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Heart-cutting two-dimensional gas chromatography–isotope ratio mass spectrometry analysis of monoaromatic hydrocarbons in complex groundwater and gas-phase samples

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ABSTRACT

Compound-specific isotope analysis (CSIA) is increasingly used to evaluate the origin and fate of petroleum hydrocarbons in the environment. However, as samples often contain a complex mixture of compounds and the method requires a full chromatographic separation, it can be challenging to obtain accurate and precise isotope values. In this study, in order to develop a method to analyze carbon isotopes in benzene, toluene, ethylbenzene, and xylenes (BTEX) in complex environmental samples, a two-dimensional heart-cutting gas chromatograph (GC) was hyphenated to an isotope ratio mass spectrometer (IRMS). The focus was placed on benzene and toluene, which are the main compounds of concern in contaminated sites. A full separation for BTEX was successfully achieved using a 60 m polar column in the first dimension and a 30 m non-polar column in the second dimension. Accuracy and precision of carbon isotope measurements of standards were not impacted by the new setup compared to classic one-dimensional (1D) GC-C–IRMS. For benzene and toluene, precision remained very good (≤0.2‰) for concentrations comprised between 5 and 20 μg/L. A high matrix load did not influence the precision and accuracy of isotope measurements. The method was tested on several samples from two different field sites. For all samples tested, full chromatographic baseline resolution was achieved for benzene and toluene. Spatial variability of isotopes values linked to biodegradation was evidenced for one field site. This new 2D-GC-C–IRMS method will broaden the spectrum of samples suitable for isotope analysis and will be therefore able to give new insights into attenuation processes of BTEX in contaminated sites or source fingerprinting.

1. Introduction

Compound-specific isotope analysis (CSIA) is a powerful tool to evaluate the origin and fate of volatile organic compounds such as benzene, toluene, ethylbenzene, and xylenes (BTEX) or chlorinated solvents, which are widespread contaminants in the environment. Therefore, CSIA is increasingly applied, for example to track the extent of biodegradation of contaminants [1,2], to distinguish between biodegradation mechanisms [3,4], or to differentiate several sources of contamination [5,6]. Isotope fractionation factors have been determined for numerous degradation pathways and provide a basis to evaluate field data [7–10]. A guidance document from US EPA outlining good practice for CSIA application is also available [11].

CSIA is commonly performed using a gas chromatograph coupled to an isotope-ratio mass spectrometer (GC–IRMS). In this instrument, the compounds eluting from the GC column are transformed into a single analyte (for example CO_2 for carbon). Thus, unlike for a GC hyphenated to a quadrupole mass spectrometer (GC–qMS) for example, where co-eluting compounds can still be identified and quantified in the selected ion monitoring (SIM) mode, excellent chromatographic resolution is crucial for GC–IRMS.

While chlorinated solvents can typically be easily resolved in samples from contaminated sites, (BTEX) present a much greater challenge because they occur as part of a complex mixture of hydrocarbons at most field sites. In gas samples from sites with nonaqueous phase liquid (NAPL), BTEX are masked by very high amounts of other compounds (paraffins, isoparaffins, olefins...) with a high vapor pressure [12]. In water samples, BTEX dissolve

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preferentially compared to other compounds. However, as spills age, the ratio between target to matrix compounds can decrease due to the higher solubility of BTEX (and consequently a fast depletion in NAPL compared to non-aromatic compounds), making their analysis more challenging.

During biodegradation of organic contaminants, molecules with light isotopes are usually degraded more rapidly than those with heavy isotopes, leading to an enrichment of heavy isotopes in the remaining fraction[2]. While for chlorinated hydrocarbons isotope enrichment factors for carbon (ε_C) can be as high as −30‰ [2], for benzene and toluene, ε_C for anaerobic degradation range from −0.8 to −3.6‰ [2] and from −0.5 [2] to −6.7‰ [13], respectively. As a result, the change in the carbon isotope ratio for a given amount of degradation is usually smaller for BTEX than for chlorinated hydrocarbons. Thus, reaching high precision of isotope values through full peak separation is particularly critical for BTEX measurements to evidence isotope enrichment or depletion.

To date, two strategies have been developed to separate compounds of interest from non-target compounds for isotope ratio measurements by GC–IRMS. The first strategy relies on the use of long GC columns. For instance, Kawashima and Murakami [14] successfully used a 150 m long column bonded with polydimethylsiloxane to analyze selected individual components in a mixture of VOCs following thermal desorption. However, a dedicated high-pressure regulator was required to maintain the pressure in such a long column and each run lasted more than two hours. Furthermore, resolution improves with an increase in column length but this improvement is proportional to the square root of column length. The second strategy implements two-dimensional heart-cutting GC hyphenated to an IRMS detector. This approach has been used to analyze flavor components [15], steroids in urine [16], wax compounds in tobacco leaf and smoke samples [17], selected congeners in polychlorobiphenyls (PCB) and polychloronaphthalene (PCN) [18], C_2 to C_5 hydrocarbons produced from biomass burning experiments [19], and raspberry aroma compounds from food products [20]. Because of the complexity of petroleum, 2D-GC has been widely applied to characterize various classes of compounds such as paraffins, olefins or aromatics [21]. However, 2D-GC was often hyphenated to a mass spectrometer to overcome difficulties in identifying compounds that were still overlapping despite a multidimensional separation. For example, Henderickx and Ramaekers [22] applied 2D-GC–MS to identify about 70 individual compounds in a C_9-C_{10} aromatic hydrocarbon pyrolysis distillate. 2D-GC has been also used to determine the concentration of aromatic and oxygenated compounds in gasoline with a quadrupole MS [23]. Although BTEX are common environmental contaminants and there is growing interest in the use of CSIA, no studies have yet been conducted on 2D-GC hyphenated to an IRMS detector for BTEX in complex environmental samples.

