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Coupling of a Headspace Autosampler with a Programmed Temperature Vaporizer for Stable Carbon and Hydrogen Isotope Analysis of Volatile Organic Compounds at Microgram per Liter Concentrations

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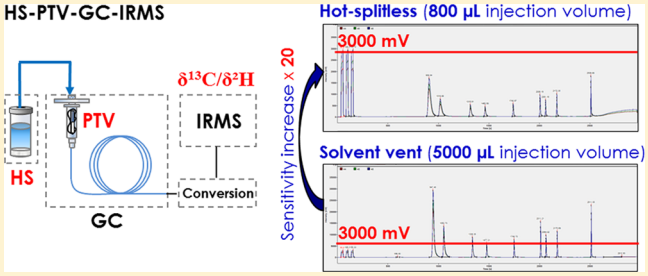
Supporting Information

ABSTRACT: One major challenge for the environmental application of compound-specific stable isotope analysis (CSIA) is the necessity of efficient sample treatment methods, allowing isolation of a sufficient mass of organic contaminants needed for accurate measurement of the isotope ratios. Here, we present a novel preconcentration technique—the coupling of a headspace (HS) autosampler with a programmed temperature vaporizer (PTV)—for carbon ($\delta^{13}C$) and hydrogen ($\delta^{2}H$) isotope analysis of volatile organic compounds in water at concentrations of tens of micrograms per liter. The technique permits large-volume injection of headspace samples, maintaining the principle of simple static HS extraction. We developed the method for multielement isotope analysis ($\delta^{13}C$ and $\delta^{2}H$) of methyl *tert*-butyl ether (MTBE), benzene, toluene, ethylbenzene, and *o*-xylene (BTEX), and analysis of $\delta^{13}C$ for chlorinated benzenes and ethenes. Extraction and injection conditions were optimized for maximum sensitivity and minimum isotope effects. Injection of up to 5 mL of headspace sample from a 20 mL vial containing 13 mL of aqueous solution and 5 g of NaCl (10 min of incubation at 90 °C) resulted in accurate $\delta^{13}C$ and $\delta^{2}H$ values. The method detection limits (MDLs) for $\delta^{13}C$ were from 2 to 60 μg/L (MTBE, BTEX, chlorinated ethenes, and benzenes) and 60−97 μg/L for $\delta^{2}H$ (MTBE and BTEX). Overall, the HS−PTV technique is faster, simpler, isotope effect-free, and requires fewer treatment steps and less sample volume than other extraction techniques used for CSIA. The environmental applicability was proved by the analysis of groundwater samples containing BTEX and chlorinated contaminants at microgram per liter concentrations.



During recent years, compound-specific stable isotope analysis (CSIA) using gas chromatography/isotope ratio mass spectrometry (GC/IRMS) has become an important tool to study the fate of organic pollutants in the environment.[1,2] One major challenge for application of CSIA is the analysis of low concentrations of organic compounds in environmental matrixes. Isotope ratio mass spectrometry (IRMS) requires sufficient material to provide an accurate measurement of isotope ratios (i.e., $^{13}C/^{12}C$, $^{2}H/^{1}H$). The limiting factor is the low natural abundance of the heavy isotopes (^{13}C, 1%, and ^{2}H, 0.01%),[3] which determines the detection limit. Absolute amounts of carbon and hydrogen required are on the order of 10 and 30 ng, respectively,[1] approximately 2 orders of magnitude higher than the amount of compound needed for conventional concentration analysis. Therefore, efficient extraction and preconcentration techniques are essential for environmental applications of CSIA. A further prerequisite of the sample treatment techniques for CSIA is that they should not impart significant isotope effects (i.e., changes of the isotope ratio compared to the true value).

GC/IRMS primarily has been applied for the carbon isotope analysis of volatile organic groundwater pollutants, i.e., benzene, toluene, ethylbenzene, and *o*-xylene (BTEX), chlorinated ethenes, chlorinated benzenes, and fuel oxygenates, which possess high environmental and health significance, and their regulatory limits in drinking water are in the low microgram per liter range in most OECD (Organisation for Economic Co-Operation and Development) countries.[4,5] Extraction methods such as purge and trap (P&T) and solid-phase microextraction (SPME) have been used for CSIA to reach these low concentrations. The limits of detection (MDLs) reported for SPME and carbon-CSIA of priority groundwater contaminants are in the range of 4−2200 μg/L.[6−11] Some studies have reported isotope effects for some compound classes due to their interaction with the polymer phase of the SPME fiber.[6,8] In samples with complex mixtures of contaminants, competition among the analytes for the limited adsorption sites on the

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coating material has been demonstrated.[10,12] To date, P&T is the most effective extraction technique for online CSIA, with MDLs for carbon CSIA of volatile compounds, including monoaromatic compounds and halogenated hydrocarbons, from 0.07 to 27 μg/L.[10,13] Despite its high preconcentration efficiency, P&T cycles are long (typically 1−4 min loop transfer time, 15−30 min purge times, and 1−5 min desorption), and high water backgrounds are observed (1000 mV amplitude for m/z 18 after 15 min of purge time[13]), which can lead to peak tailing and increase on the memory effect from previous analysis. The method requires several phase-transfer steps (volatilization, adsorption, desorption, and in most of the cases, cryofocusing), which need to be carefully optimized for minimum isotope effects.[14,15] Some attempts have been made to simplify the steps involved in the conventional P&T methodology. A single-step dynamic headspace extraction was designed, combining purging and cryofocusing, and applied for carbon stable isotope analysis of chlorinated ethenes in water at concentrations of tens of micrograms per liter.[16] However, this methodology is based on a self-made P&T device and has not been widely applied.

In this study, we investigated the possibility of applying a commercially available and novel preconcentration technique consisting of the coupling of a static headspace (HS) autosampler with a programmed temperature vaporizer (PTV) for CSIA of volatile organic compounds in water samples at low microgram per liter levels. The PTV allows the injection of large volumes of headspace sample by cold trapping of the volatile compounds in an adsorbent material packed inside the GC liner, combined with controlled elimination of the water vapor via a split line.[17,18] The HS−PTV coupling provides a high enrichment of the volatile compounds in a single step and maintains the principle of simple static headspace extraction. It is therefore simpler, faster, and requires fewer sample treatment steps and less sample volume than other preconcentration methods coupled with CSIA to date.

The method was optimized for the multielement isotope analysis (δ^{13}C and δ^{2}H) of typical groundwater pollutants [methyl *tert*-butyl ether (MTBE), BTEX] and for carbon isotope analysis of chlorinated benzenes and ethenes. There are just two studies in which a PTV injector has been satisfactorily applied for the large-volume injection of liquid organic extracts into the GC/IRMS.[19,20] However, to the best of our knowledge, this is the first time that the HS−PTV−GC/IRMS coupling has been presented. Additionally, to our knowledge, this is the first study providing a systematic evaluation of the analytical characteristics and the limits of detection for hydrogen isotope analysis of BTEX.

EXPERIMENTAL SECTION

Reagents and Stock Solutions. *Reagents.* See the Supporting Information, section 1.1, Table S-1.

Stock Solutions. Solutions of the target compounds with known isotopic composition (laboratory isotope standards used as reference compounds, see paragraph below) were prepared by dissolution of aliquots of each pure compound in pentane (for liquid injection) and in Milli-Q water (for HS analysis). Different stock solutions were prepared for carbon and for hydrogen isotope analysis. Stock solutions for carbon contained the following: methyl *tert*-butyl ether (MTBE, 99.9%); benzene, toluene, ethylbenzene (ETB) and *o*-xylene (99.8%); *cis*-1,2-dichloroethene (*cis*-1,2-DCE, 99.8%), trichloroethene (TCE, 99.5%), chlorobenzene (CB, 99.9%), and 1,2-dichlorobenzene (1,2-DCB, 99%). In the stock solutions for hydrogen isotope analysis, chlorinated compounds were not included due to the formation of HCl in the pyrolysis process preventing the precise determination of hydrogen isotope composition[1] The concentrations of target compounds were adjusted to equal molar concentrations of carbon and hydrogen of each analyte, respectively, to obtain similar signal intensities for all analytes (m/z 44 for carbon and m/z 2 for hydrogen isotope analysis). Stock solutions were stored in the refrigerator at 4 °C and were stable under these conditions over a period of at least 1 year.

Determination of δ^{13}C and δ^{2}H Values for Laboratory Isotope Standards. The δ^{13}C [‰, calibrated vs Vienna Pee Dee Belemnite (VPDB) scale][21] and δ^{2}H [‰, calibrated vs Vienna Standard Mean Ocean Water−Standard Light Antarctic Precipitation (VSMOW−SLAP)][22] of the laboratory isotope standards were determined by different reference methods. Pure substances were analyzed by elemental analyzer (EA)-IRMS for δ^{13}C determination and by high-temperature pyrolyzer (HTP)-IRMS for δ^{2}H (Supporting Information, section 1.2). Standard solutions of the isotope standards in pentane were analyzed by liquid injection into the GC/IRMS system. Standard solutions of the isotope standards in Milli-Q water were analyzed by HS−GC/IRMS (hot-splitless injection mode).

Gas Chromatography/Isotope Ratio Mass Spectrometry (δ^{13}C and δ^{2}H). The compound-specific isotope ratios in multicomponent mixtures were determined using an Agilent 7890A GC (Agilent Technologies, Waldbronn, Germany) coupled to an IRMS (MAT 253, Thermo Fisher Scientific, Bremen, Germany) via GC IsoLink (CNH) and ConFlo IV universal interface (Thermo Fisher Scientific, Bremen, Germany). The GC was equipped with a programmed temperature vaporizer (Multimode Inlet (MMI) G3510A/G3511A, Agilent Technologies, Waldbronn, Germany) and a CombiPAL autosampler (CTC Analytics AG, Zwingen, Switzerland). The target compounds were baseline-separated using a DB-MTBE capillary column (60 m × 0.32 mm i.d. × 1.80 μm f.d.; Agilent Technologies, Waldbronn, Germany). The temperature program started at 35 °C and was held for 10 min, then increased at a rate of 4 °C/min to 150 °C and then at 20 °C/min to 260 °C and held for 5 min. Once the compounds were separated on the GC column, they were transferred into the corresponding conversion furnace for carbon or hydrogen analysis.

Carbon Isotope Analysis by GC/C-IRMS. Analytes were combusted for δ^{13}C determination. The combustion oven was equipped with the new version of combustion reactor: aluminum tube filled with a NiO tube and NiO/CuO wires, and soldier joint connected with a metal capillary (designed for GC-IsoLink systems, Thermo Fisher Scientific, Bremen, Germany[23]), and maintained at 1000 °C. The helium carrier gas flow rate was 2 mL/min. To ensure accuracy and precision of the GC/C-IRMS system, a standard solution of the laboratory isotope standards (MTBE, BTEX, chlorinated benzenes, and ethenes) was analyzed every six measurements. The concentrations in the standard were adjusted to give approximately the same peak amplitude (m/z 44) for all the target compounds and for the reference gas (CO_2): 2000−2500 mV, as previously suggested to reduce the uncertainty of the measurement.[24] The NiO/CuO catalyst of the combustion reactor required oxidation after every 30−40 measurements.

Hydrogen Isotope Analysis by GC/HTC-IRMS. High-temperature pyrolysis at 1420 °C was used to convert organically

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bound hydrogen quantitatively into hydrogen gas for δ^2H measurement.[25] The carrier gas flow was 1.2 mL/min to achieve the longer reaction times required for complete pyrolysis of the analytes.[26] The reactor was an empty alumina (Al_2O_3) tube (0.5 mm i.d., 320 mm length, Thermo Fisher Scientific, Bremen, Germany). A standard solution of the laboratory isotope standards (MTBE and BTEX) was analyzed every six measurements to ensure the stability and accuracy of the measurements. The concentrations of the standards were adjusted to give approximately the same peak amplitude (m/z 2) between 3000 and 4000 mV for all the target compounds and for the reference gas (H_2). The H_3^+ factor was determined daily to monitor the overall state of the GC/HTC/IRMS system. We injected the standard solution of isotope standards at least five times to equilibrate the alumina surface. After equilibration, the δ^2H values remained stable (and in good agreement with the reference values by HTP-IRMS) for 20−25 runs. Then, the reactor was regenerated with a flow of oxygen (see further details in the Supporting Information, section 1.4.).

Headspace Sampling. The CombiPAL autosampler (CTC Analytics AG, Zwingen, Switzerland) was equipped with a gastight HS syringe (250−5000 μL) without closing valve (connected to the atmosphere) and an HS oven for six HS vials. The conditions for partitioning of the target compounds into the gas phase were optimized (Supporting Information, section 2.1). Twenty milliliter HS vials containing 5 g NaCl were filled with 13 mL of water sample and then closed with magnetic crimp caps with PTFE/butyl septa (3 mm thickness, Th. Geyer, Renningen, Germany). The samples were heated at 90 °C for 10 min while shaking at 250 rpm (Supporting Information Table S-4). After equilibration, the heated syringe aspirated the selected headspace sample volume and injected it into the PTV.

Programmed Temperature Vaporizer. The PTV used was a Multimode Inlet (MMI) G3510A/G3511A from Agilent (Waldbronn, Germany). The cooling of the injector was accomplished by liquid nitrogen, and helium was used as the carrier gas. In conventional split/splitless injectors, hot-splitless is the method of choice for samples with low concentrations. In this mode, the whole headspace sample is transferred from the injector into the GC column, providing maximum sensitivity with the limitation of the injection volume (conditioned by the internal volume of the liner). The PTV injector can be programmed in "solvent vent mode" which overcomes this limitation. This operating mode allows the injection of large volumes of headspace sample by cold trapping of the target compounds in the liner, combined with controlled elimination of the water vapor via split line.[17,18] In this study, a HS−PTV method was optimized for carbon and hydrogen isotope analysis of the target compounds and compared with the conventional hot-splitless method.

Hot-Splitless Injection. The injector was equipped with a deactivated glass liner (geometry: short taper at the bottom, 4 mm internal diameter, 800 μL internal volume—SGE analytical science, Kiln Farm, United Kingdom).[27] The injector was maintained at 300 °C, and the splitless injection time was set at 2.40 min (Supporting Information Figure S-1). The maximum injection volume was 800 μL of headspace sample, injected at a speed of 30 μL/s (Supporting Information Table S-3).

Solvent Vent Injection. A deactivated glass liner (with the same geometry as described above) was packed with a hydrophobic porous polymer, Tenax-TA (80/100 mesh, Supelco, Bellefonte, U.S.A.). For carbon isotope analysis, packing lengths of 1 and 2.5 cm were appropriate, while for hydrogen 1 cm packing length was required to avoid isotope effects. All variables involved in the solvent vent injection (initial temperature, vent time, vent flow, desorption time, and desorption temperature) were evaluated and optimized for maximum sensitivity and minimum isotope effects. Optimum conditions were as follows: the split valve was opened and the headspace sample was introduced into the injector at 0 °C, with a vent flow of 5.0 mL/min and a vent pressure of 5.00 psi (Supporting Information Figure S-1, Table S-4). The split valve was closed 0.5 min after injection, and the liner was heated at a rate of 650 °C/min to 300 °C for desorption of the analytes. The analytes were transferred splitless from the liner into the capillary column. The split valve was opened again 2.90 min after the injection (2.40 min splitless time), and the liner temperature was held at 300 °C for 10 min. It was possible to inject from 250 to 5000 μL of headspace sample. The injection time for 5000 μL headspace was 1 min (injection speed of 80 μL/s) during which the injector is operating in venting conditions (Supporting Information Figure S-1).

Determination of Method Detection Limits and Reproducibility. To determine the method detection limits (MDLs), consecutive dilutions of the aqueous stock solutions of laboratory isotope standards were analyzed by HS−PTV−GC/IRMS for δ^{13}C and δ^2H determination. The study was exclusively performed with compounds at isotopic natural abundance for both carbon and hydrogen.

Method Detection Limits and Reproducibility for Carbon. The MDLs were calculated by the moving mean procedure described by Jochmann et al.[13] The MDLs for each target compound correspond to the lowest concentration with standard deviation of δ^{13}C from triplicate analyses ≤0.5‰, and ≤0.5‰ deviation from the mean value of all analyses over a range of concentrations. The reproducibility and long-term stability of the HS−PTV−GC/C-IRMS method was demonstrated by the analysis of aqueous standards at optimum conditions over a period of 1 month.

Method Detection Limits and Reproducibility for Hydrogen. The same procedure as for carbon was used, but considering a standard deviation of ±5‰, which is the typical uncertainty for δ^2H, according to the manufacturer and published literature.[28,29] The reproducibility and long-term stability of the HS−PTV−GC/HTC-IRMS method was demonstrated by the analysis of aqueous standards at optimum conditions over a period of 2 weeks.

RESULTS AND DISCUSSION

The main scope of this work was to develop the HS−PTV−GC/IRMS coupling for CSIA of volatile organic compounds (VOCs) in water samples. A group of volatile priority pollutants comprising chlorinated and nonchlorinated compounds, with a range of physicochemical properties, was selected to cover a representative variety of compounds, allowing extrapolation of the method to further applications (Supporting Information Table S-1).

Reference δ^{13}C and δ^2H Values for Laboratory Isotope Standards. The δ^{13}C and δ^2H values of the laboratory isotope standards were determined by different reference methods (Supporting Information Table S-2). In case of carbon isotope analysis, there were small and reproducible deviations (from 0.5‰ to 1.3‰) on the δ^{13}C values of chlorinated ethenes determined by GC/C-IRMS methods and EA-IRMS. These differences have already been observed for chlorinated

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Table 1. Analytical Characteristics of the HS−PTV−GC/IRMS Method for Carbon and Hydrogen Isotope Analysis

compd	HS−PTV−GC/IRMS			GC/IRMS (ref value at optimal conditions)
δ^{13}C (‰) vs VPDB	MDL in water (μg/L)	amplitude of mass 44 at MDL (mV)	reproducibility (‰) n = 20 and 1 month[a]	STD soln in pentane (‰) n = 3[a]
MTBE ($C_5H_{12}O$)	3.4	91 ± 2	−28.5 ± 0.2	−28.4 ± 0.2
benzene (C_6H_6)	4.0	101 ± 4	−27.7 ± 0.1	−27.7 ± 0.2
toluene (C_7H_8)	5.3	200 ± 8	−24.1 ± 0.1	−23.9 ± 0.3
ETB (C_8H_{10})	8.5	421 ± 9	−28.1 ± 0.2	−28.0 ± 0.2
o-xylene (C_8H_{10})	1.9	142 ± 7	−31.0 ± 0.3	−30.6 ± 0.2
cis-1,2-DCE ($C_2H_2Cl_2$)	59.1	310 ± 10	−23.7 ± 0.3	−23.5 ± 0.3
TCE (C_2HCl_3)	50.3	400 ± 20	−26.9 ± 0.2	−26.8 ± 0.3
MCB (C_6H_5Cl)	8.5	146 ± 9	−29.5 ± 0.2	−29.0 ± 0.3
1,2-DCB ($C_6H_4Cl_2$)	4.8	190 ± 10	−30.4 ± 0.3	−30.2 ± 0.2
δ^2H (‰) vs VSMOW	MDL in water (μg/L)	amplitude of mass 2 at MDL (mV)	reproducibility (‰) n = 20 and 2 weeks[b]	STD soln in pentane (‰) n = 3[b]
MTBE ($C_5H_{12}O$)	47.8	1230 ± 20	−83 ± 5	−82 ± 1
benzene (C_6H_6)	97.1	1010 ± 10	−77 ± 5	−75 ± 2
toluene (C_7H_8)	74.7	1130 ± 10	−114 ± 3	−114 ± 2
ETB (C_8H_{10})	60.0	1643 ± 9	−64 ± 4	−63 ± 1
MTBE ($C_5H_{12}O$)	60.8	1900 ± 20	−150 ± 4	−150 ± 1

[a]Amplitude of m/z 44 in the range of 1000−3000 mV for all target compounds. [b]Amplitude of m/z 44 in the range of 3000−4000 mV for all target compounds.

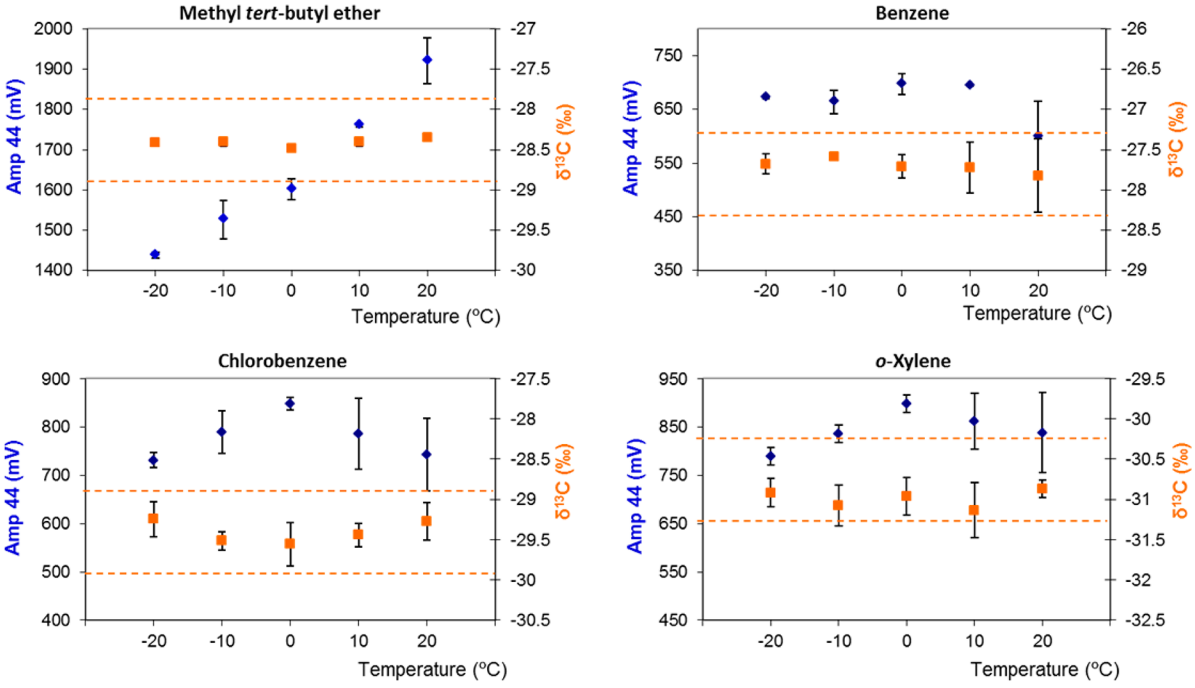

Figure 1. Effect of the temperature on the adsorption of analytes. Peak amplitude (diamonds) represents the average recovery of analytes from triplicate measurements. The δ^{13}C values (squares) are the average from triplicate measurements. The vertical bars indicate the standard deviation (1σ). The horizontal dotted lines represent the ±0.5‰ interval of the mean value from the triplicate GC/C-IRMS analysis of a standard solution in pentane. The vent flow and time were kept constant (5 mL/min and 5 min).

compounds[10,11] and were interpreted as isotope effects due to the incomplete conversion of chlorinated compounds to CO_2. However, there were no significant deviations (within the instrumental uncertainty <0.5‰ for δ^{13}C and <5‰ for δ^2H) for the results obtained by GC/IRMS with both injection techniques (liquid injection of pentane standard and HS (splitless) injection from a water standard). For the evaluation of the potential isotope effects induced by the HS−PTV method, the δ^{13}C and δ^2H values from the GC/IRMS analysis of a standard solution in pentane (n = 3) were considered as a reference (Table 1).

Optimization of HS Conditions. All conditions that have an influence on the partitioning of the analytes into the gas phase (ratio between the volume of the gas phase and the aqueous phase ($\beta = V_g/V_{aq}$), incubation temperature and time, addition of electrolyte to the medium, shaking vial during equilibration) were optimized for maximum sensitivity and tested for their effects on the isotopic composition of the compounds. A detailed description can be found in the

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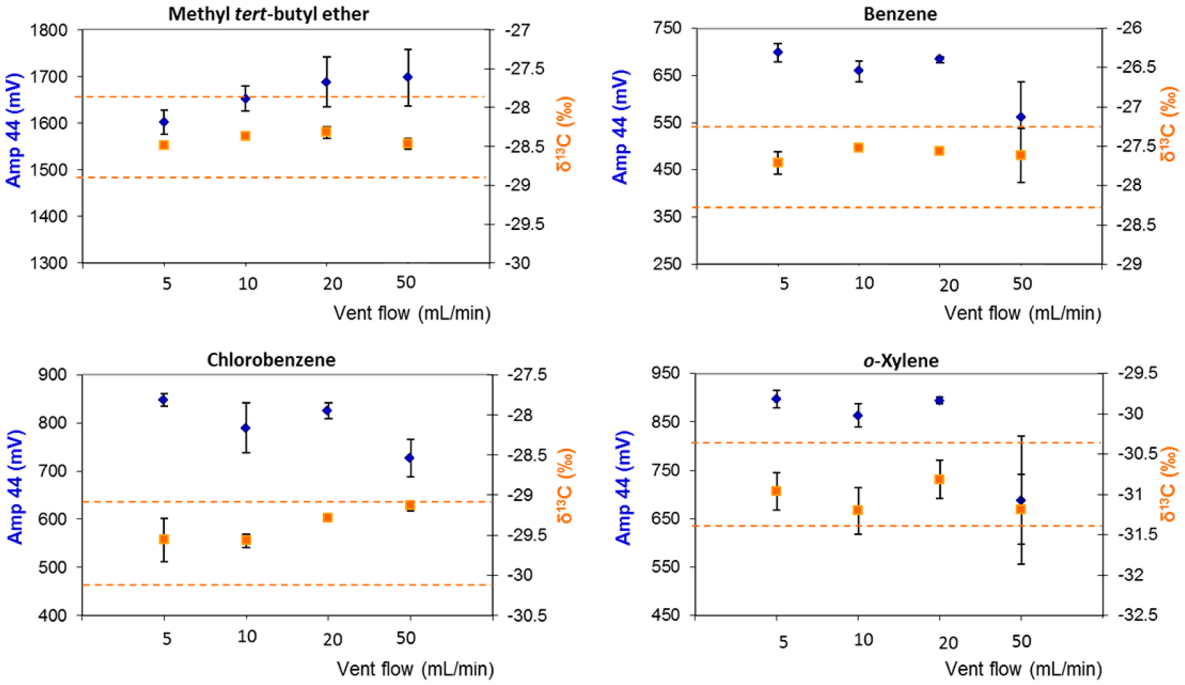

Figure 2. Effect of vent flow on adsorption of analytes. Peak amplitude (diamonds) represents the average recovery of analytes from triplicate analysis. The $\delta^{13}C$ values (squares) are the average from triplicate measurements. The vertical bars indicate the standard deviation (1σ). The horizontal dotted lines represent the ±0.5‰ interval of the mean value from the triplicate GC/C-IRMS analysis of a standard solution in pentane. The initial temperature and vent time were kept constant (0 °C and 0.5 min).

Supporting Information, section 2.1. A number of studies have reported that volatilization of organic compounds from water results only in minor carbon isotope fractionation (−0.02‰ ± 0.06‰ for benzene;[30] +0.2‰ for BTEX;[31] −0.1‰ and +0.5‰ for TCE and toluene[32]). However, measurable changes in the hydrogen isotope composition of organic contaminants have been observed for progressive vaporization (from +3.1‰ to +11.1‰ for MTBE, BTEX, heptane, and decane, +33.9‰ for n-C_{14}, and −28.9‰ for ethanol;[33] +8.9‰ for TCE[34]) and for volatilization under equilibrium conditions in aqueous solution (−9‰ ± 1‰ for MTBE, −11‰ ± 1‰ for benzene[35]). In our study, none of the conditions studied produced a significant change of the carbon and hydrogen isotope ratios of the target compounds (deviations within the instrumental uncertainty: <0.5‰ and <5‰, respectively). Conditions providing maximum sensitivity were $\beta = 0.3$ (20 mL vial with 15 mL sample), incubation temperature 90 °C for 10 min, NaCl under supersaturated conditions (5 g of NaCl in 13 mL of water—15 mL total sample volume), and continuous shaking at 250 rpm. A significant increment of the sensitivity of the overall method was achieved with the optimized conditions, especially for compounds with elevated solubility in the aqueous solution (24 times enhancement for MTBE, Supporting Information Table S-5).

Optimization of PTV Solvent Vent Injection Conditions. The solvent vent injection takes place in two different steps: (i) headspace injection, in which the compounds are retained in the liner while the water vapor is eliminated via the split valve and (ii) splitless transfer of the compounds from the liner into the GC column (Supporting Information Figure S-1). All variables involved were systematically evaluated for their potential to increase sensitivity and their effects on the isotopic composition of the analytes. The largest injection volume allowed for the CTC autosampler was selected (5000 μL headspace), and we used 2.5 cm Tenax packing inside the liner. All conditions were optimized for carbon-CSIA, and then the optimized method was tested for hydrogen-CSIA.

Evaluation of the Headspace Injection Step. The variables involved are the initial temperature of the liner, the helium flow through the split valve (vent flow), and time during which the split valve is open (vent time). The conditions were selected as a compromise to eliminate the water vapor without losing the analytes due to trap breakthrough.

The initial temperature was studied for values of −20, −10, 0, 10, and 20 °C. All the target compounds except MTBE showed a similar behavior: the highest recovery indicated by the peak amplitude corresponded to temperatures between 0 and 10 °C (Figure 1). Losses of the compounds at 20 °C were due to the lower adsorption capacity of the Tenax-TA at higher temperatures.[36] Losses were more pronounced for the more volatile compounds. For example, recovery of benzene was 14% lower at 20 °C, in comparison to its recovery at 0 °C. The lower recoveries observed for temperatures below 0 °C may be due to the ice formation blocking the pores of the adsorbent particles, which hinders the interaction of the compounds with the polymer. For MTBE, the effect of the temperature showed a different trend. Peak amplitudes increased as the temperature rose. This effect is most likely correlated with the concentration of water adsorbed to the Tenax. Although the capacity of Tenax to adsorb water is low, adsorption of water on Tenax can reduce the capacity for adsorption of polar compounds when water is present in high concentration.[37] At higher temperatures, the concentration of water on the polymer is lower, which increases the potential of the Tenax to retain polar compounds such as MTBE.[37−41] We observed an increase of MTBE peak amplitude of 1.3 times when the temperature is increased from −20 to 20 °C. The isotope composition of the target compounds experienced no significant variations (<±

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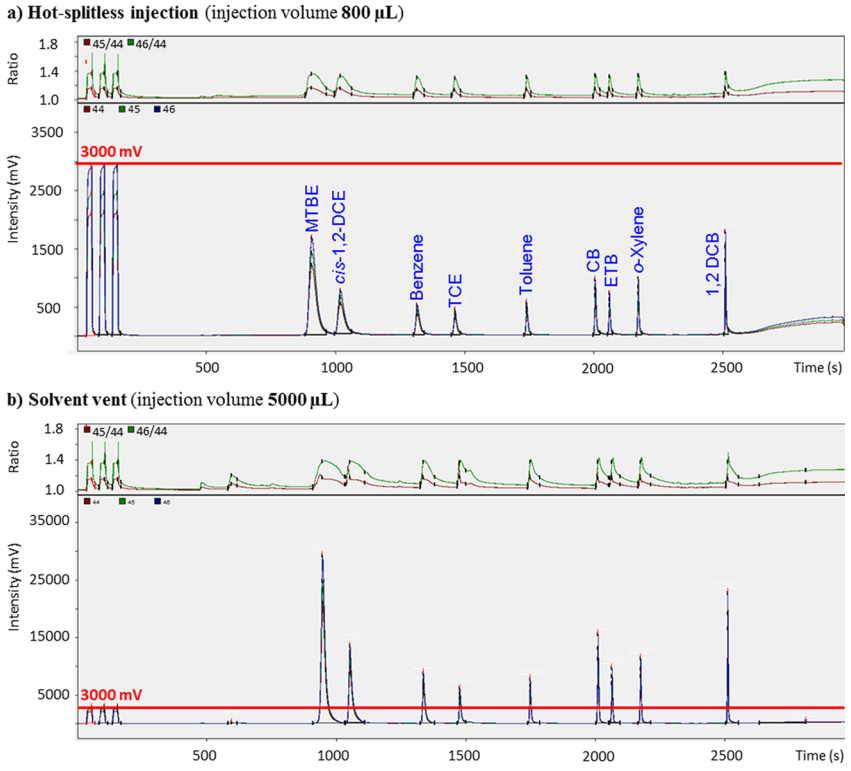

Figure 3. HS–PTV–GC/C-IRMS chromatograms of a standard solution in water containing the target compounds at concentrations between 80 and 1250 μg/L and analyzed (a) using hot-splitless method compared to (b) by solvent vent injection method.

0.5‰) for all the temperatures studied. We selected an initial temperature of 0 °C, which provided the highest peak intensity for most of the target compounds and did not cause isotope effects within the limits of instrumental uncertainty (±0.5‰).

Different vent flows, from 5 to 50 mL/min, were evaluated (Figure 2). Smaller vent flows were not considered, as low injection speeds would be required to perform the injection without risk of overloading the liner volume. Low injection speed will increase the time required for the injection unnecessarily. An injection speed of 80 μL/s, allowing the injection of a 5 mL headspace sample in approximately 1 min, was appropriate for the efficient adsorption of the target compounds in the polymer. The vent flow influences the water content in the sorbent, as dry helium passing through the polymer packing helps to remove the water.[38] The smaller concentration of water in the polymer at higher vent flows can be the reason for the increment in MTBE recovery (up to 7%). The MTBE isotope composition was not affected by varying the vent flow. The effect of the vent flow, from 5 to 20 mL/min, on other target compounds was not significant for the recoveries (relative standard deviation <4%) and the isotope ratios (variations <± 0.5‰ from the reference). With a flow of 50 mL/min, a decrease in recoveries (up to 20% losses for benzene), accompanied by a higher standard deviation of the carbon isotope ratios, was observed. This decrease in recoveries can be explained because the breakthrough volume of the trap for the compounds was exceeded. The smallest flow tested (5 mL/min) was selected to minimize the losses of target compounds.

Vent times of 1, 1.5, and 2 min (including 1 min time required for sample injection) were tested, using a vent flow of 5 mL/min. There were no significant differences in the peak amplitudes and no significant changes on the isotope composition for any of the target compounds (data not shown). A vent time of 1.5 min was selected because it allows a better stabilization of the pressure in the injector after the injection and minimizes the possible losses of target compounds.

Evaluation of the Splitless Sample Transfer into the GC Column. The compounds were transferred into the GC column by thermal desorption, heating the liner at a rate of 650 °C/min (Supporting Information Figure S-1). Desorption times of 2.40, 2.60, and 3 min showed no influence on the recoveries or on the δ^{13}C values. Final temperatures of 250 and 300 °C showed no differences in the results. A final temperature of 300 °C and a desorption time of 2.40 min (purge time 2.90 min, Supporting Information Table S-4) were selected.

Comparison of Solvent Vent Injection with Hot-Splitless Injection. In conventional injectors, hot-splitless is the method of choice for samples with low concentrations. We compared this method with the optimized solvent vent method for evaluation of sensitivity. A standard solution of the target compounds in water (concentrations from 80 to 1250 μg/L) was analyzed by the two compared methods. The maximum possible injection volume allowed by both injection techniques was used (i.e., 800 μL for hot-splitless and 5000 μL for solvent vent). With the solvent vent method, an increment in the peak areas of at least 10-fold was achieved for all the target compounds (Figure 3, Supporting Information Table S-6). However, the increment on the peak amplitudes was higher than a factor of 10 and up to a factor of 18 for MTBE (Supporting Information Table S-6). These increments in the amplitude are due to the significant sharpening of the peaks because of the cryofocusing of the compounds in the liner and the fast sample transfer into the column. This effect is more pronounced for compounds with low boiling points, which are

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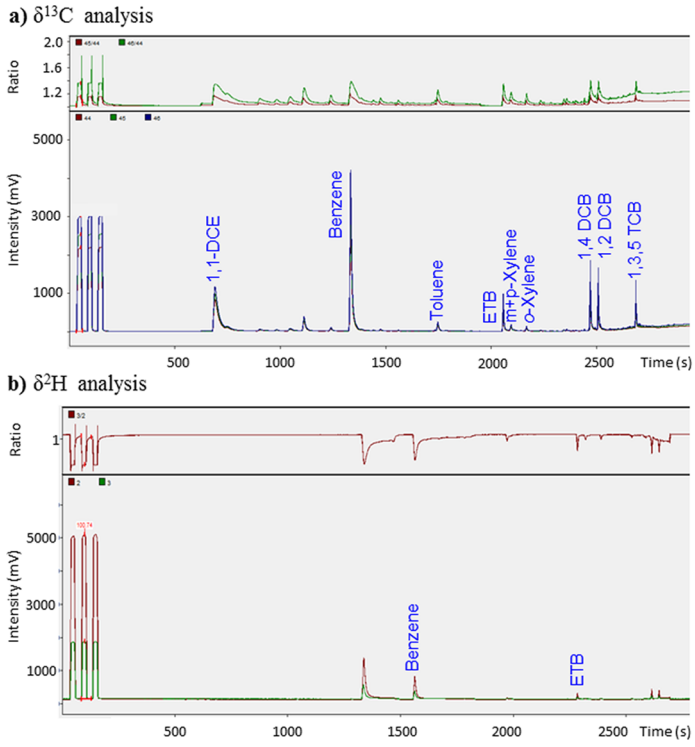

c) Concentration table

Compounds (by RT)	Conc. (µg/L)	δ13C (‰ vs. VPDB) n=3
1,1-DCE	8.35	-25.40 ± 0.09
***cis*-1,2-DCE**	<1.0	nd
Benzene	56.0	-22.4 ± 0.4
Toluene	<1.0	<DL
ETB	20.9	-33.8 ± 0.6
***m,p*-xylene**	7.55	<DL
***o*-Xylene**	8.12	-26.1 ± 0.9
1,4 DCB	32.9	-27.6 ± 0.3
1,2 DCB	28.1	-28.1 ± 0.6
1,3,5-TCB	19.2	-27.6 ± 0.3

Figure 4. HS−PTV−GC/IRMS chromatograms from the (a) $\delta^{13}C$ and (b) $\delta^{2}H$ analysis of a contaminated groundwater sample containing BTEX and chlorinated compounds at the concentrations shown in (c) the table.

the most affected by the initial peak broadening associated with hot-splitless injection. Therefore, an increment in sensitivity of approximately a factor of 20 can be achieved with solvent vent injection (5000 µL headspace sample) for volatile compounds compared to hot-splitless injection (800 µL headspace sample).

Evaluation of Different Injection Volumes with Solvent Vent Method. The autosampler (CombiPAL) was equipped with a HS syringe allowing injection volumes from 250 to 5000 µL. We tested the influence of the injection volume on the signal intensity and on the $\delta^{13}C$ values of the target compounds. Injection volumes from 250 to 5000 µL were investigated by analysis of different dilutions of the stock solution in water resulting in similar amounts of target compounds introduced into the GC column (peak amplitudes of 2000−2500 mV). The $\delta^{13}C$ values were very stable for the complete range of injection volumes and for all target compounds, with a standard deviation <0.3‰. The peak areas were also constant with a relative standard deviation <10%. This experiment demonstrates that the transfer of different volumes of headspace sample into the injector and the solvent vent injection are efficient and robust. Further tests were performed to demonstrate that the underpressure, which is generated in the HS vial when sampling 5000 µL of gas phase, did not cause isotope fractionation (Supporting Information, section 2.3).

To further assess the limits of the system, we demonstrated the possibility of analyzing up to three aliquots of headspace sample from the same sample vial. The maximum total headspace volume was 5500 µL (combining the three different aliquots) for a 20 mL HS vial containing 13 mL of water and 5 g of NaCl. We observed no losses of compounds and no significant isotope effects. This possibility provides an important advantage when analyzing samples from contaminated sites, which typically contain a highly variable concentration profile of contaminants. Precise and true CSIA of compounds at different concentrations in one sample is possible with a fast and automatic strategy, using just 13 mL of water. A complete description of this experiment can be found in Supporting Information, section 2.4.

Adaptation of the Solvent Vent Method for Hydrogen Isotope Analysis. The optimized solvent vent method was tested for its compatibility with hydrogen isotope analysis. The GC and injection conditions were the same as for carbon isotope analysis except for the carrier gas flow (1.2 mL/min for hydrogen, compared to 2 mL/min for carbon). When we used a liner with a 2.5 cm length filled with Tenax, we observed a small isotope effect and higher standard deviation for MTBE ($\delta^{2}H$: −96‰ ± 4‰, n = 3). This isotope effect was due to the amount of water retained by the sorbent,[40] resulting in a higher background level at the beginning of the chromatogram. We solved the problem by using a liner with 1 cm of Tenax packing, which provided true and reproducible isotope values for all compounds, including MTBE ($\delta^{2}H$: −82‰ ± 1‰, n = 3).

Analytical Characteristics of the Method (for Carbon and Hydrogen Isotope Analysis). The large-volume injection method evaluated in this study (HS−PTV) allows the accurate determination of the carbon and hydrogen isotope ratios of the target compounds in the low microgram per liter level. Table 1 shows the analytical characteristics of the method. The $\delta^{13}C$ and $\delta^{2}H$ values for all compounds were in agreement with the reference values from the liquid injection of the standard solution of isotope standards in pentane.

For carbon isotope analysis, the method reproducibility over a period of 1 month (n = 20, aqueous standard with peak amplitude of m/z 44 from 1000 to 3000 mV) was better than 0.3‰, which is within the typical instrument uncertainty of 0.5‰ reported by the manufacturer. The carbon MDLs were between 2 and 60 µg/L for the target compounds (Table 1).

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The peak amplitudes (m/z 44) corresponding to the MDLs range between 91 and 400 mV in agreement with previous studies.[10,13] These concentrations are 2 orders of magnitude lower than the concentrations reported for static HS analysis (from 100 to 5000 μg/L[7,9,32,42,43]), of the same order of magnitude as the lowest concentration reported for hSPME (10−125 μg/L[11]), and of the same order, or 1 order of magnitude higher, than the lowest concentrations reported for online P&T (0.07−27 μg/L[13]).

The hydrogen MDLs were from 48 to 97 μg/L corresponding to peak amplitudes (m/z 2) between 1010 and 1900 mV. To the best of our knowledge, this is the first study reporting the analytical characteristics and MDLs for hydrogen isotope analysis of BTEX. There are only three studies reporting hydrogen isotope analysis of organic compounds in water at the low microgram per liter level, and the model compounds used were fuel oxygenates.[29,35,44] With the HS−PTV−GC/IRMS method, we determined a hydrogen MDL for MTBE of 48 μg/L, which is in the same range as the values reported for P&T (20 μg/L,[44] tens of micrograms per liter,[21] 50 μg/L[29]). The hydrogen MDLs reported in this study were 1 order of magnitude higher than the carbon MDLs, also in accordance with the results published for MTBE.[29,35,44] These three studies showed significant isotope effects correlated with the P&T extraction and the pyrolysis which drifted over time and required a secondary correction for reliable δ^2H values. With the HS−PTV method and the strategy used for equilibration and regeneration of the pyrolysis reactor, no isotope effects were observed (Supporting Information, section 1.5). The reproducibility of the measurements was ±3−5‰ (1 σ) over a period of 2 weeks (n = 20, aqueous standard with amplitude for m/z 2 of 3000−4000 mV), within the typical precision of 5‰ according to the manufacturer. Therefore, no correction was required, which simplifies the determination of the δ^2H to a large extent.

The total sample treatment time for the HS−PTV method was 13 min (10 min extraction plus 3 min injection). The sample volume of 13 mL is much lower than the volumes used with P&T (up to 100 mL for the study with the best MDLs[13]), which is an important advantage when the sample amount is limited, as in the case of laboratory-scale experiments or analysis of pore water.

Demonstration of Applicability to Environmental Samples. The environmental applicability of the HS−PTV method for the determination of δ^{13}C and δ^2H was evaluated by the analysis of groundwater samples from a contaminated field site, containing BTEX and chlorinated volatile organic contaminants in the low microgram per liter range. Figure 4 shows the GC/IRMS chromatograms from the δ^{13}C (Figure 4a) and δ^2H (Figure 4b) analysis of the same sample. The accurate determination of the δ^{13}C values was possible for compounds with concentrations from 8 to 56 μg/L. The concentrations of BTEX in the sample were below the hydrogen MDLs; however, it was possible to detect the presence of benzene and ethylbenzene with concentrations >20 μg/L. This application demonstrates the potential of the HS−PTV method for CSIA of volatile organic compounds at low concentrations in a simple and automatic manner.

CONCLUSIONS

We developed a HS−PTV−GC/IRMS method for the automatic, simple, fast, and isotope effect-free analysis of VOCs in water at concentrations of tens of micrograms per liter. With the novel HS−PTV coupling, it was possible to inject up to 5 mL of headspace sample into the GC/IRMS with no significant carbon or hydrogen isotope effects for a number of target compounds comprising chlorinated and nonchlorinated compounds, with different physicochemical properties. The sensitivity of the method was 2 orders of magnitude better than the results reported with the conventional HS method. In comparison with P&T, which is the most efficient preconcentration technique coupled with CSIA to date (MDLs from nanograms per liter to micrograms per liter), the HS−PTV method provides the significant advantage of simplicity of instrumentation and minimization of the phase transfer steps involved, reducing the possible isotope effects. It provides a high enrichment of the volatile compounds in a single step (cold adsorbent trap) and maintains the simple principle of static headspace extraction. The HS−PTV coupling reaches concentrations nearly as low as P&T (MDLs in microgram per liter range), the complete sample pretreatment cycle is short (13 min), requires only 13 mL of water, and exhibits no isotope effects for any of the VOCs studied. Additionally, the HS−PTV method is highly versatile and robust, as it allows the injection of different volumes ranging from 250 to 5000 μL. Consequently, this technique is particularly well-suited for situations where the concentrations of contaminants in the samples are variable, and when small sample volumes are available. In conclusion, we have demonstrated for the first time that the HS−PTV coupling is a simple and efficient preconcentration strategy for CSIA of VOCs. The method was evaluated here for typical volatile contaminants in water samples and has the potential to be adapted for extraction of different volatile compounds and matrixes, which will help to extend the possibilities of CSIA for environmental applications.

ASSOCIATED CONTENT

Supporting Information

Experimental information, chemicals, δ^{13}C and δ^2H determination of pure phase laboratory isotope standards, δ^{13}C and δ^2H determination of laboratory isotope standards in standard solutions, analytical conditions of the HS−PTV methods, equilibration and regeneration of the pyrolysis reactor (HTC, high-temperature conversion), optimization of HS conditions, comparison of hot-splitless injection with optimized solvent vent injection, evaluation of the effect of the underpressure generated in the HS vial on the isotope composition of the compounds, analysis of consecutively taken headspace samples from the same vial. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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REFERENCES

(1) Elsner, M.; Jochmann, M.; Hofstetter, T.; Hunkeler, D.; Bernstein, A.; et al. *Anal. Bioanal. Chem.* **2012**, *403* (9), 2471−2491.
(2) Hofstetter, T. B.; Berg, M. *TrAC, Trends Anal. Chem.* **2011**, *30* (4), 618−627.
(3) Hilkert, A. W.; Douthitt, C. B.; Schlüter, H. J.; Brand, W. A. *Rapid Commun. Mass Spectrom.* **1999**, *13* (13), 1226−1230.
(4) U.S. Environmental Protection Agency National Primary Drinking Water Standards, Office of Water (4606M); EPA 816-F-03-016, 2009.http://www.epa.gov/safewater/contaminants/index.html (Accessed Jan. 7, 2015).
(5) World Health Organization Guidelines for Drinking-Water Quality: Incorporating Second Addendum; Vol. *1*, Recommendations, 3rd ed., 2008. www.who.int/water_sanitation_health/dwq/gdwq3rev/en/index.html (Accessed Jan. 7, 2015).
(6) Dias, R. F.; Freeman, K. H. *Anal. Chem.* **1997**, *69* (5), 944−950.
(7) Hunkeler, D.; Aravena, R. *Environ. Sci. Technol.* **2000**, *34* (13), 2839−2844.
(8) Hunkeler, D.; Butler, B. J.; Aravena, R.; Barker, J. F. *Environ. Sci. Technol.* **2001**, *35* (4), 676−681.
(9) Gray, J. R.; Lacrampe-Couloume, G.; Gandhi, D.; Scow, K. M.; Wilson, R. D.; et al. *Environ. Sci. Technol.* **2002**, *36* (9), 1931−1938.
(10) Zwank, L.; Berg, M.; Schmidt, T. C.; Haderlein, S. B. *Anal. Chem.* **2003**, *75* (20), 5575−5583.
(11) Palau, J.; Soler, A.; Teixidor, P.; Aravena, R. *J. Chromatogr. A* **2007**, *1163* (1−2), 260−268.
(12) Black, L.; Fine, D. *Environ. Sci. Technol.* **2001**, *35* (15), 3190−3192.
(13) Jochmann, M. A.; Blessing, M.; Haderlein, S. B.; Schmidt, T. C. *Rapid Commun. Mass Spectrom.* **2006**, *20* (24), 3639−3648.
(14) Blessing, M.; Jochmann, M.; Schmidt, T. *Anal. Bioanal. Chem.* **2008**, *390* (2), 591−603.
(15) Kopinke, F.-D.; Georgi, A.; Voskamp, M.; Richnow, H. H. *Environ. Sci. Technol.* **2005**, *39* (16), 6052−6062.
(16) Morrill, P. L.; Lacrampe-Couloume, G.; Lollar, B. S. *Rapid Commun. Mass Spectrom.* **2004**, *18* (6), 595−600.
(17) Pérez Pavón, J. L.; del Nogal Sánchez, M.; Fernández Laespada, M. E.; García Pinto, C.; Moreno Cordero, B. *J. Chromatogr. A* **2007**, *1141* (1), 123−130.
(18) Pérez Pavón, J. L.; Martín, S. H.; García Pinto, C.; Moreno Cordero, B. *J. Chromatogr. A* **2008**, *1194* (1), 103−110.
(19) Mikolajczuk, A.; Geypens, B.; Berglund, M.; Taylor, P. *Rapid Commun. Mass Spectrom.* **2009**, *23* (16), 2421−2427.
(20) Mikolajczuk, A.; Przyk, E. P.; Geypens, B.; Berglund, M.; Taylor, P. *Isot. Environ. Health Stud.* **2010**, *46* (1), 2−12.
(21) Coplen, T. B.; Brand, W. A.; Gehre, M.; Gröning, M.; Meijer, H. A. J.; et al. *Anal. Chem.* **2006**, *78* (7), 2439−2441.
(22) International Atomic Energy Agency (IAEA) Technical Note, IAEA-TECDOC-825, 1993. http://www-pub.iaea.org/MTCD/publications/PDF/te_825_prn.pdf (Accessed Jan. 7, 2015).
(23) Juchelka, D. Application Note Thermo Fisher Scientific, Note 30268. http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/CMD%20Marketing%20Material/MassSpectrometryCollateral/Application%20Notes/AN30268-Analysis-of-PAHs-EN.pdf (Accessed Jan. 7, 2015).
(24) Sherwood Lollar, B.; Hirschorn, S. K.; Chartrand, M. M. G.; Lacrampe-Couloume, G. *Anal. Chem.* **2007**, *79* (9), 3469−3475.
(25) Burgoyne, T. W.; Hayes, J. M. *Anal. Chem.* **1998**, *70* (24), 5136−5141.
(26) Bilke, S.; Mosandl, A. *Rapid Commun. Mass Spectrom.* **2002**, *16* (5), 468−472.
(27) Technical Article SGE Analytical Science, 2012. http://www.sge.com/uploads/ec/11/ec1104b52793f9383f8a726cd9b805b5/TA-0145-A.pdf (Accessed Jan. 7, 2015).
(28) Sessions, A. L. *J. Sep. Sci.* **2006**, *29* (12), 1946−1961.
(29) Kujawinski, D. M.; Stephan, M.; Jochmann, M. A.; Krajenke, K.; Haas, J.; et al. *J. Environ. Monit.* **2010**, *12* (1), 347−354.
(30) Hunkeler, D.; Andersen, N.; Aravena, R.; Bernasconi, S. M.; Butler, B. J. *Environ. Sci. Technol.* **2001**, *35* (17), 3462−3467.
(31) Harrington, R. R.; Poulson, S. R.; Drever, J. I.; Colberg, P. J. S.; Kelly, E. F. *Org. Geochem.* **1999**, *30* (8, Part 1), 765−775.
(32) Slater, G. F.; Dempster, H. S.; Lollar, B. S.; Ahad, J. *Environ. Sci. Technol.* **1999**, *33* (1), 190−194.
(33) Wang, Y.; Huang, Y. S. *Org. Geochem.* **2001**, *32* (8), 991−998.
(34) Poulson, S. R.; Drever, J. I. *Environ. Sci. Technol.* **1999**, *33* (20), 3689−3694.
(35) Kuder, T.; Philp, P.; Allen, J. *Environ. Sci. Technol.* **2009**, *43* (6), 1763−1768.
(36) Tenax TA Breakthrough Volume Data. http://www.sisweb.com/index/referenc/tenaxta.htm (Accessed Jan. 7, 2015).
(37) Ketola, R. A.; Kiuru, J. T.; Tarkiainen, V.; Kokkonen, J. T.; Räsänen, J.; et al. *Anal. Chim. Acta* **2006**, *562* (2), 245−251.
(38) Karbiwnyk, C. M.; Mills, C. S.; Helmig, D.; Birks, J. W. *J. Chromatogr. A* **2002**, *958* (1−2), 219−229.
(39) Demeestere, K.; Dewulf, J.; De Witte, B.; Van Langenhove, H. *J. Chromatogr. A* **2007**, *1153* (1−2), 130−144.
(40) von Eckstaedt, C. V.; Grice, K.; Ioppolo-Armanios, M.; Chidlow, G.; Jones, M. *J. Chromatogr. A* **2011**, *1218* (37), 6511−6517.
(41) Klisch, M.; Kuder, T.; Philp, R. P.; McHugh, T. E. *J. Chromatogr. A* **2012**, *1270* (0), 20−27.
(42) Mancini, S. A.; Ulrich, A. C.; Lacrampe-Couloume, G.; Sleep, B.; Edwards, E. A.; et al. *Appl. Environ. Microbiol.* **2003**, *69* (1), 191−198.
(43) Ward, J. A. M.; Ahad, J. M. E.; Lacrampe-Couloume, G.; Slater, G. F.; Edwards, E. A.; et al. *Environ. Sci. Technol.* **2000**, *34* (21), 4577−4581.
(44) Kuder, T.; Wilson, J. T.; Kaiser, P.; Kolhatkar, R.; Philp, P.; et al. *Environ. Sci. Technol.* **2005**, *39* (1), 213−220.

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