Method development for non-routine compound specific stable isotope analysis (CSIA) of light elements

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Preface

The presented thesis with the title ‘Method development for non-routine compound specific stable isotope analysis (CSIA) of light elements’ was prepared by Kristina L. Hitzfeld during the time from November 2009 to February 2016 under the supervision of PD Dr. Hans-Herrmann Richnow and Dr. Matthias Gehre. Data generation was conducted in the Laboratory of Stable Isotopes of the Department of Isotope Biogeochemistry at the Helmholtz-Centre for Environmental Research – UFZ in Leipzig.

Parts of this thesis have already been published or are accepted in peer-reviewed journals (see list of publications). Note that figures and text passages in the results and discussion chapters are partly taken from those publications without further indication. The original publications are to the full extend included in the appendix.

Acknowledgement

I would like to thank Dr. Matthias Gehre and PD Dr. Hans-Herrmann Richnow for the opportunity to learn from their experience while preparing this thesis and for their guidance during this time. I would also like to thank Prof. Dr. Peter Grathwohl for accepting and evaluating the presented thesis as my University supervisor. I am especially grateful for the patience, support and mutual appreciation I have received working with Dr. Matthias Gehre. I would also like to express my gratitude to Dr. Safdar Bashir, Dr. Anko Fischer, Dr. Julian Renpenning, Dr. Ivonne Nijenhuis, and Steffen Kümmel for their co-operation, shared ideas, and discussions which led to joint findings. For instrumental support and helping hands in the Laboratory of Stable Isotopes and for their open interest I would like to thank Ursula Günther and Falk Bratfisch. My acknowledgement is also directed to all the colleagues from the Department of Isotope Biogeochemistry and from other departments of the UFZ; especially to Dr. Marie Markantonis, you are a dear friend to me. Most important I want to thank my friends and family for their love, patience and support throughout my life. Dirk, I would not have come thus far without your encouragement and support and I am looking forward to every day at your side.

Abstract

Multi-dimensional compound specific stable isotope analysis (CSIA) is a promising new analytical approach wherein changes in isotope ratios are measured across multiple elements within a given compound, shedding light on reaction mechanisms and allowing for the identification and characterisation of the origin, distribution, conversion, and degradation of organic chemicals. Thus far, routine methods are available for measurements of carbon and hydrogen isotopes; however, in order to fully exploit the potential of multi-dimensional CSIA, new approaches are needed for halogen, oxygen, and hydrogen stable isotope analysis in heteroatom-bearing compounds.

In order to facilitate such an expansion, the work described in this thesis was aimed at the development of methods for CSIA for rarely-analysed chlorine and oxygen stable isotopes, as well as to improve hydrogen stable isotope analysis for halogenated compounds, which were previously inaccessible.

The presented approaches used high temperature conversion (HTC, >1200 °C) to generate HCl, CO, and H_2 analyte gas from organic compounds online after gas chromatographic (GC) separation. For respective stable isotope ratio determination of those analyte gases, the GC-HTC interface was interlinked with a gas analyser (qMS) and later an isotope ratio mass spectrometer (IRMS). For conversion characterisation an organic mass spectrometer (IonTrap MS) was used in parallel at the end of the tested reactors.

Chlorine stable isotope analysis using GC-HTC-IRMS was achieved for a set of chlorinated compounds. Hydrogen stable isotope analysis of heteroatom-bearing (Cl, N, S) substances was improved fundamentally by providing chromium powder in the reactor. The developed novel GC-Cr/HTC-IRMS method was successfully validated and implemented for hydrogen CSIA. Investigations of the commercially available reactor for oxygen GC-HTC-IRMS showed undesired by-products and HTC processes which inhibited reproducible and accurate CSIA of volatile organic compounds. For future method development and to identify and quantify interfering by-products in GC-HTC-IRMS in general, an evaluation strategy is proposed.

In summary, GC-HTC-IRMS approaches were investigated and successfully applied to extend the existing repertoire of non-routine CSIA methods for chlorine, hydrogen and oxygen stable isotopes.

Zusammenfassung

Die Aussagekraft der mehrdimensionalen komponentenspezifischen stabilen Isotopenanalyse (CSIA) hinsichtlich der Identifikation und Charakterisierung von Ursprung, Verteilung, Umwandlung und Abbau von organischen Chemikalien ist um ein Vielfaches stärker als die der herkömmlichen CSIA nur eines Elementes. Um dieses Potential auszuschöpfen, sind neben den routinemäßig zugänglichen Isotopen (^{13}C, ^{2}H) neue Methoden für Halogen-, aber auch Sauerstoff- und Wasserstoff-Isotope in Heteroverbindungen notwendig.

Ziel dieser Arbeit war die Methodenentwicklung für die CSIA von bisher nur vereinzelt untersuchten Elementen Chlor und Sauerstoff (^{37}Cl, ^{18}O), sowie für Wasserstoff (^{2}H) an halogenierten Verbindungen, die mit existierenden Methoden nicht oder nur eingeschränkt messbar waren.

Im Rahmen der vorgelegten Arbeit wurde die Hochtemperaturumsetzung (HTC, >1200 °C) zur Erzeugung von HCl-, CO- und H_2-Analyse-Gasen aus organischen Verbindungen direkt im Anschluss (online) an die gaschromatographische Trennung (GC-HTC) genutzt.

Zur Bestimmung der Isotopenverhältnisse an diesen Analyse-Gasen wurde das GC-HTC-Interface zuerst mit einem Restgasanalysator (qMS) und anschließend mit einem Isotopenverhältnis-Massenspektrometer (IRMS) gekoppelt. Für die Charakterisierung der Umsetzung wurde am Ausgang des jeweiligen Reaktors ein paralleles organisches Massenspektrometer (IonTrap MS) verwendet.

Dieser GC-HTC-IRMS Ansatz konnte für die Chlorisotopenanalyse am HCl verwirklicht und für verschiedene chlorierte Verbindungen eingesetzt werden. Durch die zusätzliche Verwendung von Chrommetall im Reaktor konnte die Wasserstoffisotopenanalyse mittels GC-Cr/HTC-IRMS für die Messung verschiedener heteroatomhaltiger (Cl, N, S) Verbindungen erfolgreich validiert und etabliert werden. Ferner zeigten die Untersuchungen des kommerziell verfügbaren HTC Reaktors für die Sauerstoffisotopenanalyse, dass unerwünschte Nebenprodukte und versteckte zusätzliche Sauerstoffquellen eine reproduzierbare und akkurate GC-HTC-IRMS Bestimmung erschweren. Es wurde eine Evaluierungsstrategie basierend auf Methoden zur Identifizierung und Quantifizierung störender Nebenprodukte und Prozesse entwickelt, die in Zukunft auch für andere GC-HTC-IRMS Methodenentwicklungen anwendbar ist.

In der vorgelegten Arbeit wurden GC-HTC-IRMS Methoden zur Bestimmung der Halogen-, Wasserstoff- und Sauerstoff-Isotopenverhältnisse untersucht und erfolgreich zur Erweiterung der CSIA heteroatom-haltiger organischer Verbindungen genutzt.

List of Publications

1. A novel online approach to the determination of isotopic ratios for organically bound chlorine, bromine and sulphur[1]

Rapid Communications in Mass Spectrometry **2011**, Volume *25*, p. 3114-3122

K. L. Hitzfeld, M. Gehre, H.-H. Richnow

K. L. Hitzfeld was substantially involved in the conception and evaluation of the entire study. Instrumental setup was assembled with the help of M. Gehre. All data were produced by K. L. Hitzfeld. The manuscript was written by K. L. Hitzfeld with helpful discussions and corrections provided by the co-authors. The developed HTC conversion approach and instrumental setup was also patented in Germany.[2]

2. Development and validation of an universal interface for compound specific stable isotope analysis of chlorine (Cl-37/Cl-35) by GC-High Temperature Conversion (HTC)-MS/IRMS[3]

Analytical Chemistry **2015***, Volume 57(5), p. 2832-2839*

J. Renpenning, K. L. Hitzfeld, T. Gilevska, I. Nijenhuis, M. Gehre, H.-H. Richnow

K. L. Hitzfeld was involved in the conception and evaluation of the study. Instrumental setup and data collection and evaluation was done by J. Renpenning with the help of K. L. Hitzfeld. K. L. Hitzfeld provided helpful discussions and corrections to the manuscript.

3. Compound specific hydrogen isotope analysis of heteroatom-bearing compounds via gas chromatography-chromium-based high-temperature conversion (Cr/HTC)-isotope ratio mass spectrometry[4]

Analytical Chemistry 2015, Volume 87(18), p. 9443-9450

J. Renpenning, S. Kümmel, K. L. Hitzfeld, A. Schimmelmann, M. Gehre

K. L. Hitzfeld was involved in the conception and evaluation of the entire study. All data were produced by K. L. Hitzfeld and co-authors. The manuscript was written by all authors together.

4. Evaluating degradation of hexachlorocyclohexane (HCH) isomers within a contaminated aquifer using compound specific stable carbon isotope analysis (CSIA)[5]

Water Research 2015, Volume 71, p. 187-196

S. Bashir, K.L. Hitzfeld, M. Gehre, H.-H. Richnow, A. Fischer

K. L. Hitzfeld was substantially involved in method development, laboratory standards establishment and the analysis and quality assurance strategy for CSIA of the field samples on which the study is based. K. L. Hitzfeld prepared parts of the manuscript, figures, method descriptions, and supporting information and revised the manuscript several times.

5. Evaluation of the performance of high temperature conversion (HTC) reactors for compound specific oxygen stable isotope analysis

Isotopes in Environmental and Health Studies 2016, accepted June 2nd 2016

K. L. Hitzfeld, M. Gehre, H.-H. Richnow

K. L. Hitzfeld substantially contributed to the ideas, conception, and evaluation of this study. Instrumental modifications and reactor constructions were done in agreement with the co-authors. All data were produced and analysed by K. L. Hitzfeld. Interpretation and manuscript preparation was performed by K. L. Hitzfeld with helpful discussions and corrections provided by the co-authors.

Abbreviations

CO	Carbon monoxide
CO_2	Carbon dioxide
Cr/HTC	Chromium based HTC
CSIA	Compound specific Isotope Analysis
DI	Dual Inlet analyte gas introduction method
EA	Elemental Analyser
EI	Electron Ionisation
eV	Electron Volt ($1.602^{*}10^{-19}$ kg m^2 s^{-2})
GC	Gas Chromatography
H_2	Hydrogen
HCH	Hexachlorocyclohexane
HCl	Hydrogen Chloride
HTC	High Temperature Conversion
IAEA	International Atomic Energy Agency
IRMS	Isotope Ratio Mass Spectrometry
MS	Mass Spectrometry
MTBE	Methyl *tert*-Butylether
mUr	milli Urey, SI-unit replacing ‰ [Brand and Coplen (2012)]
PCE	Tetrachloroethene
qMS	Quadrupole Mass Spectrometry
R	Isotope Ratio
TCE	Trichloroethene
TIMS	Thermal Ionisation Mass Spectrometry
VSMOC	Vienna Standard Mean Ocean Chloride
δ	Delta Notation [‰ or mUr], see Equation (1)

Tables and Figures

Introduction

Stable Isotopes

Various elements have multiple stable isotopes, meaning that these elements are subject to variability in the number of neutrons present in the nucleus, making certain atoms heavier and others lighter while retaining the other physical and chemical properties of these elements. Those not subject to radioactive decay are considered stable isotopes.[6] In the presented thesis stable isotopes of the light elements present in organic substances (^{2}H, ^{13}C, ^{15}N, ^{18}O, ^{37}Cl) are investigated and discussed with a focus on the analysis of stable isotopes of chlorine, hydrogen, and oxygen in organic compounds.

Isotope Ratios

During the formation of earth, the terrestrial elements were deposited with fixed ratios of their stable isotopes. The isotope ratio R of an element (E), is defined as molar ratio of the heavier isotope, that with the greater atomic mass, over the lighter isotope (E_{heavy}/E_{light}), and is used to characterise the isotopic composition.[7] Compounds with identical physical and chemical properties but different stable isotope compositions and molecular masses are known as isotopologues.[7] Localised variations in isotope ratios can be found due to processes which result in an enrichment or depletion of the heavier isotope in the residual source material and the process products. This isotope fractionation occurs via chemical, biological, and physical processes. It arises from different molecular masses, bonding energies, and oscillation frequencies which result in different reaction rates for equilibration and bond cleavage of bonds containing a heavy isotope.[8] This in turn determines reaction rates, and thereby the extent and direction of isotope fractionation.[7, 9]

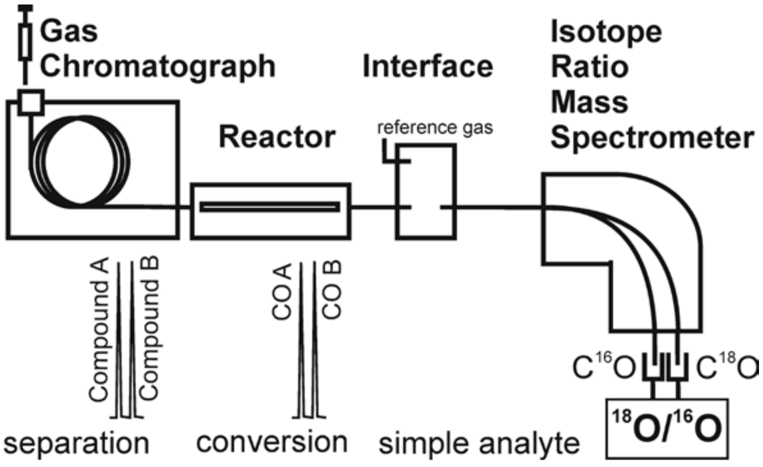
Compound specific Isotope Analysis (CSIA)

Compound specific isotope analysis (CSIA) denotes a tiered process to achieve: (i) separation of individual organic compounds of interest, here by gas chromatography; (ii) quantitative conversion of each compound to a simple analyte gas; (iii) removal of interfering by-products; and (iv) mass spectrometric analysis to designate isotopic compositions to each compound (Scheme 1).[10] By discriminating between compounds with isotope ratio differences, CSIA is able to answer research questions far beyond the possibilities offered by traditional analytical chemistry on chemical composition and properties. CSIA measures the abundance of stable isotopes of an element and relates the obtained isotope ratio E_{heavy}/E_{light} by comparison to international reference materials to defined international scales.[11] Such relative ratio differences with respect to standards allow the comparison of isotope compositions obtained by different analytical methods, different laboratories, and of historic as well as future results.[12] Standardisation is facilitated by internationally distributed reference materials of different isotope ratios which are determined in interlaboratory tests with state-of-the-art instrumentation and methods.[13] The relative isotope ratio difference to a standard is reported in the so called delta (δ) notation (Equation (1)).[7]

$$\delta^{heavy}\mathrm{E}_{\mathrm{sample}} = \frac{\left({}^{heavy}E/{}^{light}E\right)_{\mathrm{sample}}}{\left({}^{heavy}E/{}^{light}E\right)_{\mathrm{standard}}} - 1 \tag{1}$$

If an isotopologue is enriched in the heavy isotope as compared to the standard, this difference will be reflected as a positive delta value. Similarly if a compound is subject to fractionation, e.g. enzymatic degradation which discriminates against heavy isotopes, the residual fraction of the compound will be enriched in the heavy isotopes compared to the original source material. Concomitant products of the same process, e.g. metabolites, will be depleted in the heavy isotopes with respect to the original source material, respectively.

Stable isotope analysis is nowadays a versatile tool applied to a broad variety of scientific fields. From medicine, geology, biochemistry, and environmental studies to commercial applications in food authentication, contaminated site management, and forensics this technique is used to address numerous research and legal questions.[14, 15] Especially in the field of environmental forensics, CSIA is a very effective tool to identify sources, quantify biodegradation and monitor natural attenuation of contaminants in the field (as shown in a field study presented in Chapter IV).[5, 10, 14, 16, 17] Analytical methods for stable isotopes of some light elements (H, C, N) have been available for several years and are used on routine basis for CSIA of several organic compounds.[10, 18] Earlier than CSIA, elemental analysis methods have been developed and advanced techniques are available for pure compounds and bulk sample analysis today.[12, 18-20] Thus bulk analysis methods and instrumentation are only marginally explored in this thesis, mainly in the context of principles which can be transferred to the development of methods in CSIA.



Scheme 1. GC-IRMS analysis of oxygen stable isotopes

Gas Chromatography Isotope Ratio Mass Spectrometry (GC-IRMS)

Online CSIA via gas chromatography and isotope ratio mass spectrometry (GC-IRMS) denotes an instrumental setup that performs the separation, conversion and detection of specific compounds and their stable isotopes automatically along a continuous-flow helium gas stream (Scheme 1).[21] This setup is versatile and user-friendly, allowing the same functions and controlling software for the CSIA of different elements. The hyphenation of GC and IRMS by means of an interface is state-of-the-art for routine CSIA of carbon and hydrogen.[18] The interface converts the separated compounds online into a simple isotopologue (for carbon complete oxidation to CO_2 is assumed; see Equation (2)), which is then introduced to the IRMS via a system of open splits. Alternation with a suitable reference gas allows instrument calibration and results comparison. Furthermore, the interface regulates gas flow and pressure, switches the sample stream, and shields the mass spectrometer against solvents and overloads. Most importantly, it ensures that the isotopic information of the original compound is reassembled after conversion and transport in the analyte gas so it can be accurately measured by IRMS. While GC and its specifications are not different from the techniques used in conventional analytical chemistry, the IRMS was specifically developed to meet the demands in ionisation efficiency, stability, linearity, and precision for the simultaneous analysis of simple isotopologues (e.g. CO_2, H_2, N_2, CO).[8, 18] Peak integration, calibration, referencing, quality assurance, and standard operation procedures were optimised for the requirements of stable isotope analysis and are established and applied within the stable isotope research community.[7, 10, 13, 21, 22]

The crucial point for CSIA is the complete or quantitative conversion, which must itself exert no isotope fractionation effects. Commercial interfaces can be adjusted to specific elements by installing the appropriate conversion reactor. For carbon, a combustion reactor quantitatively converts the organic compounds to carbon dioxide ($^{12}CO_2$, $^{13}CO_2$; see Equation (2)) which is the simple analyte gas from which isotopic composition is measured. In the case of hydrogen a high temperature conversion (HTC) reactor facilitates the complete conversion of organic compounds to molecular hydrogen (HH, DH; see Equation (3)) which is then analysed by IRMS. To analyse oxygen stable isotopes, carbon monoxide ($C^{16}O$, $C^{18}O$; see Equation (4)) is the desired conversion product, respectively analyte gas.

$$C_xH_y + O_2 \rightarrow x\,CO_2 + {}^{y}/_{2}\,H_2O \qquad (2)$$

$$C_xH_y \rightarrow x\,C_{solid} + {}^{y}/_{2}\,H_2 \qquad (3)$$

$$C_xH_yO_z + C_{solid} \rightarrow z\,CO + {}^{y}/_{2}\,H_2 + C_{solid} \qquad (4)$$

Methods and techniques which are not GC- and MS-based as well as pre-concentration, purification and sample application measures are not discussed in this thesis. These techniques were not subject to the presented method development approaches for chlorine, hydrogen, and oxygen and comprehensive information can be found elsewhere.[10, 23]

Challenges

Leaving aside routine methods, the development of CSIA for complex compound mixtures and specific compound classes is an active and challenging research field.[18] On the one hand, existing methods are extended to lower concentration ranges, differing matrices, and sampling techniques needed for the broad application of stable isotopes in a variety of disciplines. On the other hand, the concept of multi-dimensional stable isotope analysis requires stable isotope characterisation of further elements (O, Cl, Br, S) and emerging contaminants (halogenated, persistent organic compounds) with structural features that resist or impede established conversion approaches.[18, 24] There is thus a demand for online 'continuous flow' methods or existing GC-IRMS enhancement.

Chlorine, featuring the two stable isotopes ^{35}Cl (75.78 %) and ^{37}Cl (24.22 %), is the predominant heteroatom of the important contaminant group of halogenated hydrocarbons.[25] Chlorinated compounds are found even in the most remote regions of the world due to their volatility and persistence.[26] The intensive production and ubiquitous application of these compounds in the environment, mainly as pesticides during the first half of the 20th century, has left future generations with contaminated groundwaters, soils, and biota.[27] Halogenated compounds are generally considered persistent, bioaccumulative, and responsible for severe toxic effects. Some pollutants in this group have been regulated by the Stockholm convention on persistent organic pollutants since 2001[28], but their fate and continuing distribution remains a prevalent political and research topic. The successful combination and implementation of pre-concentration, GC-IRMS method development and the interpretation of stable carbon isotopes for biodegradation quantification of hexachlorocyclohexanes (HCHs) in the field is illustrated as an example in the fourth chapter of this thesis.[29] γ-HCH (Lindane) and its isomers (α-, β-, δ-, ε-HCH) constitute one pesticide group which is often the focus of environmental research and has the potential to be studied and characterised in more detail employing a chlorine and hydrogen stable isotope analysis method.[30-32] For chlorine and its isotopes, laborious offline methods do exist; however they depend on experienced technicians and a sophisticated series of independent experiments (combustion, precipitation, conversion, and clean up) to obtain simple isotopologues that can be measured by IRMS.[1, 18, 33, 34] Unfortunately, instrumental difficulties prevent GC-IRMS solutions which convert chlorine online into suitable simple isotopologues without introducing stable isotope fractionation effects or hampering analysis on the desired ion mass traces.[1, 8, 18, 21, 35] Recently, online chlorine isotope analysis tools have been presented and are slowly gaining application (see Chapter I).[8, 18, 26]

Hydrogen stable isotope analysis of halogenated organic compounds using the established GC-HTC-IRMS reactor poses serious uncertainty due to the formation of hydrogen chloride (HCl). HCl as a by-product during conversion withdraw hydrogen atoms and thus an incorrect stable isotope ratio of the remaining hydrogen is obtained. The development of a chromium-based high temperature conversion reactor for hydrogen

GC-Cr/HTC-IRMS of heteroatom-bearing organic compounds presented in the second chapter overcomes those restrictions and can also be applied to characterise HCHs with respect to their origin and transformation.[4]

Oxygen, with the three stable isotopes ^{16}O (99.76 %), ^{17}O (0.04 %) and ^{18}O (0.20 %), is a common element in organic compounds and thus of relevance to almost all fields of forensic science.[11] Of particular environmental importance are compounds such as fuel oxygenates and pharmaceuticals, both of which are groundwater contaminants worldwide.[36, 37] The concept of multi-dimensional stable isotope characterisation has been realised, e.g. for the fuel oxygenate methyl *tert*-butyl ether (MTBE) with respect to hydrogen and carbon isotopes.[38] Using oxygen as the third dimension would complement such studies and help to characterise degradation reactions in detail.[38] Although commercial reactors for the GC-IRMS analysis of oxygen have been available for several years, only a limited number of applications have been published. [18, 39] The online conversion to the simple analyte carbon monoxide (Equation (4)) should be facilitated by the available reactors, but appear to be affected by side reactions and unreliable for certain compound classes and applications (see Chapter III). Thus GC-IRMS oxygen analysis is a remaining challenge which requires more research effort in order to obtain an adequate and reliable conversion technique.

High Temperature Conversion (HTC)

HTC is used in the following as a general term for processes which take place at high temperatures (>1200°C) under a reductive (carbon deposition) atmosphere present in a helium gas stream sometimes supported by hydrogen make-up gas. This includes thermal decomposition processes which were formerly termed ‘pyrolysis’ and ‘carbon reduction’ in stable isotope analysis.[40] Under HTC conditions thermal decomposition (pyrolysis) can take place as well as reduction and oxidation reactions. Depending on the elemental composition of the compound to be converted it may undergo only pyrolysis (see Equations (3), (4)) or only reduction like water, which is converted to carbon monoxide and hydrogen during HTC, if carbon deposition is present. If elements other than C, O, and H are involved, the variety of chemical processes during HTC is much wider and less characterised.[40] To use HTC in isotope analysis the conversion into the desired analyte gas (HCl, H_2, CO) has to be complete. Especially in the case of organic compounds also containing nitrogen, sulphur or halogens, interfering processes and by-product formation can impede stable isotope analysis.[40-42] In the course of this thesis detailed investigation of HTC and the reactions present led to characterisation and application of HTC techniques for chlorine[3], hydrogen[4] and oxygen (Chapter IV) compound specific stable isotope analysis.

HTC for chlorine isotope analysis of organochlorines

Conventionally, high temperature conversion processes under reducing conditions have been applied during steam reforming, hydrolysis, cracking, and for the disposal of problematic chemicals such as chlorinated hydrocarbons.[43-46] In the absence of oxygen and hydrogen sources, as in cracking during oil refining, carbon is deposited at high temperatures, which is known to have a high surface area and is reported to catalyse methane decomposition to form elemental hydrogen according to Equation (3) during HTC reactions.[47] It is known that thermal energy cleaves bonds to form radicals according to the order of bond energies.[48] Thus chlorine-carbon bonds (73 kcal/mol) should be cleaved before carbon-carbon (83 kcal/mol) or carbon-hydrogen bonds (94 kcal/mol).[48] Dehalogenation of chlorinated hydrocarbons has been investigated with respect to the activity of plain ceramic surfaces without catalyst, using water vapour as the hydrogen donor for hydrolysis.[49, 50] The results indicate that aluminium oxide can act as a catalyst to convert carbon tetrachloride into carbon dioxide and HCl at temperatures >350 °C in the presence of water. In the absence of water, the undesired by-products chlorine, phosgene and $AlCl_3$, were formed during carbon tetrachloride dechlorination.[50] HCl as simple analyte ($H^{35}Cl$, $H^{37}Cl$) for isotope ratio determination was due to the assumed corrosivity not used except in very early geochemical offline experiments.[51] Experiences from hydrogen stable isotope HTC applications of chlorinated compounds and the knowledge about HCl by-production led to considering it for chlorine CSIA by GC-HTC-IRMS (after Equation (5)).

$$C_xH_yCl_z + n\ H_2 \rightarrow x\ C_{solid} + z\ HCl + (n+(y-z)/2)\ H_2 \tag{5}$$

Within the first chapter of this thesis, the applicability of HCl as analyte gas was evaluated and competing HTC interfering with chlorine stable isotope determination were characterised and optimised.[1-3]

HTC for hydrogen stable isotope analysis of organic material

The current reference method for hydrogen isotope analysis is a combination of a HTC in an elemental analyser device coupled to an IRMS (EA-HTC-IRMS). In such a device, the reactor consists of an outer ceramic tube and an inner glassy carbon tube filled with glassy carbon chips and nickelised carbon.[52] This technique is used for pure compounds, water and bulk samples and is the method of choice for referencing today. For mixtures CSIA via GC-IRMS for hydrogen stable isotopes has been commercialised and is a routine method for many organic compounds today, e.g. flavour components for food authentication.[53, 54] HTC reactors used for hydrogen GC-HTC-IRMS are empty ceramic tubes which facilitate the decomposition of organic compounds into deposited elementary carbon and molecular hydrogen (see Equation (3)).[54] Both techniques EA-HTC-IRMS and GC-HTC-IRMS are applied to answer various scientific questions; however there was limited understanding of the internal processes taking place within the reactors. Only recently have studies shed light on by-products, memory, and the role of the deposited carbon for the quality of the obtained hydrogen isotope compositions.[40, 41, 55-58] For measuring chlorinated hydrocarbons, difficulties were long known as HTC of those compounds produces by-products like hydrochloric acid (HCl) and thus the hydrogen isotope composition is diverted between HCl and H_2 during analysis. Several recent approaches have employed chromium to overcome this by-product formation and thus allow hydrogen isotope analysis of halogenated hydrocarbons.[42, 59-63] However, the reactor constructions and temperatures did still not allow a general GC-HTC-IRMS method for CSIA of all halogenated compounds to be established.[62, 63] To advance this chromium based approach and overcome drawbacks in former HTC hydrogen CSIA of heteroatom-bearing compounds in general, a universal Cr/HTC reactor was developed which is discussed in detail in Chapter II.[4] It was derived from the assumption that previously applied temperatures (≤1000 °C[62, 63]) were too low and that the presence of chromium over the whole temperature profile of the reactor is needed to efficiently trap heteroatoms interfering with quantitative H_2 formation.

HTC for oxygen stable isotope analysis of organic material

Pure compound and bulk analysis of oxygen isotopes in organic compounds by means of EA-HTC-IRMS is facilitated by the quantitative production of carbon monoxide ($C^{16}O$, $C^{18}O$; see Equation (4)).[20, 64, 65] The same reactor and setup is used as in hydrogen isotope EA-HTC-IRMS and likewise is the current state-of-the-art reference method for oxygen isotope determination.[20, 52] Influences of HTC reactor temperature on quantitative EA-HTC to CO have been investigated and emphasize the importance of temperatures above 1450 °C to allow precise and accurate routine oxygen isotope measurements.[41, 52, 56]

Oxygen CSIA via GC-HTC-IRMS was furthered by different approaches which led to a commercially available HTC reactor for this application.[66-68] This reactor consists of an outer ceramic tube with an inner platinum metal tube filled with nickel wires operated at 1280 °C.[67] Unfortunately, few applications have been published to date and GC-HTC-IRMS oxygen stable isotope analysis remains a challenge [18, 21] Still, there has been only one investigation comparing the commercial available HTC to different other reactor types, which did not find a better reactor design.[39] Overall they were reporting stability problems, strong amount dependencies, and ‘carbon surplus effects’, CO production upon alkane conversion, during analysis using the commercial available reactor for different compound classes.[39, 69] In spite of the knowledge about possible by-products and interfering reactions during EA-HTC of oxygen-bearing organic compounds[56], there had been no effort to use this knowledge to explain the apparent lack of the commercial available HTC reactor performance. It can be assumed that varying by-products can influence stability as well as linearity and interfere with precise oxygen CSIA. Furthermore, CO production upon injection of alkanes suggests other oxygen sources than the converted organic compound, possibly originating from reactor materials, which would impact obtained oxygen stable isotope signatures.

Those hypotheses required further investigation of reactors, by-products, and conversion performance. Results of this HTC reactor study are presented in Chapter III of this thesis and emphasise the importance of HTC process characterisation prior to GC-HTC-IRMS method development.

Objective and Structure of the Thesis

Compound specific isotope analysis (CSIA) is a versatile tool, the importance of which is increasing in fundamental research, environmental sciences, and forensic studies.[8, 10, 14] Concomitant with broad CSIA application and growing scientific recognition, demands for new routine methods utilising up-to-now hardly accessible isotopes are arising.[8, 18] Particularly the exploitation of multi-dimensional CSIA, which combines the isotopic information of several elements, holds the potential to answer many open research questions.[18] Up-to-date routine CSIA methods are available only for some elements, compound classes, and sample matrices.[21] This thesis aimed to advance method development, process knowledge, and implement applications for the following pressing challenges in environmental stable isotope analysis:

Chapter I focuses on CSIA of stable chlorine isotopes. The feasibility of hydrochloric acid (HCl) as an analyte gas for CSIA was evaluated. A fundamentally new approach facilitating online high temperature conversion (HTC) and hydrogen reactant gas was taken and its applicability investigated. Results were published in *Rapid Communications in Mass Spectrometry*, *Analytical Chemistry*, and in form of a German patent.[1-3]

Chapter II implements chromium based HTC for hydrogen stable isotope gas chromatography isotope ratio mass spectrometry (GC-Cr/HTC-IRMS). This approach allows hydrogen stable isotope analysis of heteroatom-bearing compounds like chlorinated hydrocarbons, which were only with restrictions accessible previously. Results were published in *Analytical Chemistry.*[4]

Chapter III attends to oxygen CSIA via GC-HTC-IRMS. Conversion processes in different reactor types were characterised to gain insights into reactions and provide fundamentals for future HTC method development. Results are currently in press in *Isotopes in Environmental and Health Studies.*

Chapter IV demonstrates the first application of carbon CSIA to hexachlorocyclohexanes (HCHs) in the field, aiming to characterise and quantify biodegradation. Results were published in *Water Research.*[5]

HTC of organic compounds after GC separation in ceramic reactors and under oxygen limited conditions are the conjunctive subject of the method development approaches in this thesis. Characterisation of processes, by-products and conditions were shown to be fundamental for the use of HTC for stable isotope analysis. The main target of this thesis was to make a contribution to multi-dimensional CSIA by approaching chlorine, hydrogen and oxygen. Their stable isotope analysis in organic compound mixtures remain challenging and access to their CSIA would offer a variety of possible applications.

Results and Discussion

Chapter I – Chlorine GC-HTC-IRMS Method Development

The results discussed in this chapter are available in the form of two published papers which can be found in the appendix:

'A novel online approach to the determination of isotopic ratios for organically bound chlorine, bromine, and sulphur' (2011) in *Rapid Communications in Mass Spectrometry*[1]

'Development and validation of an universal interface for compound specific stable isotope analysis of chlorine (Cl-37/Cl-35) by GC-high-temperature conversion (HTC)-MS/IRMS' (2015) in *Analytical Chemistry*[3]

The instrumental setup and HTC principle applied for chlorine, bromine or sulphur stable isotope determination of organic compounds which is presented in this study was also patented in Germany.[2]

Previous State of the Art

Chlorine stable isotope analysis is a current challenge in compound specific stable isotope analysis (CSIA).[18] Field and laboratory studies already showed the potential of multi-dimensional (^{13}C and ^{37}Cl) CSIA, but applications are limited due to the limitations of online methods available for chlorine stable isotope analysis up to the present time.[70-76] Current online methods for mass spectrometric analysis after GC separation are mainly assigning chlorine stable isotope ratios on the basis of molecular or mass fragments ions of the non-converted compound.[26, 77-79] This means that a method must be adapted for each specific compound, as well as that structurally identical reference compounds are needed. A general routine method converting organic compounds online to a simple chlorine-containing analyte gas, similar to that for carbon or hydrogen CSIA via GC-IRMS, has thus far not been available.

Hypothesis: 'Chlorine CSIA at HCl via GC-HTC-IRMS should be possible'

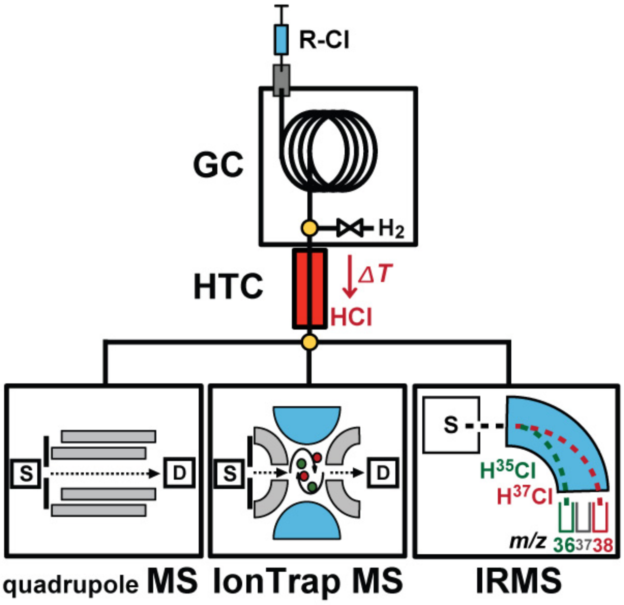
Achievements

In the presented thesis a new approach to analyse chlorine stable isotopes from HCl was pursued, implemented, and apparent conversion processes studied in detail. HCl is formed during high temperature conversion (HTC) with hydrogen reactant gas addition from chlorinated organic compounds according to Equation (5). High temperatures in an oxygen-limited (reductive) atmosphere cleave organic bonds to yield HCl. The developed instrumental setup hyphenates this HTC with GC and subsequent analysis with quadrupole

MS, IonTrap MS or IRMS were used to study HTC products and to achieve online chlorine CSIA (Scheme 2).

$$C_xH_yCl_z + H_2 \rightarrow x\ C_{solid} + z\ HCl \tag{5}$$

Proof of principle: Produced HCl was detected by means of quadrupole MS (GAM 445, Balzers AG), operated in the single ion mode to analyse *m/z* 36, 38 ($H^{35}Cl$, $H^{37}Cl$), and chlorine isotope ratios were calculated from obtained peak areas. A linear range for detection of 0.09-0.16 µmol chlorine on column could be found in which samples were analysed with a precision of less than 1 mUr. Additionally the quadrupole MS was operated in total ion mode to confirm complete conversion for chlorinated organic compounds (chlorinated ethanes, ethylenes, aromatics and cyclohexanes) in the HTC reactor. An empty ceramic (Al_2O_3) tube was used as the HTC reactor at temperatures above 1300°C and hydrogen reactant gas (~10%) was added before the reactor into the sample gas stream (Scheme 2).



Scheme 2. Instrumentation used for the GC-HTC approach to chlorine stable isotope analysis via HCl.[1, 3] By means of gas chromatography (GC) the chlorinated organic compound is separated prior to high temperature conversion (HTC) in the reactor quantitatively yielding HCl. Chlorine stable isotope determination was facilitated by mass spectrometric detection of HCl ions (quadrupole MS (gas analyser) or isotope ratio mass spectrometry (IRMS)). IonTrap MS was used for quantification and characterisation of HTC by-products.

HCl was found to be the only chlorine-bearing HTC product under those conditions. It could be shown that relative amounts of HCl produced per chlorine atom were the same for chlorinated ethylene congeners. Isotopic differences between chlorinated ethylenes with known chlorine stable isotope composition could be reproduced using the presented approach and close bracketing with standards.[1] Exemplarily, the online system was used to analyse tetrachloroethylene samples from an evaporation experiment and it could be shown that previously reported relative changes in the chlorine isotope composition of the remaining substrate were obtained. Those findings showed the potential for HTC to be an alternative approach to chlorine stable isotope analysis involving analysis of the no-converted compound.[17, 18, 26, 80, 81] The utilised setup combining GC-HTC with a quadrupole MS gas analyser, however, revealed instrumental instability causing low precision and delta shifts between measurement days. Subsequently the interface was connected to other MS systems to first analyse HTC processes and second use a more stable detector aiming to elucidate causes for the observed drawbacks and possibly overcome them.

HTC process characterisation: The combination of the GC-HTC interface with an IonTrap MS (PolarisQ, ThermoFinnigan) allowed a detailed investigation of HTC products upon conversion of chlorinated and non-chlorinated organic compounds. HTC parameters studied were reactor load, hydrogen concentration, and conversion temperature. It could be shown, that HTC conversion of non-chlorinated ethylene produced C_nH_x ($n \geq 2$) by-products, which produced molecular and fragment ions among others at *m/z* 38, thus interfering with chlorine stable isotope quantification at *m/z* 36 and 38. This overlapping of HCl and by-product signals could explain previously observed instabilities during chlorine isotope composition determination with the novel approach. HTC parameters were optimised to obtain minimal by-product formation which was found at a higher HTC temperature of 1450-1500 °C, a lower carrier gas flow (0.4 mL/min) and a H_2 gas flow of 0.1 mL/min.

Chlorine Isotope Measurements via GC-HTC-IRMS: Several chlorinated reference compounds were analysed with the optimised HTC conditions. Standard deviations of GC-HTC-IRMS measurements were below 0.5 mUr. However, severe memory effects were observed, which only could be overcome by repeated injection until stabilisation was achieved. Resulting GC-HTC-IRMS chlorine isotope composition was compared to the isotope ratios obtained with the offline dual inlet reference method DI-IRMS (Figure 1). GC-HTC-IRMS raw results had to be normalised by a linear regression and were then in very good agreement with reference values. Still, this improved method was hampered by the memory effect, which indicates detainment of chlorine in the system, most probably in the reactor. Furthermore reactor lifetime was very short, due to the ceramic tube becoming brittle upon repeated contact with HCl at those temperatures.

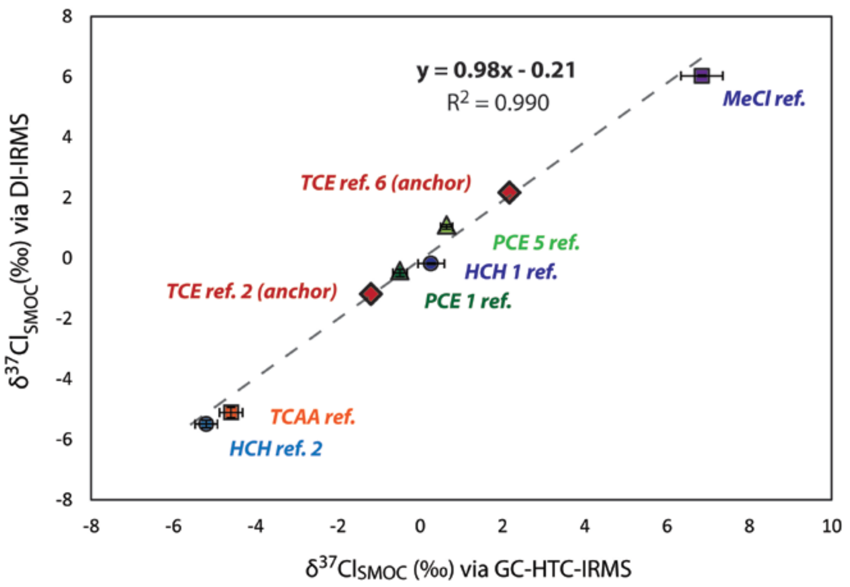


Figure 1.[3] Determination of chlorine isotope composition of several chlorinated reference compounds via GC-HTC-IRMS. Measured chlorine isotope composition was normalised to the VSMOC-scale using TCE references 2 and 6 (x-axis). Offline determined isotope composition was determined via DI-IRMS (y-axis). [MeCl (methyl chloride); TCE (trichloroethylene); PCE (tetrachloroethylene); HCH (hexachlorocyclohexane); TCAA (trichloroacetic acid)]

Discussion and Outlook

It could be shown that chlorine stable isotope analysis at HCl via GC-HTC is a feasible approach to CSIA of organochlorine compounds. However, also drawbacks like memory and reactor lifetime were observed which up to now prevent the application to chlorine CSIA in mixtures. Future development ought to focus on reactor materials, and preventing contact of HTC products with the outer ceramic tube. A possibility would be tube-in-tube reactors with an inner metal tube, however, the complete conversion to HCl and the suppression of interfering by-products have to be assured. (For detailed discussion of HTC processes and by-products see Integrated Discussion.)

So far the presented approach and instrumental setup using HTC and hydrogen reactant gas was studied in detail for the conversion of chlorinated organic compounds to HCl. Exemplarily it was also shown that the conversion of brominated and sulphur containing organic compounds to HBr and H_2S works according to Equations (6) and (7), respectively.[1] Thus, HTC could also be an alternative approach to CSIA of these elements, but further investigation would be needed to evaluate bromine and sulphur stable isotope analysis.[26, 81]

$$C_xH_yBr_z + H_2 \rightarrow x\ C_{solid} + z\ HBr \qquad (6)$$

$$C_xH_yS_z + H_2 \rightarrow x\ C_{solid} + z\ H_2S \qquad (7)$$

At the same time the need for organic reference compounds is emphasised and remains one of the main challenges and restricting parameter for chlorine CSIA after gas chromatography.[18, 77, 82] Generally the 'principle of identical treatment'[83] should be applied for referencing to the international standard VSMOC, however this implies the use of chlorinated organic reference materials. Unfortunately, there are no certified organic reference materials available yet which would allow direct relation to the VSMOC scale.[18 77, 84] Comparing chlorine stable isotope compositions given for two different trichloroethylene samples (TCE PPG and TCE Merck) in different publications illustrates this problem (Table 1). The TCE samples were measured with different methods and calibrated each according to the organic standards of the analysing laboratory, or to inorganic references used for offline conversion methods.

Table 1. δ^{37}Cl values (mUr *vs.* VSMOC) determined in different studies and with different analysis methods throughout the last years. Methods used to determine reference δ^{37}Cl values for other approaches are indicated.

	TIMS [34]	**GCqMS** [78]	**GC-HTC-IRMS** [3]	**GC-IRMS** [77, 79]	**GC-qMS** [77]	**DI-IRMS** [3, 82]
TCE **PPG**	-2.49±0.55	-2.59±0.66	-2.26±0.30	-2.7±0.11	-2.7 (standard)	-1.19±0.01
TCE **Merck**	0.00±0.67 reference method	0.54±1.04	0.01±0.24	0.48±0.09 reference method	0.58±0.44	2.17±0.2

TIMS = thermal ionisation mass spectrometry of CsCl according to [34] using a DDT (δ^{37}Cl=-4.42±0.46 mUr) as isotopic reference material (personal communication C. Aeppli).
GCqMS = gas chromatographic separation and analysis via quadrupole mass spectrometry according to [78] using a TCE (δ^{37}Cl=2.90±0.38 mUr) as isotopic standard (personal communication).
GC-HTC-IRMS = analysis at HCl according to [3] using TIMS δ^{37}Cl results for calibration.
DI-IRMS = dual inlet IRMS converting TCE offline into methyl chloride and measuring against a methyl chloride reference [3], however before optimisation of the offline conversion method [82].
GC-IRMS= analysis at molecular and mass fragment ions after [79] with TCE PPG as one of the calibration standards, results taken from [77].
GC-qMS = average of the participating laboratories (n=5, overall standard deviation given) measuring GC-qMS δ^{37}Cl values with TCE PPG as one of the calibration standards, results taken from [77].

The obtained results strongly depend on the reference values chosen for calibration, but overall are in the same range – except for the DI-IRMS results, which were obtained in an early stage of method development[82] and have to be interpreted with caution. For the other methods the overall δ^{37}Cl averages were -2.51±0.19 mUr and 0.32±0.29 mUr for TCE PPG and Merck, respectively. Regarding the physical properties of TCE, mainly its high volatility, it does not fulfil requirements usually applied for reference compounds with respect to stability and thus handling, shipping and storage. Implementation of chlorine CSIA with full comparability of measurements and referencing of δ^{37}Cl to international scales can only be achieved with suitable reference compounds available. Furthermore, due to the lack of reference material, up to now most of the referencing strategies are simple one-point calibrations, which inaccurately account for IRMS specific scale contraction.[13] In addition, the $\Delta\delta^{37}$Cl range of the reference compounds should cover the variety of expected results to allow calibration and quality assessment.

Hexachlorocyclohexanes (HCHs), which are solid at room temperature but GC amendable, might be one compound class which would fulfil stability criteria to be used as references.[82] However, HCH toxicity restrains suitability for common use as reference material. Broad application in forensics and environmental science of chlorine stable isotope analysis methods will only be possible with a universal referencing strategy and a set of reference compounds available.

Offline calibration of a reference gas against the inorganic reference VSMOC would be another referencing strategy. The calibrated gas peaks in front and after each sample then allow in-run comparison and stability monitoring. One could think of two ways to implement reference gas: HCl reference gas directly introduced into the detector, similar to the use of reference gases in CSIA of other elements, or the use of methyl chloride as reference gas to undergo HTC before analysis. Both reference gases were evaluated in subsequent experiments and did not stand the test (data not shown), thus the generation of organic reference compounds needs to be the aspired referencing strategy.

Chapter II – Hydrogen GC-Cr/HTC-IRMS Method Development

The results discussed in this chapter are available in the form of a published paper which can be found in the appendix:
‘Compound specific hydrogen isotope analysis of heteroatom-bearing compounds via gas chromatography-chromium-based high-temperature conversion (Cr/HTC)-isotope ratio mass spectrometry’ (2015) in *Analytical Chemistry*[4]

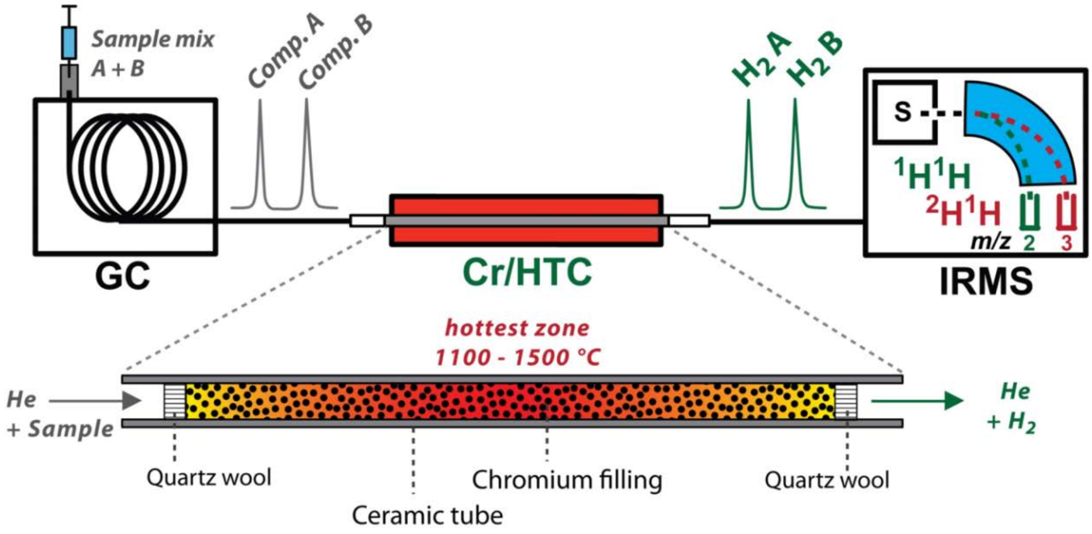
Previous State of the Art

The traditionally used GC-HTC-IRMS approach for hydrogen CSIA of organic compounds was known to be afflicted by fractionation bias for heteroatom-bearing (e.g. N, Cl, S) compounds due to the formation of hydrogen withdrawing by-products.[40, 41, 60] Chromium based HTC was recently successfully applied in elemental analysis (EA-Cr/HTC-IRMS) of organic compounds.[42] In this approach, chromium reacts with HTC by-products like HCN, HCl and H_2S to release hydrogen and thus make it accessible to stable isotope analysis. Experiments were reported using chromium for GC-IRMS, however, at lower temperatures ($\leqslant$1100 °C) and with only partial success for selected groups of compounds.[62, 63]

Hypothesis: ‘An universal accurate and precise GC-Cr/HTC-IRMS method for hydrogen CSIA using high temperatures, comparable to EA-Cr/HTC-IRMS conditions, is possible’

Achievements

Empty ceramic tubes, which are used for traditional HTC, were filled with chromium powder covering the whole heated section to obtain a Cr/HTC reactor for GC-IRMS (Scheme 3). This reactor could be installed, used and adjusted for different flows like any other GC-IRMS reactor; only a small flow restriction due to the powder filling had to be considered and peaks were a little broader than using bare HTC. Connecting the Cr/HTC reactor outlet to an IonTrap MS allowed the monitoring of conversion products and proved that heteroatoms were sequestered in the reactor forming chromium salts.



Scheme 3.[4] Setup and reactor for the GC-Cr/HTC-IRMS method to hydrogen CSIA.

Hydrogen-scarce compounds such as hexachlorocyclohexanes (HCHs), were formerly not accessible by bare HTC due to the stoichiometric formation of HCl as the major hydrogen-bearing product (HTC in Figure 2). With the Cr/HTC no HCl could be detected, instead hydrogen was produced, thus making such compound classes for the first time hydrogen CSIA available (Cr/HTC in Figure 2).

Besides a quantitative conversion, hydrogen stable isotope composition could be determined to be reproducible with standard deviations of ±5 mUr and without memory effects. By means of organic reference materials and two point calibration the performance of the GC-Cr/HTC-IRMS was compared to EA-Cr/HTC-IRMS and offline DI-IRMS results and accuracy was confirmed for a δ^2H range from -181 mUr up to 629 mUr.

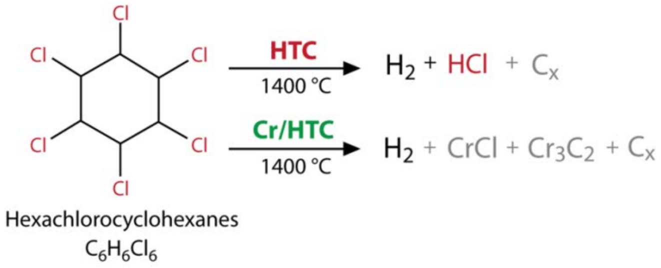


Figure 2.[4] Nonstoichiometric equations for conversions of HCH to H_2 analyte gas via HTC and Cr/HTC.

Discussion and Outlook

It could be shown that GC-Cr/HTC-IRMS targets a broad range of heteroatom-bearing compounds for which accurate hydrogen CSIA was not possible previously. Thus, the developed reactor is a promising and versatile extension of the HTC approach to other compound classes, e.g. chlorinated pesticides like HCHs or organophosphates.

One limitation on field samples, however, is the comparably high H on column amount needed (110 nmol H), which is defined by the linearity range of the GC-Cr/HTC-IRMS system. It was shown that irrespective of the compound converted, the amount dependency of the δ^2H was only acceptable for signal intensities $\geq$3V. Especially for low concentrated pollutants extraction and pre-concentration have to be adjusted to meet these required concentrations. It is still questionable where this nonlinearity in the lower concentration ranges originates from, and further experiments and modifications of the reactor design will have to show if there is possibility to extend the linear range towards lower concentrations.

Additionally, the slightly broader peak widths might challenge analysis of very complex mixtures, as baseline separation of peaks is crucial to IRMS. It could be possible that the peak widths might correlate with chromium particle size and thus flow restrictions due to the filling. Here follow-up studies with slight modifications of the chromium filling might lead to more narrow peaks. (For detailed discussion of HTC processes and by-products see Integrated Discussion.)

Chapter III – Oxygen GC-HTC-IRMS Method Evaluation

The results discussed in this chapter are presented in manuscript form and can be found in full in the appendix. The manuscript was accepted June 2nd 2016 to be printed in *Isotopes in Environmental and Health Studies.*

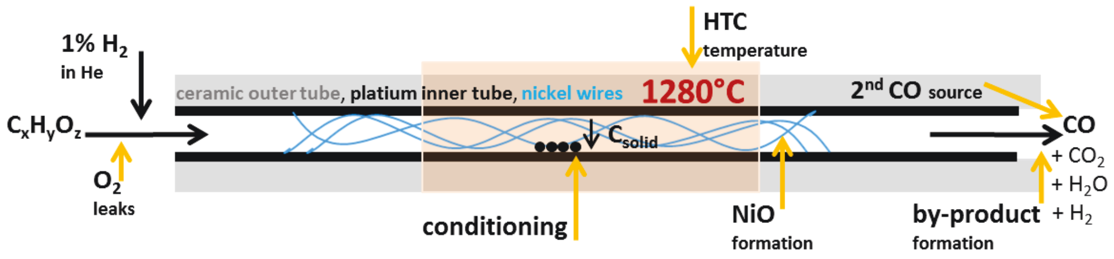
Previous State of the Art

CSIA of the $^{18}O/^{16}O$ isotope ratio in organic molecules can provide valuable insights in environmental, health, food authentication, and forensic studies. Despite available commercial reactors for oxygen GC-HTC-IRMS CSIA (see Scheme 4), applications are rare, no method has yet become a routine analytical technique, and oxygen CSIA remains challenging.[18, 39] Published and applied methods for single compound groups indicate problems with stability, linearity, reproducibility and 'carbon surplus effects'[39] without closer description and investigation of the underlying processes and their impact on the obtained $\delta^{18}O$ signatures. Little was known about the HTC reactions and products possibly restraining broader application in science. An adjustment of the available Thermo Fisher HTC for oxygen reactor[67] to analyse volatile organic compounds was not achieved by modifying existing published methods; which raised questions about processes and conditions needed for successful analysis. Furthermore, there are no methods and strategies for evaluation of HTC processes and reactors with comparable index numbers from which judgement of conversion could be drawn.

Hypothesis: 'Possible unidentified interfering by-products or HTC processes could be responsible for the observed difficulties in oxygen GC-HTC-IRMS CSIA. By means of appropriate evaluation strategies it should be possible to judge HTC reactor performance.'

Achievements

In the presented study, both commercially available and custom built tube-in-tube reactors were investigated and compared to better understand HTC processes and interfering by-products during conversion of organically bound oxygen at high temperatures (>1250°C) into carbon monoxide ($C^{16}O$, $C^{18}O$) according to Equation (4). Focus was the commercially available Thermo Fisher HTC for oxygen reactor used for CSIA via GC-HTC-IRMS.[67, 85] Reactor performance was characterised using vanillin, methyl *tert*-butyl ether (MTBE), and other volatile oxygen-containing organic compounds. By-product formation was studied by IonTrap MS while stability, linearity, precision, and accuracy were evaluated using IRMS and reference compounds with known oxygen stable isotope composition. Several adjustable parameters influencing quantitative HTC conversion to the desired carbon monoxide (CO) were identified and evaluated, and methods were developed for their monitoring (see Scheme 4). Carbon dioxide by-product formation could be identified as the major by-product hampering δ^{18}O CSIA. It could also be shown that reactor materials like the outer ceramic tube or nickel wires bear the risk of releasing 2nd source oxygen or retaining oxygen, resulting in an altered CO analyte gas δ^{18}O value. The revealed function as secondary oxygen source of ceramic material (Al_2O_3) had been hypothesised previously.[21, 52, 53] The role of nickel as reactor constituent, however, was not previously studied, and could also not be fully elucidated in the presented paper. Overall, the importance of optimal HTC conditions and how to achieve and monitor them for quality control is one of the essential findings.



Scheme 4. Nonstoichiometric visualisation of the HTC processes and parameters studied for oxygen GC-HTC-IRMS evaluation. Yellow arrows indicate adjustable parameters which were identified to interfere with δ^{18}O CSIA.

Evaluation strategy

Method development for oxygen CSIA of new compound classes with GC-HTC-IRMS should, in addition to general CSIA method parameters, also include HTC performance evaluation through:

- HTC by-product formation; CO_2/CO should be below 2%, water background and production as low as possible and stable.
- 2nd sources of oxygen; the developed 2nd source calculation method can be applied to detect possible CO contributions not originating from the compound converted.
- Reductive HTC atmosphere; water background and CO_2 production can be used to determine oxygen retention possibly in the form of NiO, which needs to be eliminated by reduction prior to analysis.
- Conditioning protocol; freshly deposited carbon in the reactor was identified as a prerequisite for analysis. Thus, information about conditioning is a basic method parameter.
- Correlation to reference δ^{18}O values; accuracy will indicate optimal measurement conditions and can be used to monitor HTC performance

Discussion and Outlook

The importance of optimal HTC conditions was underlined, prerequisites were identified, and evaluation methods proposed. The fundamental finding was that for each analyte and possible reactor quantitative CO production must be proven, and stable isotope ratios rechecked with standards. Even though the presented study is non-exhaustive with respect to investigated influences on HTC processes it could be shown that tests for possible second oxygen sources must be performed and conditioning has to be optimised prior to application of a method. (For detailed discussion of HTC processes and by-products see Integrated Discussion.)

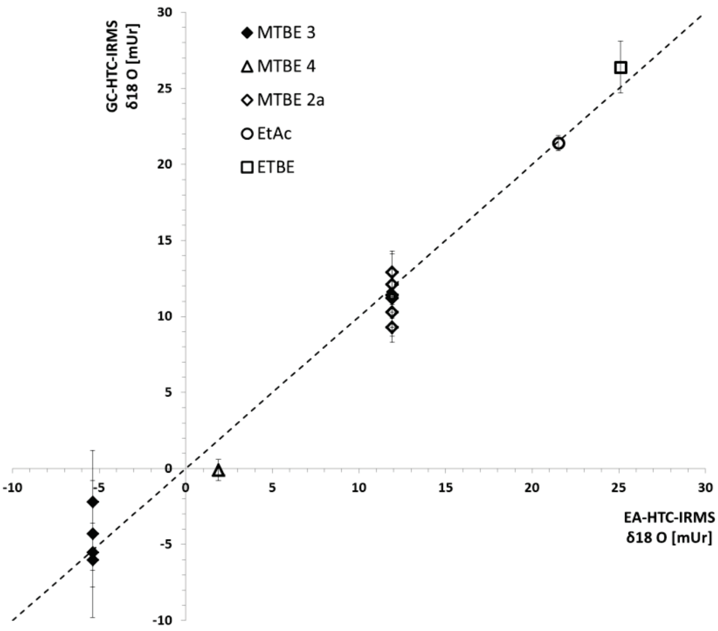


Figure 3. GC-HTC-IRMS results for different volatile compounds analysed with the Thermo Fisher HTC for oxygen reactor plotted against $\delta^{18}O$ EA-HTC-IRMS results. The dashed line indicates the ideal line of $\delta^{18}O$ GC=$\delta^{18}O$ EA values. (MTBE – methyl *tert*-butyl ether; ETBE – ethyl *tert*-butyl ether, EtAc – ethyl acetate)

Still, it was not possible to adapt a GC-HTC-IRMS method for volatile organic compounds like MTBE. Only with experienced selection of raw data was satisfying accuracy and precision of measured reference materials (Figure 3) obtained. Long term stable measurement conditions could not be established and instabilities could not be explained by the close investigation of HTC processes and reactor design. These somewhat incomprehensible findings led to the conclusion that despite its commercial availability, the basic requirements for routine CSIA application to mixtures of oxygen-bearing organic compounds are not fulfilled by this type of reactor. In contrast there is a need for oxygen CSIA via GC-IRMS to offer access to multi-dimensional stable isotope patterns. The presented study answered questions but also raised new questions and suggested possible reasons for the observed drawbacks of the existing method. Further investigations with other compound classes, improved reactors, and optimized HTC conditions are reasonable next steps. Developed evaluation methods will be of use to these subsequent studies. But existing and applied methods could be re-evaluated emphasising the question of why for some compounds reasonable oxygen GC-HTC-IRMS analysis can be established and for others not. Moreover, publishing applied conditioning measures and data selection criteria would be helpful for further development.

