

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

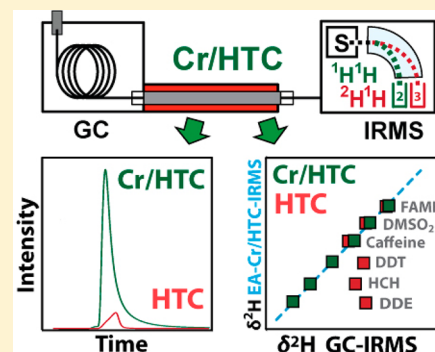
Julian Renpenning,[†] Steffen Kümmel,[†] Kristina L. Hitzfeld,[†] Arndt Schimmelmann,[‡] and Matthias Gehre^{*,†}

[†]Department for Isotope Biogeochemistry, Helmholtz-Centre for Environmental Research–UFZ, Permoserstrasse 15, D-04318 Leipzig, Germany

[‡]Department of Geological Sciences, Indiana University, Bloomington, Indiana 47405-1405, United States

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 H₂S. 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 H₂ 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 $\delta^2\text{H}$ range of -181 mUr to 629 mUr.



Hydrogen stable isotope analysis and the interpretation of resulting $\delta^2\text{H}$ 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 (H₂) analyte gas using reducing metals such as zinc, uranium, chromium, magnesium, or tungsten.^{2–4} Subsequently, $\delta^2\text{H}$ values were determined in manual dual-inlet mode using isotope-ratio mass spectrometry (DI-IRMS). Direct pyrolytic conversion of organically bound hydrogen to H₂ 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 H₂ 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 H₂ yields are

incomplete due to the formation of hydrogen-containing byproducts (HCN, HCl, and H₂S).^{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 H₂ and accurate $\delta^2\text{H}$ values. EA conversion of polyhalogenated compounds with chromium at 1000 °C, however, resulted in incomplete H₂ 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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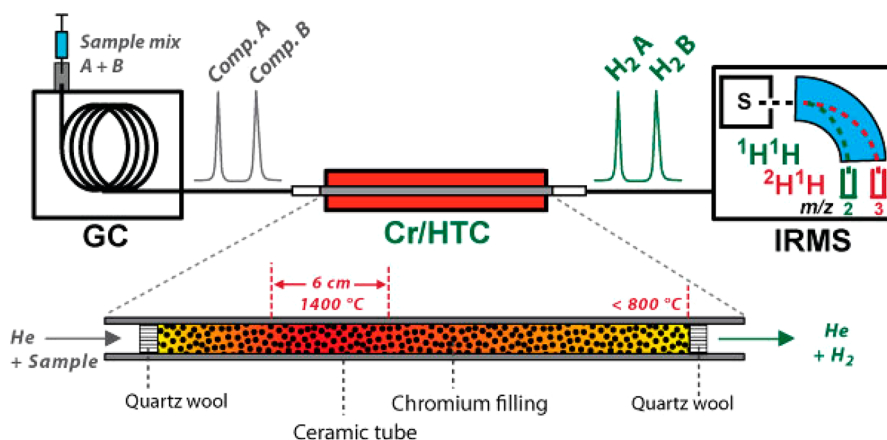


Figure 1. Schematic design of the Cr/HTC reactor incorporated into a standard GC-HTC-IRMS system for converting organic hydrogen in heteroatom-bearing samples to molecular hydrogen for subsequent determination of the hydrogen isotopic composition. All chemical elements in samples except hydrogen are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

ratio analysis of organic compounds bearing heteroelements.¹⁷ Quantitative H_2 yields and accurate $\delta^2\text{H}$ 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 Shoukar-Stash and Drimmie¹⁶ and Kuder and Philp,¹⁵ a ceramic reactor tube was packed with chromium and used as an HTC reactor at $> 1100\text{ }^\circ\text{C}$. Our system allows temperatures of up to $1500\text{ }^\circ\text{C}$ 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, $\geq 99\%$ 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; $\geq 98\%$), DDE (UFZ 2; *p,p'*-dichlorodiphenyldichloroethylene; ABCR, Germany; $\geq 99\%$), DDT (UFZ 3; 4,4'-dichlorodiphenyltrichloroethane; Supelco, USA; $\geq 97\%$), HCH (UFZ 4; γ -hexachlorocyclohex-

