Cod: 2.2022

HYDROGEN AS CARRIER GAS FOR THE ANALYSIS OF POLYCHLORINATED DIOXINS AND FURANS (PCDD/FS) BY GC-MS/MS IN FOOD AND FEED SAMPLES

S. Lang[1], F. Neugebauer[1]

[1]*Eurofins GfA Lab Service GmbH*

Introduction

The application of hydrogen (H_2) as carrier gas for GC-MS/MS analysis of hydrophobic organic compounds at ultra-trace levels had been hardly feasible to date. Drawbacks such as enhanced background, reduction in signal-to-noise ratio, changes in mass spectra and most relevant, a decrease in sensitivity related to MS/MS system performance, were the limiting factors. Novel GC-MS/MS techniques with much higher sensitivity, offer the possibility to compensate for the disadvantages of applying hydrogen as carrier gas. This is especially relevant in the field of ultra-trace PCDD/F analysis since limits of detection (LODs) and limits of quantification (LOQs) could not be reached otherwise.
The main objective of this study was to convert an existing PCDD/F analysis method for food and feed samples from helium to hydrogen as carrier gas without loss of performance and speeding up the analytical process by a reduction in runtime of 50 %. Therefore, (i) two Triple Quadrupole MS systems were compared under helium conditions as a baseline study to specifically investigate the benefits for PCDD/F analysis generated by the novel GC-QQQ technique, (ii) the PCDD/F method was converted to hydrogen as carrier gas and system performance was observed by assessing 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 system performance (iv) method performance criteria were applied to food/feed samples as part of the validation (v) the method was applied in routine analysis for 6 moth to assess the robustness and stability of the system.

Materials and Methods

An Agilent 7890B Gas Chromatograph equipped with a Gerstel MPS auto sampler system and a cold injection system (CIS), coupled to an Agilent 7000C Triple Quadrupole and an Agilent 7010 Triple Quadrupole respectively, was used for all investigations. The comparison of both MS/MS systems (7000C vs. 7010) for PCDD/Fs was performed under helium conditions. It should be noted that the same 7890 B Gas Chromatograph instrument was used, and the former 7000C MS was converted (upgraded) to the 7010 series within the study. The 7010 Triple Quadrupole system was finally used for measurements applying hydrogen as carrier gas. Measurements were carried out in Multiple Reaction Monitoring (MRM) mode. Two precursor and their respective product ions were monitored. Samples, blanks and reference material were randomly selected from the laboratory's regular analytical throughput. Sample extracts containing PCDD/Fs were particularly checked for varying types of matrices and concentrations. Matrices included food and feed, i.e. eggs, milk, fats, meat, fish, mussels, poultry and their respective products. Concentrations for all PCDD/Fs ranged approximately between 10 fg and 100 pg per sample. Calibration solutions, a test-mix solution (TMS) and a windows defining mix solution (WDM) were prepared in toluene. A thirteen point calibration curve with varying levels of native and constant levels of isotope-labelled dioxin and furan analytes was analyzed. Each point of the calibration solutions contained all 17 2,3,7,8-substituted PCDD/Fs as native as well as isotope-labelled compounds. 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 native dioxin and furan compounds critical to separate in order to assess separation efficiency of the GC-columns.

Results and discussion

(i) The comparison of the 7000C and 7010 Triple Quadrupole techniques under helium conditions generally revealed a performance improvement by a factor of 5. Assessed were linearity, sensitivity, precision, repeatability and LOQs from signal-to-noise ratios for the PCDD/F method. While stability (low residues) was reached down to the lowest calibration point of 1.73 fg μL^{-1} (2,3,7,8-TetraCDD, S/N ratio = 13) for the 7010 system, the 7000C system was only stable down to 6.33 fg μL^{-1} (2,3,7,8-TetraCDD, signal not different from noise). Mean LOQs from signal-to-noise ratio reached ~6 fg for the 2,3,7,8-TetraCDD and ~32 fg for OctaCDD (7010), which was an improvement by a factor of 3-4 compared to the 7000C. Linearity (calibration procedure), precision and repeatability exhibited lower RSDs when applying the 7010 system in most cases. The results gave substantial evidence that the 7010 Triple Quadrupole met the conditions needed to proceed with hydrogen as carrier gas. Details to the parameters tested are given in **Table 1**.

(ii) Shortly after switching from helium to hydrogen low sensitivity, intensity and signal-to-noise ratio as well as high background was observed. Contrary to our assumptions, the system was found to be stable under hydrogen after approximately one week again. Intensities were in the same order of magnitude as for helium, even though absolute abundance and peak areas decreased by a factor of ~2. Additionally, an increase in noise and a decrease of the LOQ by a factor of ~2 were found under hydrogen. Nevertheless, the LOQs ranged between 10 - 20 fg absolute for the 2,3,7,8,-TetraCDD of 10 measured replicates and improved by time. **Figure 1** shows a helium chromatogram in comparison to a hydrogen chromatogram of 2,3,7,8-TetraCDD generated 7 days after changing the carrier gas. The comparison of the two chromatograms clearly illustrates the typically narrower peak shape and peak sharpness, which is expected when switching from helium to hydrogen conditions.

Generally, the TMS standard solution showed acceptable and stable concentration levels, LOQs and an overall comparable system performance for all PCDD/F congeners after the system had adapted to hydrogen conditions. MS1 scans of OctaCDD (**Figure 2**, top helium, bottom hydrogen) showed no differences in fragmentation pattern, even though ion ratios differed. Thus, no necessity was given for changing target and qualifier ions in the method. Separation efficiency 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.

(iii) The final H_2-method for dioxins and furans was developed on a 40 m VF-Xms column (0.18 µm x 0.18 mm) applying a constant flow of 0.9 mL min^{-1} (average linear velocity: 45.9 cm sec^{-1}). Due to the physical behavior of hydrogen as GC carrier gas with the Van-Deemter minimum of theoretical plates at higher linear gas velocity it was possible to maintain separation efficiency within a runtime of 27.5 min (**Figure 4**: Comparison of a helium and hydrogen chromatogram). A reduction in acquisition time of 40 % was achieved under these conditions. LOQs on the basis of process blanks were finally determined ranging from 0.073 - 1.157 pg/sample (all analytes). Separation efficiency was basically comparable to the usually employed 60 m VF-Xms column, except for the 2,3,7,8-TetraCDF. A deterioration of 10 % separation (valley to valley ~30 %) could not be improved by method development, even though both columns show the same theoretical plate height. This phenomenon may be related to hydrogen-induced separation differences, but currently no further proof can be given. According to our best of knowledge, Lau et al. (1985) were the only group which reported on the application of hydrogen as carrier gas for PCDD/F analysis. They concluded that unusual fragments formed disturbed the isomer specific analysis on a 50 m CP SIL-88 column related to dechlorination of the PCDD/F molecules. Even though PCDDF/s are well separated in groups on a non-polar VF-Xms column it was proved in a separate experiment that no significant effects by splitting off or exchanging chlorine atoms from the dioxin and furan molecules at high temperatures in the injector or source led to formation of lower grade chlorinated molecules. Hence, no artefacts were build and possible interferences had no influence on quantitation. This is also confirmed by the good comparability of all results throughout the chlorination degrees even for the relatively higher contaminated samples.

(iv) As part of the validation 167 food and feed samples were randomly selected and measured in duplicate under helium and hydrogen conditions. The correlation of the results yielded slopes of the linear regression between y = 0.9615 x - 1.0426 x for all PCDD/F analytes, which implies good agreement of performance between He and H2 measurements (**Figure 5**). No specifically differences were found between the varying matrices. The correlation coefficient ranged between R2 = 0.8634 - 1.0675 (implies a linear relationship). Data assessment was based on all values generated, including results below the LOQ but greater than the LOD. The recovery rate ranged between 50 and 130 % under both conditions, including no larger deviation between H_2/He than up to ± 20 %.

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 in particular, the system was given enough time for rearrangement and familiarization with hydrogen as carrier gas was reached. The analytical drawbacks that may need to be faced when hydrogen application is intended are to a certain extent predictable, such as a reduction in signal-to-noise ratio. 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 are much more fragile compounds.

Being aware of these particular analytical specialties, using 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 as compared to helium if the GC/MS instrumentation is capable of detection limits at around 10 fg on column (using He) or better. Besides of an improvement of the 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 prices of hydrogen, especially considering possible helium shortages in future times. Anyway, considering its use definitely needs strict observation of GC as well as MS behavior. Also, reactions as e.g. dechlorination cannot generally be excluded, even if the presented application appear to give those effects – if at all – only to an insignificant degree.

Acknowledgements

We kindly thank Agilent Technologies for their support and collaboration.

References

Lau, B. P.-Y., Sun, W.-F. and Ryan, J.J. (1985), “COMPLICATION OF USING HYDROGEN AS THE GC CARRIER GAS IN CHLORINATED DIBENZOFURAN AND DIBENZODIOXIN GC/MS ANALYSIS”, Chemosphere, Vol. 14, No. 6/7, pp. 799-802.

Table 1: Performance comparison

Parameter tested	*7010 system*	*7000C system*
Calibration/Linearity		
Calibration procedure	*1.1 - 3.1 % RSD*	*2.4 - 5.9 % RSD*
Residues	*Better stable 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	*Definitively different from noise (S/N ratio = 13 for 2,3,7,8-TetraCDD)*	*Not different from noise*
Signal at second lowest calibration point	*Definitively different from noise (S/N ratio = 20 for 2,3,7,8-TetraCDD)*	*Not different from noise*
Signal at third lowest calibration point	*Definitively different from noise (S/N ratio = 48 for 2,3,7,8-TetraCDD)*	*Different from noise(S/N ratio = 3 for 2,3,7,8-TetraCDD)*
Peak areas	*20 times larger*	
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)*
LOQs [fg]		
2,3,7,8-TetraCDD	*mean = 6, n = 10, 11 % RSD*	*mean = 25, n = 10, 26 % RSD*
Octa-CDD	*mean = 32, n = 3, 4 % RSD*	*mean = 159, n = 3, 20 % RSD*

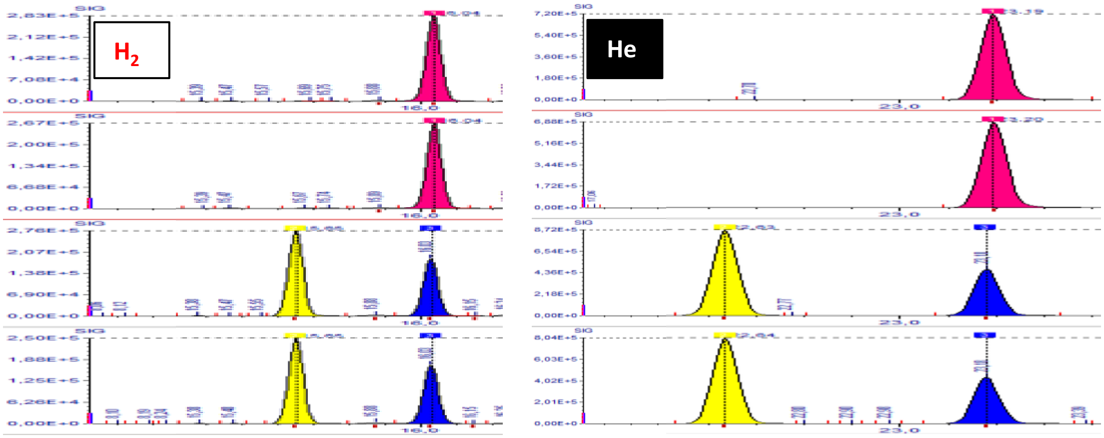

Figure 1: Peak widths using hydrogen and helium as carrier gas

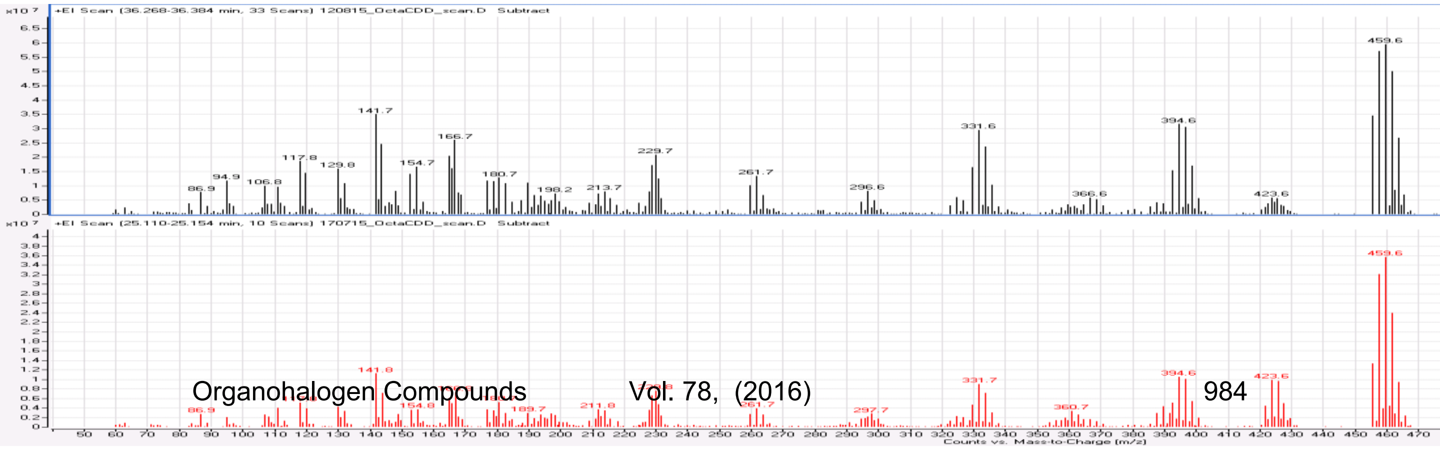

Figure 2: Mass spectra of OctaCDD using helium and hydrogen as carrier gas

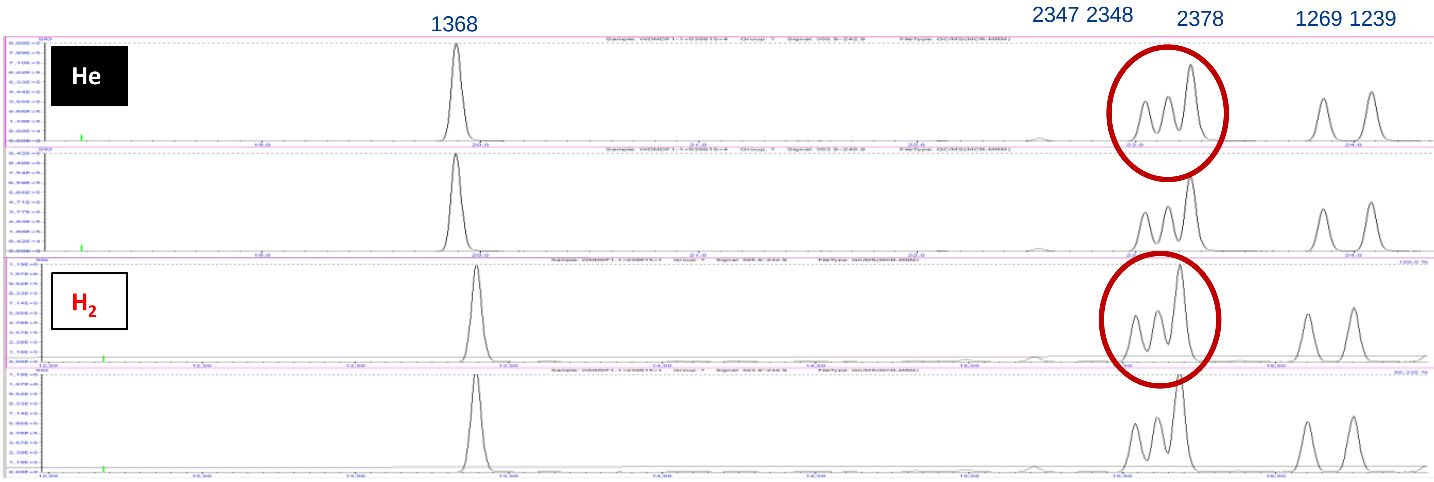

Figure 3: GC peak separation of 2,3,7,8-TetraCDD using helium and hydrogen as carrier gas

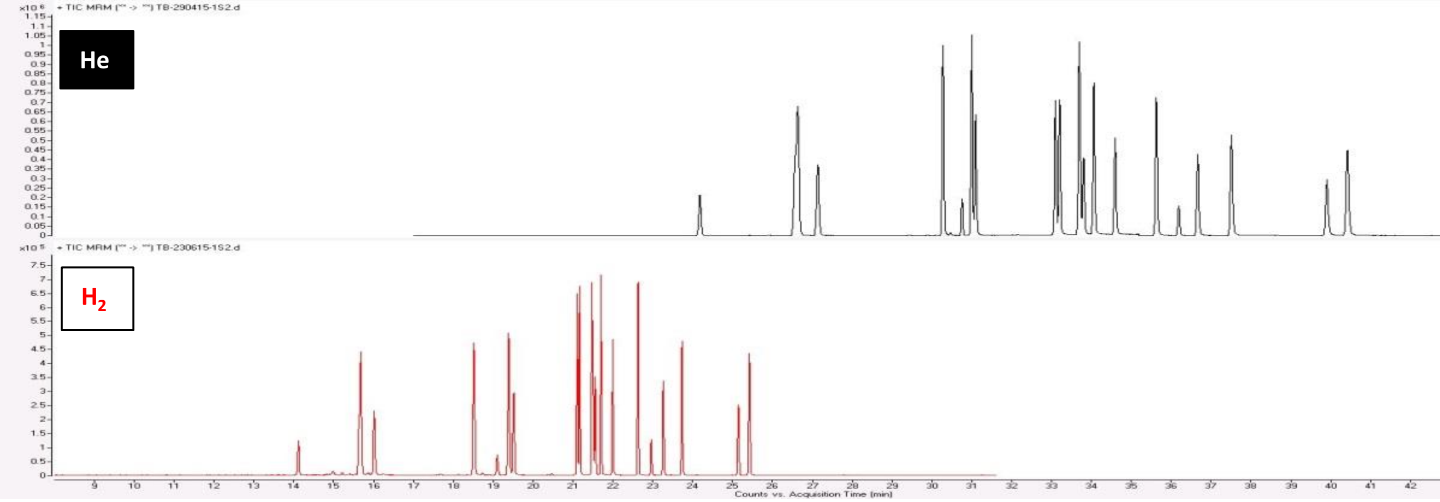

Figure 4: Comparison of total ion chromatograms for helium and hydrogen

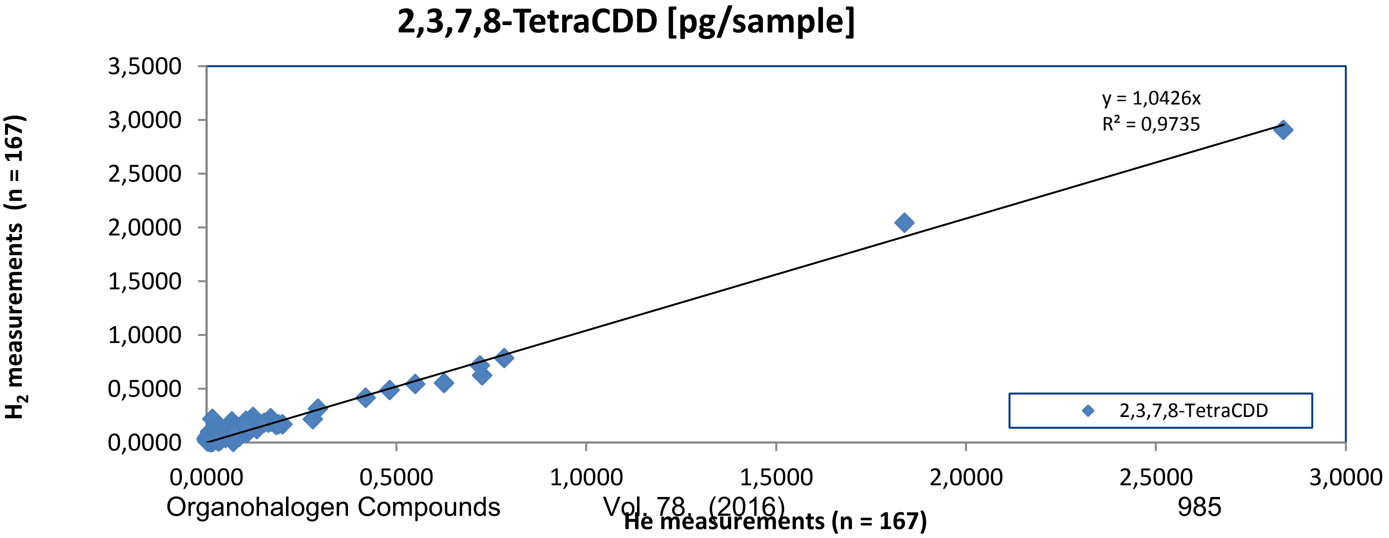

Figure 5: Correlation of helium and hydrogen measurements (n=167) of 2,3,7,8-TetraCDD