Chapter IV - Evaluating Degradation of HCH Isomers within a Contaminated Aquifer using CSIA

The results discussed in this chapter are available in detailed form in a published paper which can be found in the appendix:
'Evaluating degradation of hexachlorocyclohexane (HCH) isomers within a contaminated aquifer using compound specific stable isotope analysis (CSIA)' (2015) in *Water Research*[5]

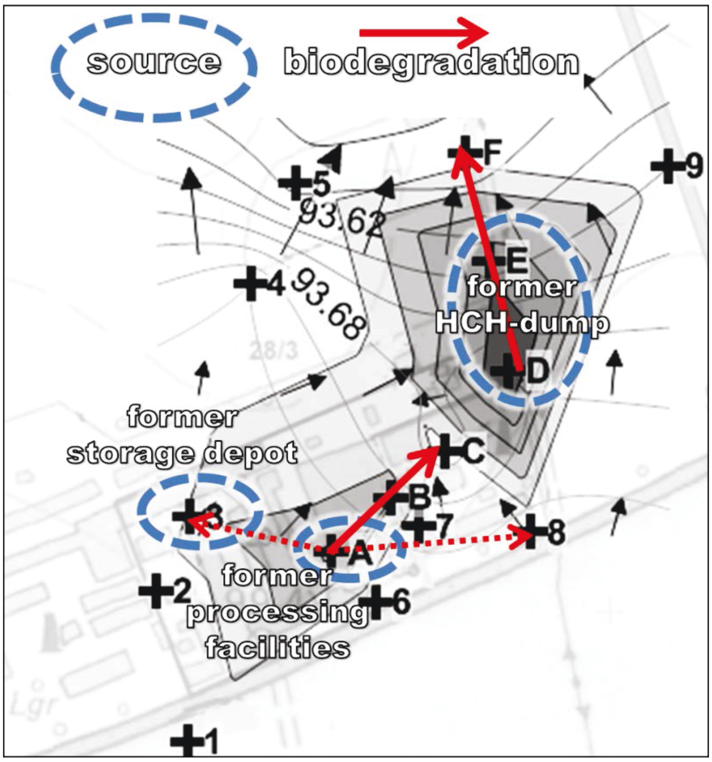
Previous State of the Art

Hexachlorocyclohexane (HCH) isomers are pollutants of particular concern because of their widespread distribution, toxicity and persistence in the environment. Out of the five HCH isomers present in technical-grade HCH, Lindane (γ-HCH) possesses insecticidal properties and has been used as agricultural and wood-preserving pesticide and as pharmaceutical treatment for infestations of lice and scabies. The intensive usage, as well as the clean-up of Lindane from technical grade HCH concomitant with waste dumping has resulted in worldwide soil and groundwater contamination with all HCH isomers.[30] Carbon stable isotope fractionation for biodegradation of HCHs was previously studied in laboratory experiments and enrichment factors were derived[32, 86], which could be used also for the quantification of HCH biodegradation in field studies. Still, the applicability for compound specific stable isotope analysis (CSIA) of carbon had not previously been demonstrated for evaluating the fate of HCHs at contaminated field sites.

Hypothesis: 'HCH biodegradation assessment within a contaminated aquifer is possible by means of carbon CSIA'

Achievements

In the presented study, the potential of carbon CSIA for assessing sources and sinks of HCHs was investigated within a contaminated aquifer at a former pesticide formulating plant. In accordance with pollutant concentration patterns and historical information carbon isotope data confirmed HCH source zones in the areas of former processing facilities, waste dump, and solvent depot (Scheme 5). Downstream of the contaminant sources significant changes in carbon isotope ratios of HCH provided evidence for their biodegradation. By means of conservative calculations using the Rayleigh equation approach[87], changes in carbon isotope ratios yield HCH biodegradation of up to 86 %. Moreover, time- and distance-dependent *in situ* first-order biodegradation rate constants were estimated. The presented study verified the applicability of carbon CSIA for source identification and assessment of HCH degradation in the environment for the first time. Furthermore, the study suggests that CSIA would be an appropriate monitoring tool and would be beneficial for the implementation and successful control of innovative management and remediation concepts in the future.



Scheme 5.[5] Conceptual site model for sources and sinks of HCHs within the contaminated aquifer of the investigated field site. Dashed ellipses show pollutant source zones. Solid red arrows illustrate HCH biodegradation, whereas dotted red arrows show expected HCH biodegradation because direct flow paths between wells are ambiguous.

HCH carbon *CSIA method*

In this study analytical methods were established and verified for extraction, pre-concentration and determination of carbon isotope ratios of HCH isomers in groundwater samples using GC-IRMS. Quality control was carried out using isotope laboratory standards of pure HCH isomers with known carbon isotope ratios determined by elemental analyser IRMS using two-point calibration with reference materials from the International Atomic Energy Agency (IAEA). All sample treatment steps were verified using HCH laboratory standards and deviations from the elemental analyser reference values were less than 1 mUr. Moreover, linearity and detection limits of the applied method were evaluated using laboratory isotope standards. CSIA of field samples were bracketed with a mixture of laboratory standards including all HCHs to assure reproducibility over monitoring campaigns of several years as well as comparability to the international reference scale and for instrumental performance monitoring. All samples were measured in at least three replicates. By means of these quality measures, the total analytical uncertainty, incorporating both accuracy and reproducibility, was in almost all cases better than ±1 mUr for the carbon stable isotope ratios of HCHs in groundwater samples. This allowed sensitive determination of changes in isotope data in space and time ensuring reliable conclusions as well as quantification of biodegradation.

Discussion and Outlook

Carbon CSIA enabled important information about HCH sources, sinks, and attenuation in the field to be obtained, as shown in the presented study. Even more insight into processes in the field would be possible using multi-dimensional isotope analysis in the future. Complementary carbon, hydrogen, and chlorine CSIA might allow new perspectives for the assessment of transformation processes of HCHs and other chlorinated organic pollutants.[8] There are preliminary studies which show the ability and advantages of such multi-dimensional CSIA approaches for chlorinated organic compounds.[72, 73, 75, 88] Within the presented thesis, methods for hydrogen and chlorine CSIA were developed, and for hydrogen isotope analysis a routine GC-Cr/HTC-IRMS method capable of analysing HCHs is now available. Even though comprehensive optimisation has been achieved for chlorine CSIA, a routine method and suitable reference compounds remain one of the major challenges for stable isotope method development in the future. Established pre-treatment, separation, and quality assurance methods developed for HCH carbon CSIA can be adapted for hydrogen and chlorine CSIA in laboratory and field studies.

Integrated Discussion and Outlook

Quantitative high temperature conversion (HTC) and its application for chlorine, hydrogen and oxygen compound specific stable isotope analysis (CSIA) via gas chromatography and isotope ratio mass spectrometry (GC-HTC-IRMS) was the central theme of the presented thesis. Though HTC had been previously applied for routine stable isotope analysis, little was known about the processes and by-products resulting from the conversion of different organic compound classes, especially heteroatom-bearing compounds. The results presented within this thesis showed that interfering by-products and sub-optimal HTC conversion conditions hampered accurate and precise stable isotope analysis and that by-product formation should not be underestimated and needs to be carefully monitored during HTC procedures for CSIA. When these effects are taken into account and appropriate work-flows followed (e.g. the developed evaluation strategy in Chapter III), and when relevant HTC processes are sufficiently well-understood, successful protocols can be found, as demonstrated in this thesis during the GC-HTC-IRMS method developments for chlorine, hydrogen, and oxygen CSIA (overview see Table 2).

Table 2. Overview of GC-HTC-IRMS method developments presented in this thesis.

Element (analyte gas)	**Starting point**	**Improvement measures**	**Results**	**Current challenges**
Chlorine (HCl)	**no** GC-HTC-IRMS method	• instrumental setup • search for reasons of instability • HTC optimisation	• **proof of principle**[1, 2] • evaluation of interfering by-products[3] • GC-HTC-IRMS measurement of reference compounds[3]	• memory effects • reactor lifetime • reference materials
Hydrogen (H_2)	heteroatom-bearing (N,S,Cl) compounds **not possible**	• evaluation of interfering by-products (HCN, H_2S, HCl) • use of chromium metal as trap	Successful GC-Cr/HTC-IRMS method development[4] **routine application**	Other heteroatom-bearing (F, Br, etc.) compounds not yet evaluated
Oxygen (CO)	commercially available method and reactor **rarely used**	• evaluation of interfering by-products • HTC process investigation	• CO_2 major by-product • variable by-product formation • strictly reductive HTC conditions / reduced reactor needed • oxidation processes cause NiO and subsequent CO_2 formation	• conditioning • instability • accuracy / precision

At the beginning of this project, the focus was chlorine CSIA method development using HTC to quantitatively generate HCl from chlorinated organic compounds (Chapter I). The idea was to use HCl analyte gas for chlorine stable isotope ratio determination. This approach was successfully realised, however, stable isotope analysis was affected by poor stability and instrumental drift.[1, 2] Initially, these effects were attributed to the used gas analyser for HCl isotope ratio determination. However, contrary to expectations, the subsequent change to GC-HTC-IRMS did not eliminate these problems. Furthermore, a higher amount of chlorinated compound-on-column was needed to reach CSIA determination limits. Those results suggested that, in contrast to initial findings with the gas analyser, the problems might originate within the HTC reactor and conversion process. Thus, the conversion interface was equipped with an additional IonTrap MS to analyse HTC products, identify limitations, and optimise HTC conversion.[3] This access allowed qualitative and quantitative characterisation of HTC products. It could then be shown that the organic by-products (C_nH_x, n>1) formed in the reactor produce mass fragments which are detected by the IRMS at ion traces used for isotope ratio determination (HCl, *m/z* 36,38), which means that their concentration influences the obtained final chlorine stable isotope ratio.[3] Consequently, parameters affecting by-product formation were investigated and optimal HTC conditions with reduced by-product formation were determined. Optimised conditions were validated with GC-HTC-IRMS measurements of chlorinated reference compounds (chlorinated ethylenes, methyl chloride, trichloroacetic acid, and HCH).[3] Still, the method was severely limited by memory effects, which at the present state of development did not allow joint CSIA determination of chlorinated compounds in a mixture. Memory effects in general are critical as they indicate that at some point in the analytical process –most probably in the ceramic reactor– retention and slow replacement of chlorine are takeing place. This is corroborated by the very short observed lifespan (less than one week) of HTC reactors used in chlorine isotope analysis.[3] The ceramic reactor was shown to influence HTC processes and interact with samples and analyte gases during oxygen HTC investigations as well. Evidence is here presented that, as previously suspected[53], ceramic material is able to leach oxygen (Chapter III) and thus contribute to water formation as hydrogen becomes available.[3] HCl has a high affinity to react with water and, though not detectable in the GC-HTC-IRMS system in our studies, a water effect cannot be definitively ruled out. Water formation and the parameters that reduce water by-product are not yet fully understood. For future method improvement or new HTC-based methods, reactor materials other than ceramic (AL_2O_3) should be considered and tested for their high temperature performance, stability, water production, and other possible interfering effects.

Another observation from the chlorine stable isotope results that demands further investigation was that the linear regression, which was used to reference raw data to the international scale, showed a relatively high expansion factor relative to other chlorine CSIA methods (Chapter I).[3] It was suggested that the high (~20 %) content of hydrogen reactant gas needed for quantitative HCl production could be the reason for this scale contraction. Improvements must be implemented, possibly by applying lower concentrations of hydrogen, possibly in the form of a diluted (e.g. hydrogen in helium) reactant gas. Considering that valid organic reference materials for chlorine stable isotope analysis are not available at this time, the use of ‘wrong’ isotope ratios, might be another source for the observed high shift resulting from linear normalisation (also see discussion on reference materials in Chapter I).

The knowledge of by-products gained during the presented chlorine HTC investigations led to detailed characterisation of conventional HTC for hydrogen stable isotope analysis of organic compound and heteroatom-bearing compounds (Chapter II).[4] From elemental analyser HTC it was shown that heteroatoms form hydrogen-containing by-products like HCl, HCN, H_2S, and that these can be eliminated by employing chromium as scavenger.[41, 42] Applying the established parallel use of IRMS and IonTrap MS, GC-HTC by-product formation could be confirmed, while for the newly developed GC-Cr/HTC reactor only hydrogen gas was detectable at the end of the reactor.[4] Applying chromium powder for the whole heated length of the reactor, thus in both the hottest and cooler zones, down to 200 °C towards the end of the reactor (see Figure 4), allows for both HTC and subsequent reaction with chromium. Which reactions take place in the reactor could not be precisely determined, but the need for temperatures of ≥400 °C was found to be necessary for Cr/HTC as well. Thus, it is reasonable that HTC processes like pyrolysis take place first, in the hottest zone of the reactor, and that the resulting conversion products then react with chromium in the cooler zones of the reactor.

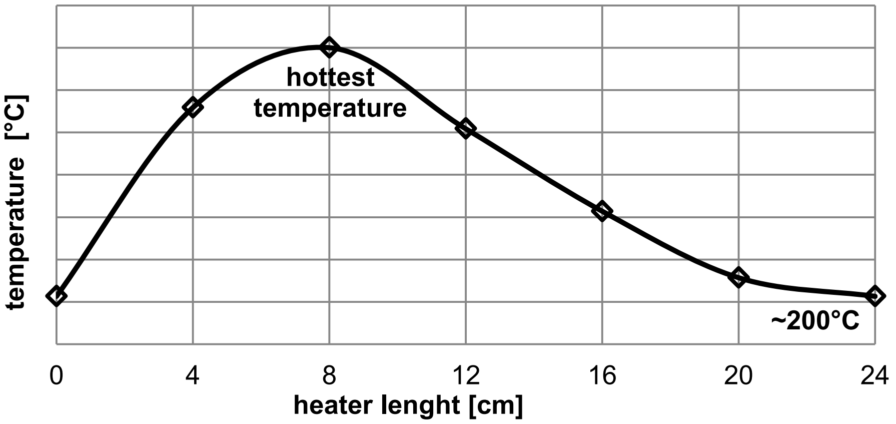


Figure 4. GC-HTC oven temperature profile (by courtesy of Dr. J. Renpenning).

Most of the undesired by-products are expected to react with chromium to form salts which are deposited in the reactor, which would also explain the observed colouration of the reactors after use with different heteroatom-bearing compounds. Hydrogen itself does not react with chromium and thus elutes unaltered, available for stable isotope analysis. This approach turned out to be very effective, even allowing hydrogen stable isotope analysis of highly chlorinated compounds like HCHs ($C_6H_6Cl_6$) which has been inaccessible with bare HTC due to major HCl formation (Chapter II).[4] This trapping of heteroatoms was also observed for nitrogen- and sulphur-containing by-products. The GC-Cr/HTC-IRMS approach therefore significantly extends hydrogen CSIA to a broad range of compound classes of interest to environmental research including pesticides, for instance chlorinated hydrocarbons, organophosphorus compounds, but also biological molecules such as amino acids. A potentially problematic environmental contaminant group could be fluorocarbons. Pyrolysis of fluorinated organics should yield the very reactive HF which might react to stable, non-reducible, volatile compounds with materials of the reactor (AlF_3) or capillaries (SiF_4). If HF is not trapped by the chromium, this reaction with other materials could interfere with hydrogen isotope ratio determination, producing H_2O as hydrogen-bearing product. The HTC conversion of fluorinated compounds therefore requires further investigation prior to GC-Cr/HTC-IRMS application for hydrogen stable isotope analysis.

Another aspect of GC-Cr/HTC-IRMS that could benefit from improvement are peak widths. These were observed to increase with temperature and possibly impede hydrogen CSIA of very complex mixtures with compounds eluting close to each other. Hydrogen diffusion in chromium particles was postulated as the reason for this broadening; however, this phenomenon is still under investigation. As for CSIA, baseline separation is always a requirement, and broadening of peaks in the reactor increases demands for gas chromatography. Here variation and optimisation of conversion parameters like carrier gas flow, chromium particle size and Cr/HTC temperatures might bear improvement potential for better separation and narrow peaks for specific compound classes.

The above discussed investigations of HTC focussing on chlorine and hydrogen stable isotope analysis raised the question whether undesired by-products could be a possible reason for limited application of the available GC-HTC-IRMS method for oxygen CSIA. Using the commercially available HTC reactor for oxygen[67] by-products were studied by parallel IonTrap MS and IRMS. Conversion parameters and by-product formation were characterised and processes partly explained (Chapter III). It was shown that the oxygen-bearing by-products carbon dioxide (CO_2) and water were indeed formed to greater extent if HTC conditions are not met with respect to reductive (oxygen limited) atmosphere. While requirements for exclusive carbon monoxide (CO) analyte gas production and minor (<2 %) CO_2 formation could be determined; until now it has not been possible to reduce water formation. Thus the detailed quantification and elucidation of HTC water by-product formation and impact on the obtained oxygen stable isotope ratio must be subjected to further investigation. Furthermore, the need for nickel wires in a platinum tube to prevent CO_2 formation was described; nevertheless, the underlying processes are not explicable with current knowledge about HTC processes. The present state-of-the-art reactor for oxygen HTC was shown to be subject to several drawbacks besides by-product formation; of these unstable HTC conditions were the most prominent (for detailed information on HTC processes present, see discussion below). Thus it would be reasonable to invest more resources into the development of a better reactor design and the search for more suitable reactor materials for oxygen GC-HTC-IRMS.

By-Products interfering with GC-HTC-IRMS

The synthesis of HTC investigations presented in this thesis is that HTC processes can result in several undesired by-products. More importantly, such by-products might be easily overlooked if IRMS is used to derive isotope ratios from the respective analyte gases. The parallel IRMS and IonTrap MS setup described above allowed for quantitative characterisation of GC-HTC products. There are four ways a by-product might interfere with stable isotope analysis, which should be considered for new method development:

(1) Element-containing by-product: Parts of the element of interest are not reacted to analyte gas, but to other volatile, or non-volatile by-products. Examples observed here are HCl, HCN, and H_2S formation or CO_2 formation instead of H_2 or CO analyte gas as observed for hydrogen and oxygen CSIA.[4] Those by-products are the most obvious and are thus usually monitored at least once during method development.

(2) By-product interfering with IRMS detection: These by-products are usually overlooked, as they are only detectable with a non-target analysis, in this thesis facilitated via IonTrap MS. For chlorine stable isotope analysis from HCl (*m/z* 36 ($H^{35}Cl$) and *m/z* 38 ($H^{37}Cl$)) recombination during HTC resulting in small organic fragments (C_nH_x, $n>2$), produced mass ions of *m/z* 36, thus hampering accurate isotope ratio determination by IRMS.[3]

(3) By-products interfering with ion source stability: For hydrogen analysis with IRMS the so called H_3^+-factor and its stability are essential for precise and accurate IRMS measurement. It is known that some compounds, e.g. SO_2, stick to isolating parts in the ion source lowering resistance and thus altering electronic properties and stability. This effect can last for several days, inhibiting accurate hydrogen CSIA, until the H_3^+-factor stabilises again. Bare HTC of sulphur-containing organic compounds showed exactly this H_3^+-factor instability, while Cr/HTC prevented the release of this by-product during hydrogen CSIA.[4]

(4) By-products altering analyte gas isotopic composition: Analyte gas should be produced from the compound of interest, however, it can also arise from a so-called 2nd source. Possible 2nd sources would be detained samples, contributions from the matrix or

background carrier, or leaching from reactor materials. During oxygen HTC investigations it was confirmed, that the outer ceramic tube of the reactor was able to react under HTC conditions with surplus carbon to form carbon monoxide. Such an effect might not influence CSIA if it contributes to the constant CO background signal. However, as shown during oxygen HTC, fresh 'activated' carbon from the compound subject to conversion resulted in a CO contribution co-eluting with the analyte gas CO peak which could thus easily be overlooked. A co-eluting by-product can only be detected if several reference compounds are available and obtained raw results are correlated with known reference values (2nd source calculation see Chapter III).

HTC Processes

Under the continuous flow conditions predominating in GC-HTC-IRMS a detailed process understanding or theoretical explanation is hardly possible. Nevertheless, the studies presented in Chapter I to III of this thesis did allow insights into processes and shed light on parameters influencing conversion. There are several, probably non-exhaustive, parameters which were determined to be exerting influence on HTC of organic compounds: temperature, type of reaction (conversion or equilibrium), compound subjected to HTC, reaction time, reaction volume, reactor design, reactor material, and auxiliary supplies like hydrogen reactant or make-up gas, deposited carbon, or metals (Cr, Pt, Ni) present in the reactor. For some of these parameters closer investigation during the presented method developments revealed information regarding importance, effects, and the supposed processes being influenced.

The importance of a high temperature (>1200 °C) was confirmed as it could be shown that by-product formation decreased with increasing temperature. In the hottest zone of the reactor (see Figure 4) thermal decomposition 'pyrolysis' of organic compounds in the presence of carbon yields the most simple and stable compounds H_2, CO, HCl, and deposited carbon (see introduction Equations (2)-(5)). This reaction is assumed to be fast and while carbon needs to be present, it does not take stoichiometric role in the reaction.[40] Only if the elemental composition of a compound itself does not allow formation of reaction products does the deposited carbon take part in the reaction, as in the case of reduction of water. For that matter, significant differences were observed in the reactivity of carbon according to the spatial and temporal point of deposition. Surplus carbon from the organic compound subjected to HTC seems to be 'activated' during conversion, even reacting with oxygen leaching from the ceramic reactor material and thus generating a 2nd, and in the case of oxygen CSIA interfering, CO source (see Chapter III). However, after some time the deposited carbon seems to migrate away from the hottest reaction zone and was no longer found to maintain the reductive atmosphere needed for oxygen HTC, as apparent through dropping CO background and increasing CO_2 by-product formation. For oxygen GC-HTC-IRMS hydrogen (1 % in helium) make-up gas is recommended[67] to maintain a reductive atmosphere and prevent NiO formation. However, evaluations showed that this small amount of hydrogen was not sufficient to reduce the reactor if it had been oxidised due to storage or use under sub-optimal HTC conditions. Conditioning with hexane was able to reduce NiO in combination with repeated reduction with a pure hydrogen stream (backflush). Additional hydrogen reactant gas is also needed for HCl formation with hydrogen-scarce chlorinated organics, and was shown to guarantee complete conversion of organically-bound chlorine to HCl (see Chapter I).[3] Using the IonTrap MS it was shown that, besides this desired and anticipated conversion process, recombination and formation of volatile organic by-products (C_nH_x, n>1) was possible, especially when hydrogen reactant gas was available, and needs to be considered during method development. A lesser hydrogen feed for chlorine and higher percentages for oxygen could potentially overcome present CSIA limitations.

Careful theoretical consideration on equilibrium processes taking place after the initial conversion in the cooler passages of the reactor (see Figure 4) indicate that e.g. the Boudouard equilibrium ($2CO \rightleftharpoons C + CO_2$) could, depending on the conditions in the reactor, impair the CO to CO_2 by-product ratio (see Chapter III). In the presence of metal constituents in the reactor, HTC conversion products were found to react further downstream. Subsequent reactions are favoured during Cr/HTC for hydrogen to remove heteroatom-bearing by-products (see Chapter II). However, undesired changes in product composition were found to occur for oxygen HTC, where platinum and nickel were thought to promote CO_2 by-product formation and oxygen detainment (NiO), respectively (see Chapter III). Not only metals but also the outer ceramic tube currently used in all HTC reactors was shown to interact. Besides its ability to leach out oxygen at temperatures around 1400 °C it also was affected by the more reactive conversion products. HCl was suspected to interact with the ceramic, causing the observed memory effect (see Chapter I).[3] Only further investigations and most probably the implementation of other materials for HTC reactors will be able to clearly indicate the origins and processes which now still limit routine GC-HTC-IRMS application for chlorine and oxygen CSIA. Another stable and constantly present HTC by-product identified in this thesis was water (see Chapter I and III). Theoretically, high temperature reduction in the presence of carbon should reduce water to hydrogen and CO. Thus, detected water at the end of the HTC reactor indicates that it was formed somewhere downstream of the hottest reactor zone requiring elucidation in subsequent experiments.

In Summary, the discussed examples illustrate the sensitivity of HTC processes to small changes in elemental composition and reductive atmosphere (especially for oxygen determination generated by frequent conditioning and hydrogen make-up gas). Moreover, the materials in the reactor and their position within the temperature profile (Figure 4) determine which subsequent reactions in addition to HTC take place. Beyond the pure reactions present, the history of the reactor with respect to previous heteroatom depositions, interactions, and fatigue determine HTC performance. Sometimes such interfering processes become apparent through signs such as memory effects, however, the lack of an obvious problem is not always synonymous with quantitative conversion.

Helpful for future method development employing HTC is the awareness that compound structure and elemental composition also seem to determine whether complete conversion and by-product formation take place. Additionally, in this thesis the importance of by-product investigation prior to method adaption for each new compound class in combination with accuracy tests using structurally similar references was underlined.

Synopsis

Multi-dimensional compound specific stable isotope analysis can reveal more information than common single element CSIA and is a promising future approach in the field of stable isotope investigations. It will aid in identifying the origin and history of a compound in applications ranging from environmental to health and forensic sciences. Heteroatom-bearing (Cl, Br, F, N, O, S) organic compounds, often emerging contaminants like pesticides or pharmaceuticals, are of great interest, for which new methods are needed to analyse isotopes of up-to-now non-routine elements. Substantial contribution to new methods for chlorine, hydrogen, and oxygen CSIA was achieved within the scope of this thesis.

For chlorine a novel GC-HTC-IRMS method was developed based on isotope ratio determination of HCl analyte gas, and promising results for chlorinated organic compounds were obtained (Chapter I). The next steps to adapt this experimental set-up to CSIA of mixtures may address memory and referencing.

Hydrogen CSIA was extended to heteroatom-bearing (Cl, N, S) compounds which were previously inaccessible due to the formation of hydrogen-bearing by-products. Employment of chromium powder in the reactor allowed the establishment of a GC-Cr/HTC-IRMS method which eliminates those interfering by-products (Chapter II). The successful application of this method for hydrogen CSIA of selected compounds has been shown and GC-Cr/HTC-IRMS is expected to be implemented for a wide range of structurally different compound classes.

Oxygen GC-HTC-IRMS reactor investigations did highlight challenges of CSIA with the present method available (Chapter III). By-products were quantified and 2nd sources detected, which interfere with accurate and precise oxygen CSIA. An evaluation strategy that can be universally used to characterise HTC conversion was developed. Considerations explaining possible underlying HTC processes allow future improvement and substantiate observations from the other GC-HTC-IRMS methods.

The application of HCH CSIA in the field and carbon stable isotope changes for assessment of biodegradation (Chapter IV) exemplarily proved the processing approach for this contaminant class. Based on the provided HCH pre-concentration and GC methods, in combination with the above-presented novel chlorine and hydrogen CSIA methods the results of this thesis provide a basis for future multi-dimensional (C, H, Cl) CSIA of HCHs.

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Appendix

To Chapter I Published paper[1]	**A novel online approach to the determination of isotopic ratios for organically bound chlorine, bromine and sulphur** *Rapid Communications in Mass Spectrometry* **2011**, Volume *25*, p. 3114-3122 K. L. Hitzfeld, M. Gehre, H.-H. Richnow and Supporting Information
Published paper[3]	**Development and validation of an universal interface for compound specific stable isotope analysis of chlorine ($^{37}Cl/^{35}Cl$) by GC-high temperature conversion (HTC)-MS/IRMS** *Analytical Chemistry* **2015**, Volume *87(5)*, p. 2832-2839 J. Renpennning, K. L. Hitzfeld, T. Gilevska, I. Nijenhuis, M. Gehre, H.-H. Richnow and Supporting Information
Patent[2]	**Verfahren und Vorrichtung zur online-Bestimmung des Isotopenverhältnisses von Chlor, Brom oder Schwefel in einer organischen Probe.** *German Patent Specification* **2010**, *Patent 10 2010 039 275* M. Gehre, K. L. Hitzfeld, H.-H. Richnow, M. Seidel
To Chapter II Published paper[4]	**Compound specific hydrogen isotope analysis of heteroatom-bearing compounds via gas chromatography-chromium-based high-temperature conversion (Cr/HTC)-Isotope ratio mass spectrometry** *Analytical Chemistry* **2015**, Volume *87(18)*, p. 9443-9450 J. Renpennning, S. Kümmel, K. L. Hitzfeld, A. Schimmelmann, M. Gehre and Supporting Information
To Chapter III accepted manuscript	**Evaluation of the performance of high temperature conversion (HTC) reactors for compound specific oxygen stable isotope analysis** *Isotopes in Environmental and Health Studies* **2016***, in press* K. L. Hitzfeld, M. Gehre, H.-H. Richnow and Supporting Information
To Chapter IV Published paper[5]	**Evaluating degradation of hexachlorcyclohexane (HCH) isomers within a contaminated aquifer using compound specific stable carbon isotope analysis (CSIA)** *Water Research* **2015**, Volume 71, p. 187-196 S. Bashir, K. L. Hitzfeld, M. Gehre, H.-H. Richnow, A. Fischer and Supporting Information

<u>**To Chapter I**</u> Published paper[1]	**A novel online approach to the determination of isotopic ratios for organically bound chlorine, bromine and sulphur** *Rapid Communications in Mass Spectrometry* **2011**, Volume *25*, p. 3114-3122 K. L. Hitzfeld, M. Gehre, H.-H. Richnow and Supporting Information
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Patent[2]	**Verfahren und Vorrichtung zur online-Bestimmung des Isotopenverhältnisses von Chlor, Brom oder Schwefel in einer organischen Probe.** *German Patent Specification* **2010**, *Patent 10 2010 039 275* M. Gehre, K. L. Hitzfeld, H.-H. Richnow, M. Seidel

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A novel online approach to the determination of isotopic ratios for organically bound chlorine, bromine and sulphur

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A novel approach for the measurement of ^{37}Cl, ^{81}Br and ^{34}S in organic compounds containing chlorine, bromine, and sulphur is presented to overcome some of the major drawbacks of existing methods. Contemporary methods either require reference materials with the exact molecular compositions of the substances to be tested, or necessitate several laborious offline procedures prior to isotope analysis. In our online setup, organic compounds are separated by gas chromatography (GC) coupled to a high-temperature reactor. Using hydrogen as a makeup gas, the reactor achieves quantitative conversion of chlorinated, brominated and sulphurated organic compounds into gaseous hydrogen chloride (HCl), hydrogen bromide (HBr), and hydrogen sulphide (H_2S), respectively. In this study, the GC interface was coupled to a quadrupole mass spectrometer operated in single-ion mode. The ion traces of either $H^{35}Cl$ (*m/z* 36) and $H^{37}Cl$ (*m/z* 38), $H^{79}Br$ (*m/z* 80) and $H^{81}Br$ (*m/z* 82), or $H_2^{32}S$ (*m/z* 34) and $H_2^{34}S$ (*m/z* 36), were recorded to determine the isotopic ratios of chlorine, bromine, and sulphur isotopes. The conversion interface presented here provides a basis for a novel method for compound-specific isotope analysis of halogenated and sulphur-containing compounds. Rapid online measurements of organic chlorine-, bromine- and sulphur-containing mixtures will facilitate the isotopic analysis of compounds containing these elements, and broaden their usage in fields of environmental forensics employing isotopic concepts.

Compound-specific isotope analysis (CSIA) is used to study natural attenuation processes, identify sources of environmental contamination, and to characterise biochemical reaction mechanisms.[1–4] Generally, the isotopic ratios of elements can be determined by offline or online methods. Briefly, for offline methods steps such as compound purification, conversion, and isotope ratio determination are conducted separately. In contrast, online methods combine sample preparation and analysis into a simplified workflow.[4] Compound mixtures are typically separated by gas chromatography, converted online via an interface into the analyte gas, and then analysed with respect to isotopic composition using isotope ratio mass spectrometry (IRMS).[3] CSIA methods are routinely used to determine the isotopic ratios of organic molecules (^{2}H, ^{13}C, ^{15}N, ^{18}O).[4,5] For organic compounds containing chlorine, bromine, or sulphur, offline and online methods have been developed (Table 1) but these contain serious drawbacks. At present, efficient online CSIA methods for these elements are not available due to instrumental limitations.[1–4] The approach presented here using hydrogen as a reactant gas to form HCl, HBr, and H_2S for use as analyte gases overcomes several current drawbacks and limitations to the further application of ^{37}Cl, ^{81}Br and ^{34}S analysis.

Chlorine isotope analysis

Isotopic analysis of chlorine remains one of the most challenging research areas in CSIA.[2] Several offline methods have been gradually improved and the first online methods were recently developed. Offline methods achieve precise results but have several limitations. The method developed by Holt *et al*. and subsequently modified by other groups involves tedious preparative steps performed to extract, enrich, and convert organochlorines into CH_3Cl.[6] This compound is then measured by dual-inlet isotope ratio mass spectrometry (DIIRMS).[6–8] This procedure determines bulk chlorine isotope ratios, but requires additional preparative separation for the analysis of individual chlorinated hydrocarbons present in complex mixtures. Similarly, thermal ionization mass spectrometry (TIMS) requires an offline conversion of compounds into CsCl, and high sample purity is essential.[9–11] The ionisation during TIMS is facilitated by a filament on which the CsCl is loaded, and from which it is ionised by filament heating.[10] Negative ion IRMS and inductively coupled plasma mass spectrometry (ICPMS) have also been used to a lesser extent for isotope ratio measurements of chlorine.[12–15] The two latter methods fail to sufficiently simplify the chlorine isotope measurements. Negative ion mode IRMS is more sensitive than positive ion mode IRMS but still requires an offline conversion into CH_3Cl.[12,13] The ICPMS method, with ionisation facilitated using a plasma torch, omits preparation by coupling a gas chromatograph directly to ICPMS but depends on high mass resolution to separate the ^{36}ArH formed in the plasma from ^{37}Cl. Recently, continuous-flow methods that omit offline sample

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Table 1. An overview of offline and online approaches for the determination of chlorine, bromine and sulphur isotopic ratios. The table has been modified from Hofstetter[4]

	Offline		Online	
	Instrumentation	Analyte	Instrumentation	Analyte
$^{37}Cl/^{35}Cl$	IRMS	CH_3Cl	GCICPMS	Cl
	TIMS	CsCl	GCIRMS	Fragment ions
			GCqMS	Molecular ion, fragment ions
			GCHTCMS*	HCl
$^{81}Br/^{79}Br$	IRMS	CH_3Br	GCICPMS	Br
	TIMS	CsBr	GCIRMS	Fragment ions
			GCHTCMS*	HBr
$^{34}S/^{32}S$	IRMS	SO_2, SF_6	GCICPMS	S
			GCHTCMS*	H_2S

IRMS – isotope ratio mass spectrometry, TIMS – thermal ionisation mass spectrometry, ICPMS – inductively coupled plasma mass spectrometry, GC – gas chromatography, qMS – quadrupole mass spectrometry, HTCMS – High-temperature conversion mass spectrometry. *Novel approach presented here.

preparation steps have been reported, but they require standard reference material mass spectra of known isotope composition: Shouakar-Stash *et al*. achieved continuous flow analysis by coupling a gas chromatograph to an IRMS instrument, but their method suffers from the need to specifically adjust the Faraday cups for each compound.[16] An attractive online method was conceived by Sakaguchi-Söder, Aeppli, and colleagues using gas chromatography coupled to quadrupole mass spectrometry (GCqMS).[17,18] However, their method involves substantial calculation to obtain isotope ratios from compound-specific mass spectrometric data and demands repeated comparison with molecularly identical reference compounds.[17,18] Although these online methods are promising, they still exclude the measurement of compounds for which a specific evaluation scheme has not been developed prior to analysis.[19] In 1961, Hoering and Parker choose hydrogen chloride gas for their investigation of chlorine stable isotope ratios due to its quantitative preparation from silver chloride and simple cracking pattern in the mass spectrometer.[20] They reported strong memory effects that restricted their method to samples with small isotopic variations. Possibly due to their findings, hydrogen chloride was abandoned from further consideration as a suitable chlorine-containing compound for direct isotopic measurements.

Bromine isotope analysis

Methods for measuring bromine isotope ratios are analogous to chlorine methods: isotope ratios are determined either offline by TIMS, via methyl bromide, or online using ICPMS or IRMS. They suffer from the same drawbacks as the respective chlorine methods. Bromine isotopes are determined by TIMS after conversion into CsBr.[21] Related to methods utilising methyl chloride for the determination of chlorine isotope measurements, organobromines can be converted offline into methyl bromide and measured via IRMS to determine bromine isotope ratios.[22,23] For ICPMS, the analyte is separated via GC and introduced into the plasma to produce ^{79}Br and ^{81}Br ions that may subsequently be detected.[24,25] The isotopic ratios for bromine, from IRMS fragment ions of whole compounds, are calculated using exactly the same procedure as for organochlorines.[23] However, few studies have investigated isotopic ratios for organically bound bromines.[24–27] This is probably due to the above-mentioned limitations of existing methods, and could be rectified by using HBr as analyte gas. It is clear that the field of bromine isotope forensics, especially for investigating flame-retardants, would certainly benefit from the development of a convenient online method.[2]

Sulphur isotope analysis

In contrast to the various instrumental approaches for chlorine and bromine isotope ratio determinations, sulphur isotope analysis is mainly facilitated by offline methods. Offline methods are geared towards the analysis of inorganic samples, while only ICPMS as an online approach is available at this time.[28,29] The historic development and establishment of methods for measuring inorganic sulphur isotopic ratios have been summarised by Mayer.[30] One possibility for determining the isotope ratios of organically bound sulphur is to covert the compound of interest into an inorganic sulphur species, and then make measurements according to the established methods using SO_2 or SF_6. Direct analysis of bulk samples can be performed by means of an elemental analyser coupled to a mass spectrometer, or via laser ablation coupled to ICPMS.[31,32] Online methods to determine compound-specific isotope ratios for organically bound sulphur species may be conducted using a gas chromatograph coupled to ICPMS.[28,29] However, the high maintenance costs certainly limit a broader application of this technique. A readily accessible online approach to determining compound-specific sulphur isotope ratios using H_2S would probably increase the application of ^{34}S analysis.

The aim of this study was to develop an online interface for the direct compound-specific determination of chlorine, bromine and sulphur isotope ratios for distinct organic compounds in complex mixtures. For accurate isotopic measurements, a complete transformation to relatively simple products is mandatory to avoid fractionation. Thus, we investigated the conversion of organically bound chlorine, bromine and sulphur to HCl, HBr and H_2S, respectively. This reaction takes place in an online ceramic reactor connected to a gas chromatograph and mass spectrometer at temperatures of 1300 to 1400 °C.[33] The proof of principle is shown for a range of organic compounds. The applicability of the interface described herein was demonstrated using an evaporation experiment, and resulted in isotope enrichment factors similar to published values obtained using established methods.

EXPERIMENTAL

Chemicals

Tetrachloroethene (PCE), trichloroethene (TCE) and 1,2-dichloroethane (DCA) were purchased from Merck (Darmstadt, Germany). Additional TCE was from PPG (Pittsburgh, PA, USA). Dichloroethene (*cis*-DCE) was purchased from Riedel-de Haën (Sigma-Aldrich, Seelze, Germany). Chlorobenzene (CB) and thiophene were obtained from Fluka (Sigma-Aldrich, Steinheim, Germany). Tetrahydrothiophene, 2-bromophenol and lindane (γ-1,2,3,4,5,6-hexachlorocyclohexane) were purchased from Aldrich (Sigma-Aldrich, Steinheim, Germany). Tribromoethene (TBE) was purchased from ABCR (Karlsruhe, Germany). Dichloromethane (DCM) and *n*-pentane were obtained from Carl Roth (Karlsruhe, Germany). Lindane was dissolved in DCM. All other compounds were dissolved in *n*-pentane.

Instrumental setup

The interface can be combined with any standard gas chromatograph and detector. Our setup consists of an A200S autosampler (CTC Analytics, Zwingen, Switzerland), a HP6890N gas chromatograph (Agilent Technologies, Böblingen, Germany), a GC/TC high-temperature oven (GC-C/TC III, ThermoFinnigan MAT, Bremen, Germany), and a gas analyser (GAM 445, Balzers AG, Balzers, Liechtenstein) (Fig. 1).[33] The gas chromatograph was equipped with a split/splitless injector and a capillary column (ZB1; 60 m × 0.32 mm, 1.0 µm film thickness; Phenomenex, Utrecht, The Netherlands). The injector temperature was set to 250 °C, the gas chromatographic flow was set to 0.8 mL/min, and 1 to 4 µL of liquid sample were injected using a split of 1:10. Commercially available ceramic tubes (0.8 mm i.d., 1.6 mm o.d., max. length 400 mm, material: aluminium oxide Degussit AL23; Friatec, Mannheim, Germany) were used as reactors without any pretreatment, at temperatures of 1300 to 1400 °C. The sample was transported by a carrier gas (He) stream through the entire setup. Fixed splits were installed (Fig. 1): The split at the hydrogen inlet joint (Split 1, Fig. 1) reduced the amount of sample for conversion in the reactor and resulted in defined, narrow peaks. An open split at the end of the reactor (Open Split, Fig. 1) prevented backpressure and ensured consistent sample flow into the gas analyser. The backflush, hydrogen, and oxygen gas flows were switched using pneumatic valves. A solvent delay using a backflush function protected the reactor from solvent. In addition, the reactor may be cleaned with a backflush stream of oxygen to remove carbon deposits from the ceramic surface (for details regarding carbon deposition, see next paragraph). Compound-specific separation of the samples was achieved by GC before hydrogen gas addition, and before the conversion process in the reactor. The conversion products were monitored using positive electron ionisation mass spectrometry (MS) (for details, see Supporting Information). The gas analyser is capable of scan mode for screening purposes, and single ion monitoring for precise measurements of specific ion traces. The linearity of the device was examined by injection of a concentration gradient of chloroform, in order to analyse the effect of HCl concentrations on the stability of the chlorine isotope ratio. The peak intensities were plotted versus the isotope ratio (Fig. 2). Chloroform in a helium gas phase was injected with split ratios ⩾1:10 to avoid fractionation in the injector. Within one order of magnitude, the system yielded relatively stable isotope ratios. This peak intensity interval was selected for further evaluation of isotope ratios. The sample concentrations were adjusted to these peak intensities within the relatively linear range of the detector. The determined intensity range of relative linearity was between 0.09 and 0.16 µmol chlorine on the column. The effluents of the column were split in a ratio of about 1 to 3 at the reactor and using an open split before the mass spectrometer (Fig. 1).

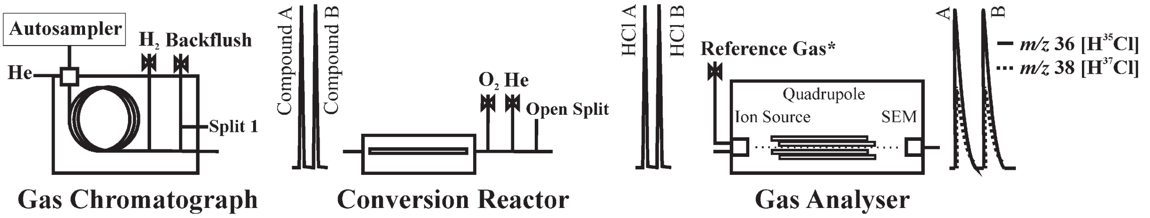

Figure 1. Instrumentation required for the presented system.[33] The conversion principle is shown for organochlorines. Sample transport via a carrier (He) gas flow from left to right. A mixture of components is injected into the gas chromatograph and separated. The solvent peak is removed using a backflush split. The sample stream is fed with ~10% hydrogen before entering the conversion reactor (ceramic tube, 1300 to 1400 °C). Online conversion of the constituents into HCl (or HBr, H_2S) occurs. The carrier gas transports the conversion products to the gas analyser [EI+, 70 V, quadrupole mass spectrometer (Balzers GAM 445)]. Ion traces of the conversion products [*m/z* 36, 38 ($^1H^{35}Cl$, $^1H^{37}Cl$), *m/z* 80, 82 ($^1H^{79}Br$, $^1H^{81}Br$) or *m/z* 34, 36 ($^1H_2^{32}S$, $^1H_2^{34}S$), respectively] are recorded. Data analysis is carried out by means of peak integration and consecutive isotope ratio calculation. *The use of reference gas compared to other CSIA methods can be implemented.

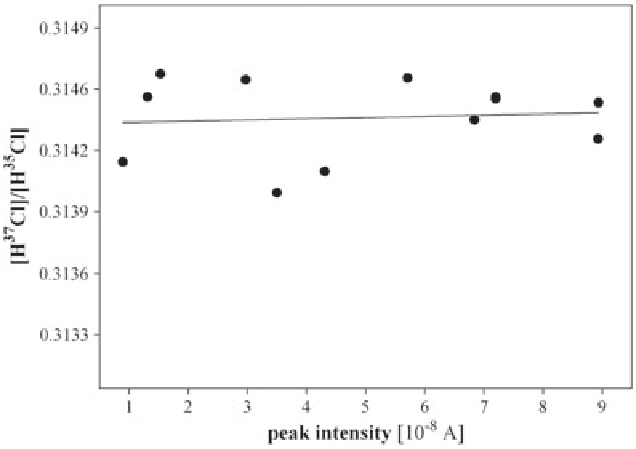

Figure 2. A linearity test of the system using a quadrupole mass spectrometer (Balzers GAM 445) as a detector. The peak intensity was plotted versus the chlorine isotope ratio. Chloroform at different concentrations was injected and converted into HCl. A linear regression slope of $6*10^{-6}/10^{-8}$ A was obtained. All samples measured during the course of this investigation were adjusted to produce peak intensities in the range of $1*10^{-8}$ A to $9*10^{-8}$ A.

Evaporation experiments

For the evaporation experiments, 200 µL of pure phase PCE was allowed to evaporate at 20 °C in a fume hood. The course of evaporation was monitored by weighing the remaining liquid PCE phase. Two parallel experiments, each using several different vials, were conducted. During the experiments a pure phase PCE sample and samples of the residual fraction at different extents of evaporation were analysed for ^{37}Cl-isotopic composition.

RESULTS AND DISCUSSION

High-temperature interface

An empty, high-temperature ceramic tube was used for the conversion of chlorinated, brominated, and sulphurated organic compounds. For the quantitative conversion of organic products into HCl, HBr and H_2S, a high-temperature reaction (1300-1400 °C) with hydrogen (~10%) was performed in a helium atmosphere. The ceramic tube was chosen due to its high temperature stability, its resistance to atmospheric oxygen diffusion, and its successful use during GC-pyrolysis methods for ^{2}H- and ^{18}O-isotope analysis.[34] This reactor is routinely employed in ThermoFinnigan GC-C/TC III interfaces (ThermoFinnigan MAT, Bremen) for hydrogen isotope measurements.[35] However, deterioration of the material takes place at high temperatures, and necessitates exchange of the reactor from time to time.

The conversion reactions are likely to occur in the hottest (1300 to 1400 °C) zone of the reactor, as shown by Gehre *et al.* during the quantitative conversion of methane at temperatures above 1300 °C.[34] Pure carbon is produced and deposited in the cooler regions of the reactor. In the zones of the reactor with temperatures above 1100 °C, the carbon reacts to a minor extent with the oxygen from the ceramic material to form gaseous carbon monoxide as a by-product.[34] This process leads to brittleness of the ceramic structure over time.[34] Oxygen depletion in the ceramic tubing can be observed as a colour change of the material from white to transparent. Visual inspection of used ceramic tubing confirms the zonation, colour change and deposition of carbon on the aluminium oxide surface of the tubing during progressive sampling. On one hand, carbon deposition indicates that damage to the ceramic material has occurred. On the other hand, it suggests that conversion of the organic analytes has occurred. Traces of oxygen in the reactor, whether molecular, bound organically, or bound in water, are converted into carbon monoxide. This reduces the amount of water in the parts of the device after the reactor to a minimum. The quantitative conversion of water into carbon monoxide in the presence of elementary carbon has been demonstrated by Gehre *et al.* at above 1300 °C.[34] Oxygen bound in organic molecules (e.g. bromophenol) results in the formation of carbon monoxide peaks in the full mass spectrum showing all the conversion products (Fig. S2, Supporting Information). Changes in the ratio of oxygen to argon during ceramic tube heating indicate that molecular oxygen is reduced to carbon monoxide.

Considering the reductive anoxic conditions, the high temperatures, and the deposition of carbon, pyrolysis supported by catalytic reactions can theoretically facilitate conversion into HCl, HBr and H_2S. Both processes have been previously described (see Supporting Information).[36–38] Gaseous conversion products from the interface were analysed by MS. The gas analyser has the capability to operate in scanning and single ion modes, and the conversion of separated products was monitored via the resulting mass spectra. The mass spectrum of PCE converted in the reactor was compared with the spectrum of pure PCE (Fig. 3). The conversion products did neither show any of the major PCE fragment ions, nor signals of other organic compounds or other chlorinated by-products. The main conversion product was HCl, with its most intense ion traces at *m/z* 36 [$^1H^{35}Cl$] and *m/z* 38 [$^1H^{37}Cl$] (Fig. 3).

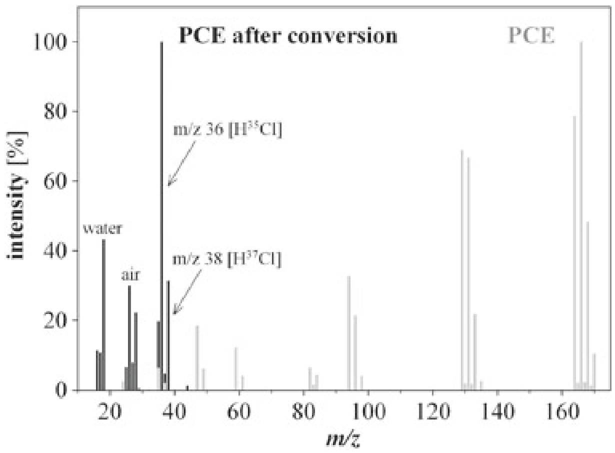

Figure 3. Quantitative conversion of PCE in the thermal interface is demonstrated. Comparison of the PCE mass spectrum with the mass spectra of the conversion products proves that HCl is the exclusive chlorine-bearing product.

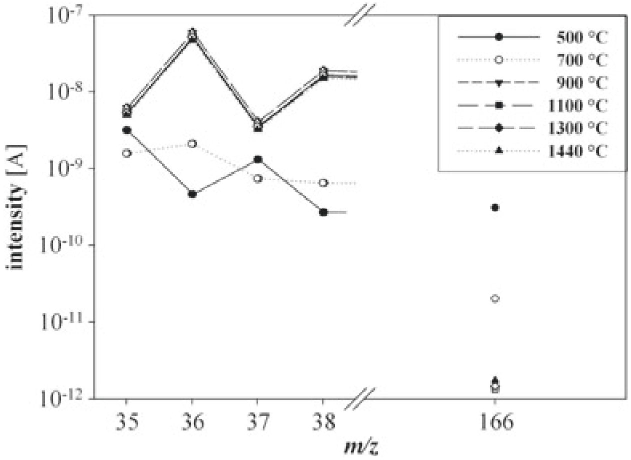

Figure 4. Analysis of selected product masses (Cl and HCl at *m/z* 35, 36, 37, 38) and the parent PCE compound mass (*m/z* 166) after the conversion at different reactor temperatures. It can be assumed that complete conversion is achieved at temperatures above 900 °C.

The optimal temperature range for conversion was determined for chlorine measurements by comparison of the PCE *m/z* 166 and HCl *m/z* 36 signal intensities at different reactor temperatures (Fig. 4). At temperatures above 900 °C, very low background *m/z* 166 intensities ($<10^{-11}$ A) compared with the *m/z* 36 intensities ($>10^{-8}$ A) were observed and this indicates that efficient conversion occurred. The conversions were conducted at 1300 °C, because not only quantitative conversion is facilitated but also molecular water is pyrolysed at this temperature.[34] In the case of organically bound sulphur, a temperature of 1400 °C was needed to ensure quantitative conversion. As quantitative conversion is the basis for reliable isotope ratios, it was verified by monitoring the parent compound masses and the background signals in the system for all compounds subjected to conversion. As an example, pure PCE has its most intense peak at *m/z* 166, followed by several peaks (e.g. *m/z* 164, 129, 131) with intensities of more than 50%, relative to *m/z* 166. The traces for ions with an intensity of more than 50% relative to the base peak of the parent compound were monitored to validate the conversion process. Quantitative conversion was achieved when the intensity of *m/z* 36 for $H^{35}Cl$ (*m/z* 80, 34 for HBr and H_2S, respectively) was at least three orders of magnitude higher than the mass traces of the parent compound, or when the mass traces of the parent compound could not be distinguished from background noise levels. All the investigated compounds (DCA, DCE, TCE, PCE, chlorobenzene, γ-hexachlorocyclohexane, tetrabromoethene, thiophene, and tetrahydrothiophene) were examined in this way to verify the efficiency of conversion for different compound classes (Table S1, Supporting Information). Specifically, the ion signals with an intensity of more than 50%, with regard to the most intense ion in a standard parent compound mass spectrum (SDBS database, National Institute of Advanced Industrial Science and Technology (AIST), Tokyo, Japan[39]), were monitored. Most parent compound ion traces showed no increase in intensity at the retention time of the conversion products. An example chromatogram for chlorobenzene conversion illustrates this procedure (Fig. S1, Supporting Information). Hydrogen chloride, hydrogen bromide, and hydrogen sulphide are produced at high temperatures from chlorine-, bromine- and sulphur-containing organic compounds according to Eqns. (1), (2) and (3), respectively. These results indicate the occurrence of reductive pyrolysis reactions in the ceramic reactor at high temperatures. Hydrogen makeup gas ensures the formation of HCl, HBr and H_2S. It is important to supply sufficient hydrogen for the quantitative conversion, and to prevent side reactions within the reactor.

$$C_xH_yCl_z + H_2 \xrightarrow{T=1300^\circ C} xC_{(s)} + zHCl \qquad (1)$$

$$C_xH_yBr_z + H_2 \xrightarrow{T=1300^\circ C} xC_{(s)} + zHBr \qquad (2)$$

$$C_xH_yS_z + H_2 \xrightarrow{T=1400^\circ C} xC_{(s)} + zH_2S \qquad (3)$$

Ion detection

The conversion products HCl, HBr and H_2S were monitored at the ion traces of their isotopologes: HCl at *m/z* 36, 38 ($^1H^{35}Cl$, $^1H^{37}Cl$), HBr at *m/z* 80, 82 ($^1H^{79}Br$, $^1H^{81}Br$), and H_2S at *m/z* 34, 36 ($^1H_2^{32}S$, $^1H_2^{34}S$). A chromatogram of cis-DCE, TCE and PCE demonstrates that GC separation, subsequent conversion, and detection of HCl from a mixture of chlorinated ethenes has occurred (Fig. 5). The calculation of area equivalents (sum area of *m/z* 36, 38) with respect to the injected amount of chlorine reveals that the detected area is proportional to the chlorine content of the converted molecule (Table 2). This has been consistently observed during three consecutive runs of a *cis*-DCE, TCE and PCE mixture (chromatogram see Fig. 5). The HCl area equivalents, in area per nmol Cl, produced from TCE and PCE are compared with the amount of HCl produced from 1 nmol Cl in *cis*-DCE. These demonstrate that the amounts of HCl produced from chlorine for the three different compounds match each other up to 96%. These are strong indications for a quantitative conversion process. However, processes occurring in the ion source need to be considered also. The low ionisation energies of the measured gases are expected to result in poor ionisation and in decomposition processes during ionisation.[40] One expected decomposition process is hydrogen abstraction

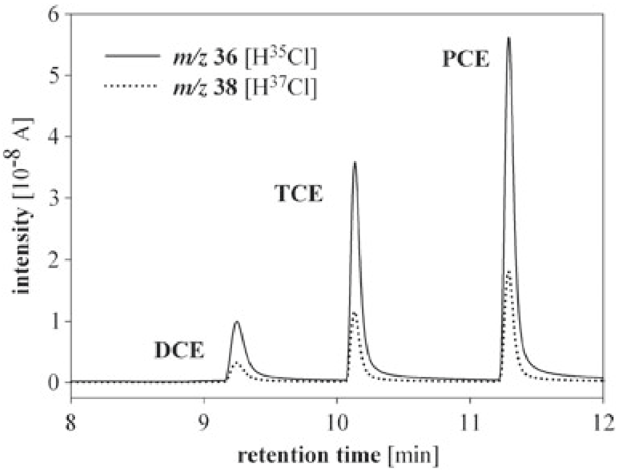

Figure 5. Chromatogram of *cis*-DCE, TCE and PCE. The mixture is separated and converted into HCl within the interface, and subsequently analysed by mass spectrometry.

Table 2. Area evaluation with respect to injected chlorine amounts. From the injected chlorine amounts and peak areas, the absolute measured areas (*m/z* 36, 38) per nmol of injected chlorine are calculated for each compound

	Injected amount	**1^{st} run** **Σarea/inj. amount**	**2^{nd} run** **Σarea/inj. amount**	**3^{rd} run** **Σarea/inj. amount**
Compound	[nmol Cl]	[10^{-8}/nmol Cl]	[10^{-8}/nmol Cl]	[10^{-8}/nmol Cl]
cis-DCE	52	1.02	1.01	0.98
TCE	67	1.02	1.02	0.99
PCE	78	1.09	1.09	1.06
RSD [%]		4	4	4

Σarea = area *m/z* 36 + area *m/z* 38; RSD – relative standard deviation.

resulting in positively charged chlorine, bromine, HS and S ions. This process was investigated for the conversion of organochlorines and HCl ionisation. In the full mass spectra, the ion traces of HCl (*m/z* 36, 38) and atomic chlorine (Cl) (*m/z* 35, 37) were found. However, evaluation of the HCl ion traces resulted in the same isotope ratio, whether assessed alone or as the sum of the HCl and Cl ion traces. For isotopic measurements of non-halides usually a closed IRMS ionisation chamber is applied to achieve maximum ionisation. For such ion sources, corrosion can be expected where HCl or HBr comes into contact with the instrument. We, in contrast, found no visible corrosion effects in the open ionisation chamber of the gas analyser, or at any other part of the ion source or detector after more than 1500 measurements. In addition, in contrast to earlier findings by Hoering and Parker,[20] we did not observe any significant memory effects for HCl, HBr or H_2S in our system. The absence of corrosion and memory effects is probably due to several characteristics of the interface, including: (i) water is avoided in the system and is pyrolysed at 1300 °C in the reactor; (ii) HCl amounts are low, approximately 15 nmol in the helium gas stream reach the mass spectrometer for analysis; (iii) the contact time is approximately 1 min for each peak, much shorter than the time in a multiple inlet system used by Hoering and Parker;[20] and (iv) the open ionisation chamber and source construction allow fast and efficient removal of non-ionised molecules from the mass spectrometer.

Isotope ratios

For chlorinated ethenes, the applicability of the presented interface for isotope ratio determination was studied in more detail. The $^{37}Cl/^{35}Cl$ ratio of PCE was determined using Eqn. (4) from the recorded ion traces *m/z* 36 and 38 ($^{1}H^{35}Cl$, $^{1}H^{37}Cl$), assuming that the hydrogen isotopic composition would not alter the ratio significantly. The high natural abundance of ^{37}Cl (24.24%), ^{81}Br (49.31%) and ^{34}S (4.25%) is contrary to the low abundance of ^{2}H (0.01%).[41] Thus, it is possible to determine the chlorine, bromine and sulphur isotope ratios directly from the ion traces of HCl, HBr and H_2S without any further corrections for ^{2}H isotopes. Hydrogen with a constant isotopic composition is provided in the reactor, and even hypothetical variation in the hydrogen isotope composition of the substrate would not have a relevant effect on the determination of ^{37}Cl, ^{81}Br, ^{34}S isotope compositions.

$$R\left(\frac{^{37}\mathrm{Cl}}{^{35}\mathrm{Cl}}\right) \approx \frac{m/z\ 38\ \text{peak area}}{m/z\ 36\ \text{peak area}} \qquad (4)$$

Peak integration was calculated using a customised algorithm based on considerations by Ricci *et al.*[42] Peak initiation was detected when a threshold was reached (usually $3*10^{-9}$ A, for *m/z* 36) and peak termination was marked when the signal fell below that threshold. The integration interval was determined by iterative slope determination from the threshold intersection backwards, for the beginning of the interval, and forwards for the end until the conditions (start: slope $> 10^{-12}$ A, end: slope $< 2*10^{-12}$ A, for *m/z* 36) were fulfilled. Both ion traces were integrated over the determined interval, using the baseline before the interval.

The resulting standard deviations for three consecutive measurements of chlorine isotope ratios were, in the majority of cases, less than ±0.00032 of the isotope ratio (*R*), representing a deviation of less than 1‰ (Fig. 6). The changes in the isotopic ratio (R) between two samples can be expressed in delta notation according to Δ [‰] = (R1 – R2)/0.00032, where R1 and R2 are the isotope ratios of the two samples.

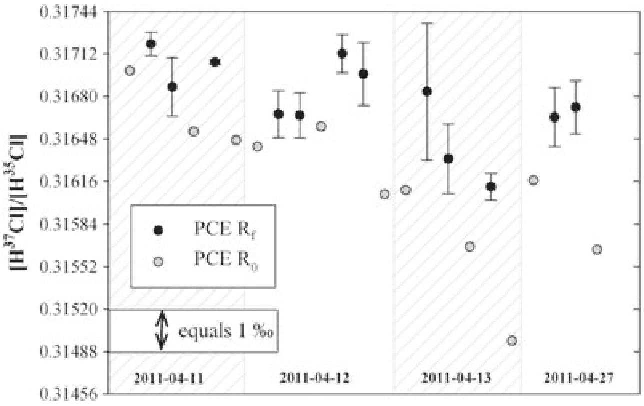

Figure 6. Instrumental drift. Data shown for one of the PCE evaporation experiments. Samples taken at different extents of evaporation (R_f, n = 3) were bracketed with a sample from the initial PCE compound (R_0, n = 2). Error bars indicate standard deviations (1σ). Measurements at three consecutive days, and at one day two weeks later are shown.

Table 3. Absolute differences in isotope ratios between a PCE and two TCE samples from different suppliers (Merck and PPG). The differences in the chlorine ratios were reported as changes in ‰ of the isotope ratio. A change of $\Delta R = 0.00032$ equals 1‰ in delta notation. TIMS and GCqMS data were kindly provided by C. Aeppli and H. Holmstrand and were determined as described elsewhere[10,18]

	TIMS[10]			GCqMS[18]			GCHTCMS*		
	δ^{37}Cl [‰ vs. SMOC]			δ^{37}Cl [‰ vs. SMOC]					
Sample		1σ	n		1σ	n			
PCE [M]	−0.72	0,3	3	−0.39	0.5	10			
TCE [M]	0.00	0,7	2	0.54	1.0	9			
TCE [P]	−2.49	0,6	3	−2.59	0.7	9			
Difference	$\Delta\delta^{37}$Cl [‰]			$\Delta\delta^{37}$Cl [‰]			$\Delta\delta^{37}$Cl [‰]	**1σ**	**n**
PCE [M] – TCE [P]	1.77			2.20			1.27	1.4	3
TCE [M] – PCE [M]	0.72			0.93			0.94	0.6	3
TCE [P] – TCE [M]	−2.49			−3.13			−3.36	0.9	3

[M] – supplied by Merck, [P] – supplied by PPG; 1σ – standard deviation; n – number of measurements.
*Novel approach presented here.

The determined absolute chlorine isotope ratios can be directly compared when the instrument stability is reasonable. We observed drifts when measuring one sample over the course of a day or several days (Fig. 6). To correct for the instrumental drift, we used the isotope ratio of a PCE laboratory reference material. The isotope ratio of this PCE was determined before and after several consecutive measurements of a sample. The sample isotope composition could then be corrected for the average of the PCE isotope ratio before and after the sample measurements. This procedure considers only results of consecutive measurements and was applied to calculate the isotope composition changes and differences. To evaluate the accuracy to determine isotope differences, a TCE sample from Merck and one from PPG with known isotopic compositions were examined (Table 3). The results demonstrate that differences obtained with our new approach are comparable with the isotope differences determined by validated methods. In the future, referencing strategies need to be implemented to assure traceability of the determined isotope ratios back to SMOC, and thus for comparability with international standards. Referencing could be realised via known reference samples, given sufficient instrument stability, or via a reference gas (Fig. 1, possibility indicated by an asterisk).

Evaporation experiments

A possible application of the conversion interface for isotope ratio monitoring was demonstrated by PCE evaporation, previously shown to induce isotope fractionation.[18] During the experiment, pure phase PCE (R_0) was evaporated and samples of the residual fraction (R_f) were analysed for ^{37}Cl-isotopic composition. The raw data show an instrumental drift in ^{37}Cl/^{35}Cl ratios (Fig. 6). The ratios determined for the initial PCE (R_0) stock, measured at regular intervals, shifted during the course of a day and over several days. Thus, the ^{37}Cl/^{35}Cl ratios of PCE (R_f) were corrected with respect to the initial PCE (R_0), to adjust for instrument instability, as described above. This correction allowed for the determination of chlorine isotope fractionation factors for the evaporation experiments. The change in weight (f, fraction remaining) was related to the change in the chlorine isotope ratio (R, according to Eqn. (4)) using the Rayleigh equation (Eqn. (5)).[43]

$$\frac{\varepsilon_{\mathrm{Cl}}}{1000}\ln(f) = \ln\left(\frac{R_f}{R_0}\right) \tag{5}$$

The chlorine isotope ratio of the PCE evaporation follows Rayleigh behaviour (Fig. 7) and an isotopic enrichment factor for chlorine ($\varepsilon_{\mathrm{Cl}}$) of -0.7 ± 0.2‰ (confidence interval, 95% of

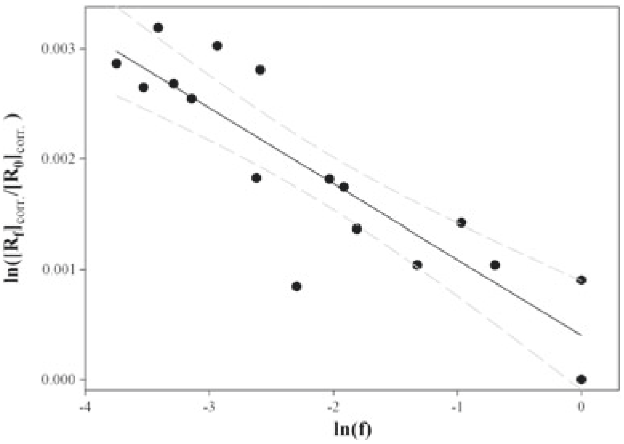

Figure 7. Chlorine isotopic fractionation during PCE evaporation from pure phase. The chlorine isotope ratio of the evaporation follows Rayleigh fractionation, the isotope enrichment factor $\varepsilon_{\mathrm{Cl}}$ was calculated to be −0.7 ± 0.20‰ (95% confidence interval of the linear regression, grey dashed lines). The measured ratios R [$R = R(^{37}\mathrm{Cl}/^{35}\mathrm{Cl})$, according to Eqn. (4)] were corrected using a PCE sample analysed at regular intervals between the other samples, to adjust for instrument instability. Remaining PCE is enriched in ^{37}Cl over the course of evaporation.

the linear regression) was determined. This value is in good agreement with the previously published value of $-1.1 \pm 0.4‰$.[18] Apparent differences in the values ε_{Cl} may be due to instrumental drift that necessitated the above-mentioned correction. In the future, variations could be reduced by using a referencing strategy. Although referencing to SMOC is not realised in our setup at this time, we were able to effectively demonstrate the applicability of the high-temperature conversion interface for chlorine isotope ratio monitoring.

SUMMARY AND OUTLOOK

We developed and evaluated a new interface for determining isotopic ratios of organically bound chlorine-, bromine- and sulphur-containing compounds. The quantitative conversion of organochlorines, organobromines and organically bound sulphur species into HCl, HBr and H_2S was demonstrated in a ceramic reactor using hydrogen gas and temperatures of 1300–1400 °C. The first results obtained with structurally different organic compounds provide evidence for complete conversion into simple products suitable for online CSIA. Fractionation experiments and the comparison of ratio differences determined with pre-existing methods demonstrate that the use of the high-temperature interface for the monitoring of changes in chlorine isotope ratios is possible, although SMOC referencing is not yet established for this system. At the moment, apparent instrumental instabilities cause variations in the measured isotopic ratios, and complicate comparability between different runs. However, this feature does not impair the principle of the applicability of the conversion interface. In the future, instrumental stability can be counterbalanced by utilising referencing measures. Furthermore, the use of a more suitable detector might improve both reproducibility and sensitivity. Possible options are isotope ratio mass spectrometry, laser absorption spectroscopy, or other detector systems.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

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The authors thank Dr. A. Götz from *In Process Instruments* (Bremen, Germany) for support regarding the GAM 445 and for programming the integration software. The authors especially thank C. Aeppli and H. Holmstrand for determining the isotope ratios vs. SMOC of one PCE and two TCE samples and providing us with the data. Further thanks to M. Seidel for initial experiments with the ceramic reactors, as well as to U. Günther and F. Bratfisch for technical laboratory assistance. We also acknowledge B. Morris for proofreading the manuscript. Three anonymous reviewers improved the manuscript and the authors thank them for their valuable comments.

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SUPPORTING INFORMATION

A novel online approach to the determination of isotopic ratios for organically bound chlorine, bromine and sulphur

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1. Detailed ion source parameters

Gas analyser: Balzers GAM 445 (Balzers AG, Balzers. Liechtenstein):

Emission:	Current:	0.8 mA	(emission current)
	Protect:	4.4 A	(maximum allowed filament current)
Voltages:	IonRef:	80 V	(ion formation potential, ion reference potential)
	Cathode:	70 V	(cathode voltage, electron acceleration voltage)
	Focus:	9.5 V	(ion lens focussing voltage)
	FieldAxis:	11.25 V	(field axis voltage)
	Extraction:	196 V	(extraction voltage, ion acceleration voltage)
	Deflection:	202 V	(internal deflection voltage)

Mass resolution: >1000 at *m/z* 500

2. Conversion of chlorobenzene

The complete high-temperature conversion of chlorobenzene into HCl in a helium/hydrogen atmosphere at 1300 °C was studied. The fragmentation pattern of the parent compound using EI+ ionisation mode was used to evaluate the transformation. The main ions (*m/z* 112, 77) of the parent chlorobenzene were practically absent, and exemplified by the three orders of magnitude difference from the signal of the main HCl ion (*m/z* 36) (Fig. S1). The measurement of chlorobenzene resulted in a chlorine isotope ratio of 0.32020 ± 0.00010 (equals 0.3 ‰, 1σ, n = 5).

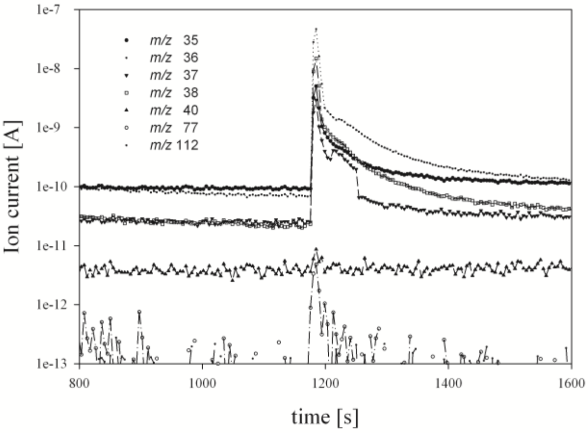

Figure S1. Chromatogram of the high temperature (1300 °C) conversion products of chlorobenzene. The following ion masses were selected for the screening: *m/z* 35, 37 (Cl ions), *m/z* 36, 38 (HCl), *m/z* 40 (argon for background information) and *m/z* 112 and 77, both most intensive (100 % and 52 %, respectively) masses of a reference chlorobenzene mass spectrum (SDBS database, National Institute of Advanced Industrial Science and Technology (AIST), Japan)). The chromatogram shows the conversion products of chlorobenzene (1200 s). The difference in the *m/z* 112, 77 and 36 intensities is more than three orders of magnitude, essentially demonstrating a complete conversion of chlorobenzene into HCl.

3. Conversion of organically bound oxygen

In order to analyse the transformation of oxygen-containing molecules to carbon monoxide in the interface, 2-bromophenol was used as a model component. The molecular ion (*m/z* 172, 174) and major fragment ions of the parent compound were essentially absent (Fig. S2). Organically bound oxygen reacts with the deposited carbon in the reactor to form carbon monoxide.

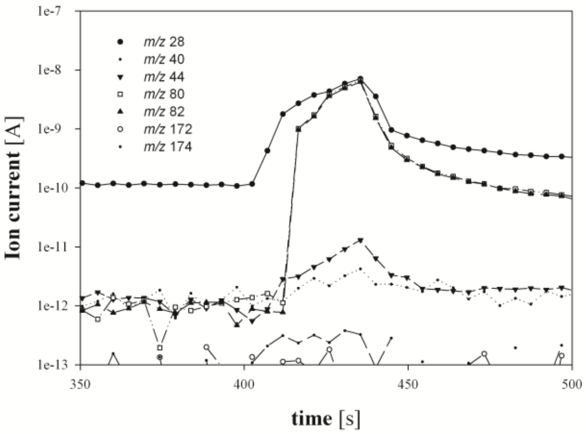

Figure S2. Chromatograms of the conversion of organically bound oxygen to carbon monoxide by means of the high temperature (1300 °C) conversion reactor. 2-bromophenol was converted and the following ion masses were selected for screening: *m/z* 28 (carbon monoxide), *m/z* 44 (carbon dioxide), *m/z* 80, 82 (HBr), *m/z* 40 (argon for background information) and *m/z* 172 and 174, both most intensive (100 % and 97 %, respectively) masses of a reference 2-bromophenol mass spectrum (SDBS database, National Institute of Advanced Industrial Science and Technology (AIST), Japan)). The chromatogram shows that the alcohol group of 2-bromophenol reacts with carbon to carbon monoxide.

4. Compounds investigated for complete conversion

Compound-specific isotope analysis (CSIA) methods are based on the complete conversion of the elements of the compound into very simple products, typically volatile compounds, used as analytes to determine the isotopic composition. Complete conversion has to be ensured to determine reliable isotope ratios. To evaluate our interface, the conversion performance was investigated employing a range of structurally diverse compounds. Almost complete transformation was observed for a number of aliphatic, olefinic, and aromatic chlorinated compounds (Table S1). In addition, the transformation of a brominated ethylene and organically bound sulphur to HBr and H_2S was investigated. The major ions formed in EI+ mode based on reference mass spectra (SDBS database, National Institute of Advanced Industrial Science and Technology (AIST), Japan (http://riodb01.ibase.aist.go.jp/sdbs/cgi-bin/cre_index.cgi?lang=eng)) were used to demonstrate the efficiency of transformation. As there were no volatile carbon compounds observed, we hypothesised that pure carbon is deposited in the ceramic tube and this was evident upon visual inspection.

Table S1. List of substances subjected to high-temperature conversion. The intensities of two main ions of the parent substance (major ions published in the reference mass spectrum (SDBS database, National Institute of Advanced Industrial Science and Technology (AIST), Japan)) are shown and compared with the intensity of the ions of the conversion product HCl (*m/z* 36), HBr (*m/z* 80) or H_2S (*m/z* 34)

Substance	main reference peaks *m/z*	maximum intensity [A]	conversion product	maximum intensity [A]
Tetrachloroethene (PCE)	166	3.2E-13	HCl	1.9E-08
	164	4.8E-13		
Trichloroethene (TCE)	95	2.5E-13	HCl	1.3E-08
	130	2.8E-13		
cis-Dicloroethene (*cis*-DCE)	61	4,4E-13	HCl	5.3E-08
	96	3.4E-13		
Lindane (γ-HCH)	181	3.9E-13	HCl	1.5E-08
	183	3.7E-13		
1,2-Dichloroethane (DCA) *	62	1.9E-11	HCl	5.0E-08
	27*	2.8E-08		
Chlorobenzene (CB)	112	3.8E-13	HCl	4.5E-08
	77	7.4E-12		
Tribromoethene (TBE)	185	2.6E-13	HBr	3.4E-09
	264	2.7E-13		
Thiophene	84	2.9E-13	H2S	4.7E-09
	58	5.3E-13		
Tetrahydrothiophene	60	4.2E-13	H2S	2.9E-09
	88	3.2E-13		

*For dichloroethane only the most intense ion of the reference mass spectra can be compared, as *m/z* 27 is present in all spectra and thus unspecific.

5. Considerations on hypothetical processes in the high temperature reactor

It is possible that catalytic processes as well as pyrolysis are involved in the transformation of components in the ceramic tube at high temperatures in our conversion interface. Usually degradation at aluminium oxide catalysts is used for the disposal of chlorinated hydrocarbons.[1–3] Reports on hydrolysis may be especially helpful for elucidating processes facilitating the conversion of organic compounds in the ceramic reactor.[4,5] Bond *et al.* investigated the hydrolysis of chlorinated methane over aluminium oxide (γ-Al_2O_3) and proposed a sequential hydrolysis of C-Cl bonds accomplished via fast but kinetically insignificant steps.[4] For molecules with a Cl/H ratio >1, the addition of water vapour resulted in complete hydrolysis with carbon dioxide and HCl being produced as the only reaction product. They observed a prolonged emission of HCl after the feeding of chlorinated compounds ceased, and they concluded that the aluminium oxide has reverted to its original hydrated state. For temperatures >500 °C and high Cl/H ratios, aluminium chloride ($AlCl_3$) formation and sublimation are reported, removing aluminium ions from the aluminium oxide and thus causing it to decompose.[4,5] Khaleel compared the catalytic activity of mesoporous aluminium oxide for the hydrolysis of carbon tetrachloride in the presence of water with a reaction in the absence of water.[5] The results indicate that aluminium oxide can act as a catalyst to convert carbon tetrachloride (in the presence of water) into carbon dioxide and HCl at temperatures >350 °C. The presence of water inhibited carbon deposition and the production of by-products and resulted in high conversion rates. In the absence of water, the undesired by-products, chlorine, phosgene and $AlCl_3$, were formed during carbon tetrachloride dechlorination.[5] However, their concentration decreased with increasing temperature. Reactive hydroxyl groups on the surface of the aluminium oxide were claimed to be responsible for hydrolysis. According to this theory, they dissociatively adsorb water molecules that interact with the carbon chloride molecules and thus facilitate the conversion.[5]

Pyrolysis or cracking reactions at high temperatures and in the absence of oxygen may proceed by other mechanisms. Hypothetically, such a destruction of the molecules and their subsequent reduction could facilitate the high-temperature conversion process. Carbon deposited at high temperatures, as found also in our conversion reactor, is known to have a high surface area and is reported to catalyse methane decomposition to form graphite and elemental hydrogen during high-temperature conversion reactions.[6] Thus, it could contribute to the overall conversion of halogenated compounds, reinforcing the reducing conditions in the reactor. However, the elucidation of the total individual processes present in the ceramic reactor during high-temperature conversion was not the subject of this study. The preliminary data indicate essentially that a complete conversion takes place and can be used to determine ^{37}Cl, ^{81}Br and ^{34}S isotope ratios from organic compounds.

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Development and Validation of an Universal Interface for Compound-Specific Stable Isotope Analysis of Chlorine ($^{37}Cl/^{35}Cl$) by GC-High-Temperature Conversion (HTC)-MS/IRMS

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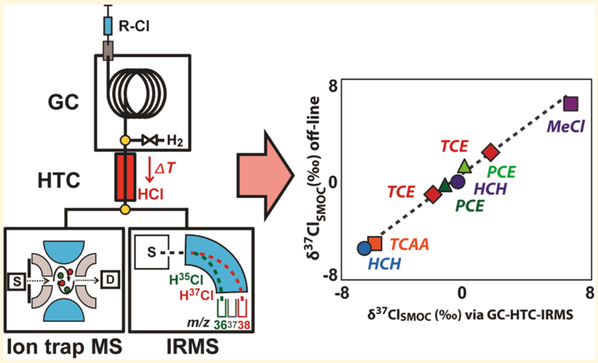
Supporting Information

ABSTRACT: A universal application of compound-specific isotope analysis of chlorine was thus far limited by the availability of suitable analysis techniques. In this study, gas chromatography in combination with a high-temperature conversion interface (GC-HTC), converting organic chlorine in the presence of H_2 to gaseous HCl, was coupled to a dual-detection system, combining an ion trap mass spectrometer (MS) and isotope-ratio mass spectrometer (IRMS). The combination of the MS/IRMS detection enabled a detailed characterization, optimization, and online monitoring of the high-temperature conversion process via ion trap MS as well as a simultaneous chlorine isotope analysis by the IRMS. Using GC-HTC-MS/IRMS, chlorine isotope analysis at optimized conversion conditions resulted in very accurate isotope values ($\delta^{37}Cl_{SMOC}$) for measured reference material with known isotope composition, including chlorinated ethylene, chloromethane, hexachlorocyclohexane, and trichloroacetic acids methyl ester. Respective detection limits were determined to be <15 nmol Cl on column with achieved precision of <0.3‰.



Identification and quantification of environmental pollutants is a field of major importance in modern science and analytical chemistry.[1−4] In addition to the identity, valuable information about source and fate of a chemical compound can be obtained from its isotopic composition. Although essential for source identification, assessment of biodegradation and characterization of (bio)catalytic reaction mechanisms for numerous chlorinated compounds, as for instance environmental pollutants, compound-specific stable isotope analysis of chlorine was thus far partly limited by the difficulty of conversion to a simple chlorine-containing gas for isotope measurements by continuous-flow isotope ratio mass spectrometry (CF-IRMS).[5−7] Currently, chlorine isotope analysis is mainly restricted to conventional off-line methods, including time-consuming conversion to either chloromethane[8,9] or cesium chloride[10] in combination with measurements via dual inlet isotope ratio mass spectrometry (DI-IRMS) or thermal ionization mass spectrometry (TIMS). Those off-line techniques, however, allow the determination of chlorine isotope composition of preoperatively isolated fractions or pure compounds only. In addition, online methods are available which do not require previous conversion of organochlorines[11] but involve the direct measurement of molecular and fragment ions via quadrupole MS (qMS)[12,13] or fragment ions via IRMS.[14] Though GC-qMS was shown to be highly sensitive (detection limit <10 pmol) and more flexible in terms of accessible compound classes, only moderate precision could be achieved ($1\sigma > 0.5‰$)[3,5,13] In contrast, GC-IRMS is less sensitive (detection limit ~10 nmol on column), but able to achieve better precision ($1\sigma = 0.1‰$).[14] However, because of specific cup configurations required, GC-IRMS is limited to a narrow range of compound for analysis. In addition, both methods are restricted in availability of reference materials, since at least two isotope standards are required for calibration, chemically identical to the analyzed compound.[5] During the past decade, application of inductively coupled plasma in combination with multicollector mass spectrometry (MC-ICPMS) coupled to a gas chromatography for chlorine isotope measurements was demonstrated in several studies.[1,15,16] Though application of MC-ICPMS is a promising universal method with a precision of $1\sigma \approx 0.1{-}0.2‰$, low chlorine ionization efficiency, interference with $^{36}Ar^{1}H$ ions, and high instrumental costs limit the use in routine application. Moreover, combination of GC for separation with MC-ICPMS requires large sample size for analysis and, thus, may restrict the analysis of environmental samples.

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Hitzfeld et al.[17] introduced a novel approach for online chlorine isotope analysis via high-temperature conversion (HTC) of chlorinated compounds into gaseous hydrochloric acid and the analysis of $H^{37}Cl/H^{35}Cl$ ions by quadrupole mass spectrometry (GC-HTC-qMS). The fundamental principles of this conversion technique were first demonstrated by Hoering and Parker[18] but were not applied for online chlorine analysis before. Briefly, chlorinated hydrocarbons undergo a chromatographic separation, followed by a subsequent high-temperature conversion at 1300 °C to hydrochloric acid (HCl). Quantitative conversion to HCl was achieved by providing additional hydrogen as reactant gas during conversion. The conversion products $H^{35}Cl$ and $H^{37}Cl$ (m/z 36 and 38) were detected via qMS (gas analyzer).[17] The presented setup, however, revealed instrumental instability ($1\sigma \leq 1.4‰$ (n = 3), delta shifts of 3.0−4.0‰ over 3 days) and referencing against the international standard (SMOC, Standard Mean Ocean Chloride) was not established. Even so, chlorine isotope analysis via HTC holds a great promise because of the universal applicability for a wide range of chlorinated compounds as well as the cost efficient incorporation into standard instrumentation for compound-specific stable isotope analysis.

The objective of this study was a detailed investigation and characterization of the HTC process using nonchlorinated and chlorinated hydrocarbons as well as isotope analysis of chlorinated hydrocarbons via conversion of the measured compounds to HCl. Therefore, the instrumental setup previously presented by Hitzfeld et al.[17] was equipped with a dual-detection system combining ion trap mass spectrometry (MS) and isotope ratio mass spectrometry (IRMS) in order to analyze HTC products and chlorine isotope composition simultaneously. Thus, we were able to identify limitations of HTC and to improve the conversion procedure. Furthermore, we were able to demonstrate the precision that can be achieved by online chlorine isotope analysis of various chlorinated compounds via high-temperature conversion and detection of $H^{35}Cl$ and $H^{37}Cl$ by isotope ratio mass spectrometry.

■ EXPERIMENTAL SECTION

Chemicals. All solvents were purchased from Merck (Darmstadt, Germany) in the highest purity available. Gases were purchased either from Linde Gas AG (Pullach, Germany) or AirProducts (Hattingen, Germany).

Reference Material for Chlorine Isotope Analysis. Tetrachloroethene (PCE) and trichloroethene (TCE) were purchased from Merck, Germany (PCE reference 1 and TCE reference 2) and from PPG, USA (PCE reference 5 and TCE reference 6). Methyl chloride reference was obtained from Linde Gas AG, Germany. Hexachlorocyclohexane (Lindane) was obtained from HiMedia, India (HCH reference 1) and Greyhound, Global (HCH reference 2). Trichloroacetic acid was obtained from Sigma-Aldrich and derivatized as described elsewhere.[19]

Determination of $\delta^{37}Cl_{SMOC}$ of Reference Material. The $\delta^{37}Cl_{SMOC}$ values of reference standards were determined by off-line analysis via dual inlet isotope ratio mass spectrometry (DI-IRMS). Prior to analysis all reference compounds were converted to methyl chloride in order to obtain accurate $\delta^{37}Cl$ values.[9,20−22] Isotope composition determined by dual-inlet mass spectrometry (DI-IRMS) was directly related to the international SMOC scale (Standard Mean Ocean Chloride; $\delta^{37}Cl = 0.0‰$, $R_{SMOC} = 0.319\,644$). The determination of $\delta^{37}Cl_{SMOC}$ was done in a triple collector gas-source dual-inlet mass spectrometer (DI-IRMS VG Optima, Isoprime Inc., U.K.) as described elsewhere.[23,24] Determined chlorine isotope composition of all utilized reference material is presented in Table 1.

Instrumental Setup of GC-HTC-MS/IRMS. The previous GC-HTC-qMS instrumental setup presented by Hitzfeld et al.[17] was modified to GC-HTC-MS/IRMS (Figure 1). The device was equipped with an autosampler (A200S, CTC Analytics AG, Switzerland). The conversion unit consists of a standard gas chromatograph (HP6890N, Agilent Technologies, Germany) and a high-temperature conversion (HTC) tubular reactor (320 mm, 0.8 mm i.d., 1.6 mm o.d.; Degussit AL23 aluminum oxide ceramic, Friatec, Germany) mounted to a high-temperature combustion oven (ThermoFinnigan, Germany). The originally installed qMS (gas analyzer) was replaced by two simultaneously operating mass spectrometers: the ion trap mass spectrometer (PolarisQ, ThermoFinnigan, Germany) and isotope ratio mass spectrometer (DELTAplus XP, ThermoFinnigan, Germany). All transfer-lines were heated (110−120 °C) to avoid condensation of H_2O and HCl by regulated heating tubing (HORST GmbH, Germany).

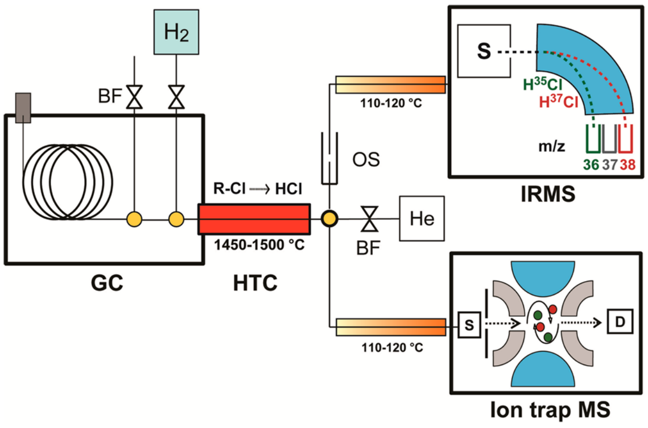

Figure 1. Schematic setup of the GC-HTC-MS/IRMS device, equipped with a gas chromatograph (GC), high-temperature conversion reactor (HTC), ion trap mass spectrometer (MS), and a multi collector isotope-ratio mass spectrometer (IRMS). OS: open split; BF: back flush.

Characterization of Conversion via MS. The high-temperature conversion was characterized and optimized via ion trap mass spectrometry. The ion trap MS was controlled by Xcalibur 1.4 software (Thermo Electron) and was able to operate within the mass range of 10−600 amu. All analytes were ionized via positive electron impact ionization (EI+ at 70 eV) at a source temperature of 225 °C and a vacuum pressure of 10^{-5} bar.

Ethylene and benzene were used as nonchlorinated model compounds for characterization of the HTC process and in particular the byproduct formation. Samples were injected manually via head space as pure compound in helium atmosphere using a split ratio of 1:10 to 1:40. The GC was equipped with a Zebron ZB-1 column (60 m, 0.32 mm i.d., 1 μm film thickness; Phenomenex Inc.) running at isothermal conditions.

For HTC characterization with ethylene as the model compound, standard conditions were applied: 27 nmol of ethylene on column, 0.8 mL/min flow rate, 0.2 mL/min hydrogen flow rate, and 1300 °C conversion temperature. The

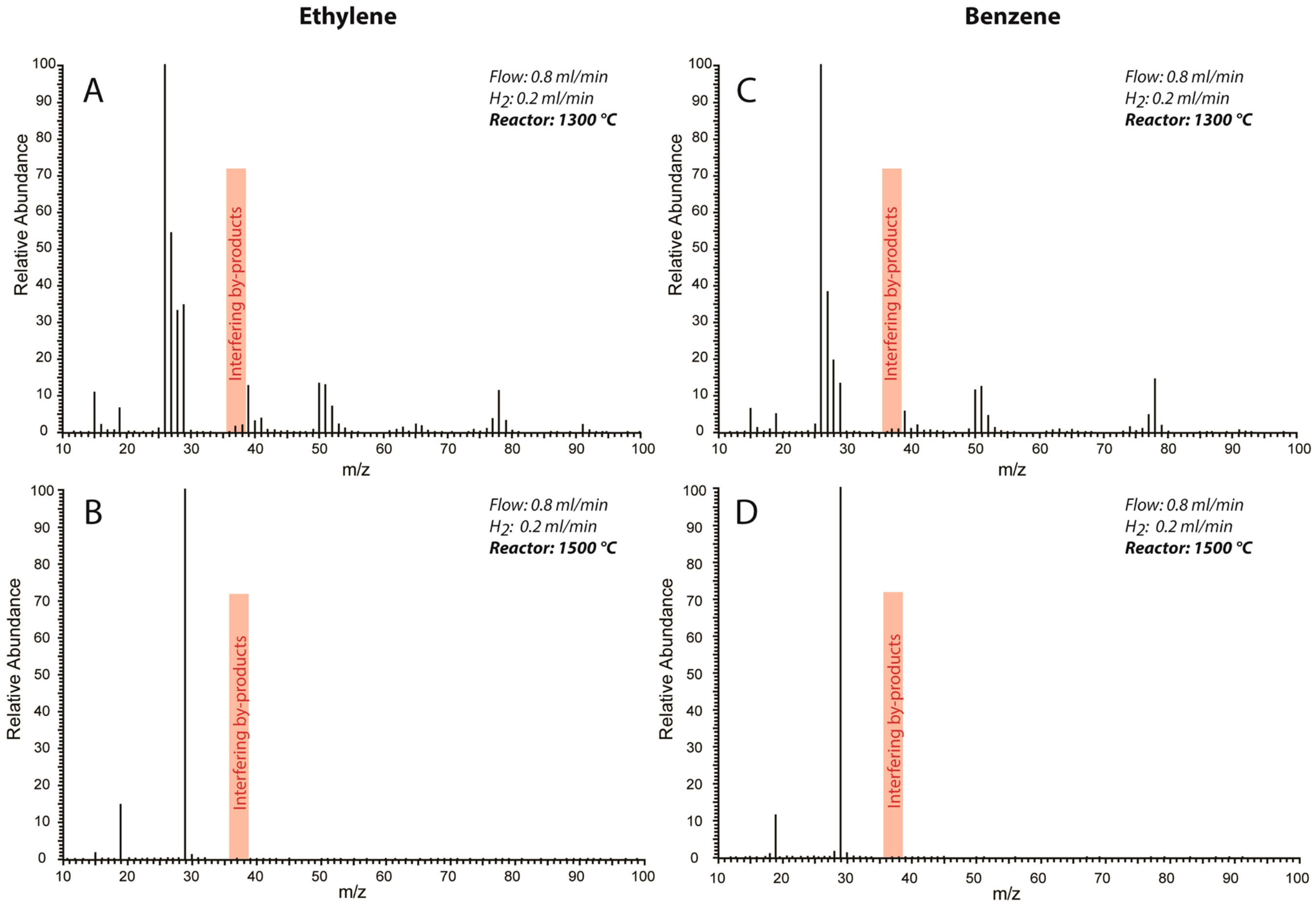

Figure 2. Byproduct formation (monitored by ion trap MS) during high-temperature conversion of the nonchlorinated ethylene ((A) 1300 °C and (B) 1500 °C) and benzene ((C) 1300 °C and (D) 1500 °C) at a GC flow of 0.8 mL/min and 0.2 mL/min reactant gas (H_2).

conversion was characterized by modifying one of the following parameters: ethylene on column (9−45 nmol on column), hydrogen gas flow (0.0−0.5 mL/min), and conversion temperatures (1000−1500 °C). All HTC products were transferred online to the ion trap MS. Conversion was characterized by quantitative analysis of molecular and fragment ions in a range of 10−200 amu produced simultaneously during HTC.

Chlorine Isotope Analysis via IRMS. Reference material was introduced into the GC via head space injection (chemicals in helium atmosphere) or liquid injection (chemicals diluted in acetone). The GC was equipped with a Zebron ZB-1 column (60 m, 0.32 mm i.d., 1 μm film thickness; Phenomenex Inc.) operating at a flow rate of 0.4 mL/min and reactant gas (H_2) flow of 0.1 mL/min. Chlorinated hydrocarbons were converted at 1450 °C. Positive electron impact ionization (EI+ at 85 eV) at a vacuum pressure of 10^{-6} bar was used and the ions m/z 36 ($H^{35}Cl$) and 38 ($H^{37}Cl$) were measured by IRMS with an amplifier gain of 1 (m/z 36), 100 (m/z 37; peak center), and 1 (m/z 38). The analyzed reference material was periodically injected in an amount of approximately 20−30 nmol of Cl on column until stabilization of the isotope values was reached. The measurements were considered stable when five repeat samples were within $1\sigma \leq 0.5$ per mil.

Calculations and Definitions. The abundance of $[H^{37}Cl]/[H^{35}Cl]$ was used to determine the relative chlorine isotope ratio R, measured by IRMS as molecular ions m/z 38 and 36 [eq 1]. For the correction from the measured mass 38/mass 36 (hydrogen chloride) to the chlorine isotopes (mass 37 and mass 35) this formula was used.

$$R(^{35}Cl/^{35}Cl)_{Sample} = area[H^{37}Cl]_{Sample}/area[H^{35}Cl]_{Sample} \quad (1)$$

Isotopic composition of chlorine isotopes was expressed as $\delta^{37}Cl_{Sample}$ in standard δ-notation in per mil (‰) relative to "Standard Mean Ocean Chloride" (SMOC) as the reference scale in delta notation [eq 2].

$$\delta^{37}Cl_{sample}[‰] = (R_{Sample}/R_{SMOC} - 1) \times 1000 \quad (2)$$

No reference gas was available in our system. Therefore, the chlorine isotope ratio of seawater (R_{SMOC} = 0.319 644 of the IAEA standard ISL354, Xiao et al.[25]) was used as an fixed value for the conversion of measured isotope ratios into delta-notation ($\delta^{37}Cl_{Sample}$).

Linear Normalization of Chlorine Isotope Composition vs SMOC Scale. In order to normalize the chlorine isotope composition to SMOC-scale, we applied a linear regression of measured (δ^M) and true (δ^T) δ-values as suggested by Paul et al.[26] The two-point normalization involved plotting the δ^M ($\delta^{37}Cl_{Sample}$ [‰] via GC-HTC-IRMS) of the reference standards on the x-axis and the δ^T ($\delta^{37}Cl$ [‰ vs SMOC] via DI-IRMS) on the y-axis. The "expansion factor" (m) and "additive correction factor" (b) were defined by linear regression ($\delta^T = m \times \delta^M + b$) as described by eq 3. For

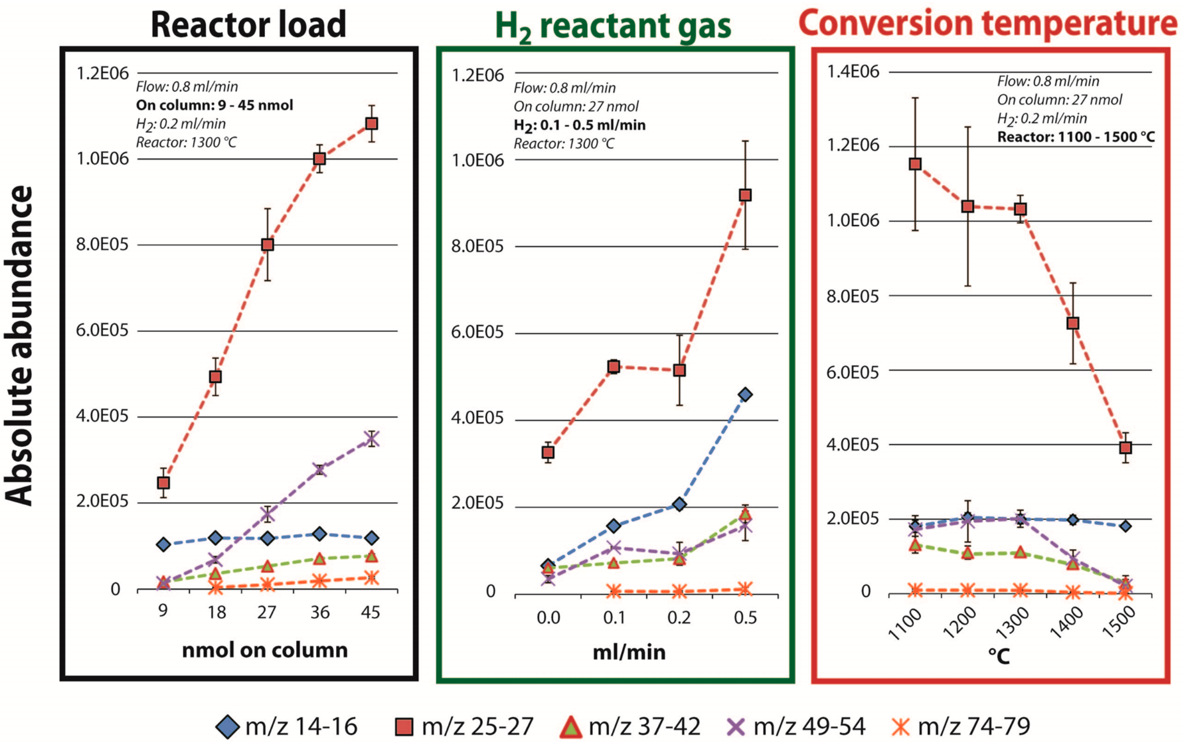

Figure 3. Characterization of byproduct formation during HTC of ethylene by considering reactor load (left), reactant gas (H_2) concentration (middle), and conversion temperature (right). Molecular and fragment ions were monitored via ion trap MS and given as absolute abundances.

normalization of sample values vs SMOC-scale, TCE references 2 and 6 were used as anchor points.

$$\delta^{37}\mathrm{Cl}[‰\ \mathrm{vs\ SMOC}] = m \times \delta^{37}\mathrm{Cl}_{\mathrm{Sample}} + b \qquad (3)$$

■ RESULTS AND DISCUSSION

Interfering Byproducts during High-Temperature Conversion. The feasibility of a compound-specific chlorine isotope analysis via HTC at 1300 to 1400 °C was already proposed by Hitzfeld et al.[17] However, the stability was insufficient for routine operation (standard deviation up to ±2.0 per mil) and efforts to establish a universal online application for chlorine isotopes analysis remained challenging. The originally applied qMS was replaced by an IRMS, as suggested by Hitzfeld et al.,[17] since instabilities of chlorine isotope measurement were related to qMS detection. Replacement of the qMS (gas analyzer) by an IRMS, however, confirmed instability of chlorine isotope ratios [$H^{37}Cl$] and [$H^{35}Cl$] (m/z 36 and 38). Therefore, we analyzed the HTC process in detail in order to understand and optimize the conversion.

For the investigation of the product spectrum, a combination of ion trap MS and IRMS was established (Figure 1) for a simultaneous analysis of HTC quality as well as the chlorine isotope composition. The analysis of high-temperature conversion at 1300 °C with nonchlorinated ethylene as model compound, however, revealed formation of several molecular and fragment ions of which some interfered with the mass of m/z 38 used for quantification of chlorine isotopes (Figure 2A and Supporting Information). Detected byproduct ions could be assigned to acetylene (C_2H_2, m/z 26), ethylene (C_2H_4, m/z 28), and benzene (C_6H_6, m/z 78). Additionally, ions in the mass range of m/z 37−43 and 49−54 were detected and associated with fragment ions of benzene, though further C_3 or C_4 hydrocarbon byproducts cannot be excluded at this point. Benzene formed during ethylene pyrolysis (Figure 2A and Supporting Information) is assumed to be a product of thermal-coupling during HTC in the presence of hydrogen, as it was previously reported for thermal-coupling of methane by Billaud et al.[27] and Gueret et al.[28] at similar conditions. The thermal-coupling upon conversion of organic material is enhanced by the partial pressure of hydrogen.[29] In our setup, thermal-coupling of ethylene was observed to occur from about 800 °C (results not shown) and reached highest intensities at 1100−1200 °C (Supporting Information). Also, hydrogen is suspected to stabilize the byproducts during pyrolysis, since benzene concentrations were observed to be about 5−10 times higher in the presence of H_2 as reactant gas at 1300 °C. Once formed, benzene could not be completely pyrolyzed at 1300 °C (Figure 2C and Supporting Information). Therefore, previous instabilities of chorine isotope composition could be assigned to overlapping ion signals of $H^{37}Cl$ and fragment ions of pyrolysis byproducts at m/z 38. In conclusion, the conversion temperature of 1300 °C was insufficient to prevent interfering byproduct formation during HTC of chlorinated hydrocarbons.

Parameters Affecting Byproduct Formation. Byproduct formation during HTC of hydrocarbons was characterized using ethylene as a nonchlorinated model compound. Effect of reactor load (9−45 nmol of ethylene on column), reactant gas (H_2) concentration (up to 0.5 mL/min), and conversion temperature (1100−1500 °C) were investigated in order to quantify and optimize HTC. Ion clusters of m/z 14−16, m/z 25−27, m/z 37−42, m/z 49−54, and m/z 74−79 were identified as the main molecular or fragment ions arising from inefficient conversion (Figure 2). Therefore, all ions associated with hydrocarbons were quantified as distinct ion clusters as described above. A special focus was put on ions

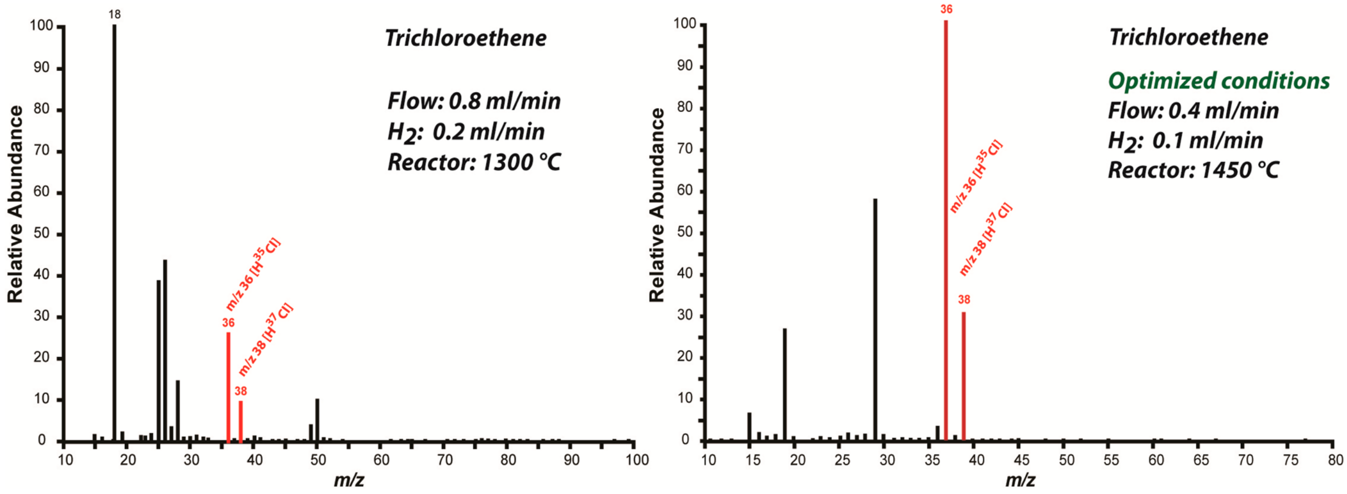

Figure 4. Conversion quality of trichloroethene (TCE) at insufficient conversion conditions (flow 0.8 mL/min, H_2 0.2 mL/min, HTC at 1300 °C) vs optimized conditions (flow 0.4 mL/min, H_2 0.1 mL/min, HTC at 1450 °C). The HTC background was monitored via ion trap MS.

which may interfere with the analysis of the chlorine isotope target compounds [$H^{35}Cl$ and $H^{37}Cl$].

All investigated parameters (reactor load, reactant gas concentration, and conversion temperature) were observed to affect byproduct formation to a certain extent (Figure 3). Increasing reactor load as well as reactant gas (H_2) concentration was observed to enhance the formation of byproducts. The increase of reactor load resulted in formation of hydrocarbons with higher molecular masses (C_4H_x and C_6H_x). In comparison, increasing H_2 concentration enhanced the formation of short hydrocarbon chains (C_1H_x and C_2H_x), which is in agreement with previous reports on thermal-coupling of methane by Billaud et al.,[27] as well as Gueret et al.[28] Increasing conversion temperature (Figure 3) resulted in a significant reduction of byproduct formation also in agreement with a previous report.[29]

According to our results, formation of byproducts interfering with the detection of HCl ions could be significantly reduced by increasing the conversion temperature from 1300 to 1500 °C (Figure 2B,D and Supporting Information). Alternatively, increasing residence time of the compound in the reactor at 1450 °C was also observed to be sufficient for a complete conversion (Figure 4 and Supporting Information). However, overload of the reactor has to be considered; therefore, analyte amount on the column has to be limited to avoid the risk of interfering byproduct formation. In order to prove complete conversion of chlorinated hydrocarbons, subsequent experiments were performed at optimized conditions (GC flow 0.4 mL/min, H_2 flow 0.1 mL/min, HTC temperature 1450−1500 °C). Quantitative conversion without interference of byproducts could be confirmed for chlorinated methane, ethylene, ethane, benzene, and acetic acid methyl ester (Supporting Information). At optimized conditions, byproduct formation for all tested compounds could be reduced, whereby no interfering fragment ions were detected (Figure 2B, D and Supporting Information). Meanwhile, formation of the HCl target ions m/z 36 and 38 was not affected by the new conversion conditions and remained in a similar range (Supporting Information).

Validation of Chlorine Isotope Measurements via GC-HTC-IRMS. Trichloroethene (TCE) and tetrachloroethene (PCE) reference materials were used for online measurement of chlorine isotope composition via GC-HTC-IRMS. All reference materials were analyzed in an amount of approximately 30 nmol of Cl on column, a helium carrier gas flow rate of 0.4 mL/min, reactant gas (H_2) flow of 0.1 mL/min, and at a conversion temperature of 1500 °C (optimized HTC conditions, see paragraph above). The corresponding chlorine isotope composition $\delta^{37}Cl_{raw}$ was determined as described in the methods part.

The stability of the GC-HTC-IRMS setup was tested by using two TCE standards (TCE references 2 and 6). The injection of 30 nmol on column resulted in a peak intensity of 1500−2000 mV (m/z 36). Both compounds were measured over 5 days under the same conditions. The corresponding chlorine isotope values ($\delta^{37}Cl_{raw}$) were observed to be very stable over 5 days and an additional replacement of the reactor tube (Figure 5). Average chlorine isotope composition ($\delta^{37}Cl_{raw}$) during this period was measured to be 23.6 ± 0.3‰ and 25.5 ± 0.3‰ for TCE reference 2 and TCE reference 6 ($n \geq 5$), respectively. In comparison, reported

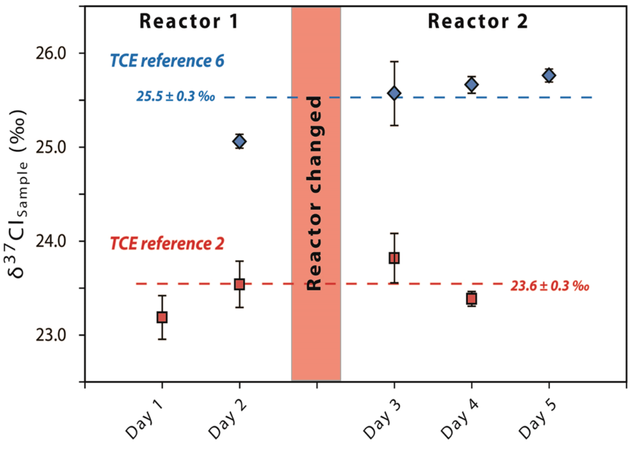

Figure 5. Evaluation of the stability of chlorine isotope analysis. Trichloroethene (TCE) reference no. 2 and 6 ($n \geq 5$) were converted at 1500 °C and total flow rate of ~0.5 mL/min (GC flow 0.4 mL/min, H_2 flow 0.1 mL/min). The stability of chlorine isotope composition was evaluated over 5 days and with two reactors.

Table 1. Chlorine Isotope Composition of Reference Compounds[a]

	DI-IRMS	GC-HTC-IRMS				
		Method validation			Analysis	
Reference compounds	δ^{37}Cl [‰ vs. SMOC]	$\delta^{37}Cl_{Sample}$ [‰]	δ^{37}Cl [‰ vs. SMOC]	n	δ^{37}Cl [‰ vs. SMOC]	n
TCE ref. 2	**-1.19 ± 0.01**	23.47 ± 0.30	**-1.20**	19	**anchor**	
TCE ref. 6	**2.17 ± 0.2**	25.65 ± 0.24	**2.10**	16	**anchor**	
PCE ref. 1	**-0.49 ± 0.12**	23.89 ± 0.37	**-0.57**	16	**-0.49 ± 0.17**	10
PCE ref. 5	**1.03 ± 0.08**	25.01 ± 0.40	**1.14**	16	**0.64 ± 0.15**	9
MeCl ref. (Linde)	**6.03 ± 0.02**				**6.85 ± 0.51**	10
HCH ref. 1 (HiMedia)	**-0.18 ± 0.03**				**0.27 ± 0.32**	9
HCH ref. 2 (Greyhound)	**-5.49 ± 0.12**				**-5.20 ± 0.27**	10
TCAA ref. (Sigma)	**-5.11 ± 0.17**				**-4.60 ± 0.28**	10

[a] Chlorine isotope composition measured off-line via DI-IRMS (δ^{37}Cl [‰ vs SMOC]) and online via GC-HTC-IRMS. Online measurements were validated (red) and the corresponding chlorine isotope composition determined as $\delta^{37}Cl_{Sample}$ [‰] and δ^{37}Cl [‰ vs SMOC]. Chlorine isotope composition of several reference compounds was analyzed (blue) and converted to SMOC-scale.

instabilities for GC-HTC-qMS were in a range of $1\sigma \leq 1.4$‰ ($n = 3$) and shifts of 3.0−4.0‰ over 3 days of measurements.[17]

Because of the observed stability of the system during analysis of the chlorine isotope composition of the TCE standards, four reference compounds (PCE reference 1, TCE reference 2, PCE reference 5, and TCE reference 6) were subsequently analyzed at similar conditions. Memory effects were observed during successive analysis of different compounds. Therefore, for each compound at least 16 data points were measured after stabilization of the corresponding isotope value. The chlorine isotope composition of the reference compounds $\delta^{37}Cl_{raw}$ is given in Table 1 and Figure 6. Linear regression of the measured chlorine isotope composition ($\delta^{37}Cl_{raw}$) to the true chlorine isotope composition ($\delta^{37}Cl_{SMOC}$) resulted in an "expansion factor" of $m = 1.52$ with an "additional correction factor" of $b = -36.83$.

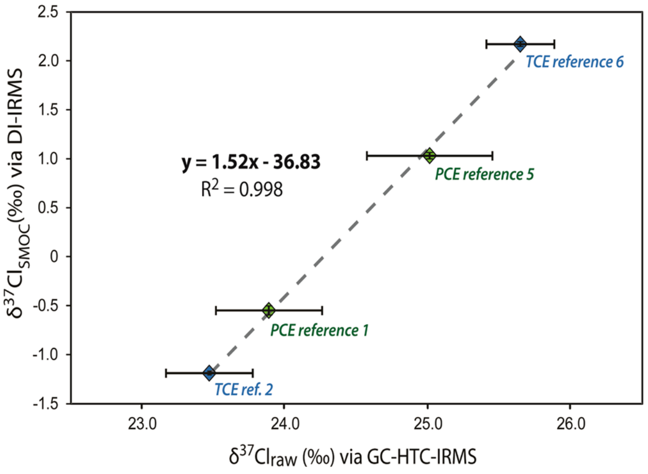

Figure 6. Validation of chlorine isotope measurement via GC-HTC-IRMS. Normalization of measured chlorine isotope composition was done using TCE and PCE reference material. True isotope composition $\delta^{37}Cl_{SMOC}$(‰) is given on the y-axis (determined off-line via DI-IRMS). Measured isotope composition $\delta^{37}Cl_{raw}$(‰) (online via GC-HTC-IRMS) is plotted at the x-axis. The "expansion factor" of the GC-HTC-IRMS setup was determined by a linear regression fit.

The normalization of the online chlorine isotope values to the SMOC-scale were in a linear agreement ($R^2 = 0.980$) to chlorine isotope composition determined via an off-line method. $\delta^{37}Cl_{SMOC}$ determined via GC-HTC-IRMS were −0.57 ± 0.37‰ ($n = 16$) for PCE reference 1, −1.20 ± 0.30‰ ($n = 19$) for TCE reference 2, 1.14 ± 0.40‰ ($n = 16$) for PCE reference 5, and 2.10 ± 0.24‰ ($n = 16$) for TCE reference 6 (Table 1). In summary, chlorine isotope composition ($\delta^{37}Cl_{SMOC}$) determined via GC-HTC-IRMS was in a very good agreement with the "true" chlorine isotope composition measured via DI-IRMS with a maximum deviation of ≤0.1‰.

Determination of Chlorine Isotope Composition of Different Compound Classes via GC-HTC-IRMS. Available materials with known chlorine isotope composition, including trichloroethylene (TCE refs 2 and 6), tetrachloroethylene (PCE refs 1 and 5), methyl chloride (MeCl ref.), hexachlorocyclohexane (HCH refs 1 and 2), and trichloroacetic acid methyl ester (TCAA ref.), were used for validation of the performance of the online chlorine isotope analysis by the GC-HTC-IRMS. The system was operating with a helium carrier gas flow of 0.4 mL/min, reactant gas (H_2) flow of ∼0.1 mL/min, and at a conversion temperature of 1450 °C. Reference compounds were injected in a concentration of approximately 30−40 nmol of Cl on column. The corresponding peak intensities were in a range of 1500−2500 mV (m/z 36) (Supporting Information). The determined chlorine isotope composition was normalized using TCE references 2 and 6 as an anchor for calibration to the SMOC-scale and are presented as $\delta^{37}Cl_{SMOC}$ (Figure 7, Table 1). The chlorine isotope composition of all reference compounds determined online via GC-HTC-IRMS was in very good agreement with chlorine isotope values determined off-line via DI-IRMS with a maximum deviation of ≤0.8‰. Further, observed precision was very satisfying with standard deviations below ±0.5‰ for all reference compounds.

Limitations and Challenges. Though online analysis of chlorine isotopes ($^{37}Cl/^{35}Cl$) via GC-HTC-MS/IRMS is a promising universal compound-specific approach, several limitations and challenges remain as discussed in the following.

Memory Effects. Online isotopes analysis of chlorine via HTC is thus far limited by serious memory effects, as shown in Figure 8. Therefore, stabilization time and repetition of measurements until stabilization of values is required for accurate chlorine isotope analysis. In order to reduce memory effects, all transfer-lines were continuously heated to avoid potential condensation of HCl or H_2O in cold sections. In addition, the ion source was heated to approximately 120 °C (personal communication ThermoFinnigan) to reduce sorption of HCl at metal surfaces. However, the success was limited;

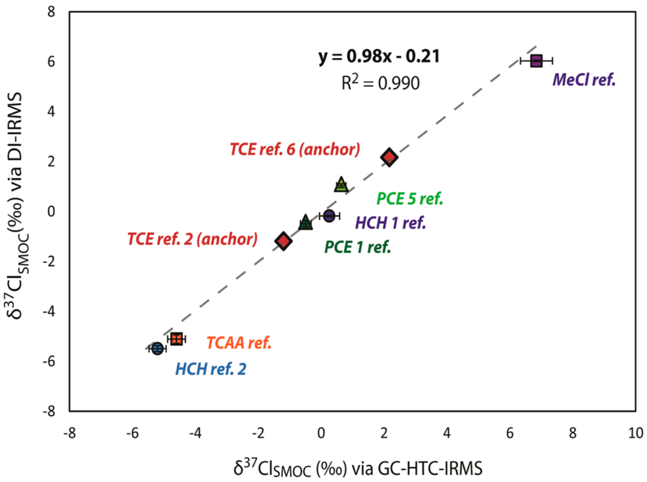

Figure 7. Determination of chlorine isotope composition of several chlorinated reference compound via GC-HTC-IRMS. True isotope composition $\delta^{37}Cl_{SMOC}$(‰) is given on the *y*-axis (determined off-line via DI-IRMS). Measured chlorine isotope composition (online via GC-HTC-IRMS) was normalized to the SMOC-scale using TCE references 2 and 6 is plotted at the *x*-axis.

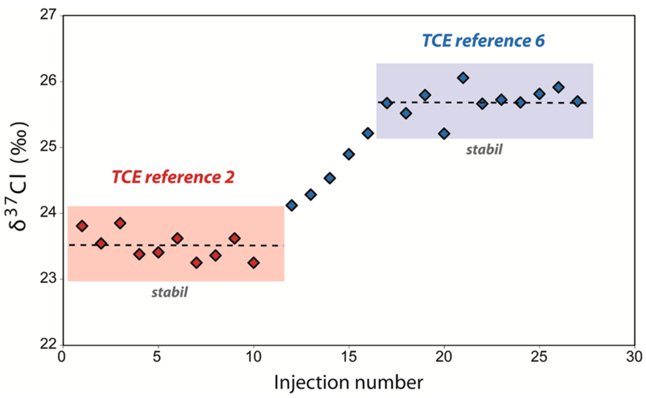

Figure 8. Memory effect during chlorine isotope measurement demonstrated for TCE reference no. 2 and 6.

conclusively, memory effects could only be assigned to the HTC reactor. Therefore, further attempts are needed to prevent memory effects, e.g., investigation of different HTC reactor materials.

Stability of the Aluminum Oxide HTC Reactor. The lifetime of the aluminum oxide ceramic was at applied conditions less than 1 week. The increasing alteration of the ceramic material resulted in leakage after a few days of operation. Therefore, a careful monitoring of the tightness of the system is needed. In our setup, the air water background was monitored online via ion trap MS.

Thus, far, however, it is not clear if only high temperature (1450−1500 °C) or high temperature in combination with HCl is responsible for the relative short lifetime of the ceramic reactor. Conclusively, reactor material with higher stability is required to overcome the limitations of aluminum oxide ceramics in the future.

Water Formation. Because of the high availability of hydrogen as reactant gas in our setup (∼20%), oxygen preferably reacted to H_2O (for details see the Supporting Information), which can potentially lead to memory effects, since it may act as a trap for hydrochloric acid. Several sources of oxygen were considered. On the one hand, oxygen was observed to penetrate the system due to leakages, small cracks of the ceramic structure due to fatigue of the material; on the other hand, oxygen was suspected to leach out permanently from the aluminum oxide ceramic reactor at high conversion temperature.[30] In both cases H_2O may be produced during HTC. However, H_2O background was observed to remain stable and did not significantly interfere with chlorine isotope measurements. Furthermore, efforts to reduce H_2O formation, including the increase of HTC temperature and carbon deposition in the ceramic rector, were of minor success. Therefore, no other efforts were undertaken to reduce the water formation. Though H_2O in a limited range was observed not to affect isotope measurements, sufficient background monitoring and maintenance remained essential to provide stable chlorine isotope analysis.

Effect of Hydrochloric Acid on Instrumental Setup. Hydrochloric acid, a highly reactive and aggressive agent, could potentially damage the IRMS ion source. Visual inspection, however, revealed no obvious effect or damage of the IRMS ion source, even after 1 year of extensive use of high-temperature conversion and HCl measurements. These findings are in accordance to the ones by Hitzfeld et al.[17] reporting no effects of HCl on the qMS source. Thus, HCl seems to be a reasonable analyte for chlorine stable isotope determination via GC-HTC-IRMS.

Expansion Factor. The expansion factor in isotope analysis is usually MS or IRMS specific. For instance, Bernstein et al.[11] reported for nine MS detectors (IRMS or qMS) an expansion factor between 0.72 and 1.31.[11] However, determined expansion for GC-HTC-IRMS was particularly high (m = 1.52). Thus far, high hydrogen content in the carrier gas flow (∼20 vol %) is suspected to affect the ionization in the ion source, similar to the H^{3+} factor in hydrogen isotope analysis.[31] This observation is not completely understood and further investigation is need in the future.

■ CONCLUSIONS

The analysis of chlorine isotopes via high-temperature conversion to HCl is a promising tool for routine analysis of chlorinated compounds. Online analysis via GC-HTC-IRMS reduces significantly sample preparation time in comparison to off-line conversion and the amount of analyte needed for chlorine isotope analysis. Furthermore, we demonstrate that a range of GC amendable chlorinated compound classes is now accessible for chlorine isotope analysis via IRMS. The performance of chlorine isotope analysis was shown for chloromethanes, chloroethenes, chlorocyclohexanes, and chloroacetic acid methyl ester. In comparison to available techniques chlorine isotope analysis via GC-HTC-IRMS showed low detection limits (∼10−15 nmol Cl on column). Lower detection limit was thus far only reported for GC-qMS (<10 pmol).[13] However, detection limit via GC-HTC-IRMS is still lower as reported for MC-ICPMS coupled to gaschromatography (≥200 nmol Cl on column),[15] TIMS (<85 nmol Cl on column)[10] and comparable to GC-IRMS (∼10 nmol Cl on column).[14] Achieved precision with our setup ($1\sigma < 0.3$‰, Figure 6) is inferior to that of GC-IRMS ($1\sigma = 0.1$‰),[14] and MC-ICPMS ($1\sigma = 0.06$‰[15]) but still sufficient for an universal online method. Quantitative conversion and HTC performance were demonstrated and characterized in detail for a range of chlorinated and nonchlorinated compounds. The parallel characterization of multi collector mass spectrometry for isotope analysis and ion trap mass spectrometry for characterization for products opens perspectives to evaluated HTC interfaces for CSIA.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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Supporting Information

Development and validation of an universal interface for compound-specific stable isotope analysis of chlorine ($^{37}Cl/^{35}Cl$) by GC-HTC-MS/IRMS

Analytical Chemistry **2015**, Volume *87(5)*, p. 2832-2839

J. Renpennning, K. L. Hitzfeld, T. Gilevska, I. Nijenhuis, M. Gehre, H.-H. Richnow

Content

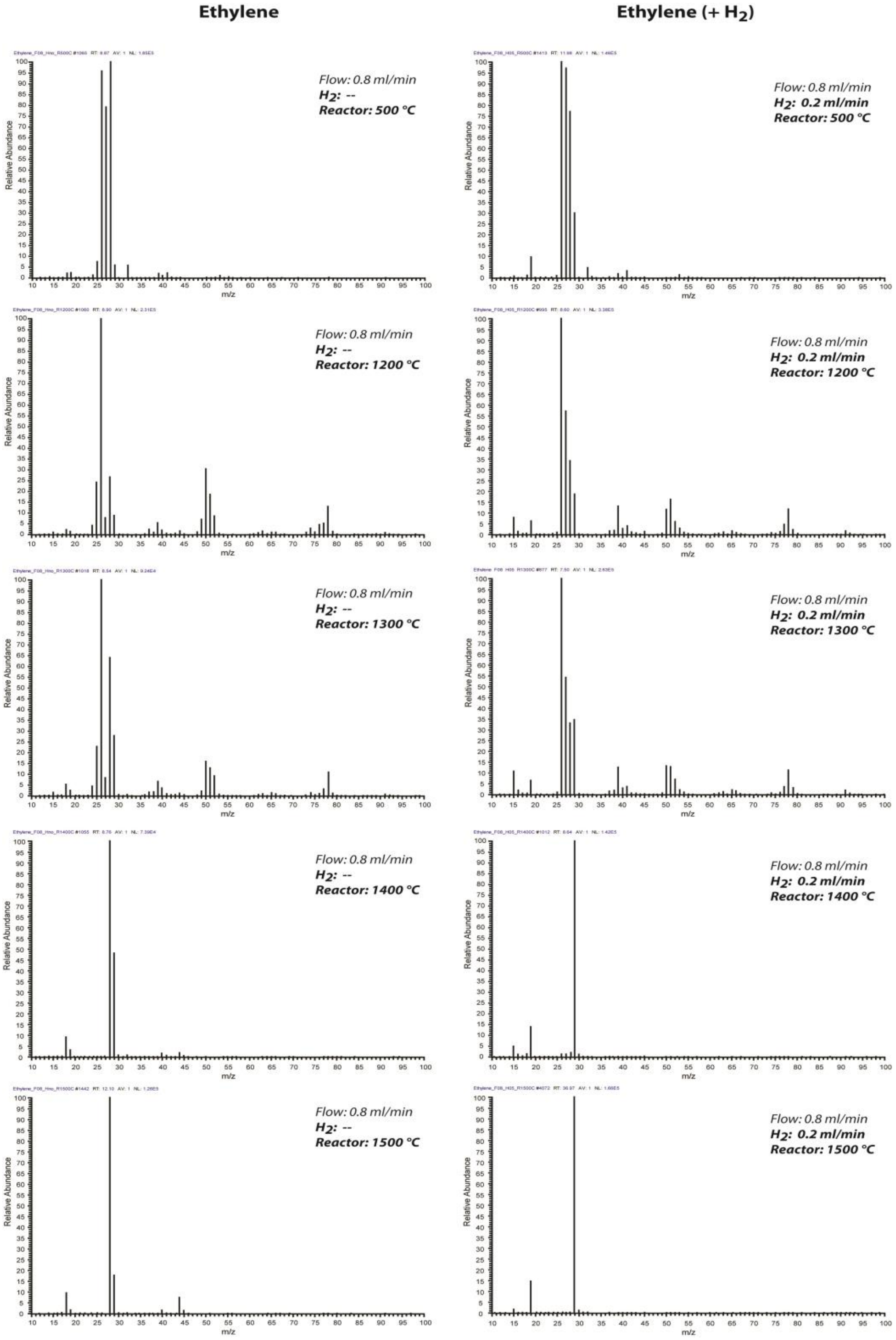

Figure S1: Evaluation of ethylene conversion at 500-1500°C with and without reactant gas (H_2), monitored via ion trap MS.

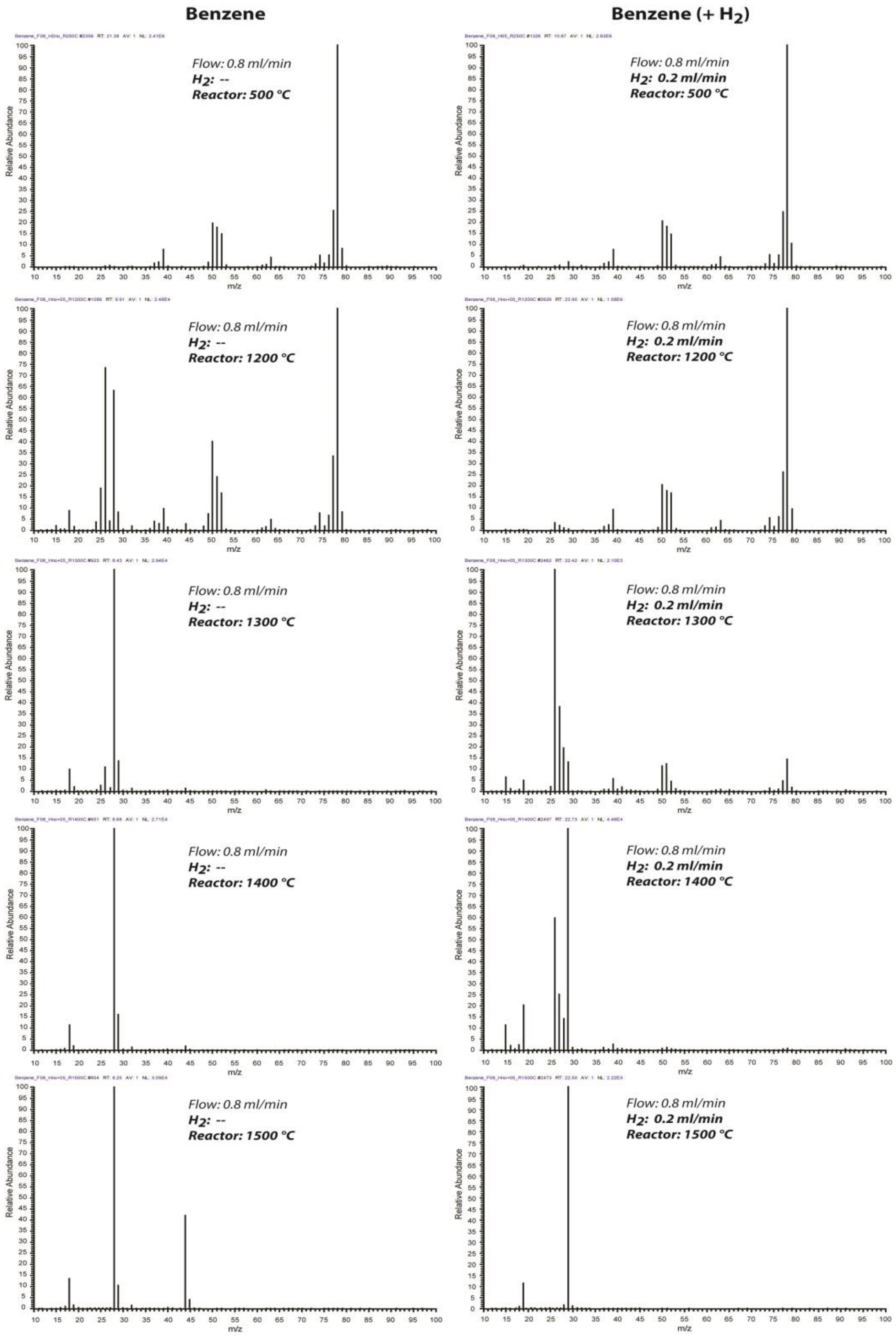

Figure S2: Evaluation of benzene conversion at 500-1500°C with and without reactant gas (H_2), monitored via ion trap MS.

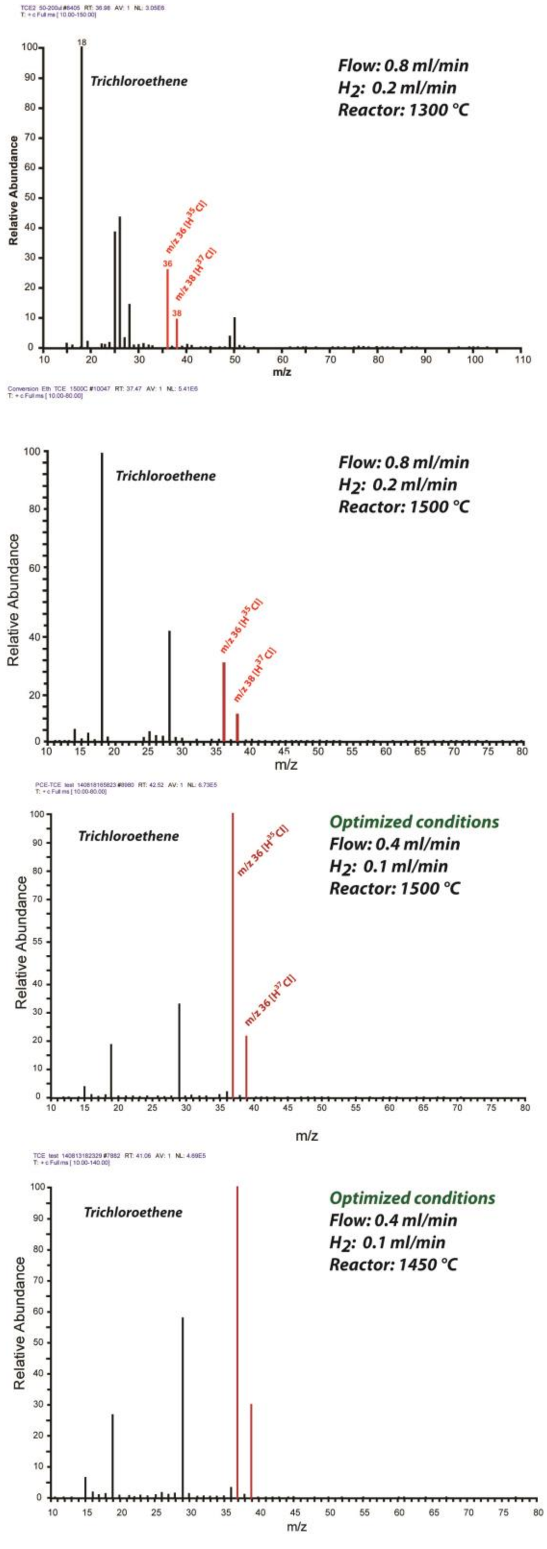

Figure S3: Evaluation of the conversion quality of trichloroethene (TCE) at 1300°C and1500°C, as well as at optimized conditions (Flow 0.4 ml/min, H_2 0.1 ml/min, HTC at 1450-1500°C). The HTC quality was monitored via ion trap MS.

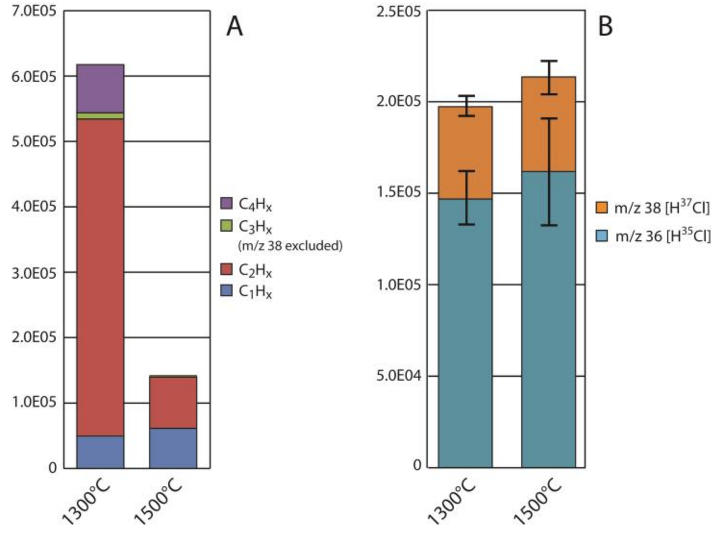

Figure S4: Evaluation of the conversion quality of trichloroethene (TCE) at 1300°C vs 1500°C by taking in account the hydrocarbon by-product formation (A) and the HCl formation (B). The abundance of products was monitored via ion trap MS.

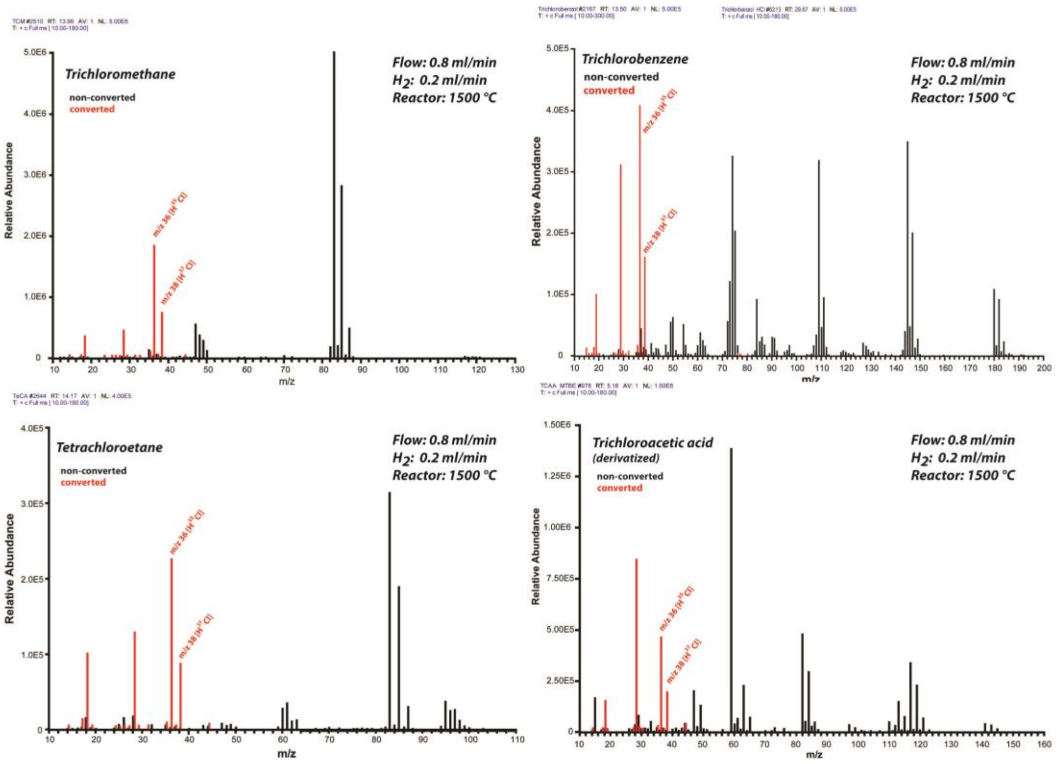

Figure S5: Evaluation of the conversion efficiency of different chlorinated hydrocarbons, demonstrated for chlorinated compounds. The corresponding mass spectra were monitored for non-converted (black) and converted (red) compound at 1500°C.

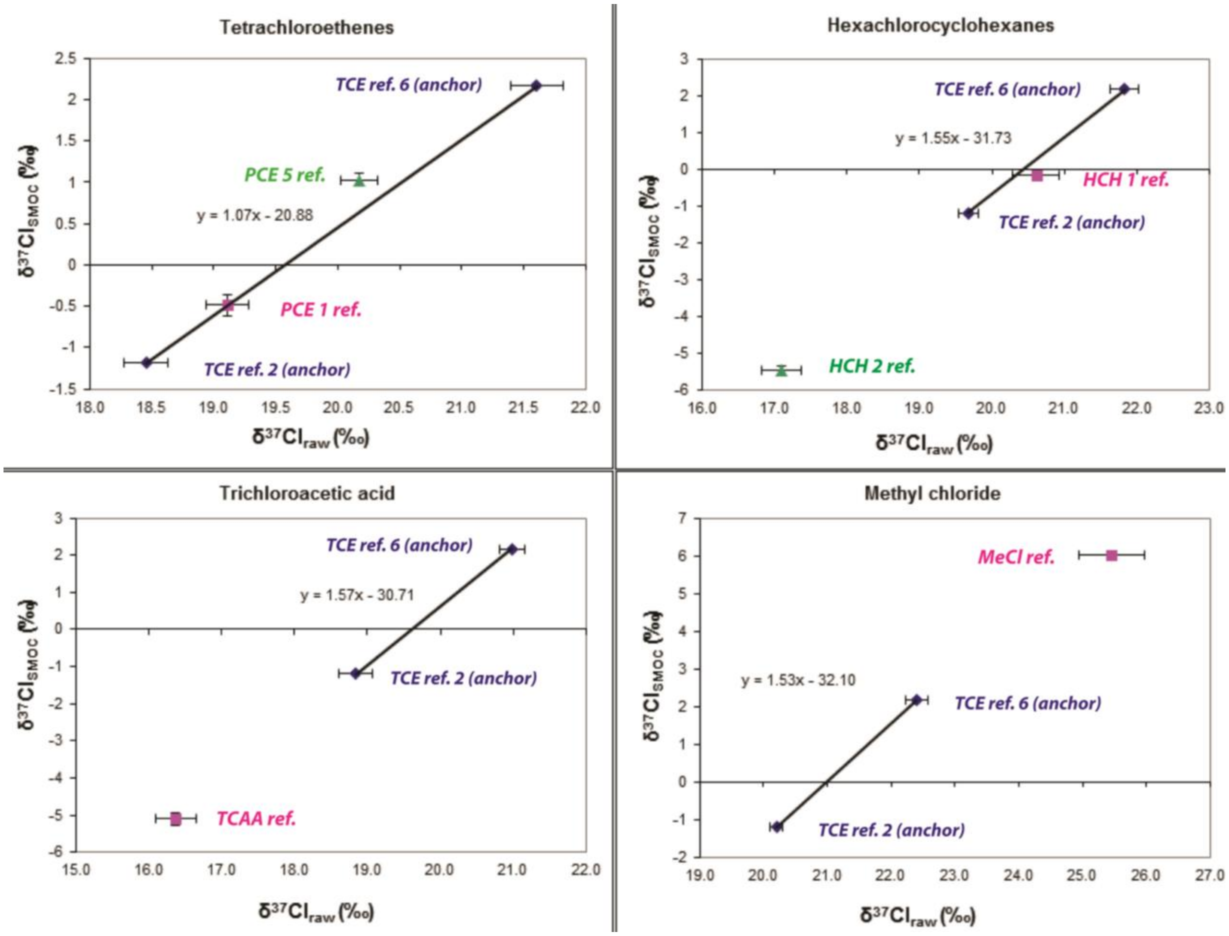

Figure S6: Determination of chlorine isotope composition of several chlorinated reference compound via GC-HTC-IRMS**.** Normalization of measured chlorine isotope composition was done using TCE reference 2 and 6. True isotope composition $\delta^{37}Cl_{SMOC}$(‰) is given on the y-axis (determined off-line via DI-IRMS). Measured isotope composition $\delta^{37}Cl_{raw}$(‰) (on-line via GC-HTC-IRMS) is plotted at the x-axis.

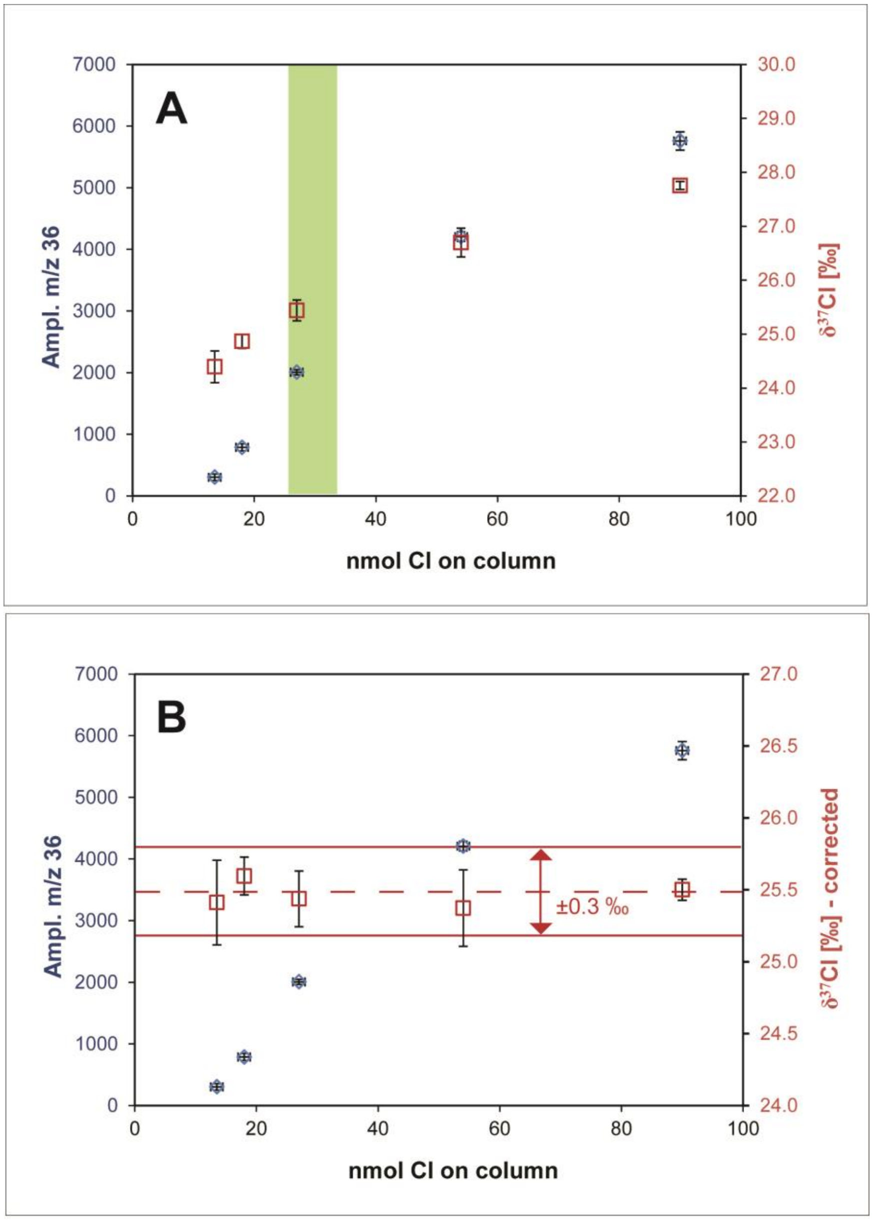

Figure S7: Sensitivity and linearity of GC-HTC-IRMS using TCE as model compound. (A) Concentration of Cl on column vs. peak intensities m/z 36 (blue) and δ^{37}Cl vaues (red) (n=5). δ^{37}Cl isotope composition is presented as raw data (A) and corrected (B).

Formation of water during HTC

Formation of water (m/z 18) was observed in the background, most probably enhanced by availability of hydrogen as reactant for the conversion. Water is suspected to condense in the non-heated parts of the transfer-lines, and therefore, generate a trap for hydrochloric acid and memory effects during chlorine isotope analysis.[18] For optimization of the HTC to improve chlorine isotope analysis a reduction of water was intended to reduce the potential memory due to condensation of water in the system.

Oxygen is known to react during HTC to either CO or H_2O if carbon or hydrogen as reactants are available, respectively.[7,31] Though oxygen was completely excluded from our instrumental system, traces of oxygen were still present. We monitored the background at different conditions in order to understand the underlying reaction dynamics. Remarkably, H_2O was observed to replace CO as main product as soon as hydrogen as reactant gas was available during HTC (Figure S8). The increase of conversion temperature to 1500°C had a minor effect on H_2O formation. To provide additional carbon during HTC the reactor was purged with methane via back flush. Deposits of carbon in the HTC reactor tube were shown to enhance conversion of O_2 to CO and reduce the amount of H_2O (Figure S9). Still, H_2O remained the main background product. Preferential conversion of O_2 to H_2O is most probably related to high availability of H_2 as reactant in our setup (H_2 ~ 20%). Though formation of H_2O may be reduced by reduction of hydrogen availability, H_2 is also required for conversion of chlorine to HCl, especially for higher chlorinated compounds. Therefore, H_2 limitation may result in isotope fractionation effects during conversion. However, monitored H_2O background levels were observed to remain stable and not significantly interfered with chlorine isotope measurements. Therefore, no further measures to reduce H_2O levels were undertaken.

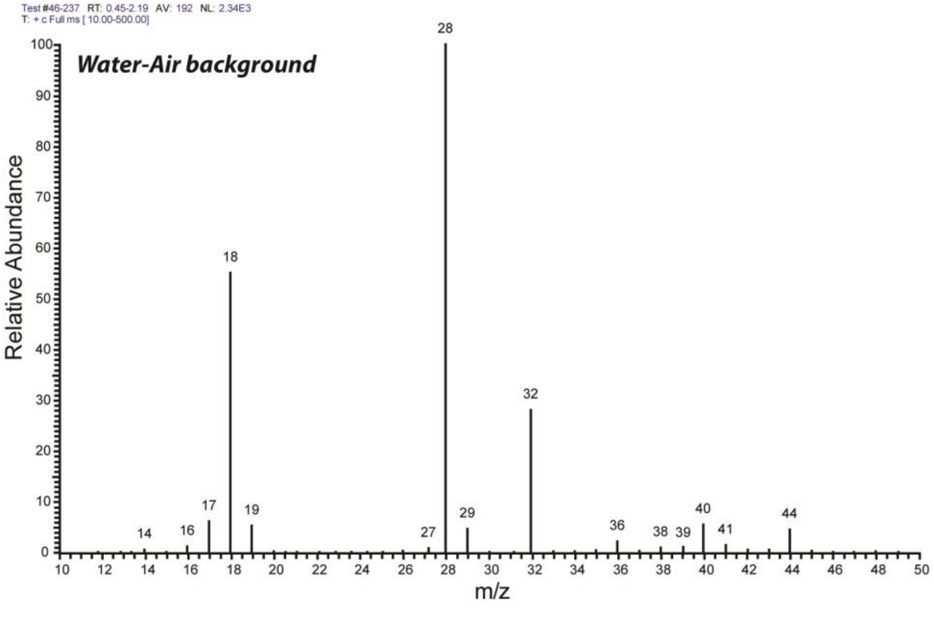

Figure S8: Water-air background of GC-HTC at 1500°C w/o reactant gas H_2. Background monitored via ion trap MS.

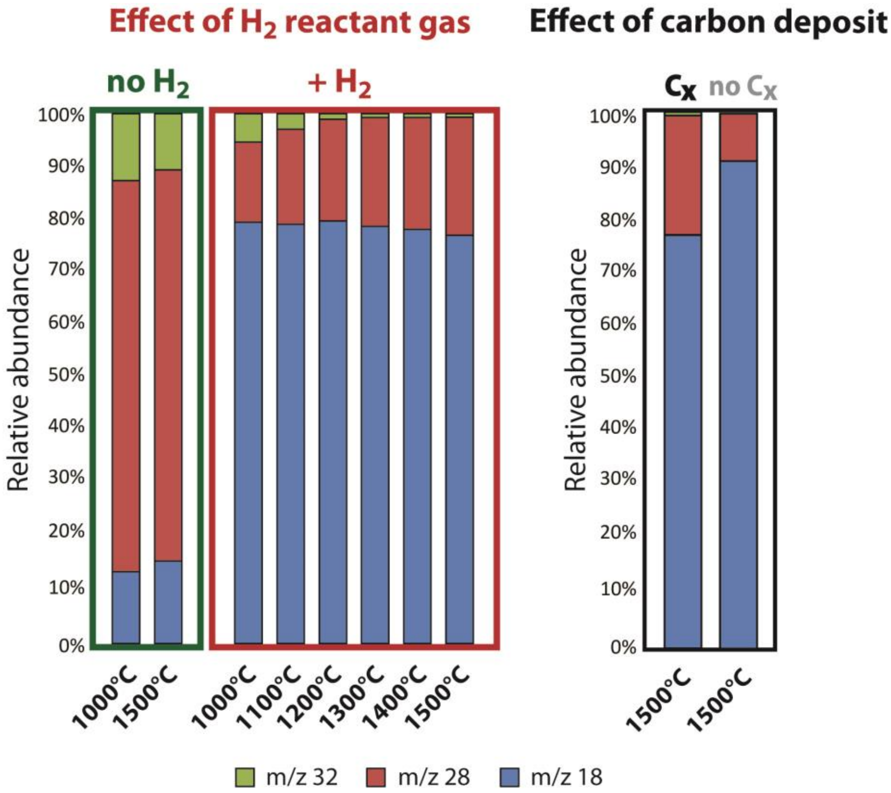

Figure S9: Characterization of water (m/z 18) formation from oxygen (m/z 32) during HTC by considering conversion temperature in combination with reactant gas (H_2) concentration (left) and carbon availability in HTC reactor (right). The range of product formation (m/z 18 [H_2O], m/z 28 [CO]) was monitored via ion trap MS and given as relative abundance.

Definition related to isotope analysis

'Continuous-flow' IRMS:

General term for analytical devices with a continuous flow delivery of an analyte to MS in a gas phase. This includes analytical devices as for instance GC-MS, GC-IRMS, EA-IRMS (Brenna et al. 1997).

Off-line methods:

Off-Line Sample Preparation for dual inlet analysis (DI-IRMS). Chemical conversion of analyte in sealed quartz tubes into appropriate compound for analysis. Approaches relying on offline sample preparation are labour-intensive, slow, and typically require large sample sizes, but can achieve high accuracy (Elsner et al. 2012).

On-line methods:

Modern techniques with chemical conversion 'on the fly'. Analytical device combines separation, conversion and MS analysis in one run, as for instance GC-C-IRMS for carbon or GC-HTC-IRMS for hydrogen isotope analysis. Continuous flow, or "online" methods, in contrast, are relatively fast, economical, and enable analysis of small samples (Elsner et al. 2012).

(19) Deutsches
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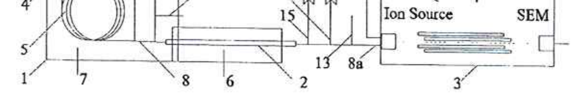
(56) Für die Beurteilung der Patentfähigkeit in Betracht gezogene Druckschriften:

DE 10 2007 031 680 A1
DE 695 20 425 T2
GB 2 270 977 A

(54) Bezeichnung: **Verfahren und Vorrichtung zur online-Bestimmung des Isotopenverhältnisses von Chlor, Brom oder Schwefel in einer organischen Probe**

(57) Hauptanspruch: Verfahren zur online-Bestimmung des Isotopenverhältnisses von Chlor, Brom oder Schwefel in einer organischen Probe, dadurch gekennzeichnet, dass die Probe in ein Trägergas eingeführt wird, das keines der genannten Elemente umfasst, und der Probenstrom durch einen Gaschromatographen (1) in einen daran direkt gekoppelten katalytischen Reaktor (2) und das im Reaktor entstehende Analysengas in ein an diesen direkt gekoppeltes Massenspektrometer (3) zur Bestimmung des Isotopenverhältnisses transportiert wird, wobei im Gaschromatographen (1) die verbindungsspezifische Auftrennung der Probe erfolgt, dann zum verbindungsspezifschen Probenstrom vor dessen Eintritt in den katalytischen Reaktor (2) Wasserstoffgas zudosiert wird, im katalytischen Reaktor (2), der Aluminiumoxid als Katalysator umfasst, die verbindungsspezifische Probe bei 800–1500°C mittels Wasserstoff vollständig zum Analysengas und Kohlenstoff umgesetzt wird, wobei das Analysengas Chlorwasserstoffgas, Bromwasserstoffgas oder Schwefelwasserstoff ist, und aus dem aufgezeichneten Massenspektrum des in das Massenspektrometer (3) gelangenden Analysengases die Häufigkeiten der jeweiligen Isotope und daraus die Isotopenverhältnisse von Chlor, Brom oder Schwefel bestimmt werden.



Beschreibung

[0001] Die vorliegende Erfindung betrifft ein Verfahren zur verbindungsspezifischen Messung des Isotopenverhältnisses von Chlor, Brom oder Schwefel in einer organischen Probe und eine Vorrichtung zur Ausführung des Verfahrens. Das Verfahren basiert auf einer gaschromatographischen Auftrennung der Probe, Umwandlung dieser in einem an den Gaschromatographen angeschlossenen Hochtemperaturofen in Chlor-, Brom- oder Schwefelwasserstoffgas und deren Nachweis im Massenspektrometer. Bei dem erfindungsgemäßen Verfahren zur Bestimmung der quantitativen Isotopenzusammensetzung von Chlor, Brom oder Schwefel in einer Verbindung der organischen Probe handelt es sich um eine online Messung, die für jede Verbindung im Gemisch spezifisch ist, also zum Beispiel zwischen verschiedenen Chlor- und/oder Bromverbindungen in einem Gemisch unterscheiden kann. Die erfindungsgemäße Methode ist einfach zu handhaben und bezüglich Genauigkeit und Sensitivität den offline Methoden vergleichbar. Da sie deutlich weniger aufwändig ist, wird für die Messungen nur ein Bruchteil an Zeit benötigt.

[0002] Online Methoden sind für die Isotopenverhältnismessung von Elementen wie Wasserstoff, Kohlenstoff, Stickstoff und Sauerstoff bekannt. Für Chlor mit seinen beiden stabilen Isotopen ^{35}Cl und ^{37}Cl, Brom mit seinen beiden stabilen Isotopen ^{79}Br und ^{81}Br sowie Schwefel mit seinen drei stabilen Isotopen ^{32}S, ^{33}S und ^{34}S gibt es bisher keine adäquaten Methoden. Im letzten Jahrzehnt wurden offline Methoden genutzt und verbessert, die präzise Resultate liefern, bezüglich der Durchführung aber mit Nachteilen behaftet sind. So beinhaltet die Methode von Holt et al. (Anal. Chem. 1997, 69, 2727–2733) sowie deren Modifikationen verschiedene aufwändige offline Schritte, um zu bestimmende organische Chlorverbindungen zu extrahieren, anzureichem und in Methylchlorid umzuwandeln, welches mittels DI-IRMS (dual inlet isotope ratio mass spectrometry) gemessen wird. Mit dieser etablierten Methode kann das Chiorisotopenverhaltnis der Gesamtprobe bestimmt werden, nicht jedoch das Chlorisotopenverhältnis in den einzelnen beinhalteten organischen Chlorverbindungen.

[0003] Die etablierte Methode der thermischen Ionisationsmassenspektrometrie (TIMS) beinhaltet zur Chlorisotopenbestimmung eine offline Umwandlung in CsCl und hat daneben hohe Anforderungen an die Probenreinheit (vgl. Holmstrand et al., Anal. Chem. 2004, 76, 2336–2342).

[0004] Eine apparativ einfache Methode zur Chlorisotopenbestimmung in einer organischen Verbindung wird von Aeppli et al (Anal. Chem. 2010, 82, 420–426) beschrieben, die die Kopplung eines Gaschromatographen an ein Quadrupol-Massenspektrometer vorsieht. Nachteilig an dieser Methode ist der immense Rechenaufwand, um über das komplette Spektrum aus den Häufigkeiten der einzelnen Peaks die verbindungsspezifischen Isotopenverhältnisse zu ermitteln. Jeder Datenlauf muss manuell komplett bearbeitet werden.

[0005] Aufgabe der vorliegenden Erfindung war es, ein verbessertes Verfahren und eine dazugehörige Vorrichtung zur verbindungsspezifischen quantitativen Bestimmung des Isotopenverhältnisses von Chlor, Brom oder Schwefel in einer organischen Probe, bereitzustellen, das die notwendige Messgenauigkeit und Sensitivität aufweist. insbesondere soll das Verfahren einfach ausführbar sein und laborintensive offline Schritte vermeiden

[0006] Die Aufgabe der Erfindung wird durch das in Anspruch 1 angegebene Verfahren und die Verwendung einer Vorrichtung gemäß Anspruch 5 gelöst. Die Unteransprüche geben vorteilhafte Ausgestaltungen an.

[0007] Erfindungsgemäß wird die Probe mittels Wasserstoff als make up-Gas in einem katalytischen Reaktor **2**, der Al_2O_3 als Katalysator beinhaltet oder daraus besteht, bei Temperaturen von 800–1500°C, vorzugsweise bei ca. 1300°C, zum Analysengas, nämlich zu HCl, HBr oder H_2S, und Kohlenstoff umgesetzt. Es findet in dem erfindungsgemäß eingesetzten Hochtemperaturofen 2 eine vollständige Umwandlung der organischen Halogen- oder Schwefelverbindungen zu Chlorwasserstoffgas, Bromwasserstoffgas oder Schwefelwasserstoffgas statt. Zunächst wird die organische Probe, die flüssig oder gasförmig sein kann, eine Einzelverbindung oder ein Verbindungsgemisch mittels Probenaufgabesystem **4** in ein Trägergas eingeführt. Als Trägergas wird erfindungsgemäß bevorzugt Helium verwendet. Um eine verbindungsspezifische Analyse zu gewährleisten, wird die zu untersuchende Probe dann in einem Gaschromatographen **1** aufgetrennt. Anschließend wird Wasserstoff als make up-Gas direkt dem Probenstrom **8** zudosiert, bevor dieser in den Reaktor **2** eintritt, in dem unter Beibehaltung der Trennung die online-Umwandlung zu HCl, HBr oder H_2S stattfindet. Das Analysengas wird dann mittels Massenspektrometer **3** analysiert. Das Massenspektrometer wird eingestellt zur Aufzeichnung der m/z-Werte (Masse-zu-Ladung-Verhältnis) der Isotope, die zu ermitteln sind. Die Messwerte werden vorzugsweise mittels einer Software verarbeitet.

[0008] Erfindungsgemäß wird am HCl über die Massenspuren 36 ($^1H^{35}Cl$) und 38 ($^1H^{37}Cl$) das Isotopenverhältnis $^{37}Cl/^{35}Cl$ bestimmt und am HBr über die Massenspuren 80 ($^1H^{79}Br$) und 82 ($^1H^{81}Br$) das Isotopenverhältnis $^{81}Br/^{79}Br$. Dabei wird der vernachlässigbare Fehler der Bestimmung von ^1H in Kauf genommen, da die natürliche Häufigkeit von ^{35}Cl zu ^{37}Cl mit ca. 75% zu 25% und von ^{79}Br zu ^{81}Br mit 51% zu 49% ungleich höher ist. Am H_2S wird über die Massenspuren 34 ($^1H^1H^{32}S$) und 36 ($^1H^1H^{34}S$) das Isotopenverhältnis $^{34}S/^{32}S$ bestimmt. Die natürliche Häufigkeit bei den Schwefelisotopen ^{32}S zu ^{34}S beträgt ca. 95% zu 4,2%.

[0009] Die genannten Isotopenverhältnisse (δ) von Chlor, Brom oder Schwefel können, vorzugsweise mit einem computerisierten Messprogramm, nach der Standardformel

$\delta = (R_{sa}/R_{st} - 1) \cdot 1000(‰)$

berechnet werden, wobei
R_{sa} das gemessene Isotopenverhältnis der Probe und
R_{st} das Isotopenverhältnis des IAEA-Referenzmaterials ist.

[0010] Das Messprogramm integriert die einzelnen Peaks und führt eine background-Korrektur durch. Das erhaltene Isotopenverhältnis stellt einen relativen Wert zum IAEA-Referenzmaterial dar.

[0011] Falls es gewünscht ist, die Ergebnisse mit einem internationalen Standard direkt zu vergleichen (z. B. für Chlor mit dem SMOC), kann das jeweilige Referenzgas HCl, HBr oder H_2S mit einem Referenzmaterial WS mit bekanntem Isotopenwert δ_{WS_PS} gegen einen Primärstandard kalibriert werden. Nach Messung der unbekannten Probe X gegen WS ergibt sich das Isotopenverhältnis der unbekannten Probe $\delta_{X\text{-}PS}$ gegen den Primärstandard in Promille gemäß nachfolgender Gleichung (vgl. Coplen, T. B. et al., Nature (London), 1983, 302, 236–238):

$\delta_{X\text{-}PS} = \delta_{X\text{-}WS} + \delta_{WS\text{-}PS} + (\delta_{X\text{-}WS} \cdot \delta_{WS\text{-}PS})/1000.$

[0012] Für das erfindungsgemäße Verfahren ist jedes für die Isotopenverhältnismessung geeignete Massenspektrometer anwendbar. Es muss in hoher zeitlicher Auflösung zwischen 2 bis 8 definierten Massen zeitnah messen und die Daten an den angeschlossenen PC übertragen. Erfindungsgemäß bevorzugt wird ein Quadrupol-Massenspektrometer oder ein IRMS (isotope ratio mass spectrometer) eingesetzt.

[0013] Gegenstand der vorliegenden Erfindung ist auch die Verwendung einer Vorrichtung, mit der das erfindungsgemäße Verfahren durchgeführt werden kann.

[0014] Kernstück der Vorrichtung ist der katalytische Reaktor **2**, der vorzugsweise als Rohr ausgestaltet ist. Er kann vollständig aus Al_2O_3 bestehen oder damit ausgekleidet sein. Sein Innendurchmesser beträgt in einer bevorzugten Ausführungsform 0,5–1 mm und sein äußerer Durchmesser beträgt vorzugsweise ca. 1,6 mm.

[0015] Der Reaktor **2** ist über die Leitung **8** mit dem Gaschromatographen **1** und über die Leitung **8a** mit dem Massenspektrometer **3** direkt verbunden. Im Ofenraum **7** des Gaschromatographen ist eine Leitung **9** für die Wasserstoffzufuhr zum Probenstrom in Leitung **8** angeordnet, der erfindungsgemäß zur Umwandlung der Probe im katalytischen Reaktor **2** in HCl, HBr oder H_2S gebraucht wird. Der Wasserstoff wird während der gesamten Messzeit als make up-Gas in den Probenstrom **8** eingemischt. Stromabwärts ist nach der Wasserstoffzufuhr **9** und vor dem Reaktor **2** eine feste Splitvorrichtung (fester Flussteiler) **10** angeordnet. Diese Splitvorrichtung **10** reduziert die Probenmenge, die im Reaktor **2** umzusetzen ist. Bevorzugt ist die Splitvorrichtung **10** eine Drosselkapillare. Auch die GC-Trennsäule und die Leitungen **8**, **8a**, **9**, **11**, **12**, **14** und **15** stellen in einer bevorzugten Ausführungsform Kapillaren dar.

[0016] Die Splitvorrichtung **10** ist mit der Leitung für die Rückspülung **11** verbunden. Um zu verhindern, dass gegebenenfalls vorhandenes Lösungsmittel aus dem Probenstrom **8** in den Reaktor **2** gelangt, wird durch Öffnen des Ventils **12a** mit Trägergas, welches mit dem Trägergas des Probenstromes identisch ist, über das gleichzeitig geöffnete Ventil **11a** der Leitung **11** rückgespült, d. h. die Ventile **11a** und **12a** werden stets parallel geschaltet. Mit dem Rückspülen (Backflush) wird der Reaktor während der Zeit des Lösungsmittels einer Probe bzw. zwischen den Messungen geschützt. In einer bevorzugten Ausführungsform werden alle Ventile der Vorrichtung (**9a**, **11a**, **12a**, **14a**, und **15a**) über eine Schaltung computergesteuert.

[0017] Die Direktkopplung des Gaschromatographen **1**, Reaktors **2** und Massenspektrometers **3** erfolgt mit einem Open split **13**, also über eine offenen Kopplung (offen gegen die Atmosphäre), wodurch sich kein Überdruck aufbauen kann. Damit ist bei allen veränderbaren Bedingungen in GC und katalytischem Reaktor ein gleichbleibender Gasstrom in die Ionenquelle des MS gesichert. Die Vorrichtung ist bevorzugt so ausgelegt, dass der Probenstrom im GC ca. 0,8–1,2 ml/min beträgt, am festen Flussteiler **10** etwas reduziert wird und das Analysengas auf die Ionenquelle des MS mit ca. 0,4 ml/min auftrifft, der Rest wird über den Open split **13** abgegeben.

[0018] In einer bevorzugten Ausführungsform weist die Vorrichtung eine Zuführung **14** für ein externes Referenzgas auf, das entweder dem Analysengas **8a** oder vorzugsweise in die Ionenquelle des Massenspektrometers zugeführt wird.

[0019] Damit ist eine genauere Rückführung der Messwerte relativ zu einem Referenzwert möglich. Die Ankopplung der Referenzgaszufuhr **14** erfolgt über einen Open split, dem ein Inertgas, zum Beispiel Helium, als Schutzgas gegen Luft zugeführt wird.

[0020] Da bei der Umsetzung im katalytischen Reaktor **2** Kohlenstoff entsteht, der sich im Reaktor **2** ablagert, ist es in einer bevorzugten Ausführungsform der Erfindung vorgesehen, den Reaktor **2** nach einer bzw. mehreren erfolgten Messungen durch Oxidation mit Sauerstoff zu reinigen. Dazu ist zwischen Reaktor **2** und Trägergaszuführung **12** eine Sauerstoffzuführung **15** angeordnet. Durch Zuführung von Sauerstoff durch Öffnen des Ventils **15a** und gleichzeitiges öffnen des Ventils **12a** der Trägergaszuführung **12** kann bei gleichzeitig offenem Ventil **11a** der Rückspülung **11** die Reinigung erfolgen. Das Zuführen von Trägergas zum Sauerstoffstrom dient dazu, das Eindringen von Sauerstoff in das Massenspektrometer zu verhindern.

[0021] Fig. 1 zeigt die erfindungsgemäß verwendete Vorrichtung in einer bevorzugten Ausführungsform, wobei die Bezugszeichen die in der Referenzliste angegebene Bedeutung haben.

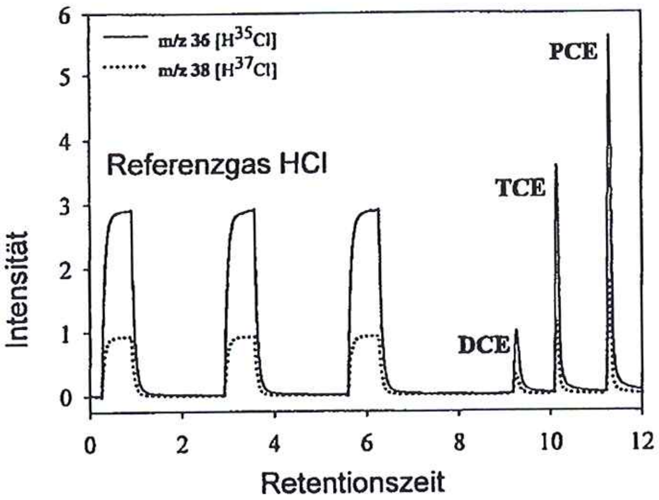
[0022] Nachfolgend wird die Erfindung am Beispiel der Bestimmung des Isotopenverhältnisses von $^{37}Cl/^{35}Cl$ näher erläutert.

Ausführungsbeispiel

[0023] 2 µl eines Probengemisches aus cis-DCE, TCE und TCA (Di-, Tri- und Tetrachlorethen) in Pentan als Lösungsmittel wird in den split/splitless Injektor des Gaschromatographen (HP 6890, Agilent) mittels eines Autosamplers (CTC AS 200) mit einer 10 µl Spritze injiziert. Auf der GC-Säule (ZB1 Phenomenex, 60 m × 0, 32 mm × 1 µm) werden über ein Temperaturprogramm im GC (40°C(5min)-10°/min–250°C(5min) die 3 Substanzen vom Lösungsmittel und untereinander getrennt. Das Lösungsmittel wird mit Hilfe des Backflush ausgeblendet (dadurch wird der Reaktor nicht mit unnötig viel Kohlenstoff belegt) und nach Umschalten in den Straigth-Modus (Backflush aus, Reaktor an Massenspektrometer direkt gekoppelt über open split) werden die drei einzelnen Komponenten direkt im Reaktor unter Zugabe von H_2 zu HCL umgewandelt Die Umsetzung zu HCl gemäß der allgemeinen Formel (I) erfolgt zu 100%. Dies wurde bei Backgroundmessungen festgestellt.

$$C_xH_yCl_z + H_2 \xrightarrow{T=1300°C,\ Al_2O_3} x\ C_{(s)} + z\ HCl \qquad (I)$$

[0024] Das nachfolgende Chromatogramm zeigt anschaulich die Trennung des Probengemisches der drei Chlorethene und drei Referenzgas-Peaks.



[0025] Am Massenspektrometer werden die Häufigkeiten der Massen 36 ($^1H^{35}Cl$) und 38 ($^1H^{37}Cl$) über den Auffänger (hier 1 SEV, oder Faraday Cup oder zwei Auffänger, für jede Masse einen) registriert und aus dem zeitlichen Verlauf entstehen zwei Integrale als Flächen. Diese werden über eine Software untereinander geteilt und ins Verhältnis zum Referenzgas (HCl aus einer Druckgasflasche, Linde) gesetzt, welche über das Ventil **14a** am Beginn der Messung dreimal für jeweils 40 Sekunden zugeschaltet wird (während Backflush eingeschaltet ist).

[0026] Mit der Formel:

$$\delta^{37}Cl = ((R^{37}{}_{sample}/R^{37}{}_{standard}) - 1)\cdot/1000$$

wird der Isotopenwert aus dem relativen Abstand der R^{37}-Verhältnisse (R^{37} = area 37/area35) zwischen Probe und Standart (hier zunächst Referenzgas) berechnet.

[0027] Über die Formel:

$$\delta^{37}Cl_{sample/SMOC} = \delta^{37}Cl_{sample/ref.gas} + \delta^{37}Cl_{ref.gas/SMOC} + (\delta^{37}Cl_{sample/refgas}\cdot\delta^{37}Cl_{refgas/SMOC})/1000$$

wird das Ergebnis an die internationale Cl-Skala angepasst

[0028] Mit dieser Technik sind reproduzierbare Isotopenverhältnisse während einer CSIA möglich. Die Standardabweichung ist im Bereich von 1‰.

[0029] Die Einzelergebnisse der Chlorisotopenverhältnisse zeigen unterschiedliche ^{37}Cl-Gehalte. Anhand der unterschiedlichen ^{37}Cl Gehalten können z. B. die Herkunft der Chemikalien oder die Prozesse, in denen diese gebildet werden, bestimmt werden.

Bezugszeichenliste

1	Gaschromatograph
2	Katalytischer Reaktor
3	Massenspektrometer
4	Probenaufgabesystem
5	GC-Trennsäule
6	Heizvorrichtung für den katalytischen Reaktor
7	Ofenraum des Gaschromatographen
8	Transportleitung für den Probenstrom
8a	Transportleitung für das Analysengas
9	Leitung für Wasserstoffzufuhr
9a	Ventil
10	Splitvorrichtung
11	Leitung für die Rückspülung (backflush)
11a	Ventil

12	Leitung für Trägergaszufuhr
12a	Ventil
13	Open split (offene Kopplung)
14	Leitung für Referenzgaszufuhr
14a	Ventil
15	Leitung für Sauerstoffzufuhr
15a	Ventil

Patentansprüche

1. Verfahren zur online-Bestimmung des Isotopenverhältnisses von Chlor, Brom oder Schwefel in einer organischen Probe, **dadurch gekennzeichnet**, dass die Probe in ein Trägergas eingeführt wird, das keines der genannten Elemente umfasst, und der Probenstrom durch einen Gaschromatographen (**1**) in einen daran direkt gekoppelten katalytischen Reaktor (**2**) und das im Reaktor entstehende Analysengas in ein an diesen direkt gekoppeltes Massenspektrometer (**3**) zur Bestimmung des Isotopenverhältnisses transportiert wird, wobei im Gaschromatographen (**1**) die verbindungsspezifische Auftrennung der Probe erfolgt, dann zum verbindungsspezifschen Probenstrom vor dessen Eintritt in den katalytischen Reaktor (**2**) Wasserstoffgas zudosiert wird, im katalytischen Reaktor (**2**), der Aluminiumoxid als Katalysator umfasst, die verbindungsspezifische Probe bei 800–1500°C mittels Wasserstoff vollständig zum Analysengas und Kohlenstoff umgesetzt wird, wobei das Analysengas Chlorwasserstoffgas, Bromwasserstoffgas oder Schwefelwasserstoff ist, und aus dem aufgezeichneten Massenspektrum des in das Massenspektrometer (**3**) gelangenden Analysengases die Häufigkeiten der jeweiligen Isotope und daraus die Isotopenverhältnisse von Chlor, Brom oder Schwefel bestimmt werden.

2. Verfahren nach Anspruch 1, dadurch gekennzeichnet, dass die Umsetzung der Probe im katalytischen Reaktor **2** bei 1300°C erfolgt.

3. Verfahren nach Anspruch 1 oder 2, dadurch gekennzeichnet, dass die Häufigkeiten der jeweiligen Isotope und die Isotopenverhaltnisse mittels eines computerisierten Messprogramms bestimmt werden.

4. Verfahren nach einem der Ansprüche 1–3, dadurch gekennzeichnet, dass das Analysengas mittels eines Quadrupol-Massenspektrometers oder eines Isotopenverhältnis-Massenspektrometers analysiert wird.

5. Verwendung einer Vorrichtung,
umfassend ein Probenaufgabesystem (**4**) zur Einführung der Probe in ein Trägergas, einen Gaschromatographen (**1**) mit GC-Trennsäule (**5**) zur verbindungsspezifischen Auftrennung der Probe, einen katalytischen Reaktor (**2**) mit Heizvorrichtung (**6**) zur Umsetzung der Probe zum Analysengas, wobei der Eingang des katalytischen Reaktors (**2**) im Ofenraum (**7**) des Gaschromatographen (**1**) angeordnet ist und der Ausgang des katalytischen Reaktors (**2**) außerhalb des Ofenraumes (**7**) liegt, und umfassend weiterhin ein Massenspektrometer (**3**) zur Bestimmung des Isotopenverhältnisses von Chlor, Brom oder Schwefel im Analysengas,
wobei Gaschromatograph (**1**), katalytischer Reaktor (**2**) und Massenspektrometer (**3**) über eine Transportleitung (**8** und **8a**) untereinander direkt verbunden sind, die Vorrichtung des weiteren im Ofenraum (**7**) des Gaschromatographen (**1**), angeordnet zwischen GC-Trennsäule (**5**) und katalytischem Reaktor (**2**), eine Wasserstoffzufuhr (**9**) zum Probenstrom (**8**) umfasst, eine Splitvorrichtung (**10**) zur mengenmäßigen Begrenzung des Probenstromes (**8**) in den Reaktor (**2**) und eine Rückspülung (**11**) zum Schutz des Reaktors (**2**) vor Lösungsmittel im Fall von flüssigen Proben, wobei die Splitvorrichtung (**10**) mit der Rückspülung (**11**) verbunden ist, die Vorrichtung des weiteren zwischen katalytischem Reaktor (**2**) und Massenspektrometer (**3**) eine mit der Rückspülung (**11**) kommunizierende Trägergaszufuhr (**12**) umfasst und zwischen dieser und dem Massenspektrometer (**3**) eine open split – Vorrichtung (offene Kopplung) (**13**) zur Gewährleistung eines gleichmäßigen Stromes des Analysengases in das Massenspektrometer (**3**) angeordnet ist,
im Verfahren zur online- Bestimmung des Isotopenverhältnisses von Chlor, Brom oder Schwefel in einer organischen Probe nach einem der Ansprüche 1 bis 4.

6. Verwendung nach Anspruch 5, dadurch gekennzeichnet, dass die Vorrichtung eine Referenzgaszuführung (**14**) aufweist, die entweder zur. Transportleitung des Analysengases (**8a**) oder in die Ionenquelle des Massenspektrometers (**3**) führt.

7. Verwendung nach Anspruch 5 oder 6, dadurch gekennzeichnet, dass die Vorrichtung zur Reinigung des katalytischen Reaktors (**2**) von Rückständen, insbesondere Kohlenstoff, zwischen katalytischem Reaktor

(**2**) und Trägergaszufuhr (**12**) eine mit der Rückspülung (**11**) und der Trägergaszufuhr (**12**) kommunizierende Sauerstoffzufuhr (**15**) aufweist.

Es folgt ein Blatt Zeichnungen

Anhängende Zeichnungen

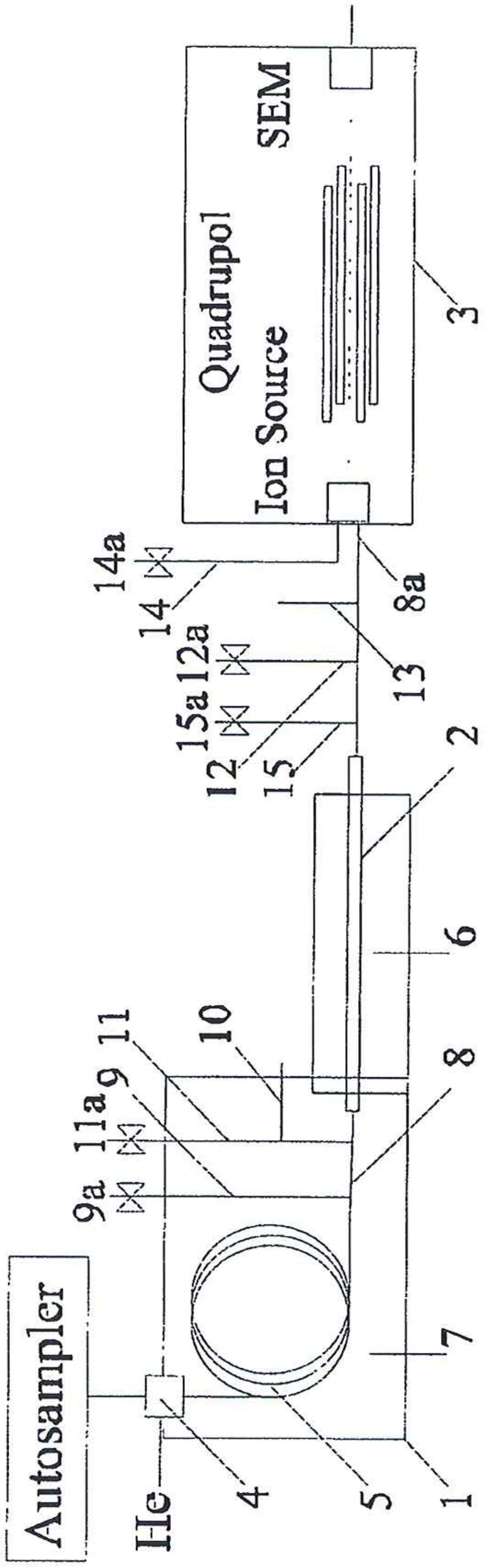


Fig. 1

To Chapter II

Published paper[4]

Compound specific hydrogen isotope analysis of heteroatom-bearing compounds via gas chromatography-chromium-based high-temperature conversion (Cr/HTC)-Isotope ratio mass spectrometry

Analytical Chemistry **2015**, Volume *87(18)*, p. 9443-9450

J. Renpennning, S. Kümmel, K. L. Hitzfeld,
A. Schimmelmann, M. Gehre

and Supporting Information

analytical chemistry

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Compound-Specific Hydrogen Isotope Analysis of Heteroatom-Bearing Compounds via Gas Chromatography−Chromium-Based High-Temperature Conversion (Cr/HTC)−Isotope Ratio Mass Spectrometry

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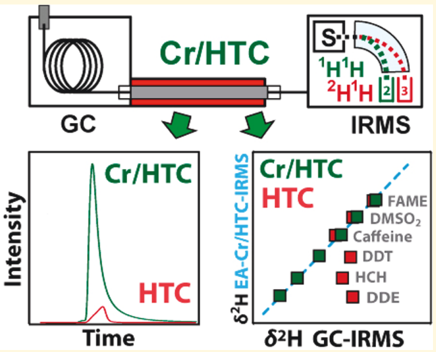
S Supporting Information

ABSTRACT: The traditional high-temperature conversion (HTC) approach toward compound-specific stable isotope analysis (CSIA) of hydrogen for heteroatom-bearing (i.e., N, Cl, S) compounds has been afflicted by fractionation bias due to formation of byproducts HCN, HCl, and H_2S. This study presents a chromium-based high-temperature conversion (Cr/HTC) approach for organic compounds containing nitrogen, chlorine, and sulfur. Following peak separation along a gas chromatographic (GC) column, the use of thermally stable ceramic Cr/HTC reactors at 1100−1500 °C and chemical sequestration of N, Cl, and S by chromium result in quantitative conversion of compound-specific organic hydrogen to H_2 analyte gas. The overall hydrogen isotope analysis via GC−Cr/HTC−isotope ratio mass spectrometry (IRMS) achieved a precision of better than ± 5 mUr along the VSMOW-SLAP scale. The accuracy of GC−Cr/HTC−IRMS was validated with organic reference materials (RM) in comparison with online EA−Cr/HTC−IRMS and offline dual-inlet IRMS. The utility and reliability of the GC−Cr/HTC−IRMS system were documented during the routine measurement of more than 500 heteroatom-bearing organic samples spanning a δ^2H range of −181 mUr to 629 mUr.



Hydrogen stable isotope analysis and the interpretation of resulting δ^2H values provide a powerful tool in many disciplines, e.g., in earth sciences, ecology, forensics, and biochemistry.[1] Hydrogen isotope analysis in organic compounds originally required two offline conversion steps, namely, first oxidation to water, and then reduction of water to molecular hydrogen (H_2) analyte gas using reducing metals such as zinc, uranium, chromium, magnesium, or tungsten.[2−4] Subsequently, δ^2H values were determined in manual dual-inlet mode using isotope-ratio mass spectrometry (DI-IRMS). Direct pyrolytic conversion of organically bound hydrogen to H_2 analyte gas via high-temperature conversion (HTC) at temperatures of > 1050 °C resulted in much-enhanced utility of continuous flow (CF) online CF-IRMS.[3,5] Modern stable isotope analysis of organic hydrogen uses isotope-ratio mass spectrometry (IRMS) where the H_2 analyte gas is generated online via (1) direct HTC at 1050 °C in an elemental analyzer (EA) or (2) via compound-specific stable isotope analysis (CSIA) in combination with GC separation of mixtures and subsequent HTC of the target compounds at 1400−1450 °C.[1,6] However, these methods yield the best results for hydrocarbons and become more challenging for nitrogen-, chlorine-, and sulfur-containing organics, where HTC-derived H_2 yields are incomplete due to the formation of hydrogen-containing byproducts (HCN, HCl, and H_2S).[7−10]

A chromium-based reactor system can overcome interferences by quantitatively scavenging heteroatoms. Chromium was first employed in hydrogen online EA−IRMS by Morrison et al.[11] and Kelly et al.[12] The analysis of water with chromium entails quantitative conversion to H_2 and accurate δ^2H values. EA conversion of polyhalogenated compounds with chromium at 1000 °C, however, resulted in incomplete H_2 yields and limited the accuracy and suitability of chromium-based reactor systems for some substrates at relatively low temperatures.[13] Efforts to trap or eliminate the byproducts with a cold trap, stainless steel, or additional reduction with hot chromium in tubular reactors at 800−1000 °C could not establish a reliable and technically simple GC−IRMS method for compound-specific hydrogen isotope analysis of polyhalogenated compounds.[7,14−16]

Gehre et al. introduced EA−Cr/HTC−IRMS (previously named Cr-EA) as an accurate tool for hydrogen stable isotope-

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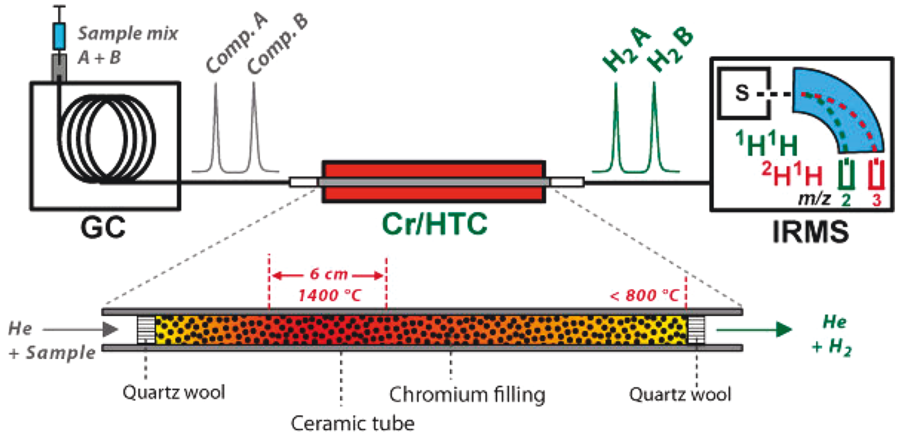

Figure 1. Schematic design of the Cr/HTC reactor incorporated into a standard GC-HTC-IRMS system for converting organic hydrogen in heteroatom-bearing samples to molecular hydrogen for subsequent determination of the hydrogen isotopic composition. All chemical elements in samples except hydrogen are irreversibly scavenged by chromium, and only H_2 is passed through as analyte gas for δ^2H determination.

ratio analysis of organic compounds bearing heteroelements.[17] Quantitative H_2 yields and accurate δ^2H values were derived from several nitrogen-, chlorine-, and sulfur-containing compounds, as documented by the comparison of several stable isotope laboratories using chromium-based reactor systems of different designs and conversion conditions as well as offline conversion and analysis by dual-inlet mode using isotope-ratio mass spectrometry (DI-IRMS).[17]

This study builds on the proven chromium-based EA reactor design and introduces an interface for compound-specific hydrogen isotope analysis using a chromium-based reactor for GC−IRMS. In contrast to earlier approaches by Shouakar-Stash and Drimmie[16] and Kuder and Philp,[15] a ceramic reactor tube was packed with chromium and used as an HTC reactor at > 1100 °C. Our system allows temperatures of up to 1500 °C and is therefore termed 'chromium-based high-temperature conversion' (Cr/HTC). Our reactor design can be implemented in existing equipment by replacing the HTC reactor with a Cr/HTC reactor, using commercially available components for GC−HTC−IRMS hydrogen isotope analysis.

■ EXPERIMENTAL SECTION

Reference Material. Organic, ≥ 99% pure reference materials (RMs) for calibration and validation included three hexadecanes (C_{16} #A, C_{16} #B, and C_{16} #C), three caffeines (1,3,7-trimethylpurine-2,6-dione) RMs (caffeine #1, caffeine #2, and caffeine #3), and three icosanic acid methyl esters (FAME #A, FAME #B, and FAME #C) that were prepared and characterized at Indiana University and collaborating laboratories, as part of a collaborative ring-test project to develop future international organic stable isotope RMs. [Note: The hydrogen isotope ratio values of hexadecanes #A, #B, and #C, caffeines #1, #2, #3, and IAEA-600 and FAMEs #A, #B, and #C are preliminary and will be updated to official consensus values later in 2015.] IAEA-600 caffeine was provided by the International Atomic Energy Agency (IAEA) in Vienna, Austria.[18,19] Eight heteroelement-bearing internal RMs (UFZ 1−8) at the Umweltforschungszentrum (UFZ, Leipzig, Germany) included DDD (UFZ 1; *p,p′*-dichlorodiphenyl dichloroethane; ABCR, Germany; ≥ 98%), DDE (UFZ 2; *p,p′*-dichlorodiphenyldichloroethylene; ABCR, Germany; ≥ 99 %), DDT (UFZ 3; 4,4′-dichlorodiphenyltrichloroethane; Supelco, USA; ≥ 97%), HCH (UFZ 4; γ-hexachlorocyclohexane; HiMedia, India; ≥ 99%), DMSO2 (UFZ 5; dimethyl sulfone; Alfa Aesar, USA; ≥ 99%), Ph2S2 (UFZ 6; diphenyl disulfide; Sigma−Aldrich, USA; ≥ 99%), TCE−PPG (UFZ 7; trichloroethene; PPG Brand, USA; ≥ 98%), and TCE−Merck (UFZ 8; trichloroethene; Merck, Germany; ≥ 99%). All RMs were isotopically characterized against international primary stable isotope measurement standards VSMOW2 and SLAP2 (i) offline at Indiana University, using combustion in quartz ampules, uranium reduction of water to molecular hydrogen, and analysis by DI-IRMS, and (ii) online at the Leipzig Laboratory for Stable Isotopes (LSI), using EA−Cr/HTC−IRMS.[17] Mean two-point-calibrated δ^2H values on the VSMOW-SLAP isotopic scale are listed in Table 2 (presented later in this work). Solvents with highest available purities were purchased from Merck (Darmstadt, Germany) and Sigma−Aldrich (St. Louis, MO, USA).

Cr/HTC Reactor Design. A commercially available GC−HTC−IRMS interface (GC−IsoLink-, GC/C III−HTC systems, Themo Fisher, Bremen, Germany) was equipped with a horizontally arranged high-temperature oven (Thermo Finnigan, Germany) maintaining reactor temperatures of up to 1500 °C. Tubular ceramic reactors (320 mm, 0.8 mm inner diameter (i.d.), 1.6 mm outer diameter (o.d.); Degussit AL23 aluminum oxide ceramic, Friatec, Germany) were mounted into the high-temperature oven. For comparative, conventional high-temperature HTC, an empty ceramic reactor tube of the same size was used in the high-temperature oven. The chromium-based reactor (Cr/HTC) represented a ceramic tube with a 240-mm-long filling of chromium powder (> 99%, particle size 250−300 μm, Cr Patinal, Merck, Germany) stretching the length of the high-temperature oven. Both ends of the bed of chromium powder were abutted by 10 mm plugs of quartz wool (HEKAtech GmbH, Germany) (Figure 1). Prior to its first use, the reactor was heated to 800 °C for 1 h and subsequently heated overnight to the operating temperature of 1400 °C in the center of the reactor. The carrier gas flow became increasingly restricted at higher temperatures of the chromium powder. For example, at 1100 °C a helium flow of 2.0 mL min^{-1} after the GC column resulted in a flow of ∼1.2 mL min^{-1} after the Cr/HTC reactor; at 1500 °C the latter flow decreased to ∼1.0 mL min^{-1}.

Analysis of Byproducts Forming in the GC−Cr/HTC−MS System. The formation of pyrolytic byproducts during HTC and Cr/HTC conversion was monitored with a PolarisQ ion trap mass spectrometer (Thermo Finnigan, Germany) coupled to a GC device (Model HP6890N, Agilent Technologies, Germany, with autosampler A200S, CTC Analytics AG, Switzerland) and mounted next to a high-temperature oven (Thermo Finnigan, Germany), as described previously.[17,20] The tubular aluminum ceramic reactor was either empty (HTC) or filled with chromium (Cr/HTC). All transfer lines were heated to 110−120 °C by regulated heating tapes (HORST GmbH, Germany) to prevent condensation. Organic compounds were injected in solution onto the GC column in split/splitless mode in concentrations that were commensurate with a hydrogen amount of ∼150 nmol H on column from each compound. The GC system was equipped with a Zebron ZB-1 column (60 m, 0.32 mm i.d., 1 μm film thickness; Phenomenex Inc., USA).. Routine operating conditions included a core oven temperature of 1400 °C and a carrier gas flow of 1.2 mL min^{-1} for both HTC and Cr/HTC.

Hydrogen Isotope Measurements. *Online Analyses via GC-Cr/HTC-IRMS.* Online analysis via GC−Cr/HTC−IRMS used an analytical train featuring a CTC CombiPAL autosampler (CTC Analytics AG, Switzerland), a GC device (HP7890, Agilent Technologies, Germany), and a GC IsoLink interface connected via a ConFlo IV open split system to a MAT 253 IRMS (Thermo, Bremen). The GC system was equipped with a Zebron ZB-1 column (60 m, 0.32 mm i.d., 1 μm film thickness; Phenomenex Inc., USA). Samples in solution were introduced into the GC via split/splitless injection in amounts that represented the hydrogen equivalent of ∼150 nmol H on column from each compound. Cr/HTC and resulting compound-specific hydrogen isotope compositions were investigated at core conversion temperatures of 1000−1500 °C. Finally, routine hydrogen isotope analyses of RMs were performed via conversion in Cr/HTC and HTC reactors at a constant core conversion temperature of 1400 °C and a post-reactor carrier gas flow of 1.2 mL min^{-1}. H_2 was transferred online through an open split into the IRMS. The H_3^+ factor was measured twice daily.

Offline Analyses via Uranium Conversion and DI−IRMS. Offline analysis via uranium conversion and DI−IRMS of the RMs was performed via combustion of organic hydrogen to water, subsequent reduction to H_2 with uranium metal,[17,21,22] and subsequent isotope ratio measurements in manual dual-inlet mode. The principle of identical treatment of international measurement standards (VSMOW2, SLAP2) and organic sample hydrogen was strictly followed by 'pre-combusting' VSMOW2 and SLAP2 with copper oxide in quartz ampules in the same fashion as organic materials to convert organic hydrogen to water.[23]

Online Analyses via EA-Cr/HTC-IRMS. Online analyses via EA−Cr/HTC−IRMS were performed as described by Gehre et al.,[17] using a conversion temperature of 1200 °C. RMs were measured as pure samples in triplicate and two-point-calibrated along the VSMOW-SLAP isotopic scale.

Calibration of Isotope Values. A preliminary one-point calibration was performed vs. C_{16} #A RM (δ^2H = −167 mUr vs. VSMOW). Subsequent two-point calibrations of raw stable isotope ratios were performed with hexadecanes C_{16} #A and C_{16} #C RMs for all GC experiments to uniformly compensate for scale compression of the mass spectrometer and arrive at corrected δ^2H values along the VSMOW-SLAP isotopic scale.[24] The abundance ratio of hydrogen isotopes $[^2\text{H}^1\text{H}]/[^1\text{H}^1\text{H}]$ was expressed in customary δ-notation (eq 1). In contrast to traditionally applied per mil (‰), all values are reported in the SI unit urey (here: mUr which is equivalent to ‰) following the recommendation of Brand and Coplen.[25]

$$\delta^2\text{H (mUr)} = \frac{R_{\text{sample}}}{R_{\text{reference}}} - 1 \tag{1}$$

General Position for Calibration Strategy. Pulses of H_2 'reference gas' from a gas cylinder were not used in this study for the calibration of δ^2H values, because such practices would violate the principle of identical treatment of sample and standard where all analyte gases must pass though the same preparative-analytical sequence.[26] 'Reference gas' pulses from an H_2 cylinder are not generated in the same fashion as H_2 analyte gas from organic matter, do not pass through a GC prior to isotopic measurement, and thus are not subject to the same potential fractionations. The availability of a wide range of hydrogen stable isotope RMs for online analytical applications eliminates the justification of using outdated and indefensible one-point calibration and the employment of H_2 'reference gas' pulses except for monitoring IRMS performance. Although one-point calibration vs. C_{16} #A RM was partly applied for CSIA of hydrogen, it failed to compensate for the scale compression of the mass spectrometer. Especially for hydrogen isotopes, a one-point calibration typically results in reduced accuracy because only two-point calibration can adequately account for the scale compression of individual mass spectrometers.[27] Therefore, two-point calibration versus calibrated RMs is recommended in order to achieve the best accuracy in hydrogen isotope analysis.

■ RESULTS AND DISCUSSION

Principles. Quantitative conversion of organic hydrogen to H_2 analyte gas is essential for avoiding fractionation and arriving at accurate δ^2H values. Incomplete conversion or partial loss of hydrogen to byproducts other than H_2 is likely to result in δ^2H values in H_2 that do not reflect total hydrogen in the organic analyte.[7,10,14,28,29] Application of the traditional HTC methodology to heteroelement-bearing organic substrates results in the formation of hydrogen-bearing byproducts such as HCl from chlorinated hydrocarbons (e.g., hexachlorocyclohexanes; Figures 2 and 3), HCN from nitrogen-bearing compounds, or H_2S from sulfur-bearing compounds (Figures S1 and S2 in the Supporting Information).[7,10,17,20,30] Some byproducts are known to influence the H_3^+ factor and can thus degrade the accuracy of hydrogen isotope measurements via IRMS.[28,31,32] Our new chromium-based reactor system overcomes the limitations of the traditional HTC system with respect to hydrogen isotope analysis of heteroatom-bearing compounds, especially of chlorinated organic materials.

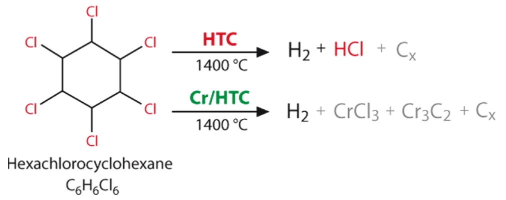

Figure 2. Nonstoichiometric equations for conversions of hexachlorocyclohexane to H_2 analyte gas via HTC and Cr/HTC at 1400 °C indicating the formation of HCl byproducts via HTC. In contrast the quantitative reaction of chlorine to chromium chloride in Cr/HTC results in quantitative H_2 yields.

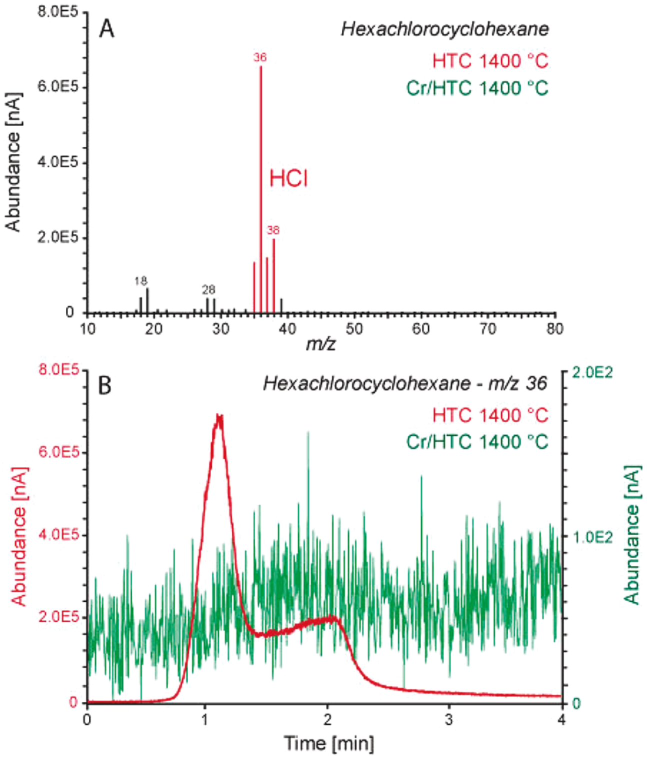

Figure 3. (A) Byproduct formation during conventional HTC (red) vs. Cr/HTC (green) monitored with an ion trap MS. HTC of hexachlorocyclohexane at 1400 °C resulted in strong formation of HCl with *m*/*z* 35–38. (B) Molecular ion *m*/*z* 36 (HCl) signal intensities of up to ~10^6 nA were observed during HTC, while no HCl formation was observed via Cr/HTC at 1400 °C.

The use of a thermally stable ceramic reactor at temperatures much higher than in quartz reactors possible ensures complete pyrolytic decomposition. The spread of chromium powder from the hottest zone to cooler temperatures near the reactor's exit provides appropriate conditions for heteroatoms to react quantitatively with chromium and remain irreversibly scavenged in the Cr/HTC reactor (Figure 3 and Figure S1). Thus, the inert carrier gas only elutes H_2 analyte gas from the Cr/HTC reactor for subsequent hydrogen isotope analysis via IRMS.

Byproduct Formation during Conversion. The molecular background scan of the HTC (1400 °C) effluent gas stream confirmed the significant formation of hydrogen-bearing byproducts from all heteroatom-containing compounds and even from hydrocarbons (Figure S1), in agreement with earlier observations.[20] The major byproduct after conventional HTC of hexachlorocyclohexane was HCl (*m*/*z* 35–38) (Figure 2). HCl formation occurred from all tested chlorine-bearing compounds, while intensities of HCl could be related to the Cl/H ratio in the molecule (data not shown). We confirmed the observation by Gehre et al.[17] of HCN (*m*/*z* 27) byproduct formation during HTC of nitrogen-bearing caffeine. Sulfur-bearing dimethyl sulfone generated H_2S, SO_2, COS, and CS_2 in different quantities (Figure S2).

In comparison to conventional HTC, our Cr/HTC resulted in a significant reduction in the abundances of byproducts. While Cr/HTC byproducts were still detected at reactor temperatures of ≤ 1000 °C, no byproducts were found at reactor temperatures of ≥ 1100 °C. At 1400 °C, Cr/HTC reduced byproduct signal intensities to the air/water background of ~10^2 nA for all heteroatom-bearing compounds (Figure 3 and Figure S2). Moreover, substantially increased H_2 yields could be confirmed using GC–Cr/HTC–IRMS in a temperature range of 1000–1500 °C, as presented in Figure 4 for hexachlorocyclohexane. The H_2 yields of conventional HTCs from hexachlorocyclohexane reached only ~10% of the respective Cr/HTC yields, both at 1400 °C (Figure 4).

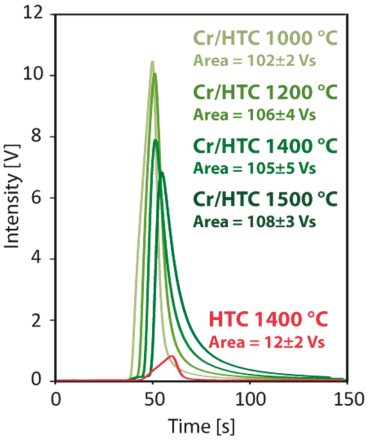

Figure 4. IRMS *m*/*z* 2 signal of H_2 from equal amounts of hexachlorocyclohexane after conventional HTC (red) and Cr/HTC (green). Conventional HTC of hexachlorocyclohexane at 1400 °C produced only ~10% of the signal strength (and thus H_2 yield) relative to Cr/HTC in the temperature range of 1000–1500 °C.

Effect of Cr/HTC Temperature on Hydrogen Isotope Analysis. A sufficiently high temperature in the Cr/HTC reactor was critical for quantitative conversion of organic hydrogen to H_2 analyte gas. Minimum temperatures of ≥ 1100 °C were required to limit byproduct formation. However, diffusion of H_2 into the hot chromium and subsequent H_2 peak broadening occurred with increasing Cr/HTC temperature (Figure 4). The overall evidence indicates a net benefit from higher operating temperatures based on (i) better H_2 yields at higher temperature (Figure 4), and (ii) a positive shift of preliminary one-point-calibrated δ^2H values for several compounds with increasing conversion temperature (Table 1). δ^2H values determined via two-point calibration using C_{16} #A and C_{16} #C RMs were accurate for all conversion temperatures with respect to the results determined with both reference methods (online via EA–Cr/HTC–IRMS and offline via DI–IRMS). The best one-point-calibrated results were achieved at conversion temperatures of 1400–1500 °C. The analytical precision of δ^2H values via Cr/HTC was not influenced by increasing conversion temperatures and was typically below ± 5 mUr. A conversion temperature of 1400 °C and two-point calibration for GC–Cr/HTC–IRMS were applied to routinely generate δ^2H values (Table 2).

δ^2H via GC-Cr/HTC-IRMS versus Alternative Techniques. The accuracy of compound-specific δ^2H values was evaluated for GC–Cr/HTC–IRMS and conventional GC–HTC–IRMS under similar conditions (i.e., flow rate ~1.2 mL min^{-1}; reactor core temperature 1400 °C; and equal amounts of H on column). All δ^2H values were compared to those of RMs that had been characterized independently via EA–Cr/HTC–IRMS (LSI UFZ), offline (Indiana University), and/or as part of a collaborative ring-test project. Measured compounds included oxygen-bearing fatty acid methyl esters

Table 1. δ^2H Values for Hexadecane (C_{16}), Caffeine, Hexachlorocyclohexane (HCH), and Dimethyl Sulfone ($DMSO_2$), Determined via Cr/HTC at 1000−1500 °C[a]

	DI-IRMS (Offline)	EA-Cr/HTC-IRMS (Online)	GC−Cr/HTC−IRMS (Online)					GC−Cr/HTC−IRMS (Online)				
T (°C)		1050	1000	1100	1200	1400	1500	1000	1100	1200	1400	1500
calibration	two-point	two-point	preliminary one-point					two-point				
slope			0.95	0.95	0.98	1.06	1.09	1.03	1.04	1.05	1.07	1.07
intercept			0	0	0	0	0	15.3	15.7	11.9	0.6	−2.5
	δ^2H	δ^2H	δ^2H					δ^2H				
C_{16} #B	−8	−11	−25	−24	n.d.[b]	−12	−12	−12	−11	n.d.[b]	−12	−14
caffeine #1	98	96	70	71	73	61	91	92	93	90	96	87
HCH	−74	−80	−81	−75	−80	−83	−78	−73	−66	−73	−82	−80
$DMSO_2$	134	123	n.d.[b]	n.d.[b]	105	121	128	n.d.[b]	n.d.[b]	124	122	118

[a] Values given in the SI unit urey (mUr, equivalent to ‰). All values were preliminarily one-point-calibrated using C_{16} #A reference material (RM) (δ^2H = −167 mUr vs. VSMOW). Proper two-point calibration relied on C_{16} #A and C_{16} #C as anchoring RMs with δ^2H values relative to VSMOW-SLAP that had been determined online via EA−Cr/HTC−IRMS, offline via DI−IRMS, and as part of a collaborative ring-test project. The precision of triplicate Cr/HTC measurements was below ± 5 mUr (equivalent to ± 5 ‰). [b] Not determined.

Table 2. δ^2H Values for Oxygen-, Nitrogen-, Chlorine-, and Sulfur-Containing RMs via GC−HTC-IRMS and G−Cr/HTC−IRMS Are Compared to Respective Data from Offline Uranium Reduction and DI−IRMS Analysis and Online EA−Cr/HTC−IRMS To Evaluate Accuracy[a]

	DI-IRMS (offline)	EA-Cr/HTC-IRMS (online)	GC-HTC-IRMS (online)	GC-Cr/HTC-IRMS (online)
	δ^2H [mUr]	δ^2H [mUr]	δ^2H [mUr]	δ^2H [mUr]
C_{16} #A	−160 ± 1	−167 ± 1	−167[b]	−167[b]
C_{16} #C	374 ± 3	387 ± 2	387[b]	387[b]
C_{16} #B	−8 ± 2	−11 ± 1	−21 ± 1	−9 ± 1
FAME #A	−177 ± 1	−186 ± 1	−192 ± 1	−181 ± 2
FAME #B	−1 ± 2	−6 ± 2	−14 ± 2	0 ± 1
FAME #C	346 ± 3	348 ± 2	338 ± 1	349 ± 2
IAEA-600	−153 ± 1	−158 ± 1	−156 ± 1	−161 ± 1
caffeine #1	98 ± 2	97 ± 1	87 ± 1	96 ± 1
caffeine #2	−152 ± 2	−157 ± 1	−157 ± 1	−162 ± 1
caffeine #3	171 ± 4	175 ± 1	180 ± 1	180 ± 2
DDD	72 ± 1	71 ± 3	252 ± 22	58 ± 1
DDE	−81 ± 2	−89 ± 2	326 ± 3	−103 ± 1
DDT	−14 ± 1	−18 ± 2	292 ± 8	−27 ± 1
HCH	−74 ± 3	−80 ± 1	771 ± 42	−82 ± 4
$DMSO_2$	134 ± 3	123 ± 1	123 ± 1	122 ± 1
Ph_2S_2	−148 ± 4	−164 ± 1	−174 ± 5	−182 ± 1
TCE-PPG	466 ± 21	463 ± 9	n.d.[c]	516 ± 2
TCE-Merck	593 ± 7	571 ± 6	n.d.[c]	629 ± 2

[a] δ^2H values are expressed in the SI unit urey (mUr, equivalent to ‰). [b] Reference materials used for two-point calibration along the VSMOW-SLAP isotopic scale, with δ^2H values determined in a collaborative ring-test project. [c] Not determined, because of low H_2 yields.

(FAME), oxygen- and nitrogen-bearing caffeine, chlorinated compounds (DDD, DDE, DDT, HCH, and TCE), and sulfur-bearing $DMSO_2$ and Ph_2S_2 (Table 2).

The analytical precision of online EA−Cr/HTC−IRMS and offline DI-IRMS data averages below ± 5 mUr (Table 2). Discrepancies are considered minor, compared to the ~800 mUr wide range of the VSMOW-SLAP scale. Volatilization of trichloroethenes during the preparation or introduction into EA is likely responsible for reduced precision of respective δ^2H values. Overall, EA−Cr/HTC−IRMS results were in accordance with DI−IRMS and/or results of the collaborative ring-test project (Table 2), while the highest deviations were obtained for C16 #C, $DMSO_2$, Ph_2S_2, and TCE-Merck. Discrepancies smaller than 16 mUr in δ^2H for both sulfur-containing compounds require further investigation.

The precision and accuracy of GC-HTC-IRMS data were dependent on the chemical characteristics of compound classes (Table 2). Oxygen-bearing FAMEs expressed excellent precision of <2 mUr and an average deviation from prescribed RM δ^2H values of ~10−15 mUr. In contrast, GC−HTC−IRMS δ^2H values of chlorinated compounds generally had a worse precision and were shifted to more positive values by several hundred mUr, as reported earlier.[7,14] The low H_2 yield of trichloroethenes precluded δ^2H determinations. Satisfying δ^2H values for nitrogen-bearing caffeines (despite HCN byproduct formation and reduced H_2 yield) contradict earlier reports and indicate variability in HTC reactions for different analytical settings such as EA and GC.[17,30,33] However, significant lowering of δ^2H with increasing signal intensity indicates that a similar trend can be expected with higher sample concentration (Figure S3 in the Supporting Information). Similarly, unexpectedly good accuracy was achieved for sulfur-bearing $DMSO_2$ and Ph_2S_2, despite byproduct formation (Figure S2 in the Supporting Information). Nevertheless, destabilization of the H_3^+ factor occurs after few sequential HTC analyses of sulfur-containing compounds, affects subsequent δ^2H measurements, and thus precludes continuous δ^2H measurements of multiple sulfur-bearing compounds via HTC.

The GC−Cr/HTC−IRMS system achieved superior precision of less than ± 5 mUr for almost all tested compounds that was coupled with good accuracy for FAMEs, caffeine, $DMSO_2$, and Ph_2S_2 (Table 2). Deviations in δ^2H values were in the range of observed differences between RM values that had been measured online via EA−Cr/HTC−IRMS and offline via DI−IRMS (Table 2). Among chlorinated compounds, excellent accuracy was achieved for HCH, whereas δ^2H values of DDD, DDE, and DDT were negatively shifted by ~10−20 mUr relative to prescribed RM δ^2H values. Trichloroethene δ^2H values expressed a large positive shift of ~50 mUr, although

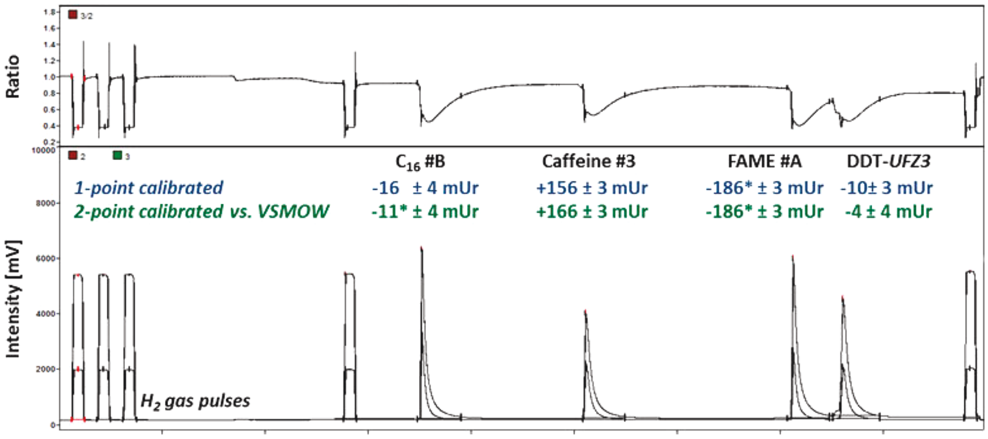

Figure 5. Compound-specific hydrogen isotope analysis of mixtures containing C_{16} #B, caffeine #3, FAME #A, and DDT converted via Cr/HTC at 1400 °C. The δ^2H values of the measured compounds cover a range of +166 mUr to −186 mUr ($\Delta\delta^2$H ≈ 350 mUr). The precision of δ^2H values for all compounds was below ± 4 mUr ($n = 10$). FAME #A was used for one-point calibration, and C_{16} #B and FAME #A were used for two-point calibration along the VSMOW-SLAP scale. (Asterisks (*) denote calibration anchors.)

ion-trap MS results indicated no byproduct formation and suggest quantitative conversion of TCE to H_2 analyte gas. Furthermore, similar $\Delta\delta^2$H isotopic differences between TCE-PPG and TCE-Merck measured via GC−Cr/HTC−IRMS (113 mUr), EA−Cr/HTC−IRMS (108 mUr) and DI-IRMS after offline conversion (127 mUr) suggest that the limited accuracy of EA-Cr/HTC-IRMS data relates to (i) being far outside of the calibration range of C_{16} #A and C_{16} #C, and/or (ii) being rooted in evaporative loss with isotope fractionation during sample preparation.[34]

Compound-Specific δ^2H Measurements of Mixtures. A mixture of four organic compounds in acetone encompassed a compound-specific isotopic range of ∼350 mUr (from +160 mUr to −186 mUr) and included C_{16} #B, caffeine #3, FAME #A, and DDT injected with a common concentration of ∼250−300 nmol H per compound on column. After GC separation, Cr/HTC at 1400 °C, and IRMS, the resulting raw δ^2H values were preliminarily 'one-point calibrated' vs. C_{16} #A. An independent two-point calibration relied on C_{16} #B and FAME #A as anchoring RMs along the VSMOW-SLAP scale. The precision for 10 replicate δ^2H measurements of each compound was below ± 5 mUr (Figure 5, as well as Table S1 in the Supporting Information). The accuracy of compound-specific δ^2H values via Cr/HTC proved to be satisfactory (Figure 5) in comparison to δ^2H values measured via both reference methods (Table 2). No memory effects were observed during sequential GC−Cr/HTC−IRMS analyses.

Opportunities and Limitations of GC−Cr/HTC−IRMS. *Reactor Lifetime.* The described GC−Cr/HTC−IRMS system quantitatively and efficiently trapped heteroatoms from more than 500 injected samples containing chlorine, nitrogen, oxygen, and sulfur. Our tests required multiple temperature changes between 1000 °C and 1500 °C that showed no effect on the Cr/HTC reactor's long-term performance. The absence of clogging of the reactor stands in contrast to EA−Cr/HTC−IRMS, where flow decreases over the reactor's lifetime as ash is accumulating. We replaced reactors merely on a precautionary basis before facing analytical problems.

Linearity and Limits of Determination. Measurements of different amounts of trichloroethene(TCE-PPG) documented a dependency of δ^2H on the injected amount and defined the required TCE sample size for $a \geq 3000$ mV signal as ∼100−120 nmol TCE on column, or 110 nmol H on column (Figure S6A in the Supporting Information). This lower size limit, in terms of H_2, was valid for all analyzed compounds, as well as for GC-injected H_2 (Figure S6B in the Supporting Information); therefore, the amount dependency at low hydrogen abundance cannot be caused by compound-dependent Cr/HTC and chromium-related efficiency. Nonlinearity may be due to the diffusion of H_2 and porosity during gas transport along the chromium-filled reactor.

Chromatography. Although peak widths for GC−Cr/HTC−IRMS were smallest at 1000 °C and increased with temperature, because of the diffusion of H_2 in chromium particles (Figure 2), it is more important to optimize chemical conversion. Improvement in chromatography at lower temperature would sacrifice accuracy. Under our chosen operating conditions of 1400 °C, GC−Cr/HTC−IRMS achieves GC baseline separation and accurate compound-specific stable isotope analysis in a simple mixture (Figure 5). More-complex mixtures may chromatographically benefit from synoptic optimizations of Cr/HTC temperature for specific compound classes, GC carrier gas flow, and chromium particle size. Therefore, baseline separation of peaks for correct peak-area integration and background correction are essential prerequisites for δ^2H measurements.

Memory Effects. No significant memory effect was observed in a continuous run of 36 H-isotopically contrasting samples (Table 3). This remarkable stability persisted throughout the analyses of various heteroatom-containing compounds spanning a 530 mUr wide isotopic range, from −172 mUr to 363 mUr, and has been corroborated by sequential compound-specific analyses of a GC-injected mixture containing C_{16} #B, caffeine #3, FAME #A, and DDT (Figure 5).

■ CONCLUSIONS

The chromium-based GC−Cr/HTC−IRMS system is a versatile and promising extension of the arsenal for compound-specific hydrogen isotope analysis. The method especially targets a broad range of heteroatom-bearing compounds for which accurate online determinations of δ^2H values was not possible by the conventional HTC approach. The introduction of tubular ceramic reactors filled with elemental chromium powder allows HTC operating temper-

Table 3. Test of Between-Sample Memory for 36 Samples of Several, H-Isotopically Contrasting and Heteroatom-Bearing Compounds That Were Measured Sequentially via GC−Cr/HTC−IRMS at 1400 °C[a]

sequence No.	sample	δ^2H [mUr]
1	DMSO$_2$	122
2	DMSO$_2$	122
3	DMSO$_2$	122
4	C$_{16}$ #A	−167
5	C$_{16}$ #A	−166
6	C$_{16}$ #B	−12
7	C$_{16}$ #B	−13
8	C$_{16}$ #C	386
9	C$_{16}$ #C	387
10	DDD	58
11	DDD	59
12	DDD	58
13	DDE	−103
14	DDE	−104
15	DDE	−103
16	DDT	−26
17	DDT	−28
18	DDT	−28
19	HCH	−78
20	HCH	−84
21	HCH	−85
22	Ph$_2$S$_2$	−181
23	Ph$_2$S$_2$	−183
24	Ph$_2$S$_2$	−183
31	C$_{16}$ #A	−167
32	C$_{16}$ #A	−169
33	C$_{16}$ #B	−10
34	C$_{16}$ #B	−14
35	C$_{16}$ #C	386
36	C$_{16}$ #C	387

[a] All values were two-point-calibrated along the VSMOW-SLAP scale using C$_{16}$ #A and C$_{16}$ #C RMs.

atures above 1100 °C. This extension of Cr/HTC significantly (i) improves the conversion of organic hydrogen to H$_2$ from heteroelement-bearing compounds and (ii) reduces the formation of hydrogen-containing byproducts that limit H$_2$ yields and lead to isotope fractionation. Optimal conversion conditions for our GC−Cr/HTC reactor were in the temperature range from 1400−1500 °C, which could be adjusted for specific compound classes and chromatographic requirements. δ^2H values of nonvolatile reference materials expressed a typical precision of less than ± 2 mUr and accuracies of approximately ± 5 mUr or ± 10 mUr when compared with δ^2H values obtained via online EA−Cr/HTC−IRMS or offline dual-inlet IRMS, respectively. GC−Cr/HTC−IRMS was not afflicted by a significant sample-to-sample memory effect. The simple design of GC−Cr/HTC reactors facilitates the handling, cleaning, and exchange of chromium. More than 500 samples containing heteroatoms (N, Cl, S, O) could be analyzed consecutively with a single reactor filling. Construction of a GC−Cr/HTC reactor takes advantage of commercially available standard equipment for routine GC−HTC−IRMS, inexpensive chromium powder, and standard ceramic tubes.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.5b02475.

> Principles of conversion to H$_2$ analyte gas; byproduct formation of hexadecane, caffeine, and dimethyl sulfone; linearity; comparability of EA-Cr/HTC-IRMS and GC-Cr/HTC-IRMS. (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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SUPORTING INFORMATION

Compound-specific hydrogen isotope analysis of heteroatom-bearing compounds via gas chromatography – chromium/HTC – isotope ratio mass spectrometry

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J. Renpennning, S. Kümmel, K. L. Hitzfeld,A. Schimmelmann, M. Gehre

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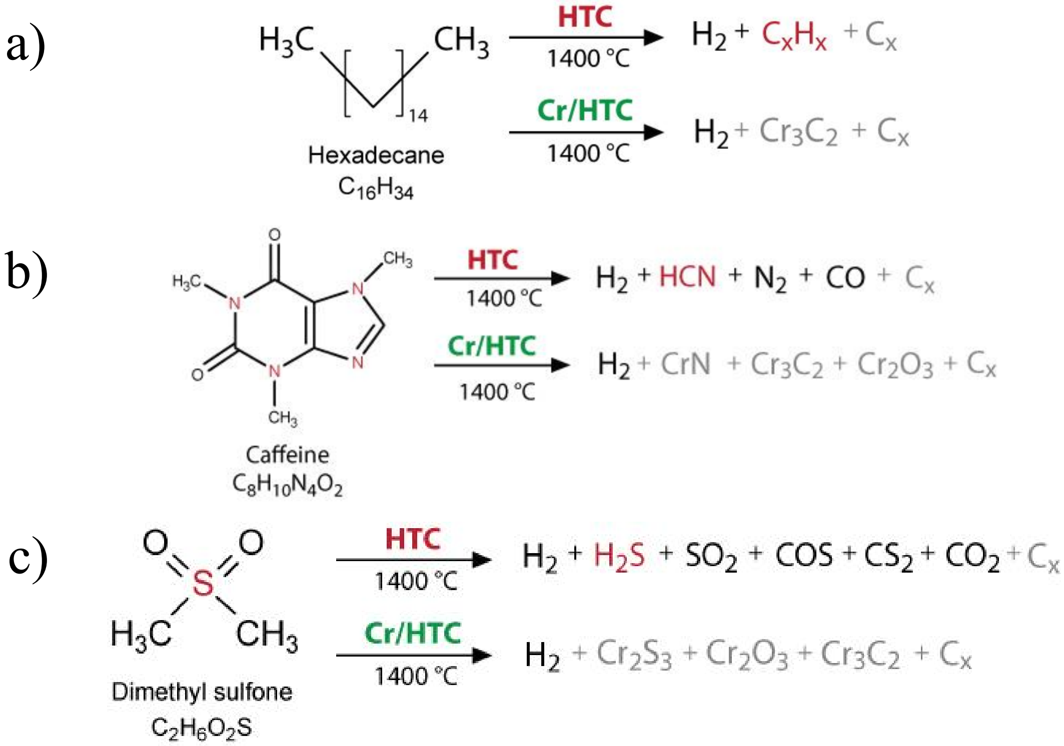

Figure S1: Non-stoichiometric formula for conversion of: a) hexadecane (C_{16}), b) caffeine (1,3,7-trimethylpurine-2,6-dione) and c) dimethyl sulfone ($DMSO_2$) to H_2 analyte gas via HTC or Cr/HTC at 1400 °C and the corresponding byproduct formation.

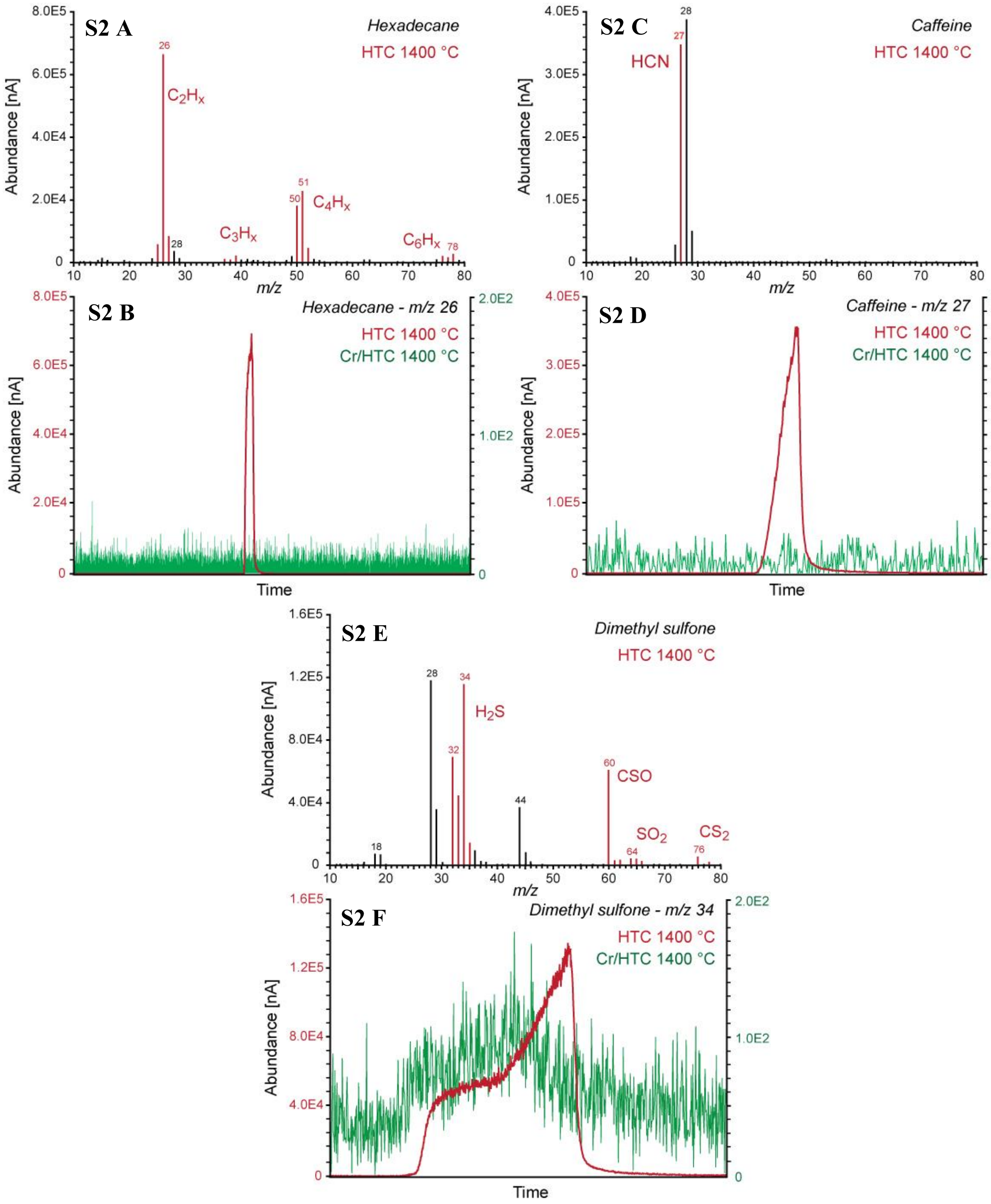

Figure S2: Byproduct formation during conversion at 1400 °C via HTC (red) vs. Cr/HTC (green) monitored with ion trap MS. Severe byproduct formation was observed for HTC, e.g., C_xH_x from hexadecane (Figure S2 A-B), HCN from nitrogen-bearing caffeine (Figure S2 C-D), or H_2S, SO_2, COS and CS_2 from sulfur-bearing dimethyl sulfone (Figure S2 F-G). Corresponding byproducts were absent for Cr/HTC when operated at sufficiently high temperature.

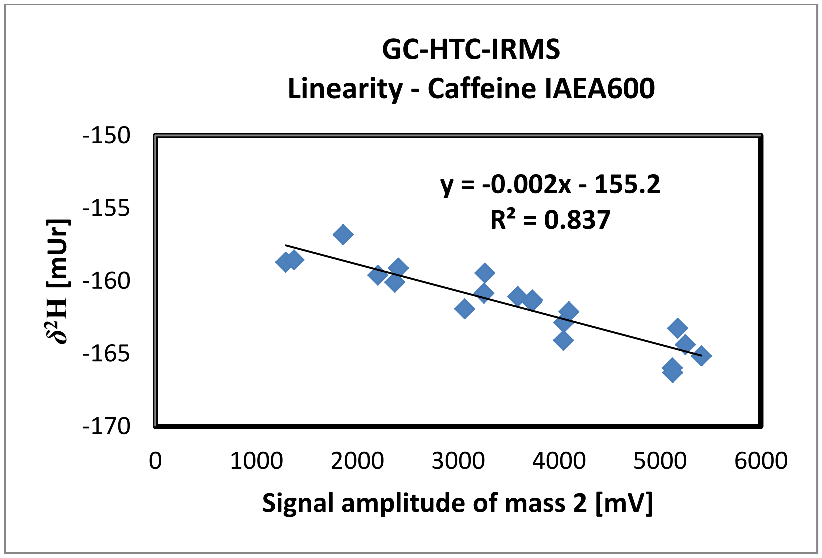

Figure S3: Linearity test of δ^2H values measured along ranges of injection sizes for caffeine (IAEA-600) via GC- HTC-IRMS at 1400 °C. Linear depletion of δ2H with increasing signal intensity was observed with a slope of about - 2 mUr/V.

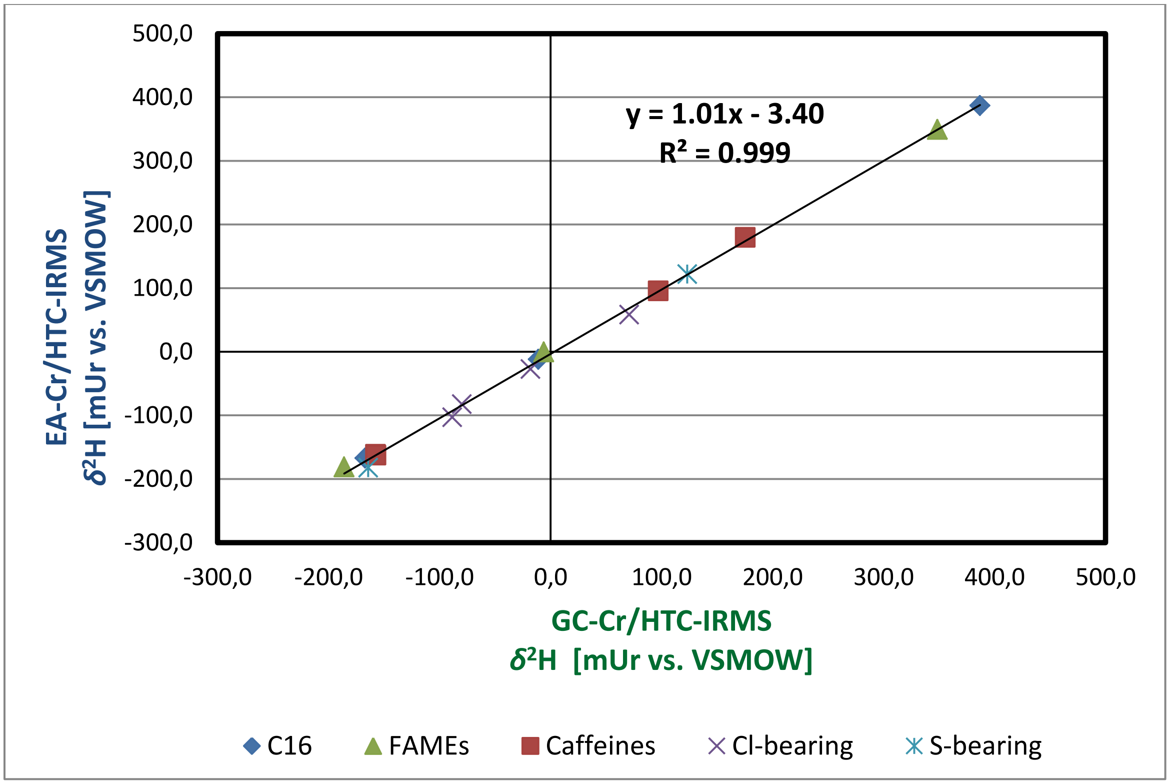

Figure S4: δ^2H values for C_{16} (#A, #B, #C), FAMEs (#A, #B, #C), caffeines (IAEA-600, #1, #2, #3), DDD-UFZ 1, DDE-UFZ 2, DDT-UFZ 3, HCH-UFZ 4, $DMSO_2$-UFZ 5 and Ph_2S_2-UFZ 6 RMs via GC-Cr/HTC-IRMS (green) are compared to respective data from online EA-Cr/HTC-IRMS (blue) to evaluate accuracy. δ^2H values are expressed in the SI unit urey (mUr, equivalent to ‰).

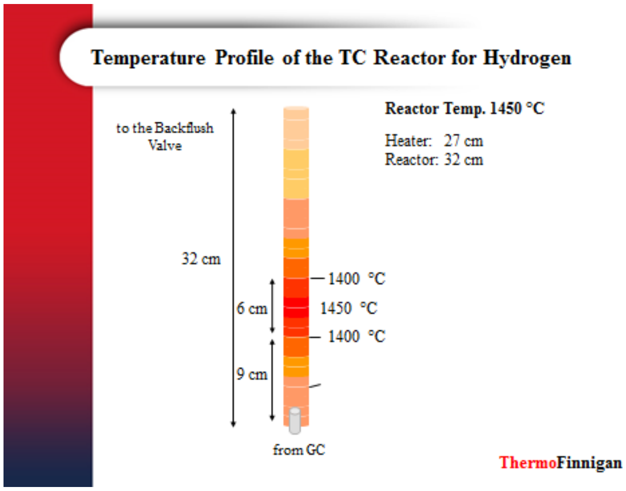

Figure S5: Temperature profile of the HTC reactor oven (ThermoFinnigan).

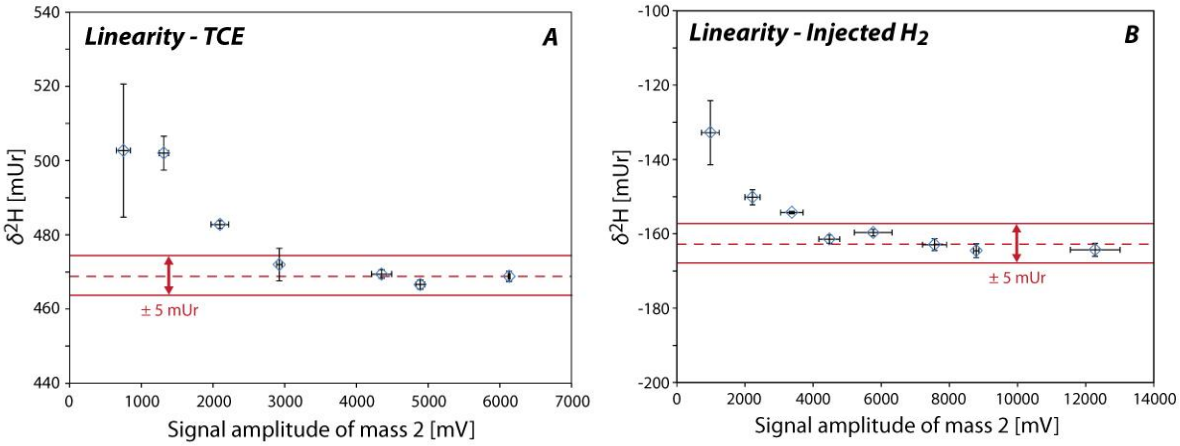

Figure S6: Linearity of δ_2H values measured along ranges of injection sizes for (A) trichloroethene (TCE-UFZ7) and (B) GC-injected H_2 gas, both processed via GC-Cr/HTC-IRMS at 1400 °C. A linear range is apparent for signals ≥ 3.000 mV.

Table S1: Compound-specific hydrogen isotope analysis of mixtures containing C_{16} #B, caffeine #3, FAME #A and DDT-UFZ3 converted via Cr/HTC at 1400 °C. The δ^2H values of the measured compounds cover a range of +166 to -186 mUr ($\Delta\delta^2$H ~ 350 mUr). The precision of δ^2H values for all compounds was below ± 4 mUr (n = 10). FAME #A was used for 1-point calibration and C_{16} #B and FAME #A were used for 2-point calibration along the VSMOW-SLAP scale. Results are compared to respective δ^2H values from online EA-Cr/HTC-IRMS (blue) to evaluate accuracy.*: calibration anchors

Compound	Peak width [s]		Amplitude mass 2 [mV]		Peak area [mV*s]		δ^2H [mUr]			n	EA-Cr/HTC-IRMS
	Mean	StDev	Mean	StDev	Mean	StDev	1- point calibration	2- point calibration	StDev		δ^2H [mUr]
C_{16} #B	84	± 3	9214	± 1607	96	± 11	-16	-11*	± 4	10	-11 ± 1
Caffeine #3	82	± 4	4985	± 503	82	± 9	156	166	± 3	10	175 ± 1
FAME #A	82	± 1	8669	± 1360	99	± 11	-186*	-186*	± 3	10	-186 ± 1
DDT - UFZ 3	96	± 3	5362	± 491	93	± 11	-10	-4	± 4	10	-18 ± 2

<u>To Chapter III</u>
accepted
manuscript

Evaluation of the performance of high temperature conversion (HTC) reactors for compound specific oxygen stable isotope analysis

Isotopes in Environmental and Health Studies **2016***, in press*
K. L. Hitzfeld, M. Gehre, H.-H. Richnow
and Supporting Information

Evaluation of the Performance of High Temperature Conversion (HTC) Reactors for Compound-specific Oxygen Stable Isotope Analysis

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Evaluation of the Performance of High Temperature Conversion (HTC) Reactors for Compound-specific Oxygen Stable Isotope Analysis

In this study conversion conditions for oxygen gas-chromatography high-temperature conversion isotope ratio mass spectrometry (GC-HTC-IRMS) are characterised using qualitative mass spectrometry (Ion Trap).

It is shown that physical and chemical properties of a given reactor design impact HTC and thus the ability to accurately measure oxygen isotope ratios. Commercial available and custom build tube-in-tube reactors were used to elucidate (i) by-product formation (carbon dioxide, water, small organic molecules), (ii) 2nd sources of oxygen (leakage, metal oxides, ceramic material), and (iii) required reactor conditions (conditioning, reduction, stability). The suitability of the available HTC approach for compound specific isotope analysis (CSIA) of oxygen in volatile organic molecules like methyl *tert*-butyl ether (MTBE) is assessed. Main problems impeding accurate analysis are non-quantitative HTC and significant carbon dioxide by-product formation. An evaluation strategy combining mass spectrometric analysis of HTC products and IRMS $^{18}O/^{16}O$ monitoring for future method development is proposed.

Keywords: High Temperature Conversion (HTC), CSIA, Oxygen 18, carbon monoxide, GC-HTC-IRMS, isotope ratios

1. Introduction

1.1 Oxygen stable isotope analysis methods

Compound specific isotope analysis (CSIA) of the $^{18}O/^{16}O$ isotope ratio in organic molecules has the potential to generate valuable results in both environmental and health studies, provided that methods can be developed which produce reliable and accurate measurements of isotope ratios. Applications for oxygen stable isotope analysis can help to answer research questions in fields ranging from geology and biochemistry to forensics and health, as well as commercial applications such as food authentication [1]. Especially in multi-dimensional CSIA the combination of oxygen isotope patterns and stable isotope information from other elements can reveal crucial correlations [2, 3]. One environmentally relevant example is methyl *tert*-butyl ether (MTBE) a highly abundant groundwater contaminant which distribution and degradation behaviour makes it a focus of environmental research [4]. Two-dimensional isotope analysis (D/H, $^{13}C/^{12}C$) has been successfully applied to elucidate abiotic and biotic degradation of MTBE [5-7]. A GC-HTC-IRMS method for $^{18}O/^{16}O$ analysis would complement these studies, allow three-dimensional process characterisation, and therefore shed light on open research questions.

Oxygen has three stable isotopes ^{16}O (99.762 %), ^{17}O (0.038 %) and ^{18}O (0.200 %), and oxygen isotope fractionation is commonly employed as an indicator for processes [8]. On-line methods for the determination of $^{18}O/^{16}O$ ratios using an elemental analyser (EA) were developed 20 years ago [9, 10]. In 1999, Kornexl *et al.* established a high-temperature conversion (HTC) procedure for the routine on-line $^{18}O/^{16}O$ determination of inorganic and organic solids and liquids [10, 11]. Two different EA-HTC systems (HTP-Hekatech, TC/EA-Thermo Fisher Scientific) have been commercially available since that time. Both systems rely on the same operating principle: converting molecularly bound oxygen into carbon monoxide (CO) at high temperature. The conversion reactor consists of an outer ceramic tube and an inner glassy carbon tube, filled with a glassy carbon grid and a layer of nickelised carbon in the reaction zone; samples are transported by a helium carrier gas and converted at 1400°C [10]. In the EA-HTC reactor, the inner glassy carbon tube prevents sample contact with the ceramic (Al_2O_3) material, resulting in lower CO backgrounds [12]. This fast, accurate and precise method has become standard, and is used for the calibration of international standards and the measurement of reference materials, laboratory standards, and pure compound samples as well as bulk samples in various applications [1, 13, 14]. Unfortunately, this EA-HTC is not applicable to compound-specific isotope analysis (CSIA) unless the compound has been highly purified prior to analysis. Methods for oxygen-CSIA from mixtures, however, are needed to comprehensively use $^{18}O/^{16}O$ to answer research questions [15]. For environmental and other compound-specific applications, the combination of gas chromatography (GC) with isotope ratio mass spectrometry (IRMS) is the only convenient solution to analyse samples from field- and laboratory experiments. Thus, an interface for the compound-specific conversion of molecularly-bound oxygen into carbon monoxide analyte gas and direct $^{18}O/^{16}O$ determination via IRMS, using high-temperature conversion, is regarded to be an appropriate solution.

There have been attempts towards CSIA methods using nickel, platinum, and carbon in ceramic tubes, analogous to reactors for the routine CSIA of D/H and $^{13}C/^{12}C$ [16, 17]. These efforts resulted in commercial oxygen-HTC reactors with a platinum tube and nickel wires combination (Thermo Fisher Scientific) or a nickel tube only (Isoprime Ltd) as constituents [17-19]. In those reactors the metal tubes are to prevent the sample gas stream from contacting the outer ceramic tube, comparably to the function of the glassy carbon tube in EA-HTC. Additionally, the use of a reducing makeup-gas (containing H_2) and regular conditioning to deposit pure carbon in the reactor is recommended to obtain strictly reductive conditions and prevent nickel oxide formation. The Thermo Fisher and Isoprime reactors use hydrogen (1 % in helium) as a make-up gas introduced into the helium carrier gas [18, 19]. However, in the case of some reported applications of the Thermo Fisher HTC reactor and reactors of similar design, this hydrogen addition was partly omitted [2, 20-24]. Although these solutions are commercially available, there are to the best of our knowledge no applications using the Isoprime reactor and only a few applications published over the last decade employing the Thermo Fisher oxygen-HTC reactor [1-3, 15, 20-32]. The HTC reaction within the Thermo Fisher HTC reactor was optimised for stable oxygen analysis of the model compound vanillin (4-hydroxy-3-methoxybenzaldehyde, $C_8H_8O_3$) where the oxygen is bound in a methoxy group, hydroxyl group, and aldehyde group [17, 18]. Measurements of vanillin from three different suppliers (Table 1) confirmed the reactor performance with regard to stability, linearity, and accuracy (see SI A).

With this Thermo Fisher HTC for oxygen reactor or similar custom build reactor types, CSIA methods were adapted for compounds mainly from the food and flavour field e.g. water [16, 33], ethanol [34], estragole [3], cholesterol [25], glycerol [21], fatty alcohols [22], and sugars [23, 24, 28]. Focussing on sample preparation they often lack a detailed characterisation of the underlying processes, high background explanations or possible by-products, and rarely question adaptability of HTC to structurally differing compounds.

1.2 High temperature conversion (HTC) principles

For stable isotope analysis, quantitative conversion of the organically-bound oxygen in a sample to CO is an essential requirement. This also holds true for GC-HTC-IRMS. The desired conversion should quantitatively yield carbon monoxide (CO) from organic oxygen containing compounds (see equation (1)[14]), to allow for oxygen stable isotope analysis from *m/z* 28 ($^{12}C^{16}O$) and *m/z* 30 ($^{12}C^{18}O$).

$$C_xH_{2y}O_z + n\,C \rightarrow z\,CO + y\,H_2 + (n+x-z)\,C \qquad (1)$$

The HTC reaction should take place under continuous flow conditions within the hottest zone of the reactor. The reaction products will be carried through the cooler parts of the reactor, however this time should not allow disproportionation of CO to CO_2 (after the Boudouard equilibrium ($C+CO_2+Energy \rightleftharpoons 2CO$)). Still, there is a set of diverse factors that can influence this conversion and vary the amounts and composition of the gained reaction products. Undesired by-products are oxygen containing compounds like carbon dioxide (CO_2) and water (H_2O) or molecules which produce ions in the IRMS that contribute to the intensities at *m/z* 28, 29 30 like nitrogen (N_2, HCN, NO) or C_xH_y recombination detected in previous studies [35-38]. Surplus carbon available in the reactor, obtained by regular conditioning with e.g. pure alkane injection, should additionally shift the Boudouard equilibrium towards CO (after the principle of Le Chatelier). However, the extent of all possible reactions under the prevalent continuous flow conditions is also dependent on temperature, pressure, and catalysts, e.g. the reactor materials [37, 39-41]. To maintain reduced reactor materials and reductive HTC conditions H_2 make-up gas can be used which is fed into the sample stream in low concentration; whether it influences water by-product formation or possible memory has not been investigated. *(For theoretical considerations on possible HTC processes see SI D)*

Reactor materials and specifications of the active reaction zone like inner diameter or temperature gradients in combination with carrier gas flow and temperature should also be considered during method development. The presence of nickel for example, as a constituent of both commercially available reactors restricts the maximum conversion temperature to below the melting point of nickel (1453°C [39]), their recommended operation temperature is 1280°C [42]. HTC, in contrast, denominates a reaction temperature above 1330°C; for EA-HTC it has been shown that only at higher temperatures is the conversion reaction quantitatively complete, in fact temperatures ≥ 1450°C are recommended to ensure full conversion and reduce by-products [35, 37, 38]. Furthermore nickel bears the risk of retaining oxygen at the nickel surface. *(For theoretical considerations on the role of nickel see SI F)*.

Zech and Glaser (2009) were the first to compare conversion performance of several custom-built reactors with platinum, nickel, graphite, and glassy carbon to approach reliable oxygen stable isotope analysis of neutral sugars in soil; however, their considerations did not lead to an improved reactor design [23]. Finally they used the commercially available Thermo Fisher HTC for oxygen reactor for their measurements of neutral sugar biomarkers [23, 28]. Although they could prove almost complete quantitative conversion (>97 % CO production), they also observed the liberation of oxygen which was traced to have originated in the reactor upon alkane injection [23]. In spite of the reactors' commercial availability, $^{18}O/^{16}O$ ratios of the measured compounds had to be corrected for drift, amount dependency, and an off-set of the measured ratios to the ratios determined by EA-HTC-IRMS of the single pure compounds [23, 24, 28]. After their corrections, the precision for their chosen sugars was from less than 0.5 mUr[#] [28] up to more than 2 mUr [23, 24] , and therefore often less than the precision for vanillin (< 1 mUr). This variation was attributed to the applied sugar derivatisation, and was considered to be acceptable for this sample class [23, 28].

The term urey (Ur), after Harold C. Urey, was proposed to overcome traditional limitations with expressing orders of magnitude differences in isotope deltas [43]. In such manner, all values in this publication are reported in the SI unit urey in contrast to traditionally applied per mil (here: mUr which is equivalent to ‰).

Apparently the use of HTC for oxygen CSIA poses problems for some chemicals while others are fairly analysable. Furthermore, the users report observations like high backgrounds, ‘carbon surplus effects’, and small linear ranges and often need to correct raw values noticeable [3, 23, 33]. Such observations are mainly not discussed in detail nor followed up by investigations of the HTC performance. Also the search for modified or improved reactor systems, which overcome those drawbacks and are better suited for compounds not amendable with the existing reactors are either not pursued or not published.

1.3 Objectives

At large the community is lacking (i) understanding of HTC processes used for oxygen CSIA and possible undesired secondary reactions, (ii) consistent criteria to determine complete quantitative conversion of organic bound oxygen to CO, and (iii) standardised evaluation procedures to assure comparability of oxygen CSIA results produced with GC-HTC-IRMS. With this study we want to contribute to the understanding of HTC processes in GC-HTC-IRMS for oxygen stable isotope determination, contribute approaches and methods to characterise HTC and its secondary reactions, and assign common observations to founded explanations. Three main questions will be in the focus:

(1) By-product formation of HTC: to which extend and under which conditions are carbon dioxide, water, and organic by-products formed?

(2) Other oxygen sources than from the compound of interest: where is oxygen available during HTC and how can interfering CO production be supressed?

(3) Optimal HTC conditions: when are optimal conditions present and what parameters (conditioning, H_2-reduction, linearity, temperature) determine them?

2. Material and Methods

2.1 Mass spectrometry

To study processes and products of HTC within the GC-HTC- reactor system, the resulting effluent could be split at the end of the HTC reactor and products be analysed with IRMS (DELTAplus XP, ThermoFinnigan, Bremen, Germany) and IonTrap MS (PolarisQ, ThermoFinnigan, Bremen, Germany) in parallel, as previously reported for other conversion reactions [36, 44]. In addition GC-HTC-IRMS measurements only were conducted using a GC in combination with a GC-IsoLink interface connected via a ConFlow IV (both Thermo Fisher Scientific, Bremen, Germany) open split and reference/working gas split system to a MAT 253 IRMS (Thermo Fisher Scientific, Bremen, Germany). The isotope composition of the carbon monoxide analyte gas was recorded via the ion traces *m/z* 28, 29, and 30. For monitoring of the background from air and the status of the system, other ion traces were recorded in the carbon dioxide configuration (*m/z* 44, 45, 46) or by setting the IRMS to *m/z* 40 and *m/z* 18.

2.2 Gas Chromatography

A GC-IsoLink interface (Thermo Fisher Scientific, Bremen, Germany) coupled to an Agilent 7890 A series gas chromatograph (Agilent Technologies, Böblingen, Germany) equipped with a CombiPAL auto sampler (CTC Analytics, Zwingen, Switzerland), was used for GC-HTC-IRMS. Injector temperature was 250°C and He (5.0, Air Liquide) carrier gas column flow 1.2 mL/min (determined to be optimal flow by preliminary experiments, data not shown). For the gas chromatographic separation of vanillin, a HP-5 column (30 m, 0.32 mm, 0.25 µm; Agilent Technologies, Böblingen, Germany) was used, and a ZB-1 column (60 m, 0.32 mm, 1 µm; Phenomenex, Utrecht, The Netherlands) was used for all other more volatile compounds.

After liquid injection vanillin dissolved in dichloromethane (DCM) was analysed with the following GC temperature programme: 40°C, 10°C min°C min^{-1} to 200°C, 20°C min^{-1} to 300°C for 2 min. MTBE, and ethyl acetate dissolved in toluene were analysed using the following GC temperature programme: 40°C at 2 min, 8°C min^{-1} to 100°C, 25°C min^{-1} to 300° for 3 min. Headspace injections from vaporised volatile compounds and mixtures in helium filled vials were analysed isothermally at 70°C.

2.3 HTC reactors

To evaluate HTC processes and variate different parameters, a set of custom build tube in tube reactors (synoptic table see SI B) and the commercially available Thermo Fisher HTC for oxygen reactor [18] were used. This allowed the development of approaches to answer questions after the main impediments and present strategies to detect and quantify drawbacks upon GC-HTC-IRMS oxygen CSIA.

2.4 Standards and Samples

Vanillin (4-hydroxy-3-methoxybenzaldehyde, $C_8H_8O_3$, 99 %) was purchased from three different producers: Sigma-Aldrich (Steinheim, Germany), Merck (Hohenbrunn, Germany), and ABCR (Karlsruhe, Germany). For the GC-HTC-IRMS analysis, vanillin, 0.025 M in dichloromethane (Carl Roth, Germany), was injected via liquid injection (1 µL) with varying split ratios (1:3 to 1:100) to determine reactor performance and linearity.

Methyl *tert*-butyl ether (MTBE, $C_5H_{12}O$) was purchased from Merck (Darmstadt, Germany) (MTBE 2a and 3) and from Sigma-Aldrich (Munich, Germany) (MTBE 4) and ethyl *tert*-butyl ether (ETBE, $C_6H_{14}O$) was purchased from Fluka (Sigma-Aldrich, Steinheim, Germany). Ethyl acetate (EtAc, $C_4H_8O_2$) of analytical grade (p.a.) was purchased from Th. Geyer (Renningen, Germany). Those volatile compounds had purities of > 97 % and were dissolved in toluene (Merck, Darmstadt, Germany) for liquid injection or vaporised at 60°C in a helium filled vial for headspace injection. Pentane (C_5H_{12}) of HPLC grade was purchased from Th. Geyer (Renningen, Germany).

Solid vanillin and liquid MTBE samples were used to obtain laboratory standards for CSIA after their oxygen isotope ratio was determined by EA-HTC-IRMS according to the procedure described in Kornexl et *al.* (1999) using the solid reference materials NBS 127, IAEA 601, IAEA SO6, and the liquid reference waters IAEA-OH 1, IAEA-OH 4, GISP for calibration [10, 14, 45, 46]. The ratios of $^{18}O/^{16}O$ are expressed in delta notation as $\delta^{18}O$ *vs.* VSMOW-SLAP scale (Vienna Standard Mean Ocean Water - Standard light Antarctic Precipitation) according to equation (1) [47], and are reported in the SI unit urey (here: mUr which is equivalent to ‰) [43].

$$\delta^{\mathbf{18}}\mathbf{O}_{\mathbf{VSMOW}} = \frac{\left(^{18}O/^{16}O\right)_{sample}}{\left(^{18}O/^{16}O\right)_{VSMOW}} - \mathbf{1} \qquad (1)$$

Two-point calibration is known to be the best choice for the normalization of measured stable isotope compositions to the international scale and was used [10, 14, 48-51]. The accuracy of the calibration was verified with the third known reference. The so-obtained laboratory standards (Table 1) were then used to monitor or if needed, calibrate the GC-HTC-IRMS raw $\delta^{18}O$ values during reactor performance tests. Within a sample run, carbon monoxide (3.7, Air Products) reference/ working gas (refgas) was used to monitor the intra-run stability and derive raw $\delta^{18}O$ values.

Table 1. The $\delta^{18}O$ composition *vs.* VSMOW-SLAP of model compounds was determined by EA-HTC-IRMS measurements. Calibration was facilitated by the reference material NBS 127, IAEA 601 and IAEA SO 6 for solid samples and the waters IAEA-OH 1, IAEA-OH 4 and GISP for liquid samples [14, 45, 46].

Compound	$\delta^{18}O$ *vs.*	$1\sigma^{\mathbf{a}}$	$n^{\mathbf{b}}$	Calibration

		VSMOW-SLAP			parameters[c]
Vanillin Merck	solid	10.37 mUr	< 0.2 mUr	5	$y= 1.02 x + 1.12$
Vanillin Sigma-Aldrich	solid	2.94 mUr	< 0.2 mUr	5	
Vanillin ABCR	solid	-2.85 mUr	< 0.2 mUr	5	
MTBE 2a	liquid	11.91 mUr	< 0.3 mUr	6	$y= 1.01 x - 1.12$
MTBE 3	liquid	-5.38 mUr	< 0.3 mUr	6	
MTBE 4	liquid	1.89 mUr	< 0.3 mUr	6	
EtAc	liquid	21.5 mUr	< 0.7 mUr	6	$y= 0.95 x - 0.81$
ETBE	liquid	25.1 mUr	< 0.3 mUr	3	$y= 1.01 x - 1.12$

[a] standard deviation [b] number of replicates. [c] two point linear normalization following the linear equation $y = mx+n$, with m being the slope and n the intercept of the regression (after Paul *et al.* [50]).

3. Results and Discussion

3.1 HTC by-product formation

By means of the IonTrap organic mass spectrometer the composition of the HTC conversion products can be online monitored and via integration (Xcalibur 1.4 software) even quantified. In this study the by-products are given in % related to the produced amount of the desired main product carbon monoxide (CO) peak area (relative abundance Σ*m/z* 28-30 [nA s]). For compounds consisting of carbon, hydrogen, and oxygen expected HTC products are CO, carbon dioxide (CO_2),water (H_2O) and small recombination products consisting of carbon and hydrogen (C_xH_y) which were shown to be produced upon HTC of organic compounds in an empty ceramic reactor [36]. Other heteroatom (e.g. nitrogen) bearing organic compounds have been omitted at this point as there are interfering by-products (e.g. N_2 *m/z* 28) expected which would need extra separation after HTC or would disturb the measurement in the IRMS (NO formation *m/z* 29).

Organic by-products. Using an empty ceramic reactor, observations of Renpenning *et al.* (2015) concerning the amount, temperature, reaction time (carrier flow), and available hydrogen dependent formation of C_xH_y by-products were confirmed upon MTBE and pentane injection [36]. Those by-products are ionised in the mass spectrometer resulting in a set of masses around *m/z* 24-29, thus interfering with isotope ratio measurements of CO (*m/z* 28-30). Taking the peak area produced by the organic by-products (Σ*m/z* 24-27) in relation to that of the obtained CO as an indicator for quantification up to 25 % of these by-products were measured under common HTC conditions (1330°C, carrier flow 1.2 mL/min, 8.4 nmol MTBE) in an empty ceramic reactor. Concomitant with organic by-product formation the standard deviation of at least three consecutive injections increased to more than 4 mUr and thus confirms that fragments of the formed organic molecules do interfere with the ion traces used for oxygen stable isotope analysis. In contrast, within tube in tube reactors, where a platinum tube prevents sample contact with the Al_2O_3 ceramic material no (< 1%) formation of organic by-products was observed. This holds true also for the Thermo Fisher HTC reactor for oxygen CSIA, which means that organic by-products are not an interference of concern for oxygen HTC with this reactor type. *(For more details on organic by-product formation in the different reactors see SI C)*

Water formation. There were no major differences observed with respect to the amount of water formed as by-product in situ during sample HTC in all investigated reactors. However, significant permanent water background signals (*m/z* 18 >2000 mV) were observed for Thermo Fischer HTC reactors which had been stored over long times before conditioning or reduction which indicates oxygen detainment at some reactor parts and is discussed in detail below. These high water backgrounds slowly decreased after 30 minutes of reduction with pure hydrogen in the backflush mode or repeated conditioning with hexane. Water backgrounds were thus taken as one measure for the conditions in the reactors. *(For more details on water formation and backgrounds see SI C)*

Carbon dioxide (CO_2). The by-product with the most variability quantified in this study was CO_2. Within the empty ceramic reactors irrespective of carrier gas flow or amount of oxygen containing organic compounds on column CO_2 amounts of less than 1 % - compared to the produced CO amount - could be determined, thus in an empty ceramic tube the only oxygen bearing organic product is the desired CO. In contrast, tube in tube reactors, with inner platinum tubes only, showed high and varying CO_2 production, confirming observations from Zech and Glaser (2009): For a custom made tube in tube reactor this resulted in the best cases around 20 % CO_2 by-product compared to the produced CO amount of a MTBE sample analysed with the IonTrap MS.

Using an IRMS for quantification is less accurate, as CO and CO_2 ion traces have to be recorded from two injections and determined peak areas (*m/z* 28-30 and *m/z* 44-46; integration via Origin software (Origin Lab Cooperation, USA)), however, it will generate a rough assessment of how much CO_2 is produced. The IRMS comparison of the modified Thermo Fisher HTC reactor without nickel wires resulted in amounts of around 5 % CO_2. Still, a significant fraction of sample oxygen that is not available for oxygen stable isotope determination at CO. Those observations imply that only platinum present - in contrast to the reactor with platinum and nickel - seems to favour the reaction by-product CO_2 *(For an elaborate discussion on possible HTC processes see SI D)*

The commercial Thermo Fisher HTC reactor developed for oxygen CSIA showed a range of CO_2 production from 1-20 % strongly depending on the reductive conditions in the reactor (Figure 1). If freshly conditioned and reductive atmosphere, either maintained by hydrogen make-up gas or due to only small oxygen on column additions, the CO_2 production was around 2 % (Figure 1). This small and acceptable CO_2 production was independent of the amount of oxygen on column for a wide range; however, higher loads of oxygen on column resulted in higher fractions of by-product formation (e.g. 7 % upon 42 nmol O). Therefrom the CO_2 by-product formation has to be tested to determine the restrictions of a method also in the upper concentration range. The influence of CO_2 production on the isotope value reflects in strong amount dependencies, respective a low linear range. Low linearity associated with higher standard deviations is a problem for measurements of laboratory or field samples. These findings imply that changes within the reactor take place and might occur unnoticed if the CO_2 production is not monitored in the course of oxygen CSIA with the Thermo

Fisher HTC for oxygen reactor. *(For tables and figures on CO_2 formation in the different reactors see SI C)*

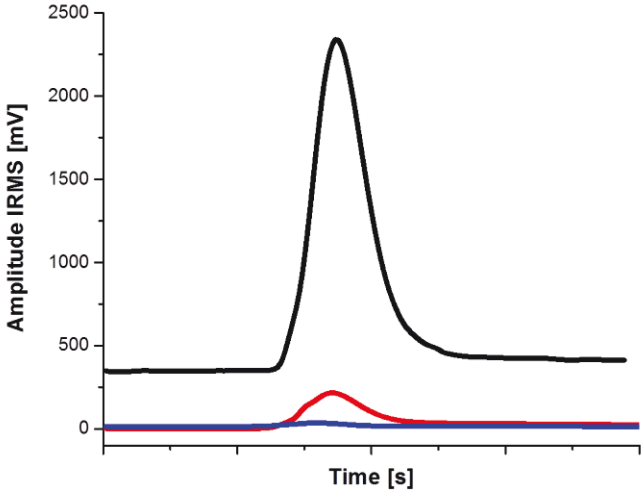


Figure 1. CO_2 by-product formation in the Thermo Fisher HTC reactor for oxygen CSIA. Upon conversion of 15 nmol oxygen on column from MTBE the main product is CO (black line). A major by-product is CO_2 (related to the amount of CO, quantification by IRMS): before conditioning (red line, 16 %) and after conditioning (blue line, 2 %).

3.2 Oxygen from other sources

On the one hand oxygen CSIA methods should quantitatively produce CO from oxygen bearing compounds; on the other hand a compound not containing oxygen (blank sample, e.g. an alkane) should also not yield CO after conversion. Notably, CO production from alkanes was reported previously and explained as ‘carbon surplus effect’ [23, 24], mobilising oxygen in the reactor. In this study distinct peaks upon alkane injection were observed for all tested reactors, albeit in differing extents. One reason for previously reported and also in this study observed $\delta^{18}O$ instability and poor reproducibility could be contamination from non-analyte oxygen sources. It is therefore necessary to determine whether the HTC-process of CO formation is being affected by an additional oxygen source.

Thus, we hypothesise that there are possible other oxygen sources besides the desired analyte which could contribute to the measured CO isotope composition of a sample. Consequently, interfering oxygen sources need to be at first identified, at second quantified, and at third eliminated, if possible. Possible sources are oxygen impurities in the carrier gas (i.e. from a leak), oxygen arising from the reactor materials, and retained oxygen from former samples. The latter case should be obvious in so called 'memory effects', defined as $\delta^{18}O$ shifts according to the value of the previously measured sample, and were not observed upon injection of samples in neither the empty ceramic tube, nor the Thermo Fisher HTC reactor, where expected $\delta^{18}O$ differences were almost met by the obtained raw values. Hence, retained oxygen exclusively from the previous sample is rather unlikely or to such a minor extend that non detectable as memory. Additionally, MTBE ($C_5H_{12}O$) contains a molecular excess of carbon available for reaction with the oxygen in the molecule.

Background oxygen from the carrier gas as a possible source was minimised by tightening joints, and frequent monitoring of the argon background (*m/z* 40 <100mV). Such a small but constant additional oxygen source would rather contribute to a permanent CO background assuming that surplus carbon is deposited in the reactor after conditioning and sample runs. In contrast, injection of organics without oxygen (hexane, pentane) could provide '*in situ'* carbon during conversion which could be more active than carbon already deposited during conditioning probably in cooler parts of the reactor. The empty ceramic reactor produced defined peaks upon pentane and hexane conversion, corresponding to about 5 % of the CO amount produced from an equivalent 8 nmol MTBE injection on column. This is a strong indication that the carbon which is produced '*in situ'* can activate oxygen present in the reactor and makes the existence of an additional oxygen source likely.

Within the Thermo Fisher HTC reactor for oxygen CSIA the extent of the alkane *m/z* 28-30 peak varied, corresponding to 0.5% - 10 % of the equivalent MTBE area. The variation in the Thermo Fisher HTC reactor for oxygen indicates that conditions for measurements without an additional oxygen source are obtainable, however seem to be unstable. In cases of 0.5 % the influence on the $\delta^{18}O$ is negligible in cases with higher percentages one would expect an additional oxygen source to contribute to $\delta^{18}O$ results of CSIA. *(For further information on memory and alkane conversion see SI E)*

Ceramic reactor material as a second oxygen source. An empty ceramic reactor consists of a ceramic (Al_2O_3) tube and thus leaves only oxygen either enclosed in or dissolved away from the ceramic lattice to react with active '*in situ'* carbon at high temperatures [12, 13, 52]. An additional finding supporting this hypothesis is that the measured $\delta^{18}O$ differences ($\Delta\delta^{18}O$) of e.g. two MTBEs in an empty ceramic reactor varies with the reactor temperature and almost vanishes at 1420°C (data not shown), which could be explained by a second source. The reaction of carbon mobilising oxygen from the ceramic would be expected to increase with higher temperatures. This should result not only in higher background values, but alsoin higher CO contributions, which superimpose CO formed by the sample conversion in the empty ceramic reactor.

To verify the hypothesised oxygen contribution from the ceramic reactor material, raw $\delta^{18}O$ values of vanillin and MTBE which had been calibrated to obtain $\delta^{18}O$ *vs*. VSMOW-SLAP (Table 1) were subjected to analysis of oxygen sources in the reactor system. The measured sample isotope composition was therefore correlated to the known isotope composition of the sample and the unknown contribution via 2nd source calculated. *(For a step by step 2nd source calculation see SI D)* With this calculations we present an feasible approach to detect 2nd contributions only on the basis of obtained raw (GC-IRMS) and reference (EA-IRMS) isotope signatures. A set of compounds analysed will result in a set of functions plotting hypothetical $\delta^{18}O$ values of a 2nd source against the possible CO fraction the 2nd source could contribute (0=no contribution, CO only from analyte; 1= only CO from the 2nd source), both obtained by the 2nd source calculation.

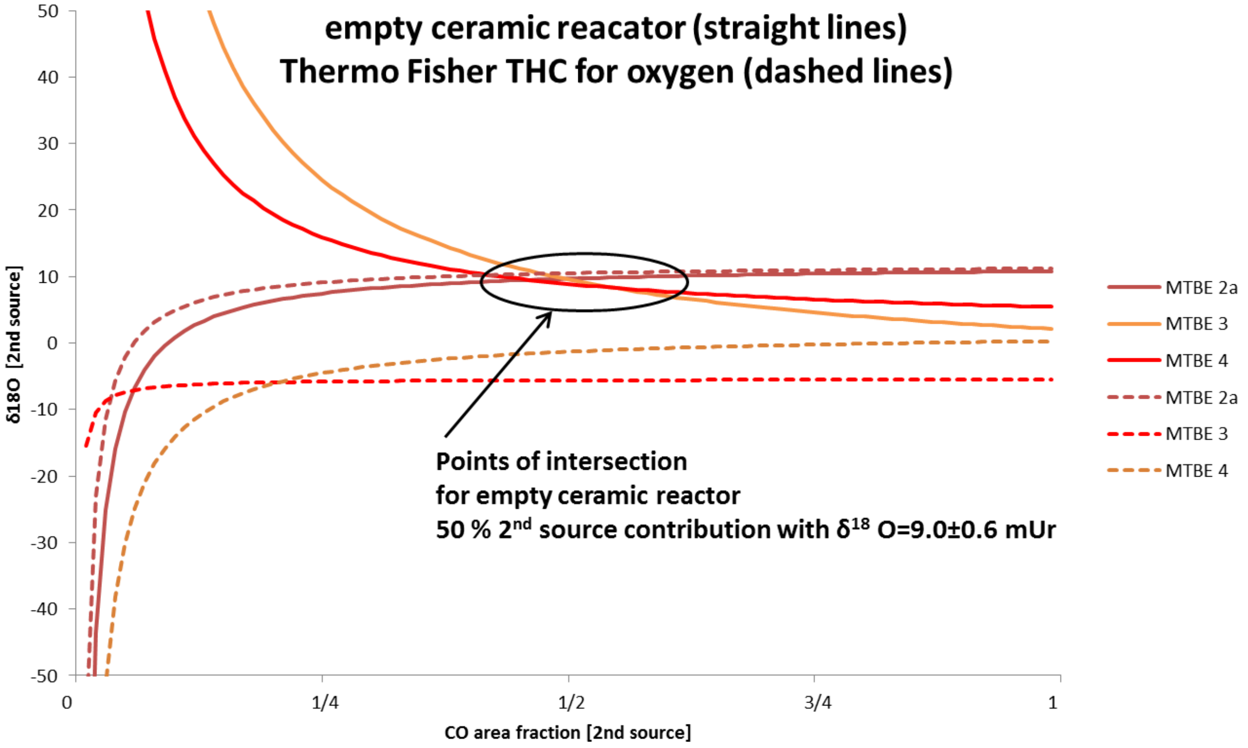


Figure 2. 2nd source calculation results for three MTBE (Table 1) for the empty ceramic reactor (straight lines) and the Thermo Fisher HTC for oxygen reactor (dashed lines). For detailed explanation on calculation and interpretation see SI E.

For an empty ceramic reactor it can be shown, that a second oxygen source is probable as the obtained raw $\delta^{18}O$ value of MTBE (C/O=5/1) can be explained with a fraction of 50 % 2nd source contribution and a $\delta^{18}O$ values of this 2nd source of 9.0±0.6 mUr (Figure 2). At the same time a fraction of 30 % 2nd source contribution is obtained for vanillin (C/O=8/3) which would correspond to the amount of surplus active carbon possibly formed during HTC of the two compounds (Table SI E1). In an empty ceramic reactor, even though very symmetrical peaks are produced at low background signals and reasonable $\Delta\delta^{18}O$ are obtained, a 2nd source contribution is present which will be easily overseen if correction measures, e.g. a two point calibration, are applied. These findings for the empty ceramic tube reactor confirm previous findings from EA-HTC, where an inner glassy carbon tube is needed to prevent sample contact with the outer ceramic tube [10].

Using a platinum tube in a ceramic tube to supress the contact of surplus carbon from the sample with the ceramic material, like in the custom made tube in tube reactor is a similar approach. However, it is obvious from the 2nd source calculation procedure and obtained CO peak pairs and shapes that this approach of manually sliding the platinum tube into the ceramic tube does not succeed.

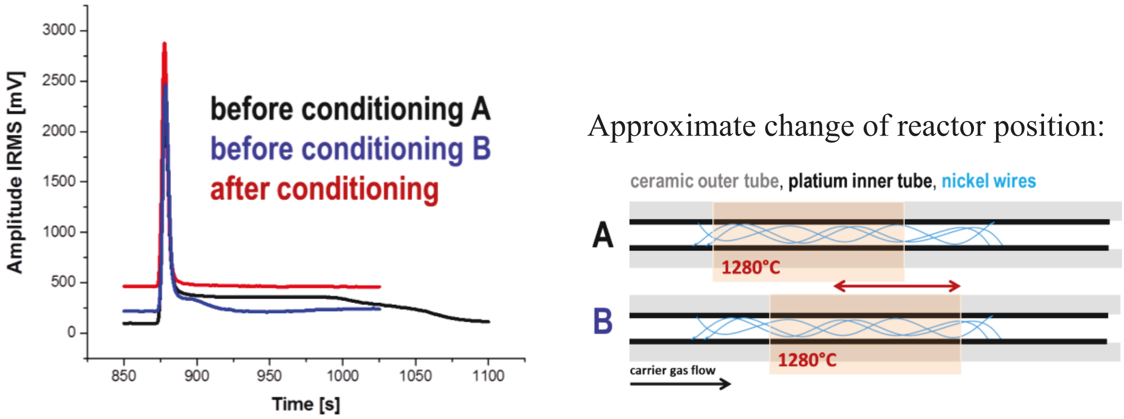
In contrast, the commercially manufactured Thermo Fisher HTC for oxygen reactor seems to efficiently prevent sample and outer ceramic contact. For the Thermo Fisher HTC for oxygen reactor there is no clear point of intersection for the obtained functions, which indicates that there is no common contribution of a 2nd source altering obtained raw $\delta^{18}O$ values (Figure 2). *(For detailed results of the 2nd source calculation for the different reactors see SI E)*

With the established 2nd source calculation it was possible to visualise and approximately quantify a possible 2nd source contribution as well as to rule out its presence.

Nickel restraining oxygen. Even though with the Thermo Fisher HTC for oxygen reactor no 2nd source contributions from the ceramic are detectable, peak shapes indicate that the CO produced originates not only from the sample (Figure 3). Especially before conditioning the CO peak shows a stretched shoulder (Figure 3). The extent of this shoulder varies with the position of the reactor in the heating device, respectively the temperature gradient of the reactor filling (nickel wires) due to their position in the hottest (1280 °C) zone of the heating device. Within the reactor there are 15 cm long nickel wires, however not covering the full length of the reactor and ending approximately 7 cm after the hottest zone. Moving the reactor into the heating element to the position furthest into the GC (against flow direction), therewith shortening the length of nickel wires after the hottest zone resulted in a less stretched shoulder (Figure 3). This change in peak shape supposes the nickel wires to interfere with the CO formation if present after the hottest zone of HTC, leading to the hypothesis that nickel wires might release additionally CO which is for whatever reason temporally delayed. The role of nickel as a reactor constituent could not be fully elucidated; however, it seems to provide surplus carbon needed to prevent CO_2 production, which occurred in all platinum-only containing reactors without nickel, as described above. Again, the most probable explanation is that oxygen reacts with the nickel wires and is released upon active carbon formation during sample HTC. This would explain the small but still present CO peaks derived from alkanes (‘carbon surplus effect’) and their variation, as well as the peak shoulder and change in shoulder upon positioning the nickel wires to a greater extend into the hottest zone (Figure 3). Furthermore after conditioning of the reactor with hexane (surplus carbon deposition) the overall CO background is raised (from *m/z* 28 <200 mV to >500 mV), thereby diluting the shoulder CO contribution and making it less recognisable for the user of a method (Figure 3). Nickel itself, as reactor constituent able to retain oxygen, is a risk in oxygen CSIA. Those findings underline the importance of possibly oxygen free HTC conditions and frequent conditioning as well as reduction with hydrogen. As leakage and positioning of the nickel wires was shown to be very critical, the treatment or storage at the manufacturer and vendor of

HTC reactors might be crucial as well.*(For detailed considerations about reactions of nickel during HTC see SI F)*

Figure 3. CO peak shape (left, *m/z* 28) of a MTBE injection (15 mmol O on column) after conversion in the Thermo Fisher HTC reactor for oxygen CSIA. Before conditioning and installed as suggested (black, A) and with the reactor moved as far as possible into the GC, thus the nickel wires are possibly positioned to a greater extent in the hottest zone (blue, B)(see right scheme). After conditioning a peak without shoulder but at high CO background is obtained (red). *(For detailed reactor assembly and heating device specifications also see Figure SI F1)*



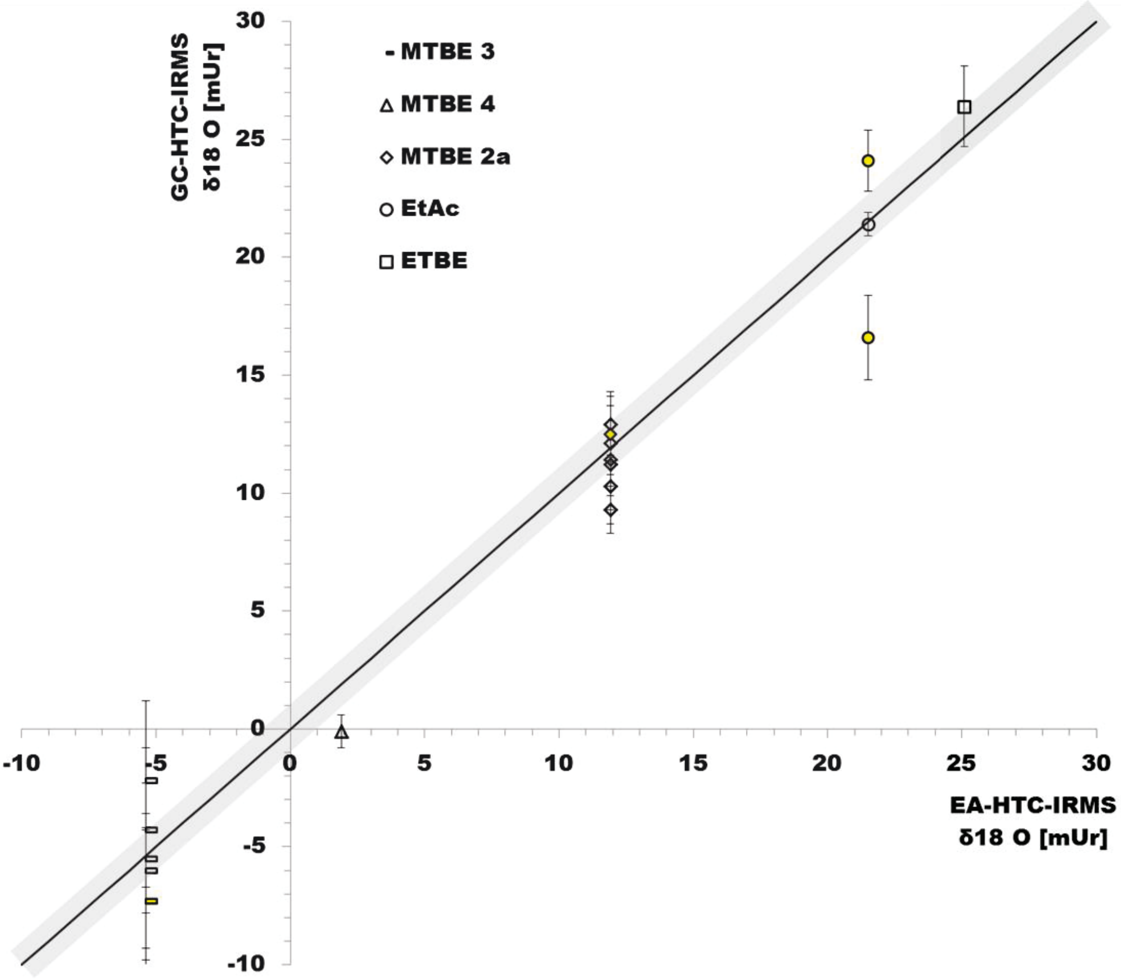
3.3 Analysis conditions

Conditioning and reduction. The above discussed findings indicate that an overall reduced and freshly conditioned system is an essential prerequisite for accurate and precise measurements. Similar requirements were found for hydrogen GC-HTC-IRMS [40]. Reduction of possibly formed nickel oxide (NiO) can be either achieved via pure hydrogen flushed in the backflush mode (at 600°C, 30 min) or via injection of alkanes (hexane 1µL, Split 1:20). To surely obtain reduced conditions, in this study both measures were combined and repeated until the water background in the online mode was below 2000 mV and peak shapes showed no shoulder (Figure 1). Recently, Zech *et al.* (2013) reported improvements using frequent conditioning with respect to the similarity of raw GC-HTC-IRMS to EA-HTC-IRMS $\delta^{18}O$ values, without further elucidating underlying processes [24]. Nonetheless, they were not able to find optimised conditioning parameters which would supersede drift, amount dependency, and post-calibration of the obtained results [24]. Our observations indicate that the reduction state of the reactor will also be reflected in linearity performance, accuracy, and precision of the measurements, which could be determined and compared by analysing compounds of known $\delta^{18}O$ (EA-HTC-IRMS) signatures. Conditioning had to be repeated frequently during measurements. A lack of surplus carbon was indicated by a steady decrease of the CO background (*m/z* 28, 29, 30), appearance of peak shoulders, and the shift of obtained $\delta^{18}O$ signatures towards more negative values. Still, the conditioning did only last for several measurements and due to falling background values often no stability was obtained or results showed clear trends. Conditioning effect even faded away and background decreased steadily if no measurements were undertaken in the backflush mode (e.g. overnight), which indicates that the active carbon deposited is either reacted completely to CO, not confined to the reactive zone anymore or deactivated due to sintering processes [40]. Also standard deviations of consecutive measurements increase from 0.3 mUr to 3 mUr and more. This underlines the importance of determining the optimal means and intervals of conditioning for each analyte and to publish them along with methods parameters to assure comparable oxygen stable isotope analysis. *(For further illustration of conditioning effect and frequency see SI G)*

Accuracy. Due to the lack of stable phases after conditioning, obtained $\delta^{18}O$ raw values were highly dependent on the background and distance to last conditioning (Figure SI F1). Only by the comparison with a lab reference optimal conditions could be assessed, the consequent application of identical treatment [53] should be complemented by the proof of quantitative conversion to the analyte gas. These conditions are hardly to be reached, and are only lasting for a couple of injections showing the fragility of the system. In spite of this instability the results also show that in principle it is possible to reproduce reference $\delta^{18}O$ signatures for the tested volatile compounds (Figure 4). If raw data is evaluated measurement by measurement taking precision, background, peak shape and alternating reference compounds into account the Thermo Fisher HTC for oxygen reactor possibly can be used to determine $\delta^{18}O$ of unknown MTBE in headspace (Figure 4). Nevertheless this data selection is highly subjective, biased and precisions up to 2 mUr deviation have to be accepted. This emphasises the need for structurally similar reference material to be analysed along with samples, as practiced by some successful applications of the Thermo Fisher HTC for oxygen reactor [23, 24].

Furthermore, accurate MTBE isotope signature findings are true for single compound headspace injections only. For mixtures, like MTBE and EtAc (Figure 4, yellow marked), standard deviations of the later eluting substance (EtAc) were even higher and $\delta^{18}O$ less precise, possibly due to disturbance of the conditions due to MTBE conversion directly before EtAc HTC. To what extend this effect influences $\delta^{18}O$ results obtained for complex, environmental samples with additional matrix effects, and peaks eluting very closely needs to be investigated during method development and should be considered when discussing stable oxygen isotope ratios determined via GC-HTC-IRMS.

Figure 4. GC-HTC-IRMS results for different volatile compounds analysed with the Thermo Fisher HTC for oxygen reactor within 10 days (17th to 26th August 2015), plotted against $\delta^{18}O$ EA-HTC-IRMS results. The black line indicates the ideal line of $\delta^{18}O$ values differing not between GC and EA (gray range ±1 mUr). Yellow filled symbols are results from mixed samples (MTBE and EtAc).



4. Conclusions

4.1 Possible interferences with GC-HTC-IRMS $\delta^{18}O$ analysis

This study identifies carbon dioxide (CO_2) by-product formation as the major interference hampering accurate and precise $\delta^{18}O$ CSIA via GC-HTC-IRMS. Furthermore it could be shown that the effective separation of sample stream and outer ceramic tubing is essential to avoid a 2nd CO source contribution from GC-HTC reactors. In the commercially available Thermo Fisher HTC for oxygen reactor this separation is successfully achieved by a platinum tube. However the used inner nickel wires bear the risk of nickel oxide formation within the reactor. The for oxygen analysis needed reduced atmosphere during HTC is impeded by the presence of nickel oxide though. Using the example of volatile organic compounds like MTBE, the strong dependence of accurate $\delta^{18}O$ signatures upon optimal reduced HTC conditions and frequent conditioning of the reactor was revealed. The observed instability and unpredictable performance of the Thermo Fisher HTC for oxygen reactor limit its application and illustrate its complicate use for $\delta^{18}O$ GC-HTC-IRMS in environmental and health studies.

4.2 GC-HTC-IRMS evaluation strategy

Method development for oxygen CSIA with GC-HTC-IRMS, should - besides general CSIA method parameters - include an intense effort to evaluate HTC conditions and processes present. In this study several considerable processes were investigated and approaches for their monitoring are presented. Even though probably not exhaustive the following parameters are needed to judge GC-HTC-IRMS performance of a reactor and method:

- **HTC by-products.** Especially the formation of CO_2 as product detracting oxygen from the desired analyte gas CO needs to be below 2 %.
- **Water background.** It could be shown that water formation as a by-product is not that important than the overall water background generated by a reactor. High (>2000mV, *m/z* 18), increasing water backgrounds indicate that the reactor is not in the reduced state and needs reduction. The formation of nickel oxide in the reactor plays an important role with respect accuracy and precision of obtained $\delta^{18}O$ signatures.
- **2nd sources of oxygen.** It could be shown that the ceramic material of GC-HTC reactors can contribute to the overall CO production. A successful calculation procedure to detect such 2nd sources was developed and should be applied prior

to analysis with a new reactor. This visualisation (Figure 2) can be easily implemented also for other stable isotope measurements. Particular attention should also be paid to peak shapes and CO formation upon injection of oxygen free alkanes to assess performance and condition of the reactor used.

- **Conditioning.** The prerequisite for GC-HTC-IRMS analysis, also of substances with surplus molecular carbon, is freshly deposited carbon in the reaction zone and strictly reductive conditions. By means of frequent conditioning e.g. with hexane (1 µL, 1:20), reference values from EA-HTC-IRMS could be reproduced and reasonable precision and amount dependency obtained. Thus, conditioning protocols and recommendations should be included in method reporting for comparability.
- **Data selection criteria.** Based on the presented findings from volatile organic compounds like MTBE, accuracy and precision are only acceptable for some injections of a repeatedly analysed sample due to instabilities and very short time intervals of optimal HTC conditions. If an experienced user selects raw data according to e.g. peak shape, background intensity, reference measurement or other criteria, it is still possible to obtain accurate $\delta^{18}O$ results (Figure 4). Hence applied criteria after which raw data is selected and obtained results revised, need to be disclosed to allow quality assessment published $\delta^{18}O$ by the community.
- **Adaptability of methods to other compounds.** From the presented experience with volatile organic compounds in contrast to vanillin it is strongly recommended to repeat all evaluation steps for a new analyte, as chemical structure and oxygen content seem to influence HTC processes.

4.3 Outlook

To our best knowledge we provide in this work the most comprehensive evaluation of the commercially available Thermo Fisher HTC for oxygen reactor for of a $\delta^{18}O$ GC-HTC CSIA. It could be shown that under optimal conditioning conditions the complete conversion of volatile organic compounds to carbon monoxide is achieved with this reactor. However, severe drawbacks and instabilities were revealed, which up to now prevent its reliable routine-use for pure headspace samples of volatile compounds like MTBE, ETBE and EtAc.

In contrast to the presented results, there is a need for oxygen CSIA via GC-HTC-IRMS to answer research questions with three dimensional stable isotope patterns. Taking into account, that samples for research usually contain several compounds, matrix effects, and possibly compounds with other heteroatoms additionally making analysis difficult, the authors doubt the use of the present methods and reactors for routine applications. Nevertheless, despite limitations, there is currently no alternative to this reactor as an initial approach to new compound-specific oxygen stable isotope analysis.

The evaluation approaches presented here are required for quality control during method development and reactor testing. In addition new reactor concepts need further investigation and elucidation of GC-HTC processes for a better understanding of the exploited HTC process.

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Supporting Information

A - Vanillin measurements; B - Overview to all reactor types used; C - By-product formation; D – HTC processes; E - Evaluating 2nd oxygen sources; F – Nickel as a reactor constituent; G - Analysis conditions

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Supporting Information

Evaluation of the Performance of High Temperature Conversion (HTC) Reactors for Compound-specific Oxygen Stable Isotope Analysis

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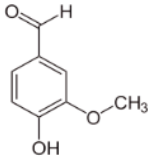
Supporting Information

Evaluation of the Performance of High Temperature Conversion (HTC) Reactors for Compound-specific Oxygen Stable Isotope Analysis

Content:

A - Vanillin measurements Thermo Fisher HTC for oxygen reactor [1, 2]



Vanillin (4-hxdroxy-3-methoxybenzaldehyde, $C_8H_8O_3$) is a white solid at room temperature but amenable to GC-HTC-IRMS and EA-IRMS analysis and was thus used as a laboratory standard for the $\delta^{18}O$ determination. The reactor was freshly conditioned and vanillin was measured over 60 times to monitor stability and reproducibility (Figure SI A1). The reproducibility of the vanillin measurements (n=3) was better than 1 mUr. The ability to differentiate between the three different vanillin samples based on their oxygen stable isotope composition ($\Delta\delta$) was well-defined, with average differences of >5 mUr (Figure SI A1). This $\Delta\delta$ allows for two-point calibration, via two of the three vanillin samples, to correct for drifts. Two vanillin samples (Merck and ABCR) were therefore used to determine the calibration parameters and were measured preceding and succeeding the third vanillin sample, for which the $\delta^{18}O$ vs. VSMOW-SLAP was calculated. The calibrated value was in agreement with the value determined by EA-HTC (Figure SI A2). The standard deviation of all calibrated values (n=10) was 0.2 mUr, which was quite satisfactory given the long measurement period. The calibration parameters were a slope of 1.1 and an intercept of -0.5 (Figure SI A2). The raw $\delta^{18}O$ values of vanillin from Sigma vs. the reference gas measured before calibration were very similar (3.52 ± 0.3 mUr) to the $\delta^{18}O$ values determined by EA-HTC-IRMS (2.94 ± 0.2 mUr)(Figure SI A2, open triangle). The calibration only slightly (to 3.25 ± 0.2 mUr) corrects the results (Figure SI A2, open circle). This means that the Thermo Fisher oxygen-HTC reactor, which was specifically designed for oxygen analysis, did indeed perform very well for vanillin analysis. The dependence of the isotope signal on the concentration of vanillin was investigated with a linearity test. Concentrations were adjusted by split variation upon vanillin injection with resulting signal intensity (*m/z* 28) from 0.1 to 7.6 V, being equivalent to 8-240 nmol of oxygen for conversion.

Figure SI A1. Raw δ^{18}O *vs. VSMOW-SLAP* (vs. reference gas,) average isotope value for the long term measurements of vanillin laboratory standards with the Thermo Fisher oxygen-HTC reactor (n=3 replicates).

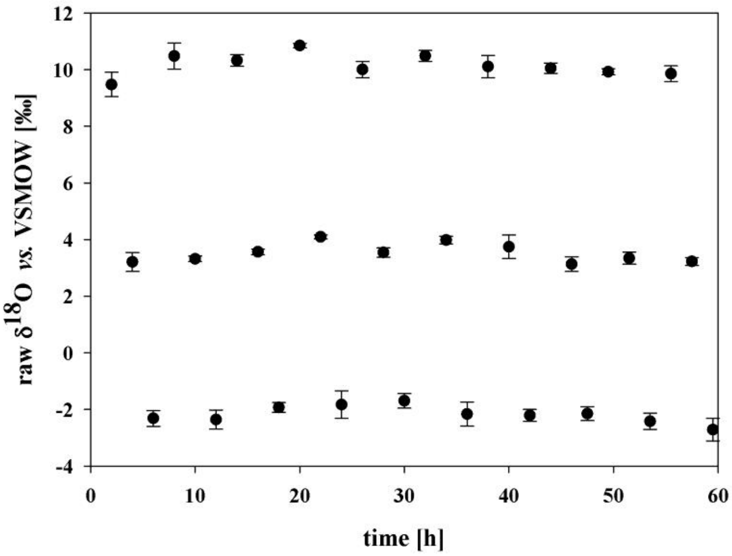


Figure SI A2. The δ^{18}O isotope composition of vanillin (Sigma) vs. VSMOW-SLAP. Calibrated values over time (filled circle) and their average (open circle) are compared to the EA-HTC-IRMS average (filled triangle) and the raw (vs. reference gas, open triangle) average isotope composition (figure SI A1).

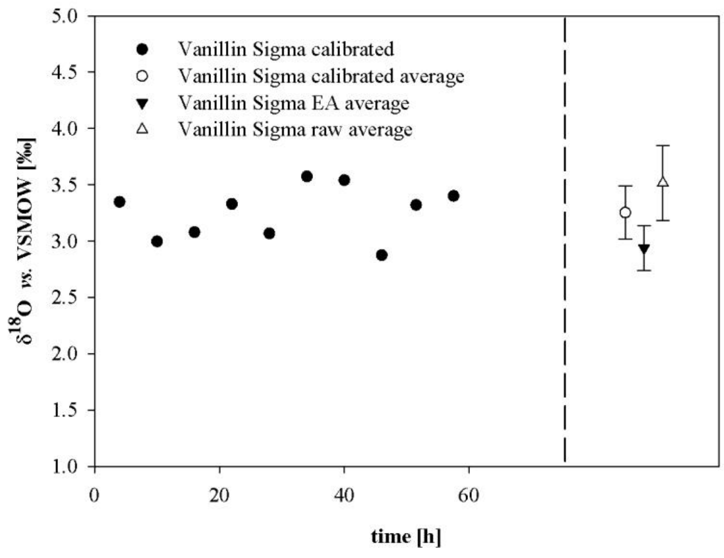
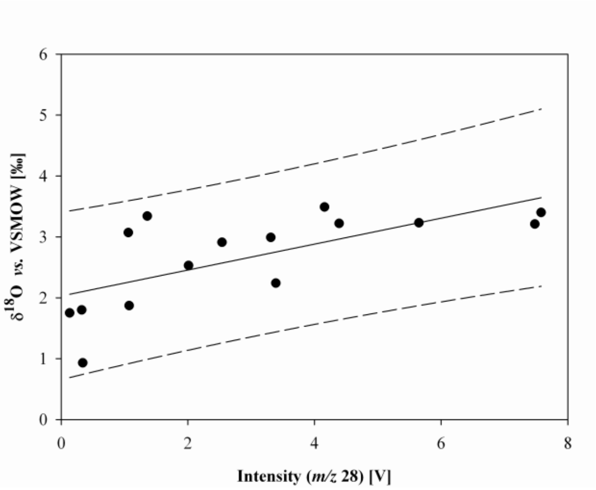


Figure SI A3. Dependency of vanillin oxygen isotope composition on the concentration. δ^{18}O plotted vs. the signal intensity (*m/z* 28) to obtain the regression curve (black line) and 95 % confidence interval (dashed line). The linearity derived from the linear regression was 0.21 mUr $*V^{-1}$.



B - Overview to all reactor types used for investigations in the course of the presented study.

Designation in main manuscript	Reactor design	°C	Conditioning	Manufacturer/Vendor	Specifications
Thermo Fisher HTC for oxygen	Platinum tube in ceramic tube, nickel wires	1280	1µL octane or hexane, 1:20	part no. 1149050, Thermo Fisher Scientific, Bremen, Germany [2]	ceramic: 320 mm, 0.8 mm i.d., 1.55 mm o.d.; platinum tube: 0.8 mm o.d.; nickel wires: 150 mm, 0,125 mm i.d.
	Empty ceramic tube	≥1330	Methane 1µL octane or hexane, 1:20	part no. 1255330, Thermo Fisher Scientific, Bremen, Germany	Commercial D/H; ceramic: 320 mm, 0.8 mm i.d.; soldered metal capillary fitting
	Empty ceramic tube	≥1330	Methane 1µL octane or hexane, 1:20	Degussit AL23; Friatec, Mannheim, Germany	320 mm, 0.8 mm i.d., 1.6 mm o.d.
	Platinum tube in ceramic tube – custom made	≥1330	Methane 1µL octane or hexane, 1:20	Pt007130, rigidity: as drawn, Goodfellow, Bad Nauheim, Germany Degussit AL23; Friatec, Mannheim, Germany	Al2O3: 320 mm, 0.8 mm i.d., 1.6 mm o.d. Pt: 310 mm, 0.57 i.d., 0.65 mm o.d.
	Platinum tube in ceramic tube	≥1330	1µL hexane, 1:20	Commercially made Thermo Fisher HTC for oxygen – nickel wires REMOVED	Tight tube in tube

C – By-product formation within the different HTC reactors

Qualitative and quantitative assessment of HTC products is possible with the used instrumental setup (figure SI C1). In this study by-products are given in % related to the amount of produced CO (Σm/z 28-30); Identification and quantification was done via:

- IonTrap MS: identification (mass spectrum), simultaneous monitoring (single ion traces), and quantification (integration (Xcalibur 1.4))
- IRMS: isotope ratio determination, consecutive monitoring (CO, CO_2 and H_2O) via instrumental control and integration (Origin) for quantification

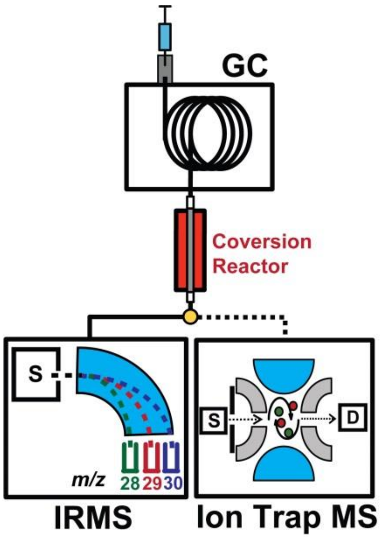


Figure SI C1. Schematic instrumental setup for the parallel analysis of HTC products with IRMS and IonTrap MS as described elsewhere [3].

HTC products in empty ceramic reactors

A low water production and minimal CO_2 formation were observed (Table SI C1). Organic by-product formation (NP; C_2H_x) with empty ceramic reactors confirms amount, flow, and temperature dependency that was first described by Renpenning *et al.* (2015) [3].

Table SI C1. Empty ceramic reactors: By-product assessment at different reactor temperatures, carrier gas flows, and amounts of MTBE on column. By-products are given in % related to the produced amount of CO. NP stands for organic recombination products (C_2H_x, Σ*m/z* 24-27). The standard deviation of at least three consecutive measurements (IRMS) is given as a measure for isotope ratio determination precision.

Flow	Temperature	MTBE on column	H2O/CO	NP/CO	CO2/CO	stdev (n≥3)	
mL/min	°C	nmol O	%	%	%	mUr	
0.8	1330	8.4	5	0.4	0.2	0.7	
0.8	1330	12.6	4	33.4	0.2	0.8	
1.2	1250	4.2	7	103.1	0.7	4.8	
1.2	1250	8.4	6	152.3	0.3	n.d.	
1.2	1330	4.2	7	0.4	0.8	0.7	
1.2	1330	8.4	5	20.9	0.4	1.6	
1.2	1400	4.2	6	0.3	3.7	2.4	
1.2	1400	8.4	4	84.3	2.0	1.0	
1.6	1330	4.2	8	0.3	0.4	0.3	
1.6	1330	6.3	6	7.1	0.3	4.3	Trend
1.6	1330	8.4	5	25.1	0.5	4.0	Trend

HTC products in platinum tube in ceramic tube reactors

A low organic by-product (NP; C_2H_x) formation, but significant CO_2 formation was observed for reactors where a platinum tube was used to separate sample stream and ceramic outer material (Table SI C2). Observed high and varying CO_2 formation impedes oxygen stable isotope determination as no quantitative conversion to CO takes place, thus confirming results for platinum-tube only test in Zech and Glaser (2009)[4].

Table SI C2. Platinum tube in ceramic tube reactors: By-products given in % related to the produced amount of CO. NP stands for organic recombination products (C_2H_x, Σ*m/z* 24-27). Reactors were: (a) custom made platinum tube in ceramic tube (CO_2 quantification IonTrap MS); (b) Thermo Fisher HTC for oxygen reactor **without** nickel wires (CO_2 quantification IRMS).

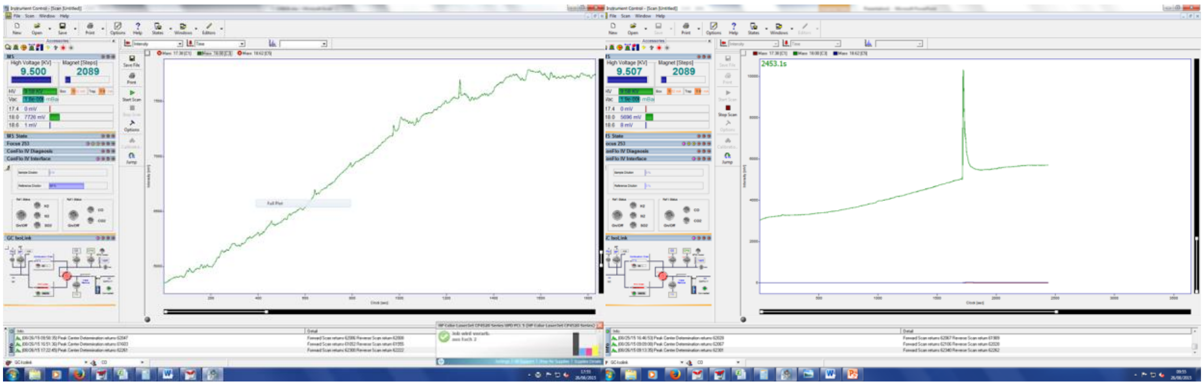
Flow	Temperatu	MTBE on cc	H2O/CO	NP/CO	CO2/CO	stdev (n≥3)	quantification
mL/min	°C	nmol O	%	%	%	mUr	
1.2	1330	4.2	11	0.4	23.9	11.0	IonTrapMS (a)
1.2	1330	7.3	-	-	6.5	0.7	IRMS (b)
1.2	1330	7,7 (EtAc)	-	-	4.7	2.0	IRMS (b)

Water formation during sample HTC

An empty ceramic reactor on average produced 6 % of water in relation to the produced CO (Table SI C1), while a platinum tube in a ceramic tube reactor on average had 11 % of water production (Table SI C2). Similar the water production in the Thermo Fisher HTC reactor ranged from 8-11%, additionally depending on the amount of hydrogen make-up gas fed into reactor (data not shown). This observed water production was already described as a main HTC by-product previously and is thus not surprising.[3] Within this study, parameters reducing water production could not be determined, thus the observed and stable water formation needs to be accepted as a critical factor in the HTC processes. It could be assumed that the water formation is statistical, thus representing the same oxygen stable isotope ratio as the produced CO. In that case, the assumed influence on the obtained $\delta^{18}O/^{16}O$ would be insignificant, which is partly indicated from the raw GC-HTC-IRMS values which agree with the reference results by EA-HTC-IRMS for the Thermo Fisher THC for oxygen reactor (Figures SI A2, E1, F1). However, it is possible that produced water withdraws oxygen affecting the CO isotope composition. Before IRMS analysis water is removed by a membrane water trap implemented in the IsoLink device, thus those water isotope ratios cannot be easily determined to prove one or the other possibility. As a future task the dependence and influence of water by-product formation on accuracy during oxygen GC-HTC-IRMS should be an issue.

Background water formation, in contrast to relatively stable water by-product formation, reached levels using the Thermo Fisher HTC for oxygen reactor, which restrained proper IRMS source functioning (*m/z* 18 >10 V). Reduction of the reactor with pure hydrogen and subsequent conditioning with hexane (Figure SI C1) lowered permanent background water to acceptable levels (*m/z* 18 <2 V), however indicate oxygen detainment at some reactor parts (see also SI E).

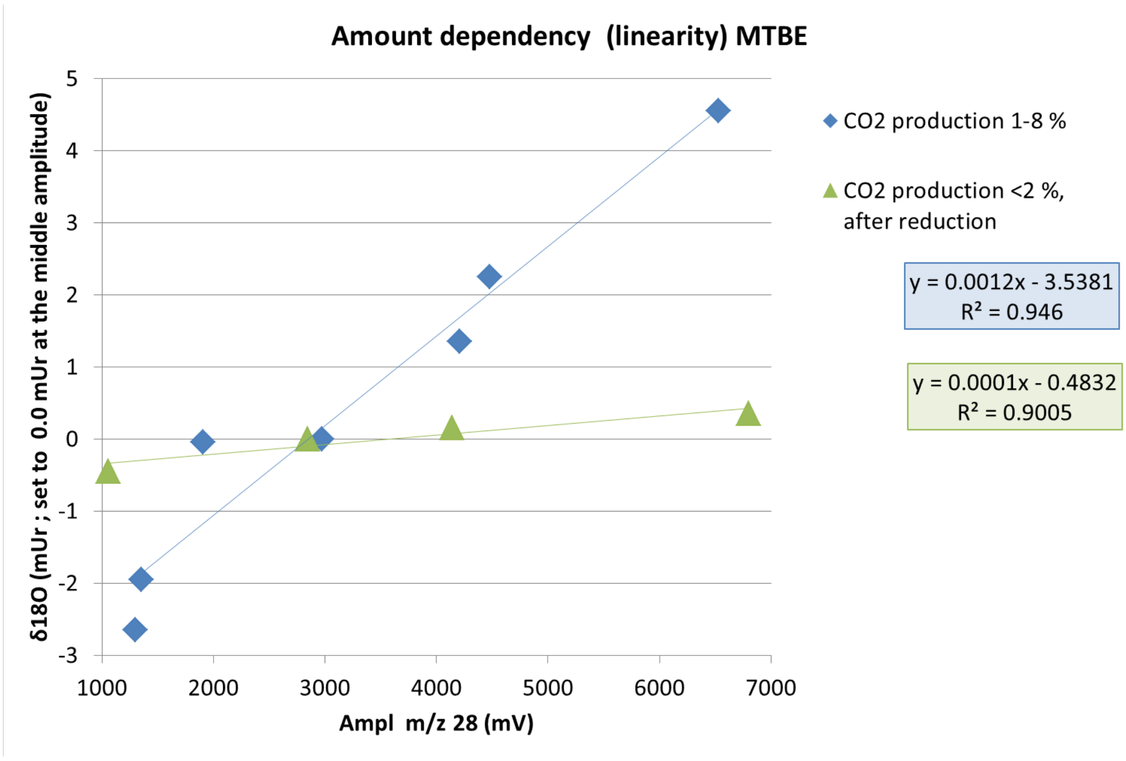
Figure SI C2. Thermo Fisher HTC for oxygen reactor: Water background (IRMS) after a measurement steadily increasing to approximately 7 V signal intensity (*m/z* 18) (left); and water background after 30 min of reduction (hydrogen flushing at 600°C), then heating of the reactor and conditioning (1280 °C, 1µL hexane, split 1:20) (right).



Linearity and CO2 by-product within Thermo Fisher HTC for oxygen reactor

Amount dependency was determined twice before and after reduction of the reactor with pure hydrogen to visualise influence of CO_2 by-product formation on the obtained $\delta^{18}O/^{16}O$ results (Figure SI C2). It can be concluded, that varying and high (>2% with respect to CO) CO_2 amounts preclude reliable oxygen stable isotope analysis with GC-HTC-IRMS.

Figure SI C3. Thermo Fisher HTC for oxygen reactor: Linearity of the CO isotope ratio compositon in the range of 4.2 – 31.5 nmol O on column MTBE. MTBE headspace injections were compared to analyse interference by CO_2 by-product formation before (blue) and after (green) reduction with hydrogen. Isotope values were set to 0.0 mUr for the 12.6 nmol O concentration and CO_2 amounts were quantified by parallel IonTrap MS measurements and are given in % related to the produced CO amounts.



D – HTC processes

Possible HTC reactions

Our model compound MTBE may be converted by several possible reactions leading to the HTC products CO, water, and CO_2. For an approximate comparison of the possible reactions and the respective thermodynamically favoured reactions the free energy (ΔG, Gibbs function) can be determined from the standard molar enthalpy (ΔH at 298 K) and the standard molar entropy (ΔS at 298 K) (Table SI F1). When the initial and the final states of a system have the same temperature the relation $\Delta G=\Delta H-T*\Delta S$ applies for changes in state. Disregarding the temperature dependency of enthalpy and entropy the free energy at different temperatures (650°C, 1280°C, 1400°C) can be calculated using stoichiometric reaction equations and standard molar values from thermodynamic tables (Table SI F1). The extent of negative ΔG indicate the most favoured reaction, which suggest that HTC of MTBE favours the production of CO as oxygen bearing product (Table SI D1 first reaction) compared to the other two reactions at high temperatures; whereas reactions producing CO_2 and water as products are favoured at lower conversion temperatures. This finding is in accordance with experimental HTC studies showing less by-product formation at higher HTC temperatures [3, 5].

Table SI D1. Calculated free energy (ΔG) of possible reactions at different temperatures. Reactions which will rather not take place spontaneously (endergonic; ΔG>0) are indicated in red. Data for calculations were taken as indicated in Table SI F1.

	ΔG=ΔH-T*ΔS [kJ mol^{-1}]		
	650 °C	1280 °C	1400 °C
HTC of MTBE			
MTBE$\rightarrow$CO+6H2+4C	-509	-975	-1064
MTBE$\rightarrow$0.5CO+0.5H2O+5.5H2+4.5C	-513	-937	-1018
MTBE$\rightarrow$0.5CO2+6H2+4.5C	-514	-925	-1003
Boudouard single direction reactions			
0.5CO2+0.5C$\rightarrow$CO	5	-50	-61
0.5CO2+0.5C$\leftarrow$CO	-5	50	61

Influence of the Boudouard equilibrium 2CO$\rightleftharpoons$ C + CO2

The observed possible HTC products CO and CO_2 are known to interact according to the so called Boudouard equilibrium, which is temperature dependent. The equilibrium position at different temperatures and at 1 bar pressure can be found in textbooks and should be completely at the side of CO (>99%) at temperatures above 1000°C [6]. Taking the carrier gas pressure (10.7 psi, ~0.8 bar) as indicator for the pressure present in the GC-HTC reactor, the equilibrium should be still at the side of CO. This assumption is reinforced by free energy calculations (Table SI D1), which indicate that the reaction towards CO at high temperatures is clearly favoured.

The Boudouard equilibrium is furthermore affected by the Le Chatelier principle, which can be used to predict the effect of a change in conditions on a chemical equilibrium. The equilibrium affected by a force like a change in pressure, temperature or concentration of the reaction compounds will react by a change of equilibrium in a way that the force is reduced [6]. This means low temperature and high pressure will shift the equilibrium to the side of CO_2 while high temperature, low pressure and the presence of surplus deposited carbon will favour CO production. The importance of deposited carbon for HTC of organic compounds for GC-HTC-IRMS of hydrogen is known [7], thus it can be assumed that it also is an important factor for oxygen stable isotope analysis. Overall, present knowledge about HTC processes indicates that conditions present in GC-HTC reactors should be able to maintain the quantitative conversion to CO.

Besides the already mentioned factors influencing HTC processes, time for completing reactions and equilibrium (see above) could be a critical factor as continuous flow conditions are determining the residence time of compounds and HTC products in the hot zone of the reactor. Assuming a length of 4 cm for the hot zone (platinum tube inner diameter: 0.57 mm) and neglecting the volume of the nickel wires the volume of the hot reaction zone can be calculated to 0.01 mL. Applying a carrier gas flow of 1.2 ml/min (neglecting the make-up gas flow) the resulting residence time in the hottest zone (hot zone volume divided by carrier gas flow) can be determined to be approximately half a second. This means, that HTC processes and subsequent hypothesised equilibration should take place in less of half a second. For the high temperature conversion time should be less influencing, as this thermal reaction should be only dependent of the temperature and then fast and irreversible. Whether the time in the reactor is sufficient to allow equilibrium establishment of the conversion products is in dispute but rather unlikely. Additionally one can take Boudouard equilibrium constants ($K_p=p^2(CO)/p(CO_2)$) determined electrochemically as an additional measure for the equilibrium position [8]. The obtained equilibrium constants are 5.2 (at 777 °C) and 1048 (at 1180 °C) also indicating the predominance of CO at high temperatures.

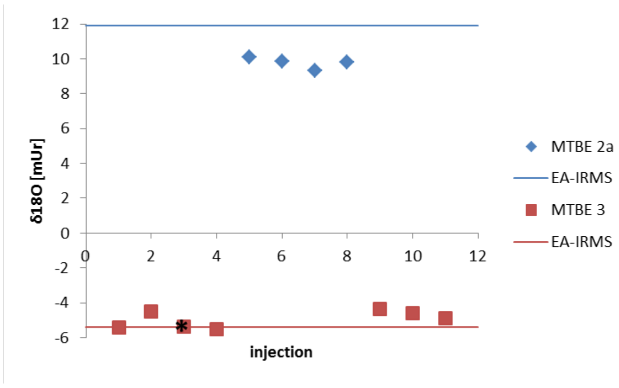
After the hottest zone passage of approximately half a second, the overall conditions will not fulfil HTC criteria anymore with respect to temperature (<1000 °C), thus hypothetically other processes could take place, e.g. the back reaction of CO to CO_2 and carbon according to the Boudouard equilibrium. This reverse reaction is thermodynamically favoured (Table SI D1 last reaction) at lower temperatures (ΔG=-53 kJ mol^{-1} at 100°C) which are present at the end of the reactor. However, CO disproportionation is kinetically hindered, which means that CO is metastable at low temperatures [6]. This also means that no change in product composition is expected in between the end of the reactor and IRMS analysis due to equilibration. Unfortunately one could speculate about an exception: a catalyst could activate CO and thus allow CO_2 formation also under these conditions or a catalysing reaction to CO2 at lower temperatures. Platinum in the cooler zones of the reactor might function as a catalyst and activate disproportionation of CO, which might explain why reactors with platinum inner tube only (Table SI C2) reveal such high CO_2 levels as HTC by-products.

Still it is not clear, what the mechanism is which prevents hypothesised disproportionation of CO in the cooler zones of a reactor additionally containing nickel wires, like the Thermo Fisher HTC for oxygen reactor. The presence of nickel carbide (Ni_3C) and thus the availability of surplus reactive carbon could be crucial, however calculated free energy of this carbon release reaction is small (-7 kJ mol^{-1}, Table SI F2) This reactor was shown to produce low CO_2 by-product fractions if conditioned properly. Here deeper investigation an further understanding of GC-HTC processes in the presence of metal surfaces is needed.

E- Evaluating 2nd oxygen sources

Test for classical memory effect

Figure SI E1. Thermo Fisher HTC for oxygen reactor: Alternating injection of MTBE with different δ^{18}O signatures. The 3rd MTBE peak (asterisk) was set to the EA-HTC-IRMS value (see Table 1) to reference the obtained GC-HTC-IRMS results.



CO production upon alkane HTC

Figure SI E2. Empty ceramic reactors: Chromatogram (*m/z* 28, 29, 30) of pentane (first peak, t_R=501 s) and hexane (second peak, t_R=617 s) after gas chromatographic separation and HTC. The peak areas correspond to 5 % of the amount of CO produced from an equivalent amounts of MTBE based on carbon concentration (quantification with IRMS).

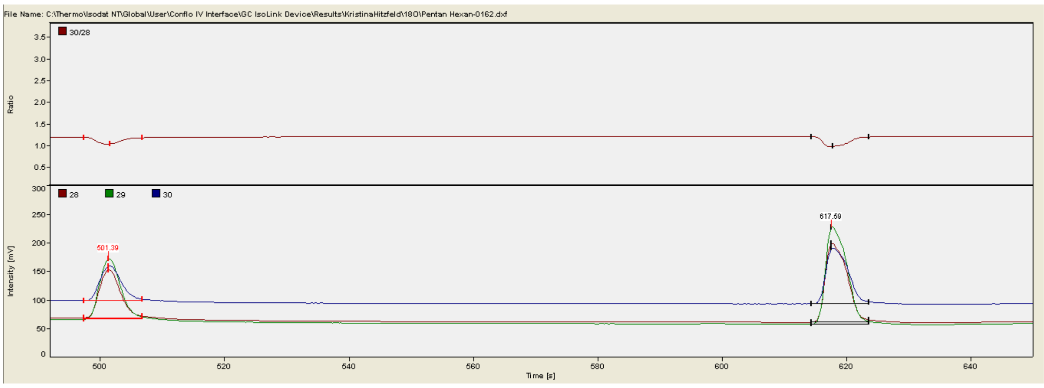
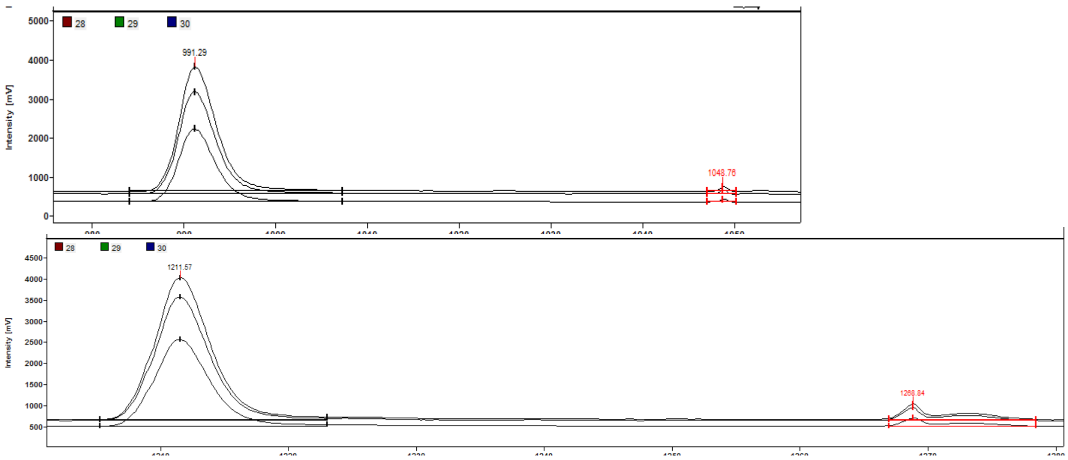


Figure SI E3. Thermo Fisher HTC for oxygen reactor: Different chromatograms of MTBE (first peak) and hexane (second peak) in a mixed injection of equivalent nmol C of each on column. The distinct hexane peak quantifies to approximately 0.5 % (upper panel) and 9 % (lower panel) of the MTBE CO area.



2^{nd} source calculation

To verify the hypothesised oxygen contribution from the ceramic reactor material [9], raw $\delta^{18}O$ values of vanillin and MTBE from GC-HTC-IRMS measurements were correlated to the known isotope composition (EA-HTC-IRMS, Table 1) of the sample to reveal the assumed unknown 2nd source contribution. This 2nd source calculation was derived by modification of the equation usually used for blank correction [10]. The main assumption is that the measured peak area only consists of the CO produced from the sample (1-x) and the contribution of the 2nd source (2S; (x)). Then the measured raw $\delta^{18}O$ value ($\delta_{measured}$) is a combination of the sample $\delta^{18}O$ value (δ_{sample}) and the 2nd source (δ_{2S}) (equation (1)).

	equation	variables
area	CO area measured=1=x+(1-x)	x= CO area fraction from the 2nd source
(1)	$\delta_{measured} = \delta_{2S}*(x) + \delta_{sample}*(1-x)$	$\delta_{measured}$= GC-HTC-IRMS raw $\delta^{18}O$ δ_{sample}= EA-HTC-IRMS
(2)	$\delta_{2S}= (\delta_{measured} - \delta_{sample}*(1-x))/x$	

To elucidate the contribution of the 2nd oxygen source eq. (1) was solved for δ_{2S} (equation (2)) and then plotted for x between 0 (no 2nd source) and 1 (only 2nd source CO). The resulting points of intersection of the different vanillin and MTBE sample curves allow an estimate of the extent (fraction of CO) and $\delta^{18}O$ signature of the 2nd source contribution (Figures SI E4, E6, E7). Assuming that the contribution of the 2nd source is stable for the same compound, the average of the intersection points is a good approximation of the 2nd source contribution (Tables E1, E2). Differences in 2nd source contribution and isotope composition were determined for different reactors (Figures SI E4, E6, E7), but also in the same reactors between vanillin and MTBE (Table SI E1). For an empty ceramic reactor it can be shown, that a second oxygen source is probable as the obtained raw $\delta^{18}O$ values of MTBE and Vanillin are composite isotope signatures and can be explained with a fraction of 30 % and 50 % 2nd source contribution and $\delta^{18}O$ values of this 2nd source of -1.2±0.2 mUr and 9.0±0.6 mUr, respectively (Figure SI E4 and Table SI E1). Moreover, these findings show, that the 2nd source contribution is neither stable with respect to the fraction of CO produced nor to the 2nd source isotopic signature. While the latter difference cannot be explained by our present understanding of the processes, the differing fraction of CO produced from MTBE and Vanillin is explainable. Assuming that 'in situ' carbon is needed to form CO (see above) the carbon to oxygen ratio, and thus the available carbon to react with oxygen from a 2nd source differs. While for MTBE (C:O=5:1) 4 moles carbon per molecule are possibly forming active carbon, for vanillin (C:O=8:3) less than 2 moles carbon are available. This theoretical assumption could explain the higher fraction of 50 % for MTBE versus 30 % for vanillin of CO contribution from the 2nd source.

Using a platinum tube in a ceramic tube to supress the contact of surplus carbon from the sample with the ceramic material, like in the custom made tube in tube reactor is was tested however it is obvious from the 2nd source calculation procedure and obtained CO peak pairs and shapes that this approach does not succeed. The 2nd source calculation for vanillin indicates still a fraction of 30 % of CO contribution from a 2nd source with a 16.4±2.5 mUr oxygen isotope signature (Table SI E2). Additionally, a pair of peaks is obtained from injection of MTBE and pentane, which has to be generated from the single compound, as headspace injection and subsequent GC separation successfully isolate the compounds. Moreover, intensity pattern of the first to the second peak is changed towards a higher second peak if pentane (C:O=5:0) is converted instead of MTBE (Figure SI E5). These are clear indications for a parallel sample flow, respective a division of the GC eluting compound peak, which allows simultaneous contact with the ceramic. Thus, it can be concluded, that easy manual sliding of the platinum tube into the ceramic tube does not tight enough to prevent parts of the sample to mobilise 2nd source oxygen from the outer ceramic tube.

Figure SI E4. Empty ceramic reactor: 2nd source calculations, plotted according to equation (2).

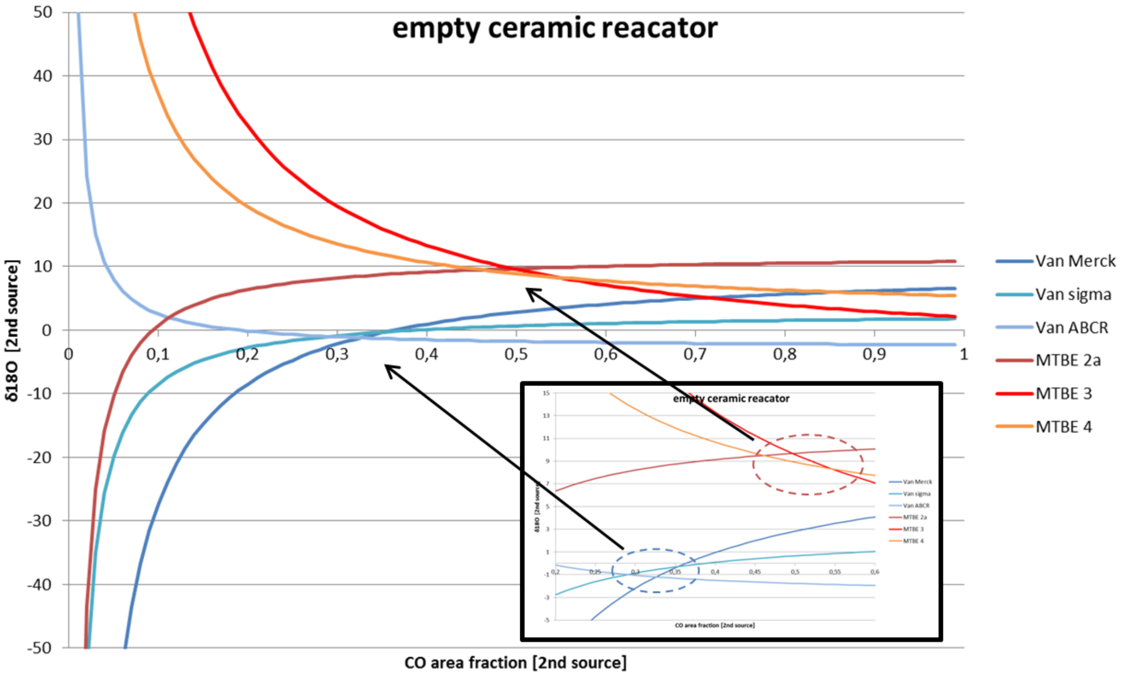


Table SI E1. Empty ceramic reactor: Raw data and 2nd source calculation results (average of the interception points, Figure SI E1) for vanillin and MTBE, measured at different measurement campaigns.

Empty ceramic reactor	$Area_{2S}$ (x) calculations	δ_{2S} [mUr] calculations	$\boldsymbol{\delta_{measured}}^{a}$ **[mUr]**	$Area_{sample}$ (1-x) calculations	EA-HTC-IRMS $\boldsymbol{\delta_{sample}}^{b}$ **[mUr]**
Vanillin Merck	0.3	-1.2±0.2	**6.6**	0.7	**10.37**
Vanillin Sigma-Aldrich			**1.8**		**2.94**
Vanillin ABCR			**-2.2**		**-2.85**
MTBE 2a	0.5	9.0±0.6	**10.8**	0.5	**11.91**
MTBE 3			**2.1**		**-5.38**
MTBE 4			**5.4**		**1.89**

[a] n >3 for all averages [b] reference value determined by EA-HTC-IRMS (see Table 1)

Figure SI E5. Custom made platinum tube in ceramic tube reactor: CO peak shape of MTBE (left) and pentane (right) injections of 21 nmol C (4.2 nmol O for MTBE) on column analysed with IonTrap MS (*m/z* 28-30, 100% = 7.4*10^4signal intensity).

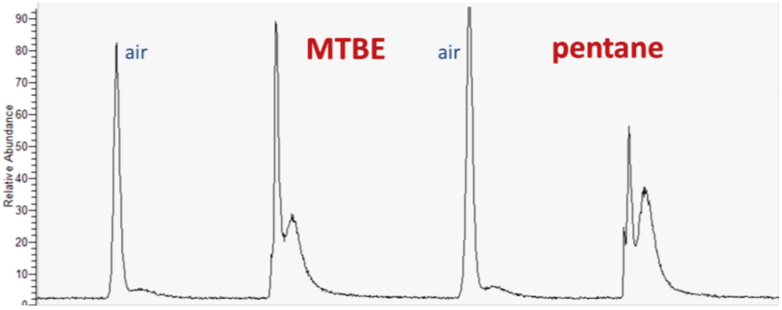


Figure SI E6. Custom made platinum tube in ceramic tube reactor: 2nd source calculations, plotted according to equation (2).

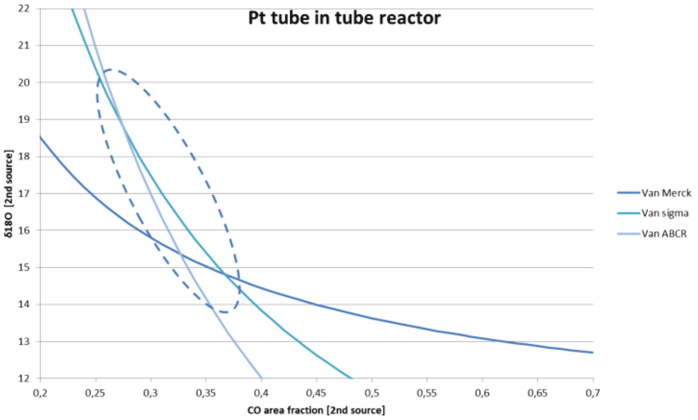
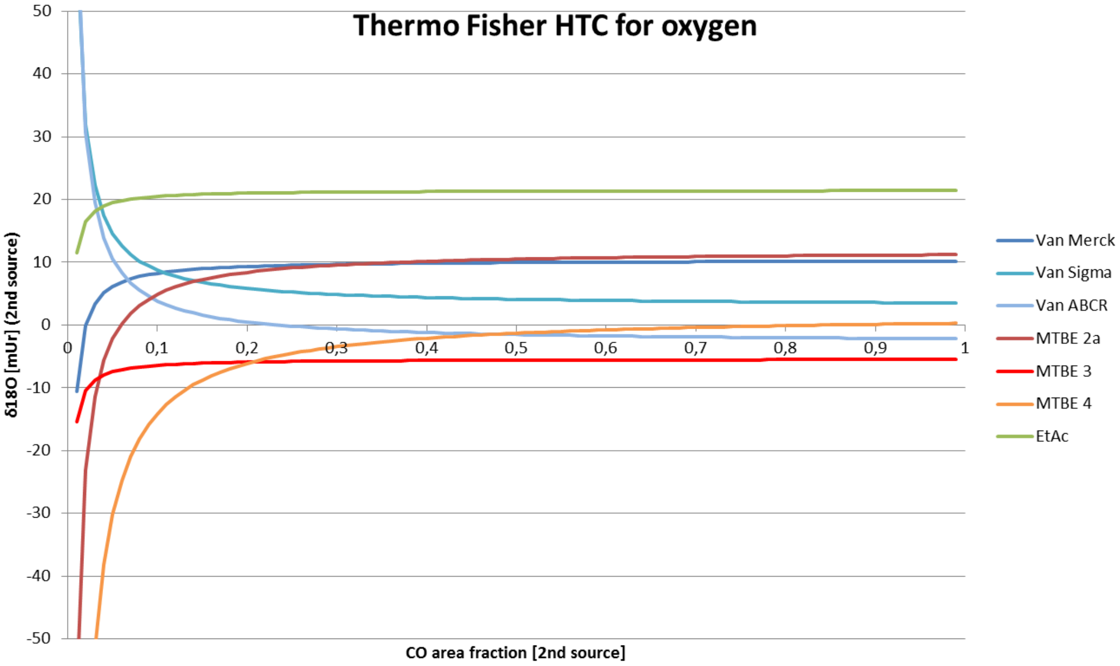


Table SI E2. Custom made platinum tube in ceramic tube reactor: Raw data and 2nd source calculation results (average of the interception points, Figure SI E6) for vanillin.

Custom made platinum tube in ceramic tube reactor	Area$_{2S}$ (x) calculations	δ_{2S} [mUr] calculations	**$\delta_{\mathrm{measured}}$[a] [mUr]**	Area$_{\mathrm{sample}}$ (1-x) calculations	**EA-HTC-IRMS δ_{sample}[b] [mUr]**
Vanillin Merck	0.3	16.4±2.5	**12**	0.7	**10.37**
Vanillin Sigma-Aldrich			**7.3**		**2.94**
Vanillin ABCR			**3.1**		**-2.85**

[a] n >3 for all averages [b] reference value determined by EA-HTC-IRMS (see Table 1)

Figure SI E7. Thermo Fisher HTC for oxygen reactor: 2nd source calculations, plotted according to equation (2). The lack of three interception points indicates no 2nd source contribution.



F - Nickel as reactor constituent

Use of nickel in oxygen HTC

Under the prevalent HTC conditions in GC-IRMS reactors nickel seems to be able to fulfil two diametrical functions: on the one hand the formation and decomposition of nickel carbide (Ni_3C) positively influences carbon availability in the reactor. On the other hand nickel bears the risk of retaining oxygen at the nickel surface.

According to thermochemical considerations the latter function as an oxygen sink and source seems to be more relevant, as the calculated free energy indicates preference of nickel oxide formation (Table SI F2). In addition, the presence of nickel as a constituent of a reactor restricts the maximum conversion temperature to below the melting point of nickel (1453°C) [11]. Nickel oxide nucleation on nickel surfaces is known to occur upon oxygen exposure at room temperature [12]. Preliminary tests showed a colour change to turquoise if a nickel wire or tube in contact with a ceramic tube is used (data not shown), indicating nickel monoxide deposition [11]. Nickel tubes only, filled and empty, could not be used as oxygen conversion reactors and showed embrittlement and leakages upon contact with air [4]. Oxygen in the system can originate either from organics, air leaks in the GC system, or from the ceramic (as discussed above) and thus it is almost impossible to store and install and operate the reactor in an oxygen free atmosphere.

The commercially available oxygen reactor by Isoprime consists of a whole nickel inner tube (615 mm length, 0.5 mm inner diameter) in a ceramic outer tube which is then conditioned by injection of organics to obtain carbon deposition in the reaction zone (personal communication)[13]. With this reactor design, additional hydrogen make-up gas is needed to prevent nickel oxide formation over the entire length of the reactor. Due to its length, this reactor requires a special heating device.

Nickel oxide can be reduced to nickel by hydrogen gas at temperatures above 200°C [11, 14]. Thus, to obtain strictly reductive conditions and prevent nickel oxide formation, the Thermo Fisher and Isoprime reactors use hydrogen (1 %) as a make-up gas introduced into the carrier gas, resulting in an approximate H_2 addition of 0.3 % into the sample stream. Organic compounds usually contain an excess of bound hydrogen compared to bound oxygen and hydrogen is released during HTC, which should serve to maintain reducing conditions. This should be possible as the setup without the hydrogen make-up gas has been demonstrated in other applications [4, 15-19]. However, if the reactor is not in a reduced condition to start with, e.g. parts of the nickel are already oxidised, it is questionable whether this hydrogen from the sample as well as the one from the make-up gas will sufficient to establish reducing conditions.

Possible reactions

For an approximate comparison of the possible reactions and the respective thermodynamically favoured reactions the free energy (ΔG, Gibbs function) can be determined from the standard molar enthalpy (ΔH at 298 K) and the standard molar entropy (ΔS at 298 K) (Table SI F1). When the initial and the final states of a system have the same temperature the relation $\Delta G = \Delta H - T * \Delta S$ applies for changes in state. Disregarding the temperature dependency of enthalpy and entropy the free energy at different temperatures (650°C, 1280°C, 1400°C) can be calculated using stoichiometric reaction equations and standard molar values from thermodynamic tables (Table SI F1).

Table SI F1. Thermochemical data of elements and compounds needed for the calculation of the free energy of a reaction. All data taken from [20] except MTBE taken from http://www.update.uu.se/~jolkkonen/pdf/CRC_TD.pdf

	ΔH at 298 K [kJ mol^{-1}]	**S** at 298 K [J K^{-1} mol^{-1}]
NiO (s)	-239,7	38
Ni (s)	0	29,9
H_2 (g)	0	130,7

H_2O (g)	-241,8	188,8
C (s)	0	5,7
O_2 (g)	0	205,1
CO (g)	-110,5	197,7
CO_2 (g)	-393,5	213,8
C_6H_{14} (g) - hexane	-167,2	388,5
CH_4 (g)	-74,9	186,3
$C_5H_{12}O$ (g) - MTBE	-283,7	265,3

The resulting free energy for a reaction indicates whether the reaction will take place spontaneously and result in the products (exergonic; $\Delta G<0$) or would rather not take place and the initial state of the educts would be preferred (endergonic; $\Delta G>0$). For hypothetical reactions of nickel and nickel oxide the resulting free energy was calculated and is shown in Table SI F2. From these calculations it can be concluded, that nickel oxide formation from oxygen and oxygen bearing organic compounds is more likely than from the HTC products CO and CO_2 (Table SI F2). However, organic compounds like MTBE can at the same time reduce nickel oxide and these reactions seem to be more exergonic (more negative ΔG values, Table SI F2) than the nickel oxide formation. It can be assumed that the nickel oxide formation in the presence of organic compounds is thus not relevant. These free energy considerations are in accordance with the observation that no classical memory effect could be detected. It is though probable that nickel oxide formation takes place during storage at room temperature and due to leaks in the GC system ($Ni+O_2$ reaction at low temperatures, Table SI F2). The tightness of the system as well as a reduction prior to measurements seem essential for oxygen CSIA. Reduction of nickel oxide can be facilitated by a hydrogen stream ($NiO+H_2$, Table SI F2) at lower temperatures - the maximal experimental reduction efficiency was shown to happen around 400 °C [21]; and was done in this study at 600 °C to save time during reactor cooling and heating. In contrast to hydrogen reduction the free energy calculation shows that reduction with organic compounds like hexane and MTBE is much more exergonic (more negative ΔG values). Thus recommended conditioning serves not only to deposit carbon in the reactor, but also reduces nickel oxide. The calculations for the reduction reaction with MTBE as organic compound even releases little more free energy than the actual intended HTC of MTBE (Table SI F2), which could indicate a preferred CO_2 formation if NiO is present during MTBE HTC. Besides, the recommended hydrogen make up gas will steadily reduce possible nickel oxide and thus permanently add to the water background, which might explain observed high water backgrounds before reduction and conditioning (Figure SI C1). The calculation results underline the need of a reduced reactor system to start with and maintain and the need to avoide NiO present in the reactor. Comparing the free energy (negative ΔG) in the upper two sections of Table SI F2, resembling a reactor system without NiO present, HTC of organic substances is the thermodynamically favoured reaction and no formation of NiO from MTBE is to be expected. As MTBE HTC to CO or CO_2 are calculated to almost the same ΔG, it can be speculated that other factors e.g. the Boudouard equilibrium (see also SI D) determine the amount of CO_2 by-product formation.

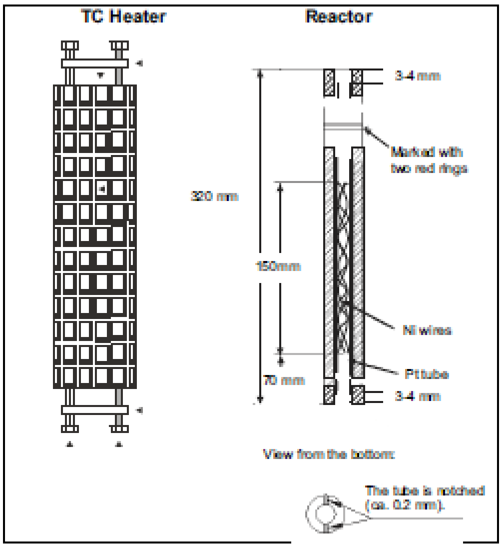
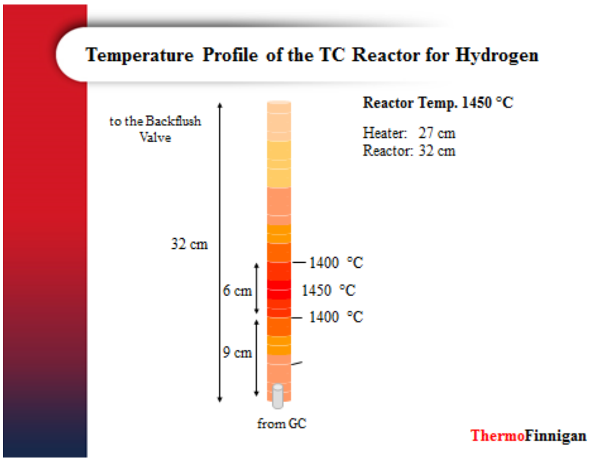
Altogether a free energy consideration can only indicate possibly thermodynamic favoured reactions, as continuous flow and equilibrium conditions present in the GC-HTC reactor are barely assessable by these basic estimations.

Table SI F2. Calculated free energy (ΔG) of possible reactions at different temperatures. Reactions which will rather not take place spontaneously (endergonic; ΔG>0) are indicated in red.

	ΔG=ΔH-T*ΔS [kJ mol^{-1}]		
	650 °C	1280 °C	1400 °C
Formation of NiO			
$Ni+0.5O_2 \rightarrow 1NiO$	-153	-93	-82
$Ni+CO \rightarrow NiO+C$	55	181	205
$Ni+CO_2 \rightarrow NiO+CO$	51	56	57
$2Ni+CO_2 \rightarrow 2NiO+C$	86	203	226
$Ni+MTBE \rightarrow NiO+6H_2+5C$	-469	-819	-885
$0.5Ni+MTBE \rightarrow 0.5NiO+0.5CO+6H_2+4.5C$	-489	-897	-975
HTC of MTBE			
$MTBE \rightarrow CO +6H_2+4C$	-509	-975	-1064
$MTBE \rightarrow 0.5CO_2+6H_2+4.5C$	-514	-925	-1003
Reduction of NiO			
$NiO+H_2 \rightarrow Ni+H_2O$	-48	-80	-86
$NiO+C \rightarrow Ni+CO$	-41	-156	-178
$NiO+0.5C \rightarrow Ni+0.5CO_2$	-46	-106	-118
$NiO+CH_4 \rightarrow Ni+CO+2H_2$	-40	-207	-239
$NiO+CO \rightarrow Ni+CO_2$	-51	-56	-57
$NiO+MTBE \rightarrow Ni+CO_2+6H_2+4C$	-560	-1031	-1121
$NiO+MTBE \rightarrow Ni+2CO+6H_2+3C$	-550	-1132	-1242
$NiO+C_6H_{14} \rightarrow Ni+CO+7H_2+5C$	-391	-860	-949
Nickel carbide [22]			
$3Ni+C \rightarrow Ni_3C$	7	6	5
$Ni_3C \rightarrow 3Ni+C$	-7	-6	-5

Reactor assembly and heating device specifications

Figure SI F1. Thermo Fisher HTC for oxygen reactor: Schematic composition of the reactor and heating oven.

	
Design of the Thermo Fisher HTC for oxygen reactor [2]	Temperature profile of the HTC reactor oven (provided by ThermoFinnigan, 2001) [23]

G – Analysis conditions Thermo Fisher HTC reactor for oxygen

Conditioning with hexane

Analysing compounds of known oxygen isotope composition (Table 1), allows obtaining information about reactor performance and efficiency of measures undertaken to approach optimal HTC conditions. Mentioned in existing methods, however not closer specified or scientifically reasoned is the intensity, frequency and effect of conditioning with e.g. hexane (1µL, 1:20). Furthermore, historical experiments with different carbon sources in a reactor suggest that formed graphite (carbon deposition in the reactor) can build up a memory and thus even worsen the quality of the HTC [24]. Our experiments underline the importance of conditioning and that accurate oxygen stable isotopes can only be obtained under hardly reached conditions, showing the fragility of the GC-HTC-IRMS system. Main observations found in conditioning experiments in this study were:

- Conditioning shifted $\delta^{18}O$ raw values, which were too negative (CO depleted in ^{18}O), towards the reference EA-HTC-IRMS values (Figure SI F1)
- The effect of conditioning was not lasting very long (Figure SI F2), had to be repeated every day at least (Figure SI F1), and was fading even if no measurements were run.
- Standard deviations of consecutive measurements were higher before than after conditioning (Figure SI F1)
- Even upon fresh conditioning not always EA-HTC-IRMS reference values were met and partly very high standard deviations could be observed (Figure SI F2)
- Background levels after conditioning were not stable (Figure SI F2)

Figure SI G1. Thermo Fisher HTC for oxygen reactor: GC-HTC-IRMS analysis of MTBE 2a over the time range of 10 days: 17th -26th August 2015. Reference $\delta^{18}O$ (EA-HTC-IRMS) value is indicated by the orange line. Conditioning (1µl hexane, 1:20) is symbolised by green arrows.

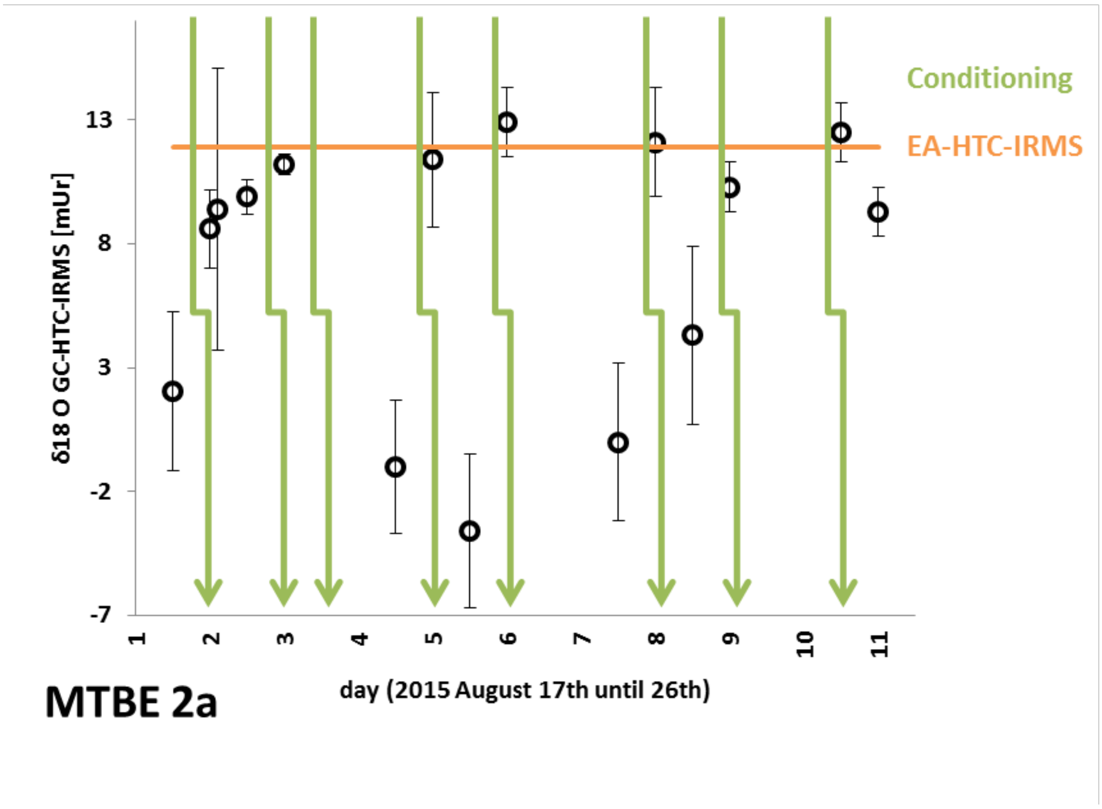
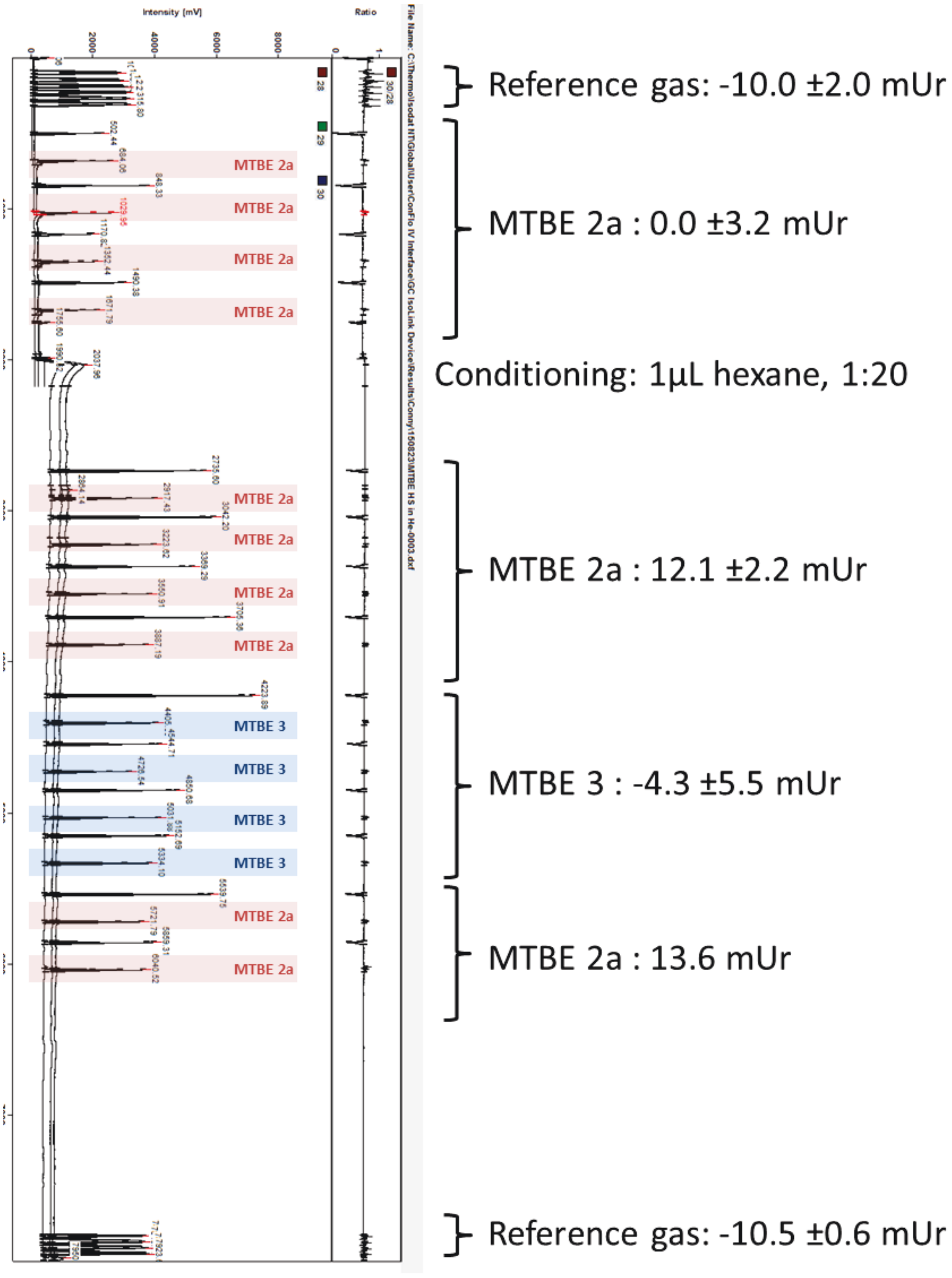


Figure SI G2. Thermo Fisher HTC for oxygen reactor: Analysis of MTBE 2a and 3 (15 nmol O on column) before and after conditioning with hexane. MTBE injection from headspace, thus an air peak elutes before the MTBE peak.



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To Chapter IV

Published paper[5]

Evaluating degradation of hexachlorcyclohexane (HCH) isomers within a contaminated aquifer using compound specific stable carbon isotope analysis (CSIA)

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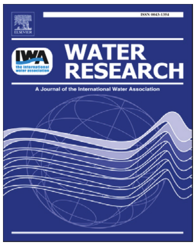

Evaluating degradation of hexachlorcyclohexane (HCH) isomers within a contaminated aquifer using compound-specific stable carbon isotope analysis (CSIA)

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ABSTRACT

The applicability of compound-specific stable carbon isotope analysis (CSIA) for assessing biodegradation of hexachlorocyclohexane (HCH) isomers was investigated in a contaminated aquifer at a former pesticide processing facility. A CSIA method was developed and tested for efficacy in determining carbon isotope ratios of HCH isomers in groundwater samples using gas chromatography — isotope ratio mass spectrometry (GC-IRMS). The carbon isotope ratios of HCHs measured for samples taken from the field site confirmed contaminant source zones at former processing facilities, a storage depot and a waste dump site. The ^{13}C-enrichment in HCHs provided evidence for biodegradation of HCHs especially downstream of the contaminant source zones. CSIA from monitoring campaigns in 2008, 2009 and 2010 revealed temporal trends in HCH biodegradation. Thus, the impact and progress of natural attenuation processes could be evaluated within the investigated aquifer. Calculations based on the Rayleigh-equation approach yielded levels of HCH biodegradation ranging from 30 to 86 %. Moreover, time- and distance-dependent *in situ* first-order biodegradation rate constants were estimated with maximal values of 3×10^{-3} d^{-1} and 10×10^{-3} m^{-1} for α-HCH, 11×10^{-3} d^{-1} and 37×10^{-3} m^{-1} for β-HCH, and 6×10^{-3} d^{-1} and 19×10^{-3} m^{-1} for δ-HCH, respectively. This study highlights the applicability of CSIA for the assessment of HCH biodegradation within contaminated aquifers.

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1. Introduction

Persistent organic pollutants (POPs) are a major contaminant group of subsurface compartments and pose substantial environmental and health risks (Minh et al., 2006; Weber et al., 2011). Hexachlorocyclohexane (HCH) isomers are globally dispersed POPs as a result of the production and application of HCH containing pesticides. HCHs cause substantial environmental impacts and are widespread in soil and groundwater (Bhatt et al., 2009; Vijgen et al., 2011). Due to their combination of toxicity and environmental persistence, the commercial production and use of HCHs has been regulated by the Stockholm Convention on POPs since 2004 and the production of the three main HCH isomers (α, β & γ-HCH) has been banned since 2009 (Vijgen et al., 2011). This measure may limit future risk from HCH contamination. However, significant impact still exists due to the extensive use of HCHs in the past, as well as the ongoing production and application of Lindane (γ-HCH) in several countries. Stockpiles from previous manufacturing and leachates from dump sites into groundwater lead to serious point source contaminations of HCHs which require management strategies (Bhatt et al., 2009; Vijgen et al., 2011). An appropriate strategy might be *Monitored Natural Attenuation* (MNA) consisting of regular evaluation of the extent and sustainability of intrinsic processes leading to the reduction of the risk and amount of a contamination (US-EPA, 1999). Since biodegradation can cause a sustainable and efficient decrease in pollutant mass, it is an important natural attenuation process and needs to be taken into account for MNA strategies (Bombach et al., 2010; Illman and Alvarez, 2009; Wiedemeier et al., 1999).

Biodegradation is a sustainable removal process of HCHs in the subsurface (Bhatt et al., 2009) and a cost efficient alternative to physico-chemical remediation of HCH contaminated groundwater and soils (Alvarez et al., 2012; Langenhoff et al., 2013; Phillips et al., 2006). The molecular structure of the HCH isomers plays a key role in biodegradation as it has been shown that α and γ isomers are degraded faster than β and δ isomers (Lal et al., 2010; Mehboob et al., 2013). However, concentration-based assessment of *in situ* pollutant biodegradation within contaminated aquifers is limited, because the concentration of contaminants can also be significantly decreased by physical processes like volatilization, sorption, dilution and dispersion. Thus, the decline of pollutant concentrations can only provide indirect indication for *in situ* pollutant biodegradation. In order to directly prove pollutant biodegradation, advanced tools are recommended (Bombach et al., 2010; Illman and Alvarez, 2009).

One of the most promising tools for monitoring *in situ* biodegradation of organic contaminants in aquifers is compound-specific stable carbon isotope analysis (CSIA) (Meckenstock et al., 2004). Molecules with light carbon isotopes (^{12}C) in the reactive position require less energy for bond cleavage and, thus, tend to be degraded faster than molecules containing a heavy carbon isotope (^{13}C), resulting in an ^{13}C-enrichment in the remaining stock of the pollutant. This process is called stable isotope fractionation and can be detected via changes in carbon isotope ratios (^{13}C/^{12}C, most commonly given as δ^{13}C) of a pollutant toward more positive δ^{13}C-values. Therefore, CSIA allows for the assessment of pollutant biodegradation based on the degree of carbon isotope fractionation observed at a contaminated field site.

In laboratory studies, significant changes in carbon isotope ratios have been observed for HCH biodegradation under both oxic and anoxic conditions. Anaerobic degradation exhibited higher carbon isotope fractionation of HCHs than aerobic degradation (Badea et al., 2011, 2009; Bashir et al., 2013). However, the applicability of CSIA has not previously been demonstrated for the evaluation of *in situ* biodegradation at a HCH-contaminated field site.

In this study, CSIA was applied for assessing biodegradation of HCHs in a contaminated aquifer. Besides hydrogeochemical parameters and pollutant concentrations, carbon isotope ratios of HCHs were measured for three monitoring campaigns taking place in 2008, 2009 and 2010, in order to determine the progress and sustainability of HCH biodegradation. To our best knowledge, this is the first study which addresses the use of CSIA for assessing the fate of HCHs within a groundwater system.

2. Materials and methods

2.1. *Field site*

2.1.1. *Site history*

The field site is located in the area of a former pesticide formulating plant that included both a formulation site and a packaging facility. The pesticide formulation began in 1935 and continued for more than five decades. The pesticide products prepared at the site were mainly HCH-based but also included other pesticides, e.g. dichlorodiphenyltrichloroethane (DDT). As known from historical information, HCH was not produced on-site, but technical HCH was purchased from suppliers and γ-HCH was purified for use in pesticide formulation. HCH contamination of soil and groundwater were mainly caused by losses of HCH-containing raw materials and products during purification, pesticide formulation and storage as well as irrigation and dumping of production-related wastes.

2.1.2. *Hydrogeology*

The highest pollutant concentrations were found within the upper quaternary aquifer, which consists of 12–15 m thick (glacio-) fluvial sand and gravel deposits. This aquifer is largely separated from the underlying tertiary aquifer by a 30 m thick clay- and coal-bed layer. The lower aquifer exhibits almost no contamination. The mean effective groundwater flow velocity of the upper aquifer was estimated at 0.3 m/d. The matrix of the upper aquifer displayed a low organic carbon content of only 0.014 %, thus minor retardation of HCHs is expected (Lotse et al., 1968).

In the early decades of the 20th century, drainage measures were initiated to facilitate nearby mining activity, which led to a lowering of the groundwater table and a reversal of groundwater flow direction in the area of the field site. Groundwater resurgence and realignment to the previous groundwater flow direction have occurred since the termination of these measures in the early 1990s. During the last decade, the groundwater table has been relatively stable with slightly increasing tendency. The main groundwater flow direction was largely constant toward north/northeast to north/northwest (Fig. 1). Hence, the contaminant plume in the upper aquifer was established in the main groundwater flow direction and has achieved quasi-stationary conditions (Fig. 1).

2.2. *Sampling*

For the CSIA monitoring, 13 groundwater wells were sampled in 2008 and 15 wells in 2009 and 2010, respectively, covering the upstream groundwater flow (well 1), the central groundwater flow (wells A–F), and the western (wells 2–5) and eastern fringe (wells 6–9) of the contaminant plume (Fig. 1). Wells 1 and 3 were established in 2009, in order to monitor the pollutant distribution in more detail within the groundwater upstream flow and the western fringe of the contaminant plume.

Groundwater sampling was carried out by an authorized contractor based on standard procedures given in the Supporting Information (SI). During groundwater monitoring campaigns in autumn 2008, 2009 and 2010, samples were collected from wells with filter screens spanning the entire depth of the water-saturated zone within the upper aquifer using a submersible electrical pump. Samples for concentration analyses were sent to an analytical laboratory where they were processed immediately. For CSIA of HCHs, two 1 L glass bottles (Schott, Germany) were filled with groundwater and sealed with Teflon-coated caps (Schott, Germany) without headspace, in order to avoid evaporation of the samples. The groundwater samples were adjusted to a pH of 2 using hydrochloric acid (HCl; 25 %, Carl Roth GmbH & Co. KG, Germany) to inhibit microbial activity. After sampling, they were stored in the dark at 4 °C until extraction.

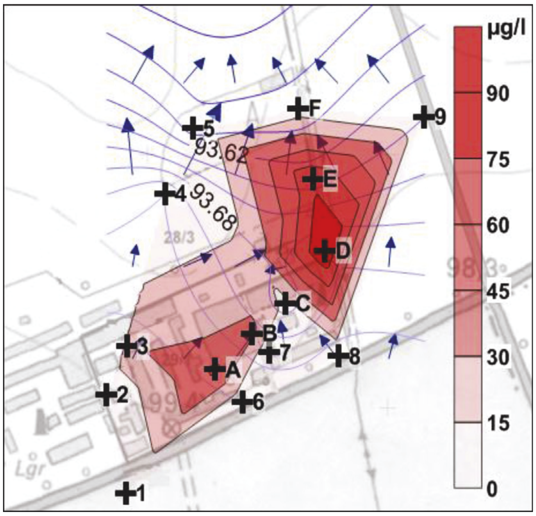

Fig. 1 – Distribution of HCHs (sum of concentrations of HCH isomers [µg/L] in 2010) and groundwater flow direction (blue arrows) within the upper aquifer of the investigated field site. The main parts of the contaminant plume are: upstream flow (well 1), central flow (wells A–F), western fringe (wells 2–5), eastern fringe (wells 6–9). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.3. *Analytical procedures*

2.3.1. *Concentration analysis*

Concentration of dissolved oxygen, temperature, pH, redox potential, and electrical conductivity were measured during sampling using appropriate electrodes (CellOx® 325, SenTix® 41, SenTix® ORP, KLE 325; WTW GmbH, Germany).

Concentration analyses of contaminants and hydrogeochemical parameters were performed according to analytical standard procedures summarized in SI.

2.3.2. *CSIA*

For CSIA of HCHs, two 1 L groundwater samples of each well were extracted three times with 30 mL dichloromethane (DCM; ≥ 99.8 %, Carl Roth GmbH & Co. KG, Germany) in a separating funnel. The DCM extracts obtained from the two groundwater samples were combined and dried with anhydrous sodium sulfate (Na_2SO_4; ≥99 %, Bernd Kraft GmbH, Germany). The combined DCM extracts were reduced to approximately 1 mL using a rotary evaporator. The extraction procedure did not result in significant changes in carbon isotope ratios of HCHs, as described in SI.

CSIA of HCHs were performed by gas chromatography - isotope ratio mass spectrometry (GC-IRMS), using a system described elsewhere (Badea et al., 2011, 2009). Detailed information on the GC-IRMS analysis of HCHs can be found in SI. Quality control was carried out using isotope laboratory standards consisting of pure HCH isomers (97–99 %, Sigma-Aldrich Chemie GmbH, Germany) with carbon isotope ratios determined by elemental analyzer - isotope ratio mass spectrometry (EA-IRMS) using reference materials from the International Atomic Energy Agency (IAEA–CH–6, IAEA–CH–7) (Coplen et al., 2006). The carbon isotope ratios of HCHs measured by EA-IRMS and GC-IRMS were reported in the delta notation ($\delta^{13}C$) relative to the international standard Vienna Pee Dee Belemnite (VPDB) according to Eq. (1) (Coplen, 2011).

$$\delta^{13}C_{sample} = \frac{R_{sample}}{R_{standard}} - 1 \tag{1}$$

R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ ratios of the sample and VPDB, respectively. The $\delta^{13}C$-values were reported in per mil (‰). All samples were measured at least in triplicates. The analytical uncertainty was ≤±0.6‰ in nearly all cases and, thus, in good agreement with the typical uncertainty for CSIA of organic groundwater pollutants (≤±0.5‰) (US-EPA, 2008). Higher uncertainty of CSIA (≤±0.9‰) was only given for few $\delta^{13}C$-values (4 out of 57). Since method-related isotope effects were negligible (as described in SI), higher uncertainty seemed to be caused by random impairment of measurement.

2.4. *Quantitative interpretation of isotope data*

The Rayleigh equation can be applied to mathematically describe isotope fractionation of biodegradation processes, as shown in Eq. (2):

$$\frac{(\delta_t + 1)}{(\delta_0 + 1)} = \left(\frac{C_{Bt}}{C_0}\right)^{\varepsilon} \tag{2}$$

where δ_t is the isotope ratio of the substrate at a certain time t of biodegradation, δ_0 is the initial isotope ratio of the substrate, C_{Bt}/C_0 is the fraction of substrate remaining during biodegradation at a certain time t and ε is the isotope enrichment factor (Mariotti et al., 1981).

The degree of contaminant biodegradation can be calculated as the percentage of the initial contaminant concentration decreased due to biodegradation (B [%]), as shown in Eq. (3).

$$B[\%] = \left(1 - \frac{C_{Bt}}{C_0}\right) \cdot 100 \tag{3}$$

Combining Eq. (3) with the Rayleigh equation (Eq. (2)) allows for the quantification of contaminant biodegradation over a time or distance interval (e.g., a groundwater flow path) (Thullner et al., 2012). Required data inputs are the initial isotope ratio of the contaminant at a starting point in either time or in space (e.g., the location of the contaminant source), and the isotope ratio of the remaining contaminant at a temporal or spatial observation point (e.g., a well downstream of the source). The amount of contaminant biodegradation between the starting point (0) and the observation point (x) is then given by Eq. (4) (Thullner et al., 2012).

$$B[\%] = \left(1 - \frac{C_{Bx}}{C_0}\right) \cdot 100 = \left[1 - \left(\frac{\delta_x + 1}{\delta_0 + 1}\right)^{\left(\frac{1}{\varepsilon}\right)}\right] \cdot 100 \tag{4}$$

Moreover, distance-dependent *in situ* first-order biodegradation rate constants (λ_s) can be estimated by changes in isotope ratios over the distance between the initial and observation points (s) using a Rayleigh-equation based approach (Hunkeler, 2008):

$$\lambda_s = -\frac{1}{\varepsilon \cdot s} \ln\left(\frac{\delta_x + 1}{\delta_0 + 1}\right) \tag{5}$$

Time-dependent *in situ* first-order biodegradation rate constants (λ_t) can be determined by taking into account the travel time of the pollutants along the groundwater flow path (t):

$$\lambda_t = -\frac{1}{\varepsilon \cdot t} \ln\left(\frac{\delta_x + 1}{\delta_0 + 1}\right) \tag{6}$$

where the travel time (t) can be approximated using the groundwater flow velocity (v) and the distance between the initial and observation points (s):

$$t \approx \frac{s}{v} \tag{7}$$

First-order biodegradation rate constants can be used to calculate biological half-life distances ($s_{1/2}$) or times ($t_{1/2}$), representing the distance or time needed for the biodegradation of half of the initial pollutant concentration (Wiedemeier et al., 1999):

$$s_{1/2} = \frac{\ln 2}{\lambda_s} \tag{8}$$

$$t_{1/2} = \frac{\ln 2}{\lambda_t} \tag{9}$$

Uncertainties for B [%], λ_s, λ_t, $s_{1/2}$ and $t_{1/2}$ were estimated by error propagation as described in SI.

3. Results and discussion

3.1. *HCH distribution and hydrogeochemical conditions*

3.1.1. *Concentration of HCHs*

Well A exhibited significant higher concentrations of HCHs (sum of α-HCH, β-HCH, γ-HCH, δ-HCH; 2008: 68 μg/L, 2009: 48 μg/L, 2010: 48 μg/L) compared to well 1 (2009: 0.1 μg/L, 2010: 0.1 μg/L) located upstream of the contaminant plume. This indicated a contaminant source in the vicinity of well A (Fig. 1). The concentrations of HCHs decreased within the central groundwater flow of well A at wells B (2008: 38 μg/L, 2009: 47 μg/L, 2010: 33 μg/L) and C (2008: 57 μg/L, 2009: 30 μg/L, 2010: 10 μg/L), respectively (Fig. 1). A significant increase in concentrations of HCHs was detected within the further central groundwater flow at wells D (2008: 111 μg/L, 2009: 100 μg/L, 2010: 91 μg/L) and E (2008: 196 μg/L, 2009: 149 μg/L, 2010: 72 μg/L), implying an additional source zone at these wells (Fig. 1). Concentrations of HCHs significantly decreased within the central groundwater flow of wells D and E at well F (2008: 7 μg/L, 2009: 5 μg/L, 2010: 3 μg/L) (Fig. 1). Compared to the wells of the central groundwater flow (A–F), wells of the western and eastern part of the contaminant plume exhibited lower concentrations of HCHs, illustrating the lateral distribution of HCHs in the aquifer (Fig. 1).

The highest concentration levels were observed for δ-HCH. The concentrations of the other HCH isomers were at least three times lower than those of δ-HCH except of well 3 where γ-HCH yielded highest concentrations (Fig. 2, Fig. SI4–SI6). Due to the high abundance of γ-HCH at well 3 (2009: 99 μg/L, 2010: 15 μg/L) (Fig. SI6), a distinct source zone could be expected in the western part of the contaminant plume in the vicinity of well 3. Besides δ-HCH, β-HCH was generally more frequently detected compared to α- and γ-HCH.

Based on the differences in concentrations and patterns of HCHs, indications could be derived for distinct source zones and natural attenuation of HCHs. However, it was not possible to gain information on specific natural attenuation processes which led to the observed changes in HCH concentrations and on the extent of sustainable removal by pollutant degradation. Therefore, CSIA was applied in order to refine the conceptual site model and to evaluate the prerequisite for the implementation of a MNA concept at the field site. Moreover, CSIA should provide validation of source zones indicated by concentrations and patterns of HCHs.

3.1.2. *Hydrogeochemical parameters*

The central groundwater flow of the contaminant plume (wells A–F) exhibited anoxic conditions while wells A, B and C were affected by stronger reducing conditions than wells D, E and F located in the northern part of the contaminant plume (described in detail in SI, Fig. SI3). Concentration data of hydrogeochemical parameters indicated that methanogenesis and sulfate reduction were prevalent redox processes at wells A, B and C as well as nitrate reduction at wells D and E (Fig. SI3).

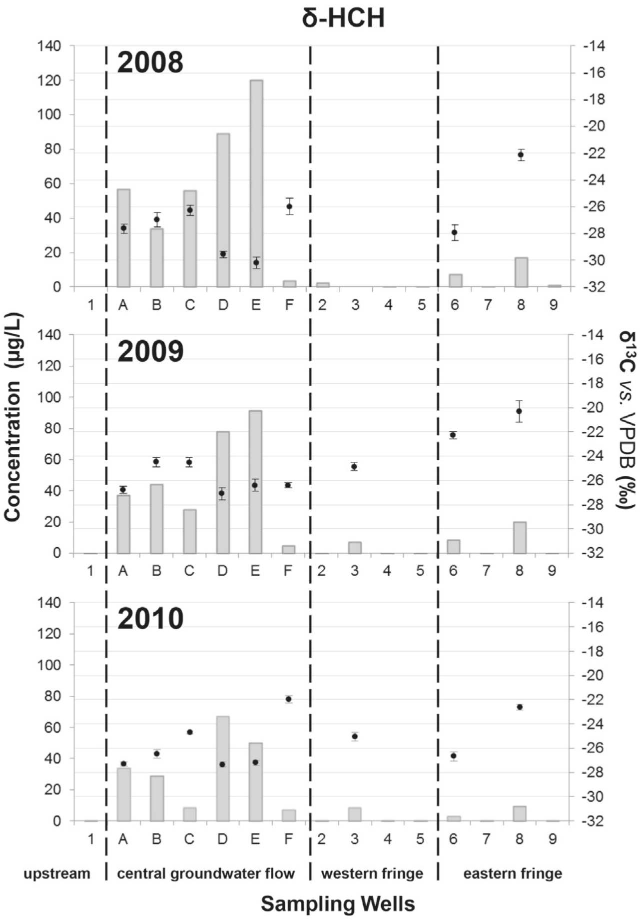

Fig. 2 – Concentrations (bars) and carbon isotope ratios (dots) of δ-HCH for the monitoring campaigns in 2008, 2009 and 2010. Wells 1 and 3 were established in 2009, thus, concentration and isotope data are not available for the monitoring campaign in 2008. Uncertainty of concentration analysis is <10 % in all cases. Errors of δ^{13}C-values are indicated as error bars.

Wells 2, 5 and 7, 9 located at the western and eastern fringe of the contaminant plume, respectively, as well as well 1 located upstream of the contaminant plume showed oxic conditions. However, sulfate reduction and methanogenesis seemed to be relevant at wells 3, 4, 6 and F. Thus, heterogeneous redox conditions could be expected in some areas of the fringe of the contaminant plume.

3.2. *Qualitative assessment of HCH degradation*

The isotope data obtained from the monitoring campaigns in 2008, 2009 and 2010 were used to evaluate natural attenuation of HCHs within the contaminant plume as well as temporal variations over the three-year period. Differences in carbon isotope ratios >+1‰ to ≤+2‰ were considered to indicate HCH biodegradation while differences >+2‰ were considered to provide evidence for HCH biodegradation (US-EPA, 2008).

3.2.1. *δ-HCH*

The δ-HCH concentrations at well 1 located upstream of the contaminant plume were too low (0.1 µg/L) for measuring carbon isotope ratios of δ-HCH ($\delta^{13}C_{\delta\text{-HCH}}$-values). Thus, it was not possible to derive information on δ-HCH biodegradation in this part of the aquifer.

At well A located in the expected source zone of the southern central groundwater flow, δ-HCH exhibited relative consistent carbon isotope ratios (2008: −27.6‰, 2009: −26.8‰, 2010: −27.3‰) (Fig. 2). The similarity of the $\delta^{13}C_{\delta\text{-HCH}}$-values implied that the decrease in concentration over time (2008: 57 µg/L, 2009: 37 µg/L, 2010: 34 µg/L) was primarily caused by physical processes rather than biodegradation. Along the central groundwater flow, carbon isotope ratios at well B were similar to well A in 2008 (−27.0‰) and in 2010 (−26.5‰), respectively. Thus, δ-HCH biodegradation at well B could be neglected for these two years. A significant difference in $\delta^{13}C_{\delta\text{-HCH}}$-values (>+2‰) was observed in 2009, providing evidence of δ-HCH biodegradation at well B in this year. Within the further central groundwater flow, δ-HCH at well C was slightly more ^{13}C-enriched compared to well A in 2008 (−26.3‰) and significantly more ^{13}C-enriched in 2009 (−24.5‰) as well as in 2010 (−24.7‰) (Fig. 2). This ^{13}C-enrichment was associated with decreasing δ-HCH concentrations (2008: 56 µg/L, 2009: 28 µg/L, 2010: 9 µg/L), indicating a temporally linked δ-HCH biodegradation at well C (Fig. 2).

Within the further central groundwater flow, δ-HCH concentrations increased significantly at wells D (2008: 89 µg/L, 2009: 78 µg/L, 2010: 67 µg/L) and E (2008: 120 µg/L, 2009: 91 µg/L, 2010: 50 µg/L). In 2008, δ-HCH was more ^{13}C-depleted at wells D (−29.6‰) and E (−30.2‰) compared to well A (−27.6‰). The more ^{13}C-depleted δ-HCH and its higher concentrations revealed a second discrete HCH source in the area of wells D and E (Fig. 2). The decrease in δ-HCH concentrations at wells D and E from 2008 to 2010 was accompanied by ^{13}C-enrichment (Fig. 2). The temporal difference in $\delta^{13}C_{\delta\text{-HCH}}$-values was >+2‰ and, thus, provided evidence that biodegradation contributed to the natural attenuation of δ-HCH in the expected source zone of wells D and E. At well F, δ-HCH showed lower concentrations (2008: 4 µg/L, 2009: 5 µg/L, 2010: 7 µg/L) and significant ^{13}C-enrichement compared to wells D and E (Fig. 2), demonstrating δ-HCH biodegradation within the northern part of the central groundwater flow.

For the western fringe of the contaminant plume, δ-HCH concentrations were only high enough for measuring reliable $\delta^{13}C_{\delta\text{-HCH}}$-values at well 3 (2009: −24.9‰, 2010: −25.1‰). δ-HCH was significantly more ^{13}C-enriched compared to wells A and D/E located in expected source zones (Fig. 2). Assuming the $\delta^{13}C_{\delta\text{-HCH}}$-values obtained at wells A and D/E as source carbon isotope ratio for well 3, the difference in $\delta^{13}C_{\delta\text{-HCH}}$-values at well 3 (>+2‰) could be attributed to δ-HCH biodegradation.

For the eastern fringe of the contaminant plume, $\delta^{13}C_{\delta\text{-HCH}}$-values of well 6 (2008: −27.9‰, 2010: −26.7‰) were similar to the carbon isotope ratios obtained at well A (Fig. 2), indicating that δ-HCH biodegradation was negligible at well 6 in these two years. Significantly more ^{13}C-enriched δ-HCH (−22.3‰) provided evidence of δ-HCH biodegradation at well 6 in 2009. Compared to other wells, ^{13}C-enriched δ-HCH was observed at well 8 (2008: −22.1‰, 2009: −20.3‰, 2010: −22.6‰) located within the further downstream flow of the eastern fringe of the contaminant plume, proving δ-HCH biodegradation at this well.

Based on carbon isotope data of δ-HCH, distinct contaminant source zones could be confirmed at well A and wells D/E, respectively. Evidence of δ-HCH biodegradation was provided downstream of these source zones and within the eastern and western fringe of the contaminant plume. A temporal increase in δ-HCH biodegradation was revealed for wells within the source zones (A, D, E), whereas variable δ-HCH biodegradation could be expected for wells B and 6.

3.2.2. *α-HCH*

The α-HCH concentrations were only high enough for the measurement of reliable $\delta^{13}C_{\alpha\text{-HCH}}$-values at wells D and E as well as partially at A and B.

α-HCH exhibited the highest concentrations (2008: 25 μg/L, 2009: 20 μg/L, 2010: 6 μg/L) and ^{13}C-depletion (2008: −29.6‰, 2009: −28.1‰, 2010: −28.7‰) at well E (Fig. SI4), confirming the discrete source in the vicinity of this well. The difference in $\delta^{13}C_{\alpha\text{-HCH}}$-values of well E was >+1‰ but <+2‰ between 2008 and 2009, indicating an increase in α-HCH biodegradation between this two years. In 2010, α-HCH was more ^{13}C-depleted than in 2009, which could be attributed to a decline in α-HCH biodegradation. Thus, the decreasing α-HCH concentration between 2009 and 2010 seemed to be mainly caused by physical processes at well E.

At well D, α-HCH exhibited lower concentrations (2008: 8 μg/L, 2009: 7 μg/L, 2010: 6 μg/L) and was more ^{13}C-enriched (2008: −23.5‰, 2009: −25.9‰, 2010: −27.2‰) compared to well E (Fig. SI4), indicating α-HCH biodegradation at well D. However, the α-HCH concentration slightly decreased at well D and α-HCH became more ^{13}C-depleted over time. Thus, natural attenuation seemed to rise mainly due to physical processes, while the impact of biodegradation decreased. Probably, the recharge of α-HCH from the unsaturated zone into the groundwater slightly declined and the microbial α-HCH removal contemporaneously diminished at well D.

Compared to well E, α-HCH was more ^{13}C-enriched and had lower concentrations in the source zone at well A (2008: −28.3‰, 2010: −22.4‰) and in its direct groundwater flow at well B (2010: −20.4‰) (Fig. SI4). Assuming that the source isotope ratio for wells A and B was similar to $\delta^{13}C_{\alpha\text{-HCH}}$-values obtained at well E, evidence can be provided for α-HCH biodegradation at wells A and B. The difference in $\delta^{13}C_{\alpha\text{-HCH}}$-values of >+5‰ at well A from 2008 to 2010 could be attributed to an increase in α-HCH biodegradation at this well.

In summary, $\delta^{13}C_{\alpha\text{-HCH}}$-values confirmed the distinct source zone at well E and provided evidence for α-HCH biodegradation within the source zone of well A and its direct groundwater flow at well B. While an increase in α-HCH biodegradation was evident at well A over time, α-HCH biodegradation obviously decreased at well D.

3.2.3. *β-HCH*

Due to the low β-HCH concentrations, $\delta^{13}C_{\beta\text{-HCH}}$-values could only be measured for wells A, B, D, E and 7.

At well A, β-HCH concentrations increased over time (2008: 4 μg/L, 2009: 7 μg/L, 2010: 9 μg/L), while similar $\delta^{13}C_{\beta\text{-HCH}}$-values of −25.4‰ and −25.8‰ were determined in 2008 and 2010, respectively. In contrast, more ^{13}C-depleted β-HCH (−27.3‰) was found at well A in 2009 (Fig. SI5). Thus, β-HCH at well A seemed to be affected by biodegradation obviously stronger in 2008 and 2010 compared to 2009. Within the further central groundwater flow, a difference in $\delta^{13}C_{\beta\text{-HCH}}$-values of >+7‰ could be observed at well B (Fig. SI5), providing evidence of β-HCH biodegradation at this well.

Compared to wells A and B, higher β-HCH concentrations were found at well D (2008: 10 μg/L, 2009: 11 μg/L, 2010: 15 μg/L). This increase in β-HCH concentration along the central groundwater flow could be attributed to the distinct source zone in the area of well D, which was confirmed by the ^{13}C-depleted β-HCH at well D (2008: −29.8‰) (Fig. SI5). Changes in $\delta^{13}C_{\beta\text{-HCH}}$-values from 2008 to 2010 (>+4‰) suggested, despite slightly increasing β-HCH concentrations, that β-HCH biodegradation proceeded at well D. The slight rise in β-HCH concentrations might be caused by an increase in β-HCH recharge from the unsaturated zone into the groundwater. However, carbon isotope ratios revealed an enhancement of β-HCH biodegradation, which probably prevented an even higher increase in β-HCH concentrations at well D. Compared to well D, β-HCH was more ^{13}C-enriched and exhibited lower β-HCH concentrations at well E, indicating β-HCH biodegradation on the flow path between those two wells. The decrease in β-HCH concentration at well E was accompanied by a shift in carbon isotope ratios of >+6‰, which provided evidence that biodegradation contributed to the temporal decline in β-HCH concentration.

Within the eastern fringe of the contaminant plume, a ^{13}C-enrichment for β-HCH of >+4‰ was observed between 2008 and 2010 (Fig. SI5). Thus, biodegradation contributed to the decrease in β-HCH concentrations at this well over time.

In summary, carbon isotope data of β-HCH confirmed the distinct pollutant source at well D and provided evidence for β-HCH biodegradation along groundwater flow paths from well A to B and from well D to E. Moreover, an increase in β-HCH biodegradation was revealed at wells D and 7 over time.

3.2.4. *γ-HCH*

Concentrations of γ-HCH were only high enough for measuring reliable $\delta^{13}C_{\gamma\text{-HCH}}$-values at few wells (3, A, D, E).

The highest γ-HCH concentration (99 μg/L) and a $\delta^{13}C_{\gamma\text{-HCH}}$-value of −27.4‰ were measured at well 3 in 2009 (Fig. SI6). The γ-HCH concentration indicated a distinct source zone at well 3 (see 3.1.1) which could be confirmed by the relative ^{13}C-depleted γ-HCH at this well in 2009. The decrease in γ-HCH concentration to 15 μg/L in 2010 was accompanied by a shift in $\delta^{13}C_{\gamma\text{-HCH}}$-values of +1.7‰, indicating that biodegradation contributed to the temporal removal of γ-HCH at well 3.

Within the central groundwater flow, γ-HCH was more ^{13}C-enriched and exhibited lower concentration at well A (2008: −24.4‰, 3 μg/L) compared to well 3, indicating γ-HCH biodegradation within the source zone at well A.

Compared to wells A and 3, ^{13}C-depleted γ-HCH was observed at well E (2008: −29.7‰, 2009: −27.9‰, 2010: −30.1‰), which confirmed the discrete HCH source zone at this well. Similar $\delta^{13}C_{\gamma\text{-HCH}}$-values indicated that physical processes seemed to lead to the decrease in γ-HCH concentration at well E (2008: 41 μg/L, 2009: 29 μg/L, 2010: 10 μg/L). Compared to well E, γ-HCH exhibited lower concentration and was more ^{13}C-enriched at well D (2008: 5 μg/L, −25.7‰), which could be attributed to γ-HCH biodegradation.

Based on $\delta^{13}C_{\gamma\text{-HCH}}$-values, a distinct contaminant source zone for γ-HCH could be confirmed at well 3. The discrete HCH source zone at well E was approved as well. Indications for γ-HCH biodegradation were found at wells A and D.

3.2.5. *Discussion of CSIA results*

The carbon isotope ratios of HCHs confirmed three distinct contaminant source zones at the field site which were indicated by concentration data. Based on historical information, the source at well A can be considered to result from contamination at former processing facilities and the source at wells D and E from contamination at the former dump site of HCH wastes (Fig. 3). Moreover, a distinct γ-HCH source at well 3 seemed to originate from contamination at a former storage depot (Fig. 3). Since γ-HCH was the predominant HCH isomer, it can be concluded that it was stored in the vicinity of well 3 after purification from technical HCH.

Carbon isotope data of HCHs provided evidence of HCH biodegradation downstream of the HCH source zones at the former processing facilities and dump site, revealing that biodegradation contributed to the natural attenuation of HCHs within the investigated aquifer (Fig. 3). However, in some cases the decrease in concentration of HCHs was caused by physical processes. Since sorption and evaporation of HCHs can be neglected due to both the low organic matter content in the aquifer's matrix (0.014 %) and the low tendency of volatilization of HCHs from water (Sahsuvar et al., 2003), dispersion, dilution and HCH recharge from the unsaturated zone into the groundwater are likely the most relevant physical processes influencing the concentration of HCHs within the aquifer.

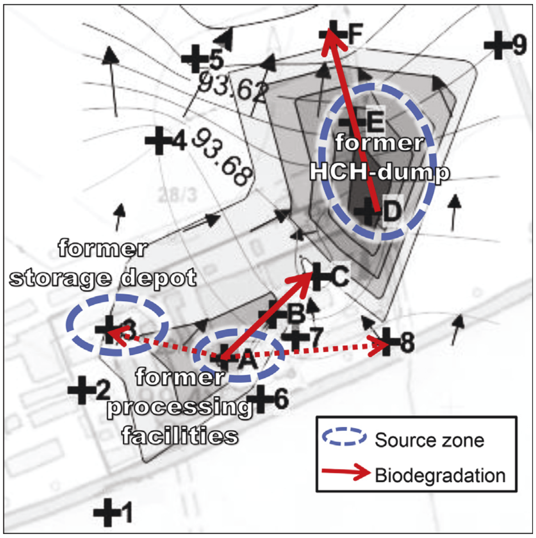

Fig. 3 – Conceptual site model for sources and sinks of HCHs within the upper aquifer of the field site. Dashed ellipses show pollutant source zones. Solid red arrows illustrate HCH biodegradation, whereas dotted red arrows show expected HCH biodegradation because direct flow paths between wells are ambiguous. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

From 2008 to 2010, HCHs showed a decrease in concentrations concomitant with ^{13}C-enrichment at most wells, indicating that the contribution of biodegradation to natural attenuation of HCHs increased over time. At few wells, HCHs displayed an inconsistent relationship between changes in carbon isotope ratios and changes in concentrations. In those cases, trends of concentrations of HCHs could provide information on the overall natural attenuation or recharge of HCHs but only limited indications for biodegradation. In addition, CSIA could more precisely reveal whether biodegradation contributed to natural attenuation of HCHs and, therefore, provided considerable information on the fate of pollutants at the field site.

3.3. *Quantification of HCH biodegradation*

Using the Rayleigh-equation approach (Thullner et al., 2012), calculations of the percentage of biodegradation (B [%]), distance- and time-dependent *in situ* first-order biodegradation rate constants (λ_s [1/m], λ_t [1/d]) as well as half-life distances and times ($s_{1/2}$ [m], $t_{1/2}$ [d]) were carried out for flow paths within the main groundwater flow direction. In order to evaluate the sustainability of microbial removal, HCH biodegradation was quantified based on isotope data obtained for monitoring campaigns in 2008, 2009 and 2010. Due to low γ-HCH concentrations, only a limited number of $\delta^{13}C_{\gamma\text{-HCH}}$-values were obtained and, thus, it was not possible to quantify γ-HCH biodegradation.

The use of appropriate isotope enrichment factors (ε) is required for the quantification of HCH biodegradation based on the Rayleigh-equation approach. The detailed selection of ε-values used for calculating HCH biodegradation is described in SI. A ε-value of −3.7±0.8‰ was applied for the quantification of anaerobic α-HCH biodegradation and a ε-value of −3.9±0.6‰ for the quantification of anaerobic β- and δ-HCH biodegradation.

3.3.1. *Percentage of biodegradation*

Biodegradation of α-HCH could be calculated for the flow path from well A (source zone of the former processing facilities) to well B (A→B) in 2010 and revealed a value of 42 % (Table 1). For the same flow path, β-HCH biodegradation increased from 34 % in 2008 to 86 % in 2010 (Table 1). Also for this flow path (A→B), δ-HCH exhibited a biodegradation of 46 % in 2009, while biodegradation in 2008 and 2010 was negligible (Table 1). Increasing δ-HCH biodegradation was determined for the flow path A→C (2008: 30 %, 2009: 45 %, 2010: 50 %) (Table 1).

Downstream of the source zone of the former HCH dump (E→F), δ-HCH biodegradation of 67 % in 2008 and 75 % in 2010 were obtained, while it was negligible in 2009 (Table 1), revealing temporal variability in the contribution of biodegradation to natural attenuation of δ-HCH at the northern fringe of the contaminant plume. β-HCH exhibited a quite constant extent of biodegradation for the flow path D→E in 2008 (38 %) and 2010 (41 %), but its biodegradation was negligible in 2009. Thus, temporal variability in the extent of β-HCH biodegradation was obvious along this flow path.

Table 1 – Percentage of biodegradation (B [%]), distance-dependent and time-dependent *in situ* first-order biodegradation rate constants (λ_s [1/m], λ_t [1/d]) as well as half-life distances and times ($s_{1/2}$ [m], $t_{1/2}$ [d]) for HCHs calculated for flow paths of the central groundwater flow of the contaminant plume (wells A–F) in 2008, 2009 and 2010. Uncertainties were calculated according to error propagation described in SI.

Pollutant	Flow path	Distance [m]	Residence time [d]	Year	B [%]	λ_s [1/m]	$s_{1/2}$ [m]	λ_t [1/d]	$t_{1/2}$ [d]
α-HCH	A→B	52	175	2008	n.d.				
				2009	n.d.				
				2010	42[a] ± 11	(10 ± 4) × 10^{-3}	67 ± 24	(3 ± 1) × 10^{-3}	223 ± 80
β-HCH	A→B	52	175	2008	34[a] ± 9	(8 ± 3) × 10^{-3}	86 ± 30	(2 ± 1) × 10^{-3}	287 ± 100
				2009	n.d.				
				2010	86[b] ± 5	(37 ± 6) × 10^{-3}	19 ± 3	(11 ± 2) × 10^{-3}	62 ± 10
	D→E	72	240	2008	41[b] ± 8	(7 ± 1) × 10^{-3}	44 ± 10	(2.2 ± 0.2) × 10^{-3}	148 ± 35
				2009	n.s.				
				2010	38[a] ± 10	(7 ± 2) x 10^{-3}	36 ± 6	(2 ± 1) x 10^{-3}	120 ± 20
δ-HCH	A→B	52	175	2008	n.s.				
				2009	46[b] ± 9	(12 ± 3) × 10^{-3}	60 ± 16	(4 ± 1) × 10^{-3}	199 ± 52
				2010	n.s.				
	A→C	98	327	2008	30[a] ± 12	(4 ± 2) × 10^{-3}	190 ± 78	(1.1 ± 0.5) × 10^{-3}	632 ± 309
				2009	45[b] ± 9	(6 ± 2) × 10^{-3}	112 ± 30	(1.9 ± 0.4) × 10^{-3}	374 ± 91
				2010	50[b] ± 7	(7 ± 1) × 10^{-3}	99 ± 17	(2.1 ± 0.4) × 10^{-3}	331 ± 61
	E→F	71	237	2008	67[b] ± 8	(16 ± 4) × 10^{-3}	44 ± 10	(5 ± 1) × 10^{-3}	148 ± 35
				2009	n.s.				
				2010	75[b] ± 6	(19 ± 3) × 10^{-3}	36 ± 6	(6 ± 1) × 10^{-3}	120 ± 20

n.d. – not determinable because carbon isotope ratio could not be measured due to low concentration.

n.s. – biodegradation is not significant.

[a] Biodegradation is indicated as δ^{13}C-values used for calculation differ by >+1‰ to ≤+2‰

[b] Proof of biodegradation as δ^{13}C-values used for calculation differ by >+2‰

In general, highest biodegradation was estimated for most of the flow paths in 2010, indicating a general trend of increasing HCH biodegradation over time.

3.3.2. *In situ first-order biodegradation rate constants*

Time- and distance-dependent *in situ* first-order biodegradation rate constants (λ_t, λ_s) for α-HCH were estimated at 3×10^{-3} d^{-1} and 10×10^{-3} m^{-1} for the flow path A→B in 2010 (Table 1). For the same flow path and year, a higher rate constant was obtained for β-HCH (11×10^{-3} d^{-1} and 37×10^{-3} m^{-1}), indicating a preferential biodegradation of β-HCH compared to α-HCH. For the flow path A→B, biodegradation rate constants of δ-HCH were mainly lower than those of α- and β-HCH (Table 1). Thus, it can be inferred that δ-HCH was the most recalcitrant HCH isomer with respect to calculated biodegradation rate constants and half-life values (Table 1). A high recalcitrance of δ-HCH under anoxic conditions has also been suggested in other studies (Lal et al., 2010; Mehboob et al., 2013).

Compared to flow path A→B, higher biodegradation rate constants for δ-HCH were estimated for flow paths A→C (λ_t: up to 2.1×10^{-3} d^{-1}, λ_s: up to 7×10^{-3} m^{-1}) and E→F (λ_t: up to 6×10^{-3} d^{-1}, λ_s: up to 19×10^{-3} m^{-1}), indicating more pronounced δ-HCH removal within the further groundwater flow of the source zone at the former processing facilities and the northern fringe of the contaminant plume, respectively. In contrast to δ-HCH, biodegradation rate constants for β-HCH increased for the flow path A→B over time and were significantly higher compared to flow path D→E in 2010 (λ_t: up to 2×10^{-3} d^{-1}, λ_s: up to 7×10^{-3} m^{-1}). Thus, β-HCH exhibited higher biodegradation rate constants downstream of the source zone at the former processing facilities compared to the source zone at the former HCH dump site.

Overall, λ_t-values obtained in this study were in the same range as rate constants for anaerobic HCH biodegradation determined for laboratory experiments (Langenhoff et al., 2001; Quintero et al., 2005).

4. Conclusions

Carbon isotope ratios of HCHs in combination with pollutant concentration patterns and historical information were used to evaluate sources and *in situ* biodegradation for HCH isomers in the investigated aquifer. Moreover, comprehensive information on the temporal progress of HCH biodegradation could be revealed employing time-resolved CSIA. The obtained knowledge is currently used for decision making whether and how a MNA concept can be implemented as sole measure or in combination with remediation of source zones at the field site.

Based on our study, we could highlight:

- the applicability of CSIA for the assessment of biodegradation and the source identification of HCHs within contaminated aquifers,
- the potential of CSIA for the quantification of HCH biodegradation,
- that time-resolved CSIA can reveal temporal variations in HCH biodegradation and provide information on the influences of various processes on natural attenuation.

Due to the intensive production of HCHs and their worldwide usage, there are a huge number of HCH-contaminated production, formulation and dump sites (Vijgen et al., 2011). At these sites, time-resolved CSIA could be applied to identify trends in attenuation of HCH isomers and help to predict the evolution of contaminant plumes, as exemplified for the first time in our study. *In situ* biodegradation rate constants could be integral in modeling the current status and future development of contaminant plumes. Thus, CSIA possesses the potential for improved prediction of HCH distribution within contaminated aquifers. The presented results suggest that CSIA constitutes a viable monitoring tool and could be beneficial for the implementation and control of innovative management and remediation concepts like *Monitored* or *Enhanced Natural Attenuation* (MNA, ENA) at HCH contaminated field sites.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2014.12.033.

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Supporting Information

Evaluating degradation of hexachlorocyclohexane (HCH) isomers within a contaminated aquifer using compound-specific stable carbon isotope analysis (CSIA)

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Content: 7 sections, 16 pages, 6 figures, 1 table

SI-1 Standard procedures for groundwater sampling and concentration analyses of pollutants and hydrogeochemical parameters

- Groundwater sampling was performed according to DIN 38402-13 [1] and DVWK-Merkblatt 245/1997 [2].
- HCHs concentration analysis was performed according to DIN 38407-F2 [3].
- Sulfate und nitrate concentration analysis was performed according to DIN EN ISO 10304-1 D 19 [4].
- Ammonium concentration analysis was performed according to DIN 38406 E 5 [5].
- Methane concentration analysis was performed according to EDI guideline, 2. Part: Surface water [6].

Uncertainty of concentration measurements was <10 % of the obtained value.

SI-2 CSIA of HCHs

Isotope laboratory standards of HCHs

α-, β-, γ- and δ-HCH were purchased as pure compounds (97-99 %, Sigma-Aldrich Chemie GmbH, Germany) and carbon isotope ratios were determined by elemental analyser - isotope ratio mass spectroscopy (EA-IRMS).[7] The carbon isotope ratios of HCHs were reported in delta notation ($\delta^{13}C$) vs. V-PDB (see Eq. 1 in the main text) based on a two-point calibration with reference materials obtained from International Atomic Energy Agency (IAEA) (IAEA-CH-6, IAEA-CH-7). The calibration was verified by an additional reference material (IAEA-CH3). EA-IRMS analyses were performed in triplicates with standard deviations < 0.2 ‰ (Table SI 1). Carbon isotope ratios of HCHs measured by EA-IRMS were used as isotope laboratory standards to monitor the instrument performance of CSIA for HCHs and to check reliability of the extraction procedure of HCHs from water samples.

GC-IRMS

CSIA of HCHs was performed by gas chromatography - isotope ratio mass spectrometry (GC-IRMS) as described elsewhere.[8] The GC-IRMS system consisted of a gas chromatograph (6890 Series; Agilent Technology, USA) coupled via a GC/C III interface to a MAT 252 mass spectrometer (both Thermo Fisher, Germany). The gas chromatograph was equipped with split/splitless injector and a ZB-1 column (60 m, 0.32 mm, 1 µm; Phenomenex, USA). Helium was used as carrier gas (2 mL/min) for the chromatographic separation. The combustion reactor containing Pt, Ni, CuO (Thermo Fisher, Germany) was operated at 980°C. The combustion oven was re-oxidised frequently and the performance of the combustion was monitored by regular (every six samples) analysis of the isotope laboratory standards of HCHs.

Evaluation of the extraction procedure of HCHs from water phase

In order to evaluate possible isotope effects of the extraction of HCHs from water samples (see section 2. Materials and methods in the main text) the whole procedure was examined for changes in carbon isotope ratios of HCHs. Glass bottles with a volume of 1150 mL (Schott, Germany) were filled almost completely with tap water. The flasks were spiked with the isotope laboratory standards of HCHs (in acetone) to a final concentration of 100 µg/L for each HCH isomer. Similar to the groundwater samples, 1ml HCl (6M) was added for preservation. The water samples were extracted 3 times with 30 mL dichloromethane (DCM) in a separating funnel (shaking time 1.5 min). DCM extracts were combined and dried with anhydrous Na_2SO_4. DCM extracts were evaporated with a rotary evaporator up to 1-2 ml solvent volume and then completely in a gentle nitrogen stream at room temperature. The dry extracts were dissolved in 0.5 mL of acetone. The extracts were measured with GC-IRMS and the obtained $\delta^{13}C$-values of HCHs were compared to $\delta^{13}C$-values of HCHs from isotope laboratory standards. The difference between $\delta^{13}C$-values of HCHs obtained for extracts and isotope laboratory standards were ≤0.6 ‰ (Fig. SI1), indicating that extraction and sample pre-concentration do not lead to significant carbon isotope effects for HCHs.

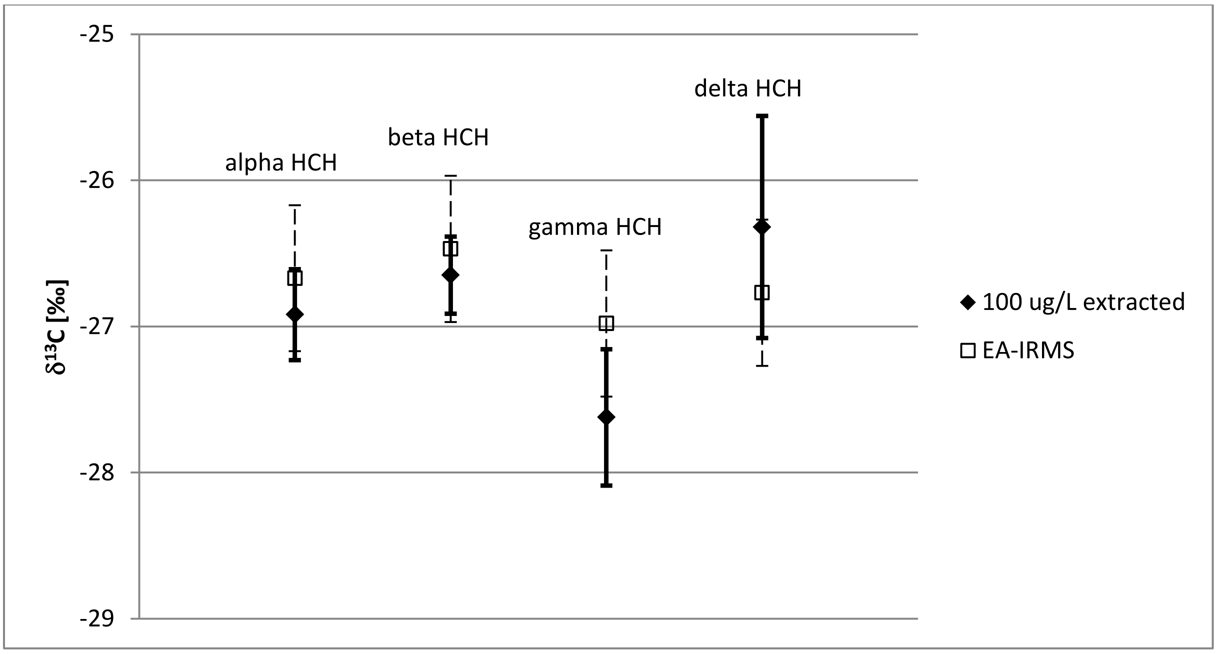


Fig. SI1: Comparison of $\delta^{13}C$-values of HCHs measured after extraction from water and pre-concentration using GC-IRMS (♦) to $\delta^{13}C$-values of HCHs obtained by EA-IRMS (□). Black bars indicate the standard deviation of $\delta^{13}C$-values of GC-IRMS analyses. Dashed bars indicate a demonstrative ±0.5 ‰ range of the $\delta^{13}C$-values of EA-IRMS analyses in order to illustrate the relation to the respective $\delta^{13}C$-values of GC-IRMS analyses.

Method validation

The linearity of the method was evaluated and the limits of detection were defined to improve the sensitivity and precision of the method compared to our previous study.[8] Mixtures of the isotope laboratory standards of HCHs in acetone at different concentrations were analysed and the results compared to the δ^{13}C-values obtained by EA-IRMS and evaluated with respect to the produced CO_2 signal intensity (*m/z* 44, in V). Exemplarily, the results for δ-HCH are shown in Fig. SI2.

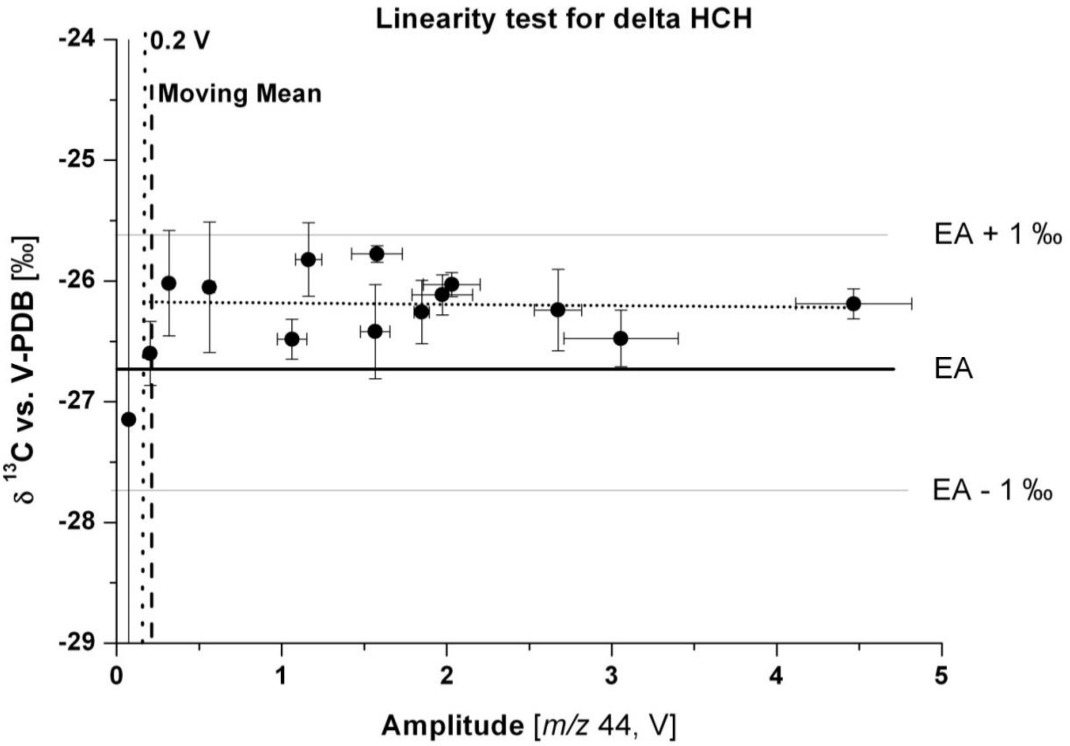


Fig. SI2: Linearity test for GC-IRMS analysis of δ-HCH with method detection limits indicated for arbitrary threshold (vertical dotted line 0.2 V criteria) and moving mean procedure (vertical dashed line). The linear regression for δ^{13}C-values of δ-HCH exceeding the method detection limits (dotted horizontal line) and the δ^{13}C-value obtained by EA-IRMS (black horizontal line) are indicated.

Method detection limits were derived from peak signals, which indicated minimal intensity for accurate and precise δ^{13}C-values. There are different possibilities to determine the lower method detection limit. In this study, we choose arbitrary threshold values of an intensity >200 mV and a standard deviation of 1σ <0.5 ‰. Another approach is the iterative moving mean procedure for which a moving average of three values has to have a standard deviation of 1σ <0.5 ‰.[9] The first average, moving from the most intensive peak of the linearity test to the lower concentration range, which cannot fulfil this criterion, defines the lower detection limit.[9] This procedure resulted in higher detection limits, however, very similar linearity slopes and average δ^{13}C-values (Tab. SI1). Linearity slopes of HCHs were <0.3 ‰/V (arbitrary thresholds) and even <0.2 ‰/V for the range of higher intensities (moving mean) (Tab. SI1). In comparison to previously published linearity [8], our approach showed that CSIA allows interpretation of carbon isotope ratios for all HCHs in the concentration range of field samples. Linearity results for δ-HCH, the main HCH isomer of this study, were better than previously published [8] and thus increased reliability for interpretation.

The comparison of results with different intensities was therefore determined to be valid. As field samples were analysed in this study, only results with intensities of at least 200 mV were accounted for data interpretation.

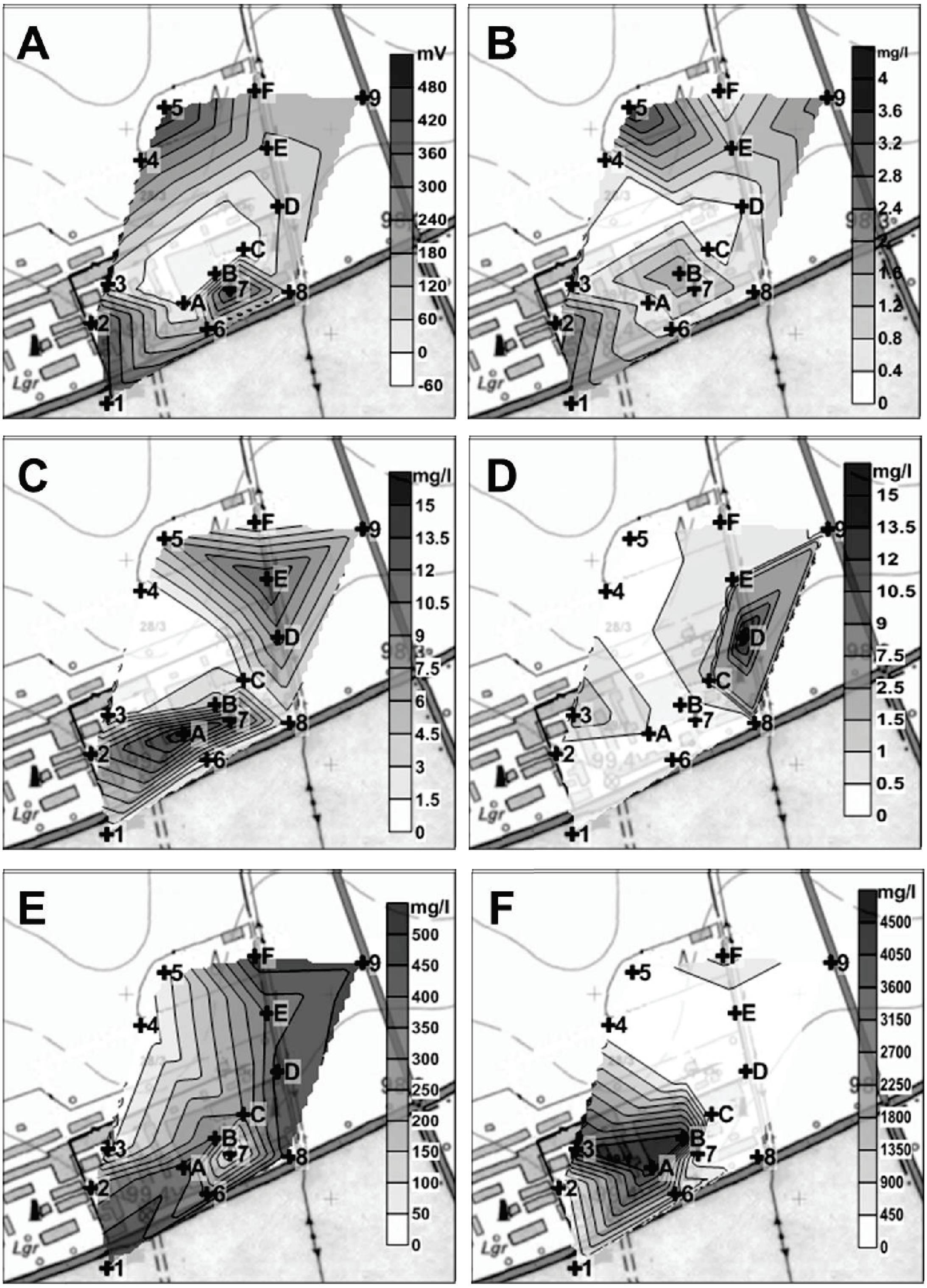
Tab. SI1: Comparison of method detection limits, average δ^{13}C-values in the linear range and linearity slopes for the two approaches of the determination of detection limits. a) Standard deviation of three replicates is <0.2 ‰.

	EA-IRMS	iterative moving mean procedure [9]					arbitrary thresholds intensity < 200 mV; σ > 0.5 ‰				
				method detection limit					method detection limit		
HCH	**δ^{13}C [‰]a)**	average δ^{13}C [‰]	1σ	min [mV]	max [mV]	Linearity slope [‰/V]	Average δ^{13}C [‰]	1σ	min [mV]	max [mV]	Linearity slope [‰/V]
α	**-26.7**	-27.1	0.3	756	4089	-0,20	-27.1	0.4	310	4089	-0.25
γ	**-27.0**	-27.8	0.1	640	4860	-0.01	-27.7	0.3	294	4860	-0.12
β	**-26.5**	-26.2	0.3	411	3243	0.08	-26.3	0.3	273	3243	0.17
δ	**-26.8**	-26.2	0.3	203	4466	-0.01	-26.2	0.3	203	4466	-0.01

SI-3 Hydrogeochemical parameters

Since hydrogeochemical parameters were quite similar for the monitoring campaigns in 2008, 2009 and 2010, redox conditions of the investigated aquifer are exemplarily described for the data set obtained in 2010. The source zone around well A as well as the central groundwater flow of this area at wells B and C exhibited redox potentials <-20 mV (Fig. SI 3A) and oxygen concentrations below the detection limit of 1 mg/L (Fig. SI 3B), indicating reducing and anoxic conditions within these aquifer zones. Redox potentials and oxygen concentrations tended to increase upstream and further downstream of the expected source zones (Fig. SI3A, Fig. SI3B), revealing more oxidizing conditions towards the fringes of the contaminant plume. At some wells (A, D, E, 2, 7), high nitrate concentrations from 8 to 15 mg/L (Fig. SI3C) suggested that nitrate seemed to be a relevant electron acceptor. A significantly high ammonium concentration of 14 mg/L was determined at well D (Fig. SI3D), which might be caused by strong nitrate reduction or an anthropogenic input. Sulfate concentrations were higher in the source zone at well A (380 mg/L) while significantly lower concentrations were observed in the direct and eastern groundwater flow downstream of this zone at wells C (252 mg/L) and 7 (52 mg/L), respectively (Fig. SI3E). The decrease in sulfate concentration could be attributed to sulfate reduction at these two wells. Within the central and eastern fringe of the plume, sulfate concentrations were relatively high (441 mg/L at well 8, 463 mg/L at 9, 486 mg/L at F) (Fig. SI3E) compared to other wells in the plume, suggesting that sulfate reduction was a negligible electron acceptor process in these zones. The north-western fringe of the contaminant plume (39 mg/L at well 3, 246 mg/L at well 4) showed an opposite trend (Fig. SI3E), which gave indication for sulfate reduction. The highest methane concentration (4500 µg/L) was measured at well A (Fig. SI3F). Thus, methanogenesis occurred within this contaminant source zone. Methane concentrations mainly decreased with increasing distance from well A (Fig. SI3F), suggesting that methanogenesis is not an important biogeochemical process at the fringe of the contaminant plume. An exception was well F, where a relative high methane concentration (1100 µg/L) was detected (Fig. SI3F), suggesting a distinct area of methanogenesis which seemed to be in the vicinity of zones with more oxidizing conditions. Thus, heterogeneous redox conditions could be expected in some areas of the fringe of the contaminant plume.

Fig. SI3: Distribution of redox potential (A), oxygen concentration (B), nitrate concentration (C), ammonium concentration (D), sulfate concentration (E) and methane concentration (F) within the upper aquifer of the field site for the monitoring campaign of 2010.



SI-4 Error propagation for quantification of biodegradation

Uncertainties of the percentage of biodegradation (B [%]) were calculated according to the error propagation described by Thullner et al. (2012)[10]. For first-order biodegradation rate constants, half-life distances and half-life times, error propagation was applied according to Eq. SI 1:

$$u_y = \sqrt{(\frac{\partial y}{\partial x_1} * u_{x_1})^2 + (\frac{\partial y}{\partial x_2} * u_{x_2})^2 + \cdots + (\frac{\partial y}{\partial x_i} * u_{x_i})^2} \; u_y =$$

$$\sqrt{(\frac{\partial y}{\partial x_1} * u_{x_1})^2 + (\frac{\partial y}{\partial x_2} * u_{x_2})^2 + \cdots + (\frac{\partial y}{\partial x_i} * u_{x_i})^2} \qquad \text{(SI 1)}$$

where x_i are the variables determining the calculated quantity (y; here rate constants, half lifes) and u_i their respective errors.

SI-5 Selection of isotope enrichment factors for quantification of HCH biodegradation

Since the contaminant plume exhibited mainly anoxic conditions, carbon isotope enrichment factors (ε) which were determined for anaerobic HCH degradation [8, 11, 12] were applied for the calculation of biodegradation of HCHs at the investigated field site. So far, ε-values have only been determined for anaerobic α-HCH biodegradation by the bacterial strain *Clostridium pasteurianum* with -3.7 ± 0.8 ‰ [11] and for anaerobic γ-HCH biodegradation by the bacterial strains *Desulfococcus multivorans* DSM 2059 and *Desulfovibrio gigas* DSM 1382 with -3.4 ± 0.5 ‰ and with -3.9 ± 0.6 ‰ [8], respectively. These carbon isotope enrichment factors were applied for the quantification of *in situ* degradation for α-HCH (-3.7 ± 0.8 ‰) and for γ-HCH (-3.9 ‰ ± 0.6 ‰). Similar reaction mechanisms can be expected for anaerobic biodegradation of all HCHs [13], which is confirmed by the similarity of carbon isotope enrichment factors determined by Badea *et al.* (2009, 2011) [8, 12]. Thus, it can be assumed that all HCH isomers should exhibit similar carbon isotope fractionation. Based on this assumption, the carbon isotope enrichment factor for anaerobic γ-HCH biodegradation exhibiting highest carbon isotope fractionation (-3.9 ‰ ± 0.6 ‰) was applied to calculate the biodegradation of β- and δ-HCH which leads to a conservative estimation of *in situ* degradation of the two HCH isomers in comparison to the application of the ε-value for α-HCH.

The ε-values for anaerobic HCH biodegradation determined so far are relative similar and, therefore, indicate a consistent trend leading to the assumption that the impact of variability of isotope enrichment factors on the quantification of HCH degradation is relative low. However, whether this trend can be expected for all circumstances of anaerobic HCH biodegradation needs to be confirmed. Further studies are required for determining ε-values especially for anaerobic β- and δ-HCH biodegradation as well as for a broader range of pure and mixed cultures which are able to degrade HCHs under anoxic conditions. The broader knowledge on isotope enrichment factors for anaerobic HCH biodegradation would be beneficial for the quantification of *in situ* HCH removal because isotope enrichment factors can be selected more properly according the prevailing degradation conditions at a contaminated field site. Thus, it can improve the modelling of the current status and future development of HCH plumes in contaminated aquifers.

SI-6 Concentrations and carbon isotope ratios of α-HCH, β-HCH and γ-HCH

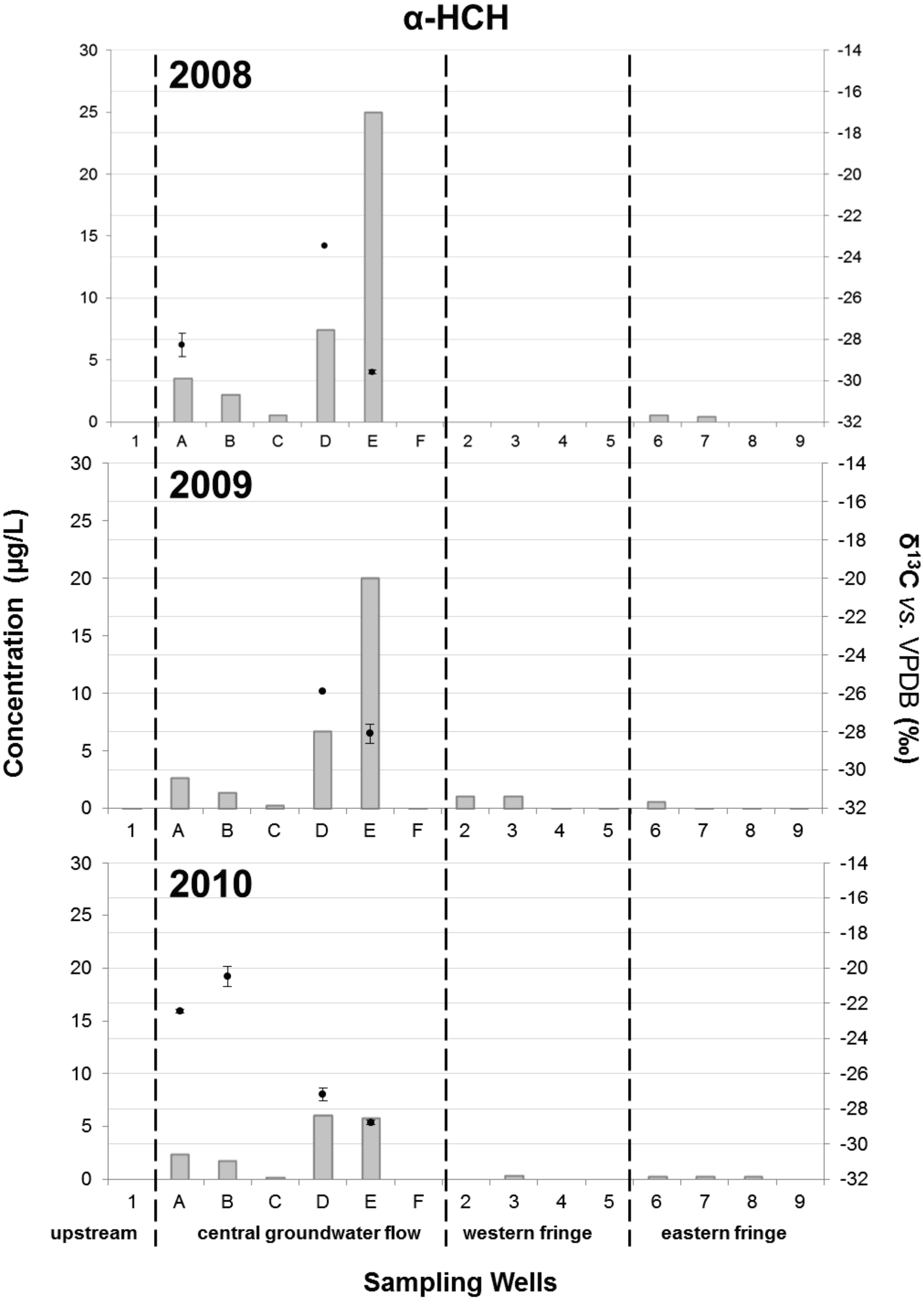


Fig. SI4: Concentrations (bars) and carbon isotope ratios (dots) of α-HCH for the monitoring campaigns in 2008, 2009 and 2010. Wells 1 and 3 were established in 2009, thus, concentration and isotope data are not available for the monitoring campaign in 2008. Uncertainty of concentration analysis is <10 % in all cases. Errors of δ^{13}C-values are indicated as error bars.

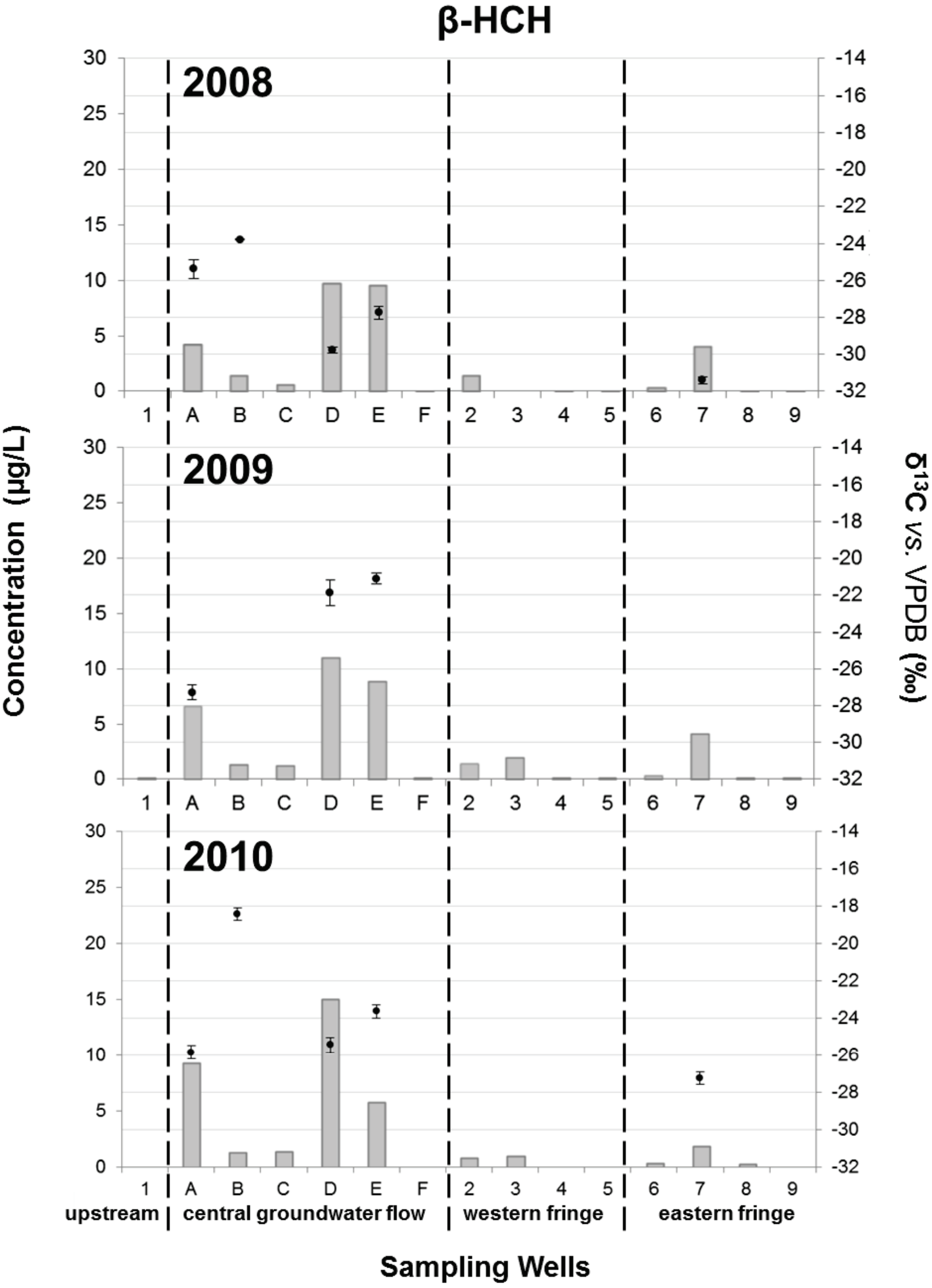


Fig. SI5: Concentrations (bars) and carbon isotope ratios (dots) of β-HCH for the monitoring campaigns in 2008, 2009 and 2010. Wells 1 and 3 were established in 2009, thus, concentration and isotope data are not available for the monitoring campaign in 2008. Uncertainty of concentration analysis is <10 % in all cases. Errors of δ^{13}C-values are indicated as error bars.

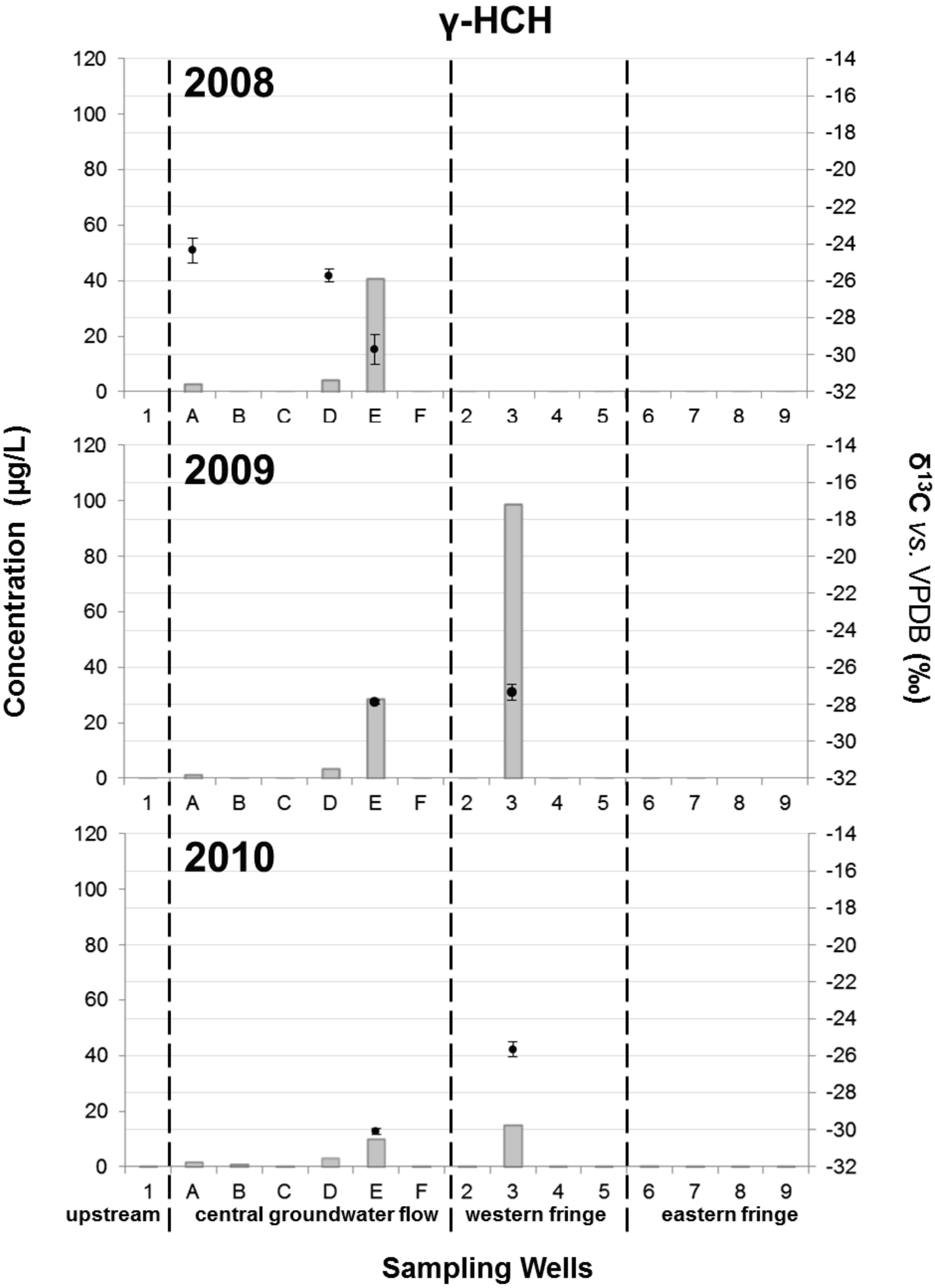


Fig. SI6: Concentrations (bars) and carbon isotope ratios (dots) of γ-HCH for the monitoring campaigns in 2008, 2009 and 2010. Wells 1 and 3 were established in 2009, thus, concentration and isotope data are not available for the monitoring campaign in 2008. Uncertainty of concentration analysis is <10 % in all cases. Errors of δ^{13}C-values are indicated as error bars.

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