ane; HiMedia, India; $\geq 99\%$), DMSO2 (UFZ 5; dimethyl sulfone; Alfa Aesar, USA; $\geq 99\%$), Ph2S2 (UFZ 6; diphenyl disulfide; Sigma-Aldrich, USA; $\geq 99\%$), TCE-PPG (UFZ 7; trichloroethene; PPG Brand, USA; $\geq 98\%$), and TCE-Merck (UFZ 8; trichloroethene; Merck, Germany; $\geq 99\%$). All RMs were isotopically characterized against international primary stable isotope measurement standards VSMOW2 and SLAP2 (i) offline at Indiana University, using combustion in quartz ampoules, 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 $\delta^2\text{H}$ 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, Thermo Fisher, Bremen, Germany) was equipped with a horizontally arranged high-temperature oven (Thermo Finnigan, Germany) maintaining reactor temperatures of up to $1500\text{ }^\circ\text{C}$. 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 $250\text{--}300\text{ }\mu\text{m}$, 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 $800\text{ }^\circ\text{C}$ for 1 h and subsequently heated overnight to the operating temperature of $1400\text{ }^\circ\text{C}$ in the center of the reactor. The carrier gas flow became increasingly restricted at higher temperatures of the chromium powder. For example, at $1100\text{ }^\circ\text{C}$ a helium flow of 2.0 mL min^{-1} after the GC column resulted in a flow of $\sim 1.2\text{ mL min}^{-1}$ after the Cr/HTC reactor; at $1500\text{ }^\circ\text{C}$ the latter flow decreased to $\sim 1.0\text{ mL min}^{-1}$.

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. H₂ was transferred online through an open split into the IRMS. The H₃⁺ 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 H₂ 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. C₁₆ #A RM (δ²H = -167 mUr vs. VSMOW). Subsequent two-point calibrations of raw stable isotope ratios were performed with hexadecanes C₁₆ #A and C₁₆ #C RMs for all GC experiments to uniformly compensate for scale compression of the mass spectrometer and arrive at corrected δ²H values along the VSMOW-SLAP isotopic scale.²⁴ The abundance ratio of hydrogen isotopes [²H¹H]/[¹H¹H] 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

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

General Position for Calibration Strategy. Pulses of H₂ ‘reference gas’ from a gas cylinder were not used in this study for the calibration of δ²H values, because such practices would violate the principle of identical treatment of sample and standard where all analyte gases must pass through the same preparative-analytical sequence.²⁶ ‘Reference gas’ pulses from an H₂ cylinder are not generated in the same fashion as H₂ 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 H₂ ‘reference gas’ pulses except for monitoring IRMS performance. Although one-point calibration vs. C₁₆ #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 H₂ analyte gas is essential for avoiding fractionation and arriving at accurate δ²H values. Incomplete conversion or partial loss of hydrogen to byproducts other than H₂ is likely to result in δ²H values in H₂ 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

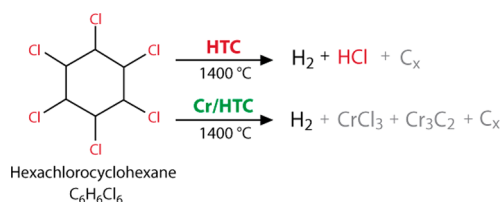


Figure 2. Nonstoichiometric equations for conversions of hexachlorocyclohexane to H₂ analyte gas via HTC and Cr/HTC at 1400 °C indicating the formation of HCl byproducts via HTC. In contrast the quantitative reaction of chlorine to chromium chloride in Cr/HTC results in quantitative H₂ yields.

compounds, or H₂S from sulfur-bearing compounds (Figures S1 and S2 in the Supporting Information).^{7,10,17,20,30} Some byproducts are known to influence the H₃⁺ 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.

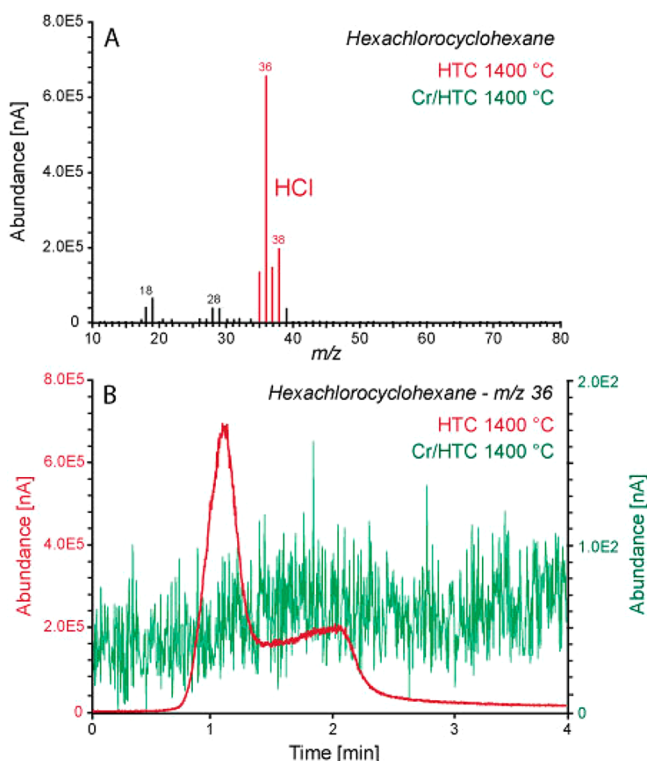


Figure 3. (A) Byproduct formation during conventional HTC (red) vs. Cr/HTC (green) monitored with an ion trap MS. HTC of hexachlorocyclohexane at 1400 °C resulted in strong formation of HCl with m/z 35–38. (B) Molecular ion m/z 36 (HCl) signal intensities of up to $\sim 10^6$ nA were observed during 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 back-

ground of $\sim 10^2$ 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 $\sim 10\%$ of the respective Cr/HTC yields, both at 1400 °C (Figure 4).

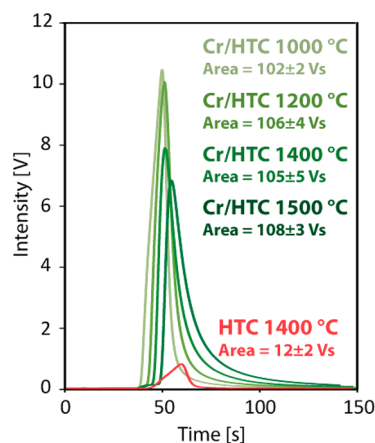


Figure 4. IRMS m/z 2 signal of H_2 from equal amounts of hexachlorocyclohexane after conventional HTC (red) and Cr/HTC (green). Conventional HTC of hexachlorocyclohexane at 1400 °C produced only $\sim 10\%$ of the signal strength (and thus H_2 yield) relative to Cr/HTC in the temperature range of 1000–1500 °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. $\delta^2\text{H}$ Values for Hexadecane (C_{16}), Caffeine, Hexachlorocyclohexane (HCH), and Dimethyl Sulfone (DMSO_2), Determined via Cr/HTC at 1000–1500 $^\circ\text{C}$ ^a

T ($^\circ\text{C}$)	DI-IRMS (Offline)	EA-Cr/HTC- IRMS (Online)	GC-Cr/HTC-IRMS (Online)					GC-Cr/HTC-IRMS (Online)				
		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
	$\delta^2\text{H}$	$\delta^2\text{H}$	$\delta^2\text{H}$					$\delta^2\text{H}$				
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

^aValues given in the SI unit urey (mUr, equivalent to ‰). All values were preliminarily one-point-calibrated using C_{16} #A reference material (RM) ($\delta^2\text{H} = -167$ mUr vs. VSMOW). Proper two-point calibration relied on C_{16} #A and C_{16} #C as anchoring RMs with $\delta^2\text{H}$ 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 ‰). ^bNot determined.

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

^a $\delta^2\text{H}$ values are expressed in the SI unit urey (mUr, equivalent to ‰).

^bReference materials used for two-point calibration along the VSMOW-SLAP isotopic scale, with $\delta^2\text{H}$ values determined in a collaborative ring-test project. ^cNot 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 $\delta^2\text{H}$ 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 C_{16} #C, DMSO_2 , Ph_2S_2 , and TCE-Merck. Discrepancies smaller than 16 mUr in $\delta^2\text{H}$ 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 $\delta^2\text{H}$ values of ~ 10 – 15 mUr. In contrast, GC-HTC-IRMS $\delta^2\text{H}$ 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 $\delta^2\text{H}$ determinations. Satisfying $\delta^2\text{H}$ values for nitrogen-bearing caffeine (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 $\delta^2\text{H}$ 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 $\delta^2\text{H}$ measurements, and thus precludes continuous $\delta^2\text{H}$ 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 $\delta^2\text{H}$ 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 $\delta^2\text{H}$ values of DDD, DDE, and DDT were negatively shifted by ~ 10 – 20 mUr relative to prescribed RM $\delta^2\text{H}$ values. Trichloroethene $\delta^2\text{H}$ values expressed a large positive shift of ~ 50 mUr, although

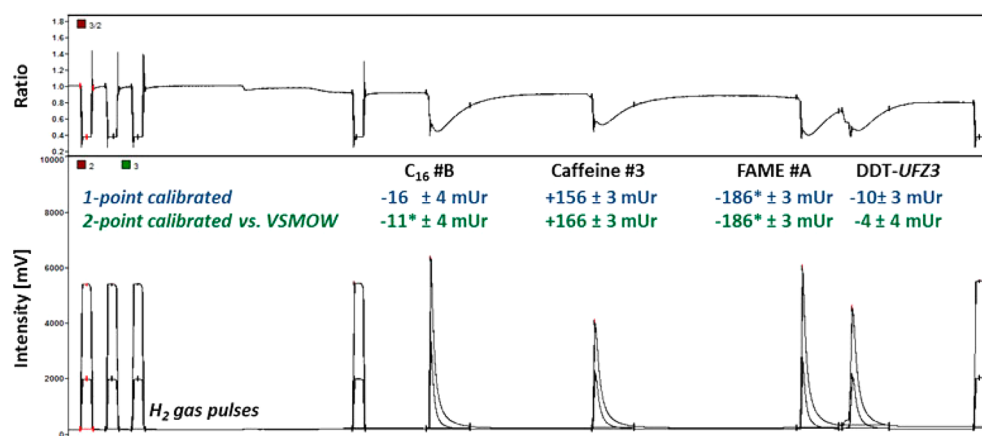


Figure 5. Compound-specific hydrogen isotope analysis of mixtures containing C_{16} #B, caffeine #3, FAME #A, and DDT converted via Cr/HTC at 1400 °C. The δ^2H values of the measured compounds cover a range of +166 mUr to -186 mUr ($\Delta\delta^2H \approx 350$ mUr). The precision of δ^2H values for 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 $\Delta\delta^2H$ 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	$\delta^2\text{H}$ [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

^aAll values were two-point-calibrated along the VSMOW–SLAP scale using C₁₆ #A and C₁₆ #C RMs.

atures above 1100 °C. This extension of Cr/HTC significantly (i) improves the conversion of organic hydrogen to H₂ from heteroelement-bearing compounds and (ii) reduces the formation of hydrogen-containing byproducts that limit H₂ 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. $\delta^2\text{H}$ 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 $\delta^2\text{H}$ 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 H₂ analyte gas; byproduct formation of hexadecane, caffeine, and dimethyl sulfone; linearity; comparability of EA–Cr/HTC–IRMS and GC–Cr/HTC–IRMS. (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Fax: +49 (0) 341 235 1443. E-mail: matthias.gehre@ufz.de.

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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