The objective of this study was to develop a 2D-GC-C–IRMS method to analyze carbon isotope in BTEX in complex environmental samples (groundwater and gas-phase samples). The method was tested with samples from field sites for which 1D-GC-C–IRMS, even under optimized conditions for baseline resolution of benzene and toluene, failed to yield sufficient chromatographic resolution. As a first step of the 2D-GC method development, two samples containing a high load of matrix were analyzed with different combinations of columns in the first and second dimensions to determine the combination of columns and oven program giving the best separation for the compounds of interest (i.e. BTEX with a focus on benzene and toluene). Then, the analytical performance of the new setup was assessed through the determination of metrics such as precision, accuracy, limit of detection (LOD), peak width, and linearity range. The influence of matrix load on these metrics and on isotope values was assessed by varying the ratio between target compound to matrix based on material from a field site. Finally, the application of this method was illustrated with samples from two field sites.

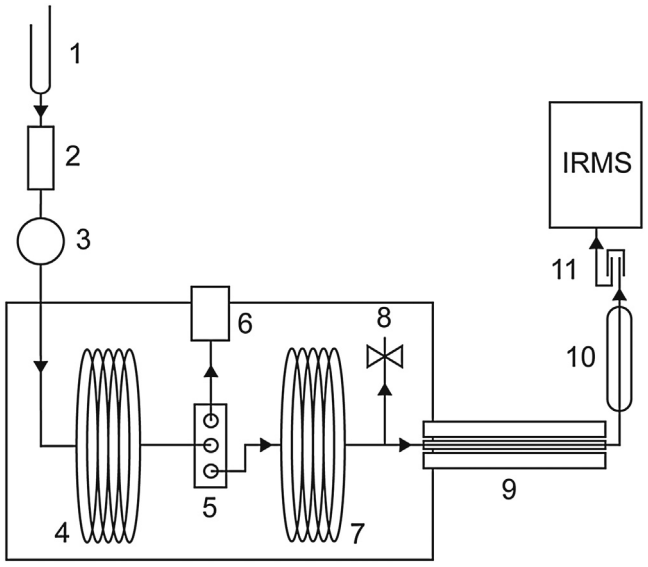

Fig. 1. 2D-GC-C–IRMS configuration. (1) sparge vessel; (2) Trap concentrator; (3) cryotrap; (4) first GC column; (5) Deans Switch; (6) FID; (7) second GC column; (8) Heart Split valve; (9) combustion furnace; (10) Nafion tube; (11) open split.

2. Material and methods

2.1. Chemicals

Reference compounds were obtained in high purity from different suppliers as follows: Ethylbenzene and *m*-xylene from Fluka (both min. 99% purity, Steinheim, Germany) toluene from Riedel-de-Haën (min 99.5% purity, Seelze, Germany), benzene from Sigma Aldrich (min 99.7% purity, Steinheim, Germany) and *o*-xylene from Alfa Aesar (99% purity, Karlsruhe, Germany).

2.2. Carbon isotope analysis

Carbon isotope ratios were determined using an Agilent 7890A gas chromatograph coupled to an Isoprime 100 IRMS via an Isoprime GC5 combustion interface (Elementar) operated at 970 °C. A scheme of the instrumentation for 2D-GC-C–IRMS is shown in Fig. 1. Water samples were preconcentrated using a purge-and-trap module (Stratum, Teledyne Tekmar); 25 mL of sample was purged with N_2 gas in a sparge vessel (40 mL/min, Fig. 1, number 1) for 10 min and the degassed compounds were absorbed on a Vocarb 3000 trap (VICI, Fig. 1, number 2). Compounds were then transferred to a cryogenic trap at −100 °C (Tekmar Dohrmann, Fig. 1, number 3) which was rapidly heated to 180 °C (ramp of 15 °C/s) to transfer the entire mass of compound to the GC. The carrier gas was helium.

2.2.1. 1D-GC-C–IRMS

In order to highlight the need for 2D-GC, 1D-GC-C–IRMS was also performed. Compounds were injected in a DB-VRX column (60 m × 0.32 mm, 1.8 μm, Agilent J&W) operated in constant flow mode (1.2 mL/min). Initially, the oven program was 60 °C (0 min), 1.5 °C/min–125 °C (0 min), 30 °C/min–230 °C (2 min). This program was then optