDD/F method because of a potential loss of sensitivity. In contrast, linearity will improve with a larger-diameter draw out plate. Concurrent with the self-cleaning effect of hydrogen, source cleaning is required less frequently, but high background was observed in the system at early stages. Therefore, a conditioning protocol for background stabilization was also applied, which included an increase of source and MS temperature (source temperature: 300 °C, MS transfer line temperature: 320 °C), a slight increase in the flow (2 mL/min), a reduction of the electron multiplier voltage (EMV) to 800 V, and filament operation for one night.[4,6] It is well known that using hydrogen can degrade the MS vacuum because it increases gas load and is more difficult to pump efficiently than helium. For that reason, the carrier gas flow should be kept suitable for the pumping system. When using hydrogen with a triple quadrupole GC/MS system, there is no need for quench gas flow in the collision cell, because hydrogen will not produce any meta-stable ions. This reduces the total flow, thus improving vacuum. Accordingly, a decrease of the vacuum was not observed and compensated for in this study. In the end, slightly better vacuum was found under hydrogen conditions.

Results and discussion

Baseline study: 7000C versus 7010 series triple quadrupole technique

The 7010 series triple quadrupole GC/MS system operating in MRM mode (MS/MS) offers lower detection limits and higher sensitivity due to an increased ionization efficiency enabled with the HES source. Higher signals were achieved with the 7010 series GC/TQ when compared to the 7000 series GC/TQ resulting in improved ion ratios providing better qualitative information. Since this was a mandatory requirement for the application of hydrogen as carrier gas in ultra-trace analysis of PCDD/Fs in food and feed samples, the performance of the 7000 series and 7010 series GC/TQs were first compared under helium conditions. Linearity, sensitivity, precision, repeatability, and LOQs for the existing analytical helium method were assessed in order to evaluate the quantitative improvement of the 7010's detector for dioxin and furan analysis (Table 4).

Excellent linearity was observed with both systems, with R^2 values > 0.995 for all analytes. The relative standard deviation of the calibration procedure was found to be 1.1–3.1% and 2.4–5.9% for the 7010 and 7000 systems, respectively. The residues of the model linear function were more stable within the 13-point calibration curve for the 7010 system; while stability (low residues) was reached down to the lowest calibration point of 1.73 fg/µL for the 2,3,7,8-TetraCDD, the 7000C system showed higher residues at the two lowest calibration points and was therefore only stable to 6.33 fg/µL (2,3,7,8-TetraCDD). The RRFs (relative response factors) of the calibration for all dioxin and furan compounds were also found to be more stable within the calibration of the 7010 system and ranged from 14–23% and 18–30% for the 7010 and 7000C systems, respectively.

The sensitivity of both triple quadrupole GC/MS systems was assessed based on the signal-to-noise ratio of the three lowest calibration points (1.73, 2.71, and 6.33 fg/µL). The 7000 series detector exhibited a clearly identifiable signal, different from noise, at the third lowest calibration point (6.33 fg/µL, S/N ratio = 3.4 for 2,3,7,8-TetraCDD). At the lowest and second lowest calibration points, no peak was detectable. In contrast, a S/N ratio of 13 (2,3,7,8-TetraCDD) was found at the lowest calibration point (1.73 fg/µL) using the 7010 series GC/TQ.

The TMS with a mean concentration of the calibration was used to evaluate the measurement precision. The TMS was consecutively injected 10 times (n = 10) and the normalized precision, based on area ratios of native to $^{13}C_{12}$-labeled compounds, was consistent across all congeners on both systems, with the same trend observed for absolute precision (peak ares). The RSD of the normalized precision was ~2% higher for the 7000 series MS/MS and vice versa for the absolute precision of the 7010 series MS/MS. The RSD of repeatability (pg absolute) ranged from 0.5–2.5% for the 7010 series detector.

Limits of quantification (LOQs) were calculated based on the signal-to-noise ratio. Lower LOQs were observed for the 7010 system, i.e., an improvement by a factor of 4 and 5 for 2,3,7,8-TetraCDD and OctaCDD, respectively. Table 4 summarizes the qualification study as a comparison of the two triple quadrupole systems for all parameters tested. The results showed that the 7010 series GC/TQ met the conditions necessary to proceed with hydrogen as carrier gas.

First experiments with hydrogen

Following the baseline study, conversion from helium to hydrogen was achieved using a previously installed cross valve (three-way valve) employed to ensure a fast change of carrier gas. As expected, reduced sensitivity, intensity, and signal-to-noise ratio as well as increased background were observed in the first few days. After one week, the system stabilized and was ready for measurements. Part of this may be due to the fact that the system and gas lines were relatively new; however, in some cases, it could take longer to see the background decrease when converting a system previously used with helium carrier gas to hydrogen, because hydrogen has a cleaning effect. At this stage of the project, the main objective was to assess whether the hydrogen system proved to be applicable for PCDD/F analysis and to evaluate the differences in method performance between hydrogen and helium. For that reason, a simple method translation and the same column used under helium (VF-Xms 60 × 0.25 mm, 0.25 µm) were deemed to be sufficient for our purposes. Finally, a GC/MS/MS hydrogen carrier gas method was created that was sufficient to prove the concept, though it was not thoroughly optimized.

First observations revealed that the absolute abundance and peak areas decreased by a factor of ~2 with hydrogen carrier gas when compared to helium. Additionally, an increase in noise and a decrease of the LOQ by a factor of ~2 were found with hydrogen carrier gas. Nevertheless, LOQs ranged from 10–20 fg absolute for 2,3,7,8-TetraCDD over 10 measured replicates and improved with time. The same was observed for OctaCDD LOQs, with a range between 20–80 fg absolute.

Table 4. Performance comparison of the 7010 and 7000C systems.

Parameter Tested	7010 System	7000C System
Calibration/Linearity		
Calibration Procedure	1.1–3.1% RSD	2.4–5.9% RSD
Residues	Better stability over the calibration	Two lowest calibration points revealed high residues (not stable)
Relative Response Factors	14–23% RSD	18–30% RSD
Sensitivity		
Signal at Lowest Calibration Point (1.73 fg/µL)	S/N ratio = 13 for 2,3,7,8-TetraCDD	Not detectable
Signal at Second Lowest Calibration Point (2.71 fg/µL)	S/N ratio = 20 for 2,3,7,8-TetraCDD	Not detectable
Signal at Third Lowest Calibration Point (6.33 fg/µL)	S/N ratio = 48 for 2,3,7,8-TetraCDD	S/N ratio = 3 for 2,3,7,8-TetraCDD
Peak Areas	20 times higher	
Precision		
Normalized	0.9% RSD (2,3,7,8-TetraCDF)	2.7% RSD (2,3,7,8-TetraCDF)
Absolute	8.4% RSD (2,3,7,8-TetraCDF)	6.3% RSD (2,3,7,8-TetraCDF)
LOQ (fg)		
2,3,7,8-TetraCDD	Mean = 6, n = 10, RSD 11%	Mean = 25, n = 10, RSD 26%
OctaCDD	Mean = 32, n = 3, RSD 4%	Mean = 159, n = 3, RSD 20%

Figure 1 compares a helium chromatogram for 2,3,7,8-TetraCDD to a hydrogen chromatogram generated 7 days after changing the carrier gas. The comparison clearly illustrates the typically narrower peak shape and peak sharpness, as is expected when switching from helium to hydrogen.

Generally, the TMS standard solution showed acceptable and stable concentration levels and LOQs and overall comparable system performance for all PCDD/F congeners after the system had adapted to hydrogen conditions. MS1 scans of OctaCDD (Figure 2) showed no differences in fragmentation pattern, though ion ratios differed. Thus, it wasn't necessary to change target and qualifier ions in the method. Separation efficiency also proved comparable (Figure 3, example 2,3,7,8-TetraCDF) between hydrogen and helium when using the same column. HexaCDD/Fs were up to 10% better separated under hydrogen conditions and achieved almost baseline separation. Moreover, a first comparison of sample concentrations (hydrogen/helium ratio) revealed an average congener concentration of 91.6% (n = 16, 17 native congeners) and an average recovery rate of 101.4% (n = 16, 17 $^{13}C_{12}$-labeled congeners). Given these promising initial results, details of each single PCDD/F congener were investigated further.

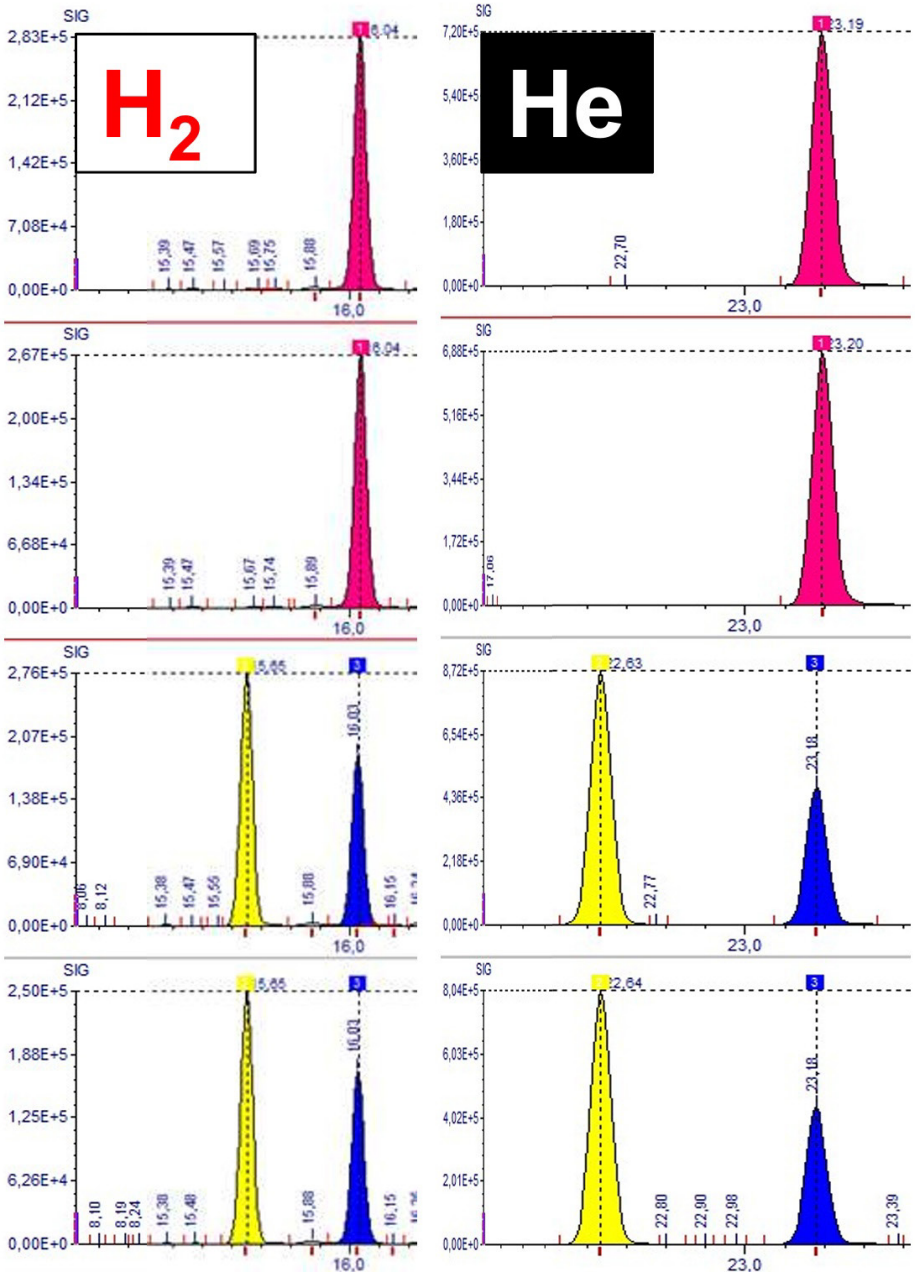

Figure 1. Hydrogen (left) and helium (right) chromatogram of 2,3,7,8-TetraCDD. The hydrogen chromatogram was generated 7 days after conversion of carrier gas. The pink peak indicates the native compound, the blue peak corresponds to the labeled compound, and the yellow peak is the injection standard.

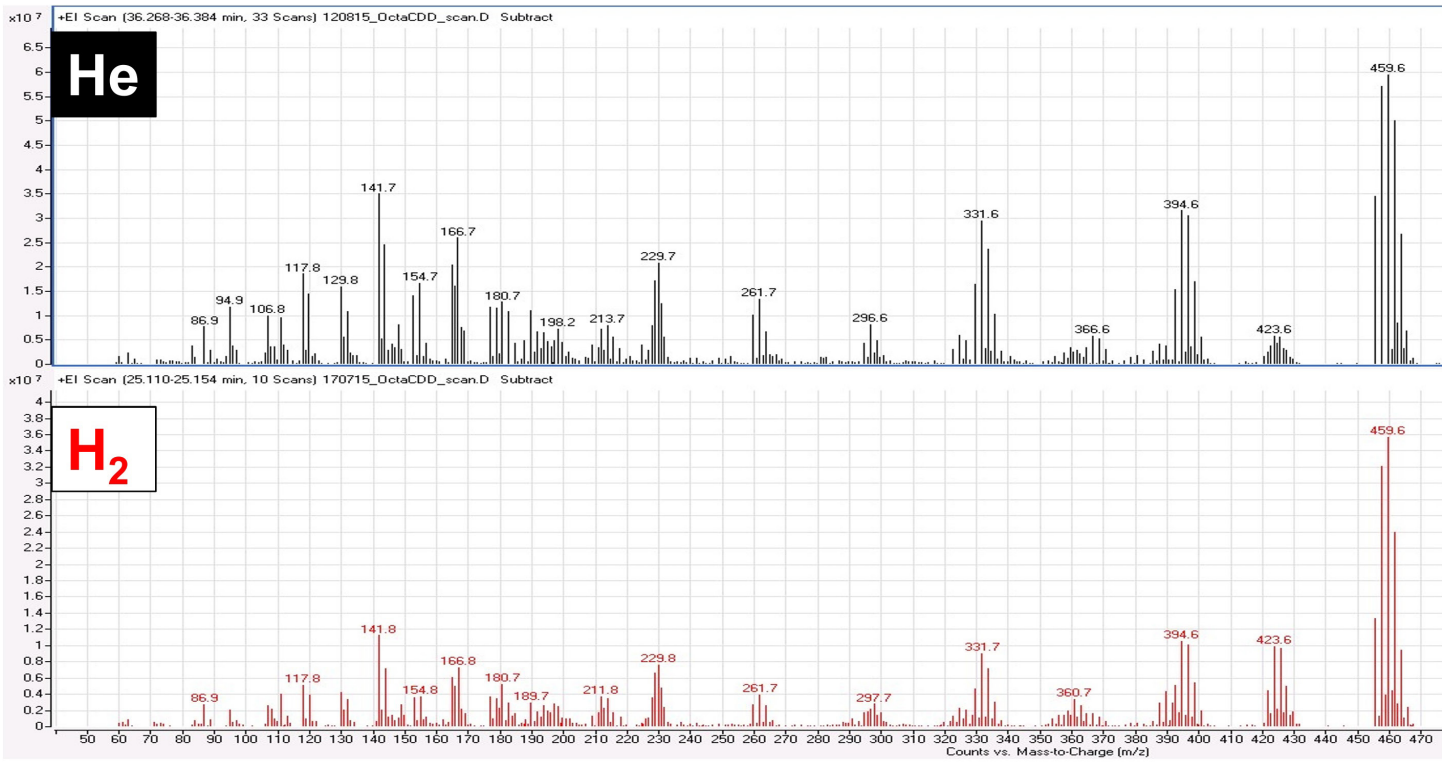

Figure 2. MS1 scans of OctaCDD (top helium, bottom hydrogen).

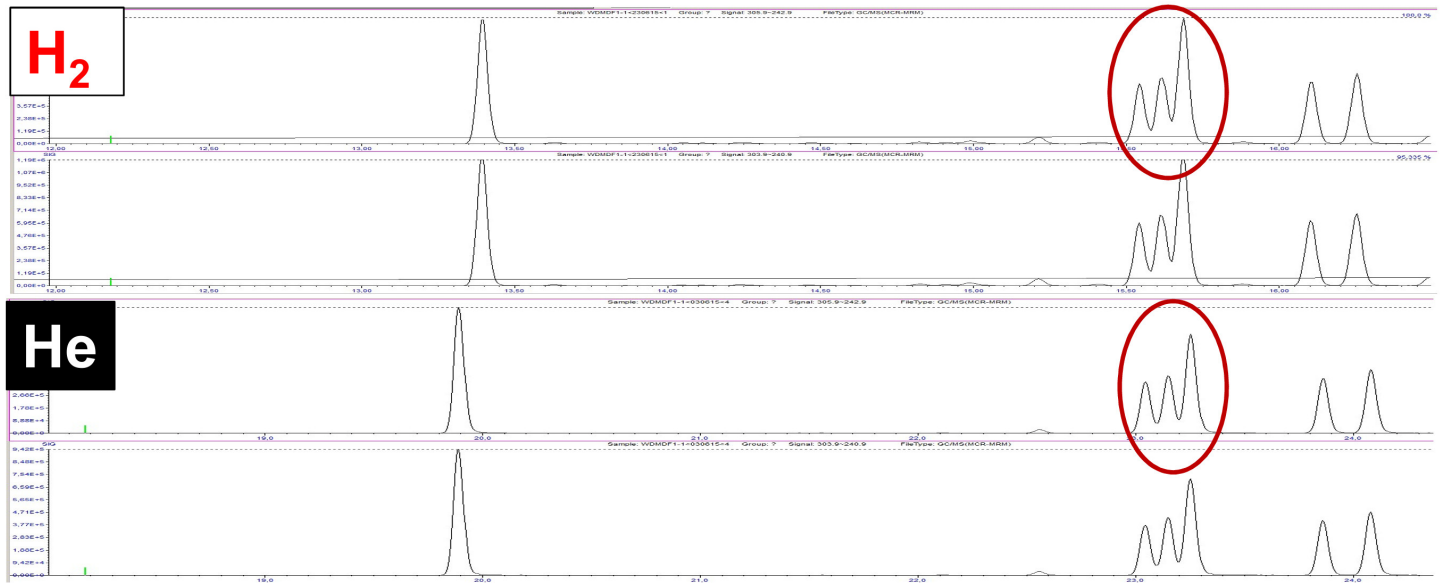

Figure 3. TetraCDF mass traces (target and qualifier ions) of the WDM solution showing the separation of 2,3,7,8-TetraCDF from 2,3,4,8-TetraCDF under hydrogen (top) and helium (bottom) conditions.

The final method and its benefits

Column choice is crucial when using hydrogen as carrier gas. The same phase ratio ensures minimum (if any) variation in the elution order of analytes, and a smaller diameter allows applying pressures appropriate for the system. The final H_2 method used here for dioxins and furans was developed on a 40 m VF-Xms column (0.18 µm × 0.18 mm, Agilent p/n CP9049). Several method parameters relevant to injection, MS settings, and GC settings were tested and optimized (details not provided here); special attention was paid to the carrier gas flow and temperature program. Regarding the vacuum, the total flow needs to be kept to an acceptable level for the pumping system; keeping head and inlet pressures constant requires raising the flow when using the same column dimensions. In this case, a hydrogen-induced pressure drop can be circumvented using shorter columns with smaller diameter when applying the same or lower flow rate. In this way, far less stress is put on the pumping system. Because hydrogen has a higher optimal linear velocity on the Van Deemter curve, we were able to preserve separation efficiency while shortening the method to a 27.5-minute run using a constant flow of 0.9 mL/min, corresponding to an average linear velocity of 45.9 cm/s. Figure 4 shows a comparison of total ion current chromatograms (TIC) generated in MRM mode with helium (60 m VF-Xms column, flow: 1.2 mL/min) and hydrogen (40 m VF-Xms column, flow: 0.9 mL/min) for PCDD/Fs. A 40% reduction in acquisition time was achieved under these conditions using hydrogen.

Method validation

Following development and creation of the final acquisition method, it was necessary to qualify the system to confirm method compliance between hydrogen and helium carrier gas. A 13-point calibration curve (measured in triplicate) showed excellent linearity for the hydrogen system, with an RSD of the calibration procedure of 1–4% for all compounds. Residue analysis (deviation of data points from the model linear function) exhibited theoretical stability down to 1–2 fg/µL; in practical application, 5.0 fg/µL was found to be a feasible and measurable concentration. Relative response factors (RRFs) derived from the calibration ranged between 0.57 and 1.0, while lowest RSD was found for the OctaCDD (5.5%) and highest for the 2,3,7,8-TetraCDD (12.5%) congener. The RSDs of RRFs for all compounds ranged from 18–30% and 14–23% under helium conditions as generated with the 7000C and 7010 sources, respectively. Contrary to our expectations, these values improved when using hydrogen. The signal-to-noise ratio for the lowest calibration point of 2,3,7,8-TetraCDD (1.73 fg/µL) decreased by a factor of ~4 compared to helium and was calculated with a ratio of 3, which is still an appropriate signal-to-noise ratio for PCDD/F detection according to US-EPA method 8280B. The LOQs generated from the signal-to-noise ratio of the TMS solution were found to be 10 and 18 fg absolute (n = 27) for 2,3,7,8-TetraCDD and OctaCDD, respectively. In contrast to the hydrogen system operated with the 60 m VF-Xms for pre-tests, a slight increase in the LOQs was observed. Presumably this effect is not related to column performance but rather to minor system aging and an increase of background noise. Separation efficiency was comparable to the normally employed 60 m VF-Xms column, with

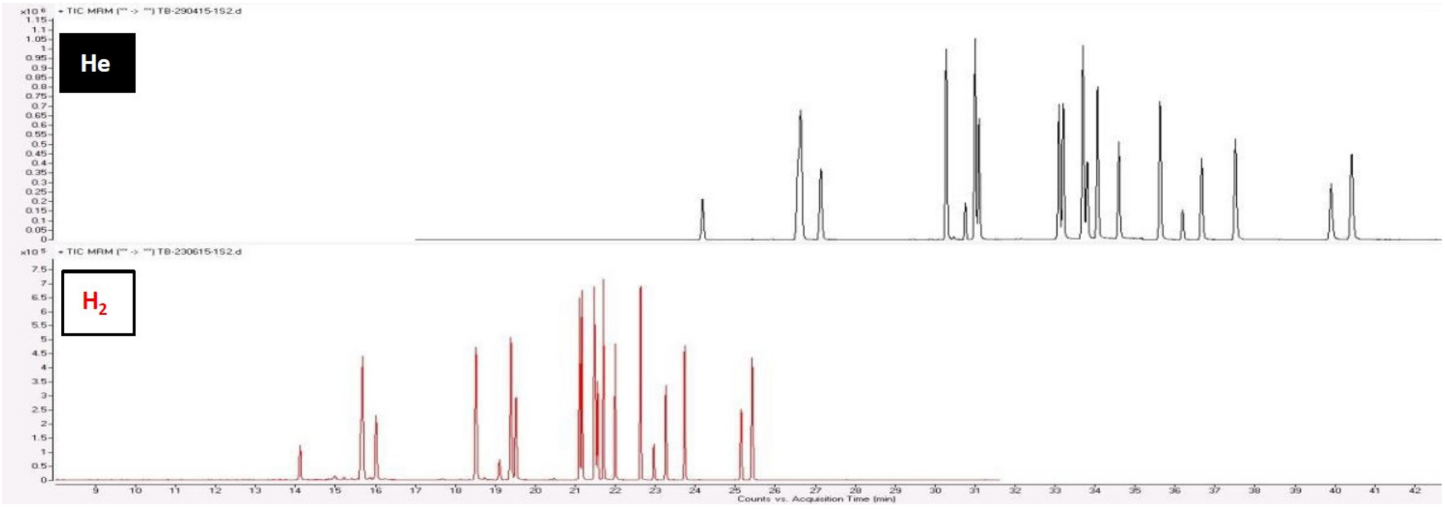

Figure 4. Total ion current (TIC) chromatograms for PCDD/Fs generated in MRM mode applying helium (top, 60 m VF-Xms, flow: 1.2 mL/min) and hydrogen (bottom, 40 m VF-Xms, flow: 0.9 mL/min) as carrier gas.

the exception of 2,3,7,8-TetraCDF. A deterioration of 10% separation (valley to valley ~30% separation) could not be improved by method development, even though both columns show the same theoretical plate heights. This phenomenon may be related to hydrogen-induced separation differences, but this was not investigated further.

In addition to the parameters covered during qualification and method validation with the hydrogen system, process blanks, LODs and LOQs, accuracy, and stability of the RRFs were further investigated. Process blanks measured in duplicate under hydrogen and helium were in good agreement. Resulting LODs and LOQs were calculated as the mean congener concentration plus 3 times (LOD) and 5 times (LOQ) the standard deviation. LOQs ranged between 0.05 and 1.15 pg/sample for the 17 native PCDD/F congeners and were in accordance with those generated under helium conditions. LOQs calculated from the signal-to-noise ratio and from the calibration procedure were up to one order of magnitude lower and were therefore discarded for sample assessment, since LOQs from process blanks are the limiting factor when evaluating PCDD/F results. Accuracy of various food/feed standard reference material was determined with a maximum RSD of ± 20%; the deviation between hydrogen and helium measurements ranged between ± 10%. Hydrogen RRFs were slightly different from helium RRFs, and minimum, maximum, control, and alert limits were adapted in the control charts. Nonetheless, RRFs were stable over time and limits were not exceeded.

Validation continued with the comparison of 167 food and feed samples, randomly selected and measured in duplicate under helium and hydrogen conditions. The correlation of results yielded slopes of the linear regression between y = 0.9615x and y = 1.0426x for all PCDD/F analytes, showing good agreement of method performance between He and H_2 measurements. Figure 5 shows the correlation of 2,3,7,8-TetraCDD data. No specific differences were found in the correlation of the data for the varying matrices. The correlation coefficient ranged between R^2 = 0.8634 and R^2 = 1.0675 (implying a linear relationship) for all congeners. The predetermined criteria of y = 0.9 to 1.1x and R^2 = 0.8 to 1.2 were met. Data assessment was based on all values generated, including negative values as "below the LOQ". As a result, no correlation was found for 1,2,3,7,8,9-HexaCDF, because there were only two positive data pairs out of 167, and linearity criteria were not met for the 1,2,3,4,7,8,9-HeptaCDF. In the latter case, a linear relationship of R^2 = 0.9757 was found for n = 25 positive data pairs. The recovery rate ranged between 50 and 130% under both conditions, including deviation of not more than ± 20% between H_2 and He.

To our knowledge, Lau *et al.* are the only group to date to have reported on the application of hydrogen as carrier gas for PCDD/F analysis.[7] They concluded that unusual fragments related to dechlorination of the PCDD/F molecules disturbed the isomer-specific analysis on a 50 m CP SIL-88 column. Dechlorination and catalytic hydration in the injector or ion source may lead to formation of lower-grade chlorinated molecules with same or different retention time and should be excluded when applying hydrogen as carrier gas. Even though PCDDF/s are well separated in groups on a nonpolar VF-Xms column, a separate experiment showed that splitting off or exchanging chlorine atoms from the dioxin and furan molecules at high temperatures in the injector or source was not associated with formation of lower grade chlorinated interference molecules. Hence, no artifacts were created, and potential interferences had no influence on quantitation. This is also confirmed by the good agreement of all results throughout the degrees of chlorination, even for the relatively more contaminated samples. Nevertheless, insignificant dechlorination was observed. Thus, it is crucial to determine whether dechlorination takes place when applying hydrogen, if the

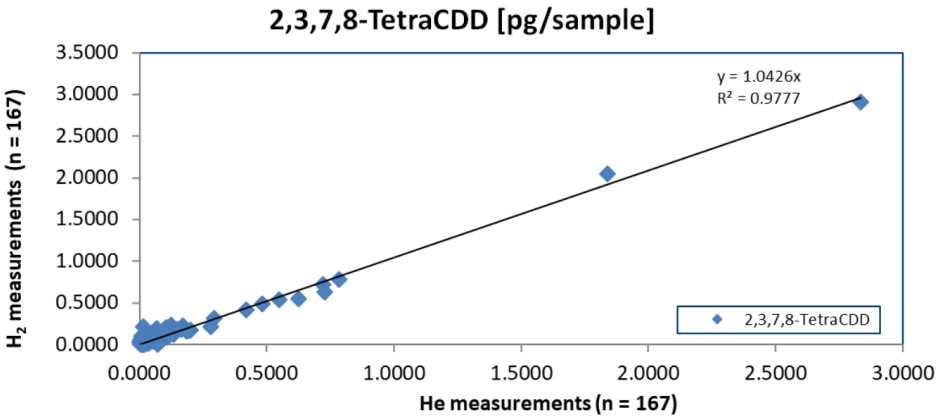

Figure 5. Correlation of 2,3,7,8-TetraCDD data, 167 samples of varying food/feed matrices.

resulting peaks with a lower degree of chlorination disturb the target analytes, and if so, to what extent. For PCDD/Fs, toxic equivalent (TEQ) considerations are critical when assessing dechlorination or interferences, because each congener's concentration is weighted by its toxic equivalency factor (TEF) before summation. Consequently, errors in measured concentrations affect TEQ in proportion to the TEF. For example, an overestimation of 2,3,7,8-TetraCDD (TEF = 1) contributes fully to the TEQ, whereas the same overestimation in 1,2,3,4,6,7,8-HeptaCDD (TEF = 0.01) contributes only 1%. This difference should be taken into account when evaluating potential analytical bias.

Method ruggedness over time: 5–10 months and 3,000 injections

Over a five-month period, approximately 2,000 food/feed sample extracts and the same order of magnitude standard solutions containing PCDD/Fs were measured with the hydrogen system. All parameters consulted for qualification and validation were reviewed and re-evaluated during this time to assess the robustness of the system. Parameters such as precision, linearity, and RRFs remained constant. Blanks yielded no differences to validation, and a variety of standard reference materials met accuracy expectations without exception during this time.

LOQs calculated from the signal-to-noise ratio increased from 10 to 20 and 16 to 30 fg absolute for 2,3,7,8-TetraCDD and OctaCDD, respectively. Separation efficiency slightly decreased by 2.5% for 2,3,7,8-TetraCDD after approximately 3000 injections in total. Sensitivity decreased by a factor of three. While the system was qualified, the lowest calibration point measurable was 2 fg/µL and in between the stress test phase, i.e., after approximately 9–10 months, the lowest point measurable increased to 6 fg/µL. This slight deterioration of system performance is assumed to be related to system aging, as the GC/TQ was new when the study had started. The use of hydrogen as carrier gas is not believed to be responsible for any of the effects observed during this robustness assessment.

In one year of experimental work, switching the carrier gas was seen to cause the most issues, not only when changing from helium to hydrogen but also vice versa. Getting the system to stabilize took time and was associated with a period of decreased sensitivity. Periodic alternation of the carrier gas is thus not recommended for a single system. Rather, performing all experiments and observations needed under helium, then switching to hydrogen, should reduce method development effort and time to a minimum.

Conclusion

This comparison of the 7000 and 7010 series triple quadrupole systems successfully demonstrates that all parameters tested showed an improvement for the analysis of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). The positive outcome of the baseline study in terms of an obvious sensitivity benefit was a fundamental requirement to proceed with hydrogen as carrier gas. Since enhanced detection of ions is facilitated with the 7010 series GC/TQ, its potential for cost and resource saving application may not solely result from the use of hydrogen as carrier gas. If appropriate conditions are met, the 7010 series GC/TQ equipped with the HES source allows for possible redesign and acceleration of the analytical process as well as improved analytical quality.

Overall, it was straightforward to convert the existing PCDD/F helium method for food and feed samples to hydrogen conditions, provided that all analytical needs were known, the system was given enough time for rearrangement, and familiarization with the best practices for using hydrogen as carrier gas was completed. The analytical drawbacks that may need to be faced when changing to hydrogen, e.g., reduced signal-to-noise ratio, are to a certain extent predictable. In this study, we successfully proved that hydrogen is applicable at ultra-trace levels provided the known decrease in sensitivity can be compensated for. Other compounds of interest may exhibit other unique challenges. For example, their mass spectra/fragmentation pattern might be different because they react with hydrogen. With proper consideration of these analytical factors, hydrogen may be applicable not only for PCDD/Fs but also for a range of other organic compounds.

For PCDD/F analysis, the use of hydrogen as carrier gas offers several advantages over helium if the GC/TQ instrumentation is capable of detection limits around 10 fg on column (using He) or better, as is the case for the 7010 series GC/TQ. In addition to improved analytical quality at comparable or slightly better separation, there is a high potential for saving analytical runtime as well as analytical costs due to lower purchase price of hydrogen, especially considering possible helium shortages in the future. In any case, the decision to use hydrogen should be based on full consideration of the possible implications on GC and MS performance. Also, reactions such as dechlorination cannot be excluded, even if the presented application appears to produce those effects to an insignificant degree.

Acknowledgment

The method development work described in this application note was performed by Eurofins GfA Lab Service, located in Hamburg, Germany.

Our thanks go to Frank Neugebauer and the Eurofins GfA Lab Service team in Hamburg.

References

1. Alvarado, J. S.; Silzer, J.; Lemley, F.; Erickson, M. D. Separation of Polychlorinated Biphenyls by Fast Gas Chromatography. *Analytical Communications* **1985**, *34*, 381–383.
2. Cramers, C. A.; Janssen, H.-G.; Van Deursen, M. M.; Leclercq, P. A. High-Speed Gas Chromatography: An Overview of Various Concepts. *Journal of Chromatography A* **1999**, *856*, 315–329.
3. Muñoz-Guerra, J. A.; Prado, P.; Vargas García-Tenorio, S. Use of Hydrogen as a Carrier Gas for the Analysis of Steroids with Anabolic Activity by Gas Chromatography–Mass Spectrometry. *Journal of Chromatography A* **2011**, *1218*, 7365–7370.
4. Agilent EI GC/MS Instrument Helium to Hydrogen Carrier Gas Conversion. User Guide. *Agilent Technologies*, publication number 5994-2312EN, **2022**.
5. L'Homme, B.; Scholl, G.; Eppe, G.; Focant, J.-F. Validation of a Gas Chromatography–Triple Quadrupole Mass Spectrometry Method for Confirmatory Analysis of Dioxins and Dioxin-Like Polychlorobiphenyls in Feed Following New EU Regulation709/2014. *Journal of Chromatography A* **2015**, *1376*, 149–158.
6. Quimby, B. D.; Prest, F. H. Conversion of Agilent EI GC/MSD Systems from Helium to Hydrogen Carrier Gas. *Agilent Technologies presentation*, **2012**. https://www.agilent.com/cs/library/usermanuals/public/user-guide-coverting-ei-gcms-instruments-5994-2312en-agilent.pdf.
7. Lau, B. P.-Y.; Sun, W.-F.; Ryan, J. J. Complication of Using Hydrogen as the GC Carrier Gas in Chlorinated Dibenzofuran and Dibenzodioxin GC/MS Analysis. *Chemosphere* **1985**, *14(6/7)*, 799–802.

www.agilent.com

DE-017265

Printed in the USA, August 25, 2026
5994-9218EN

Agilent
Trusted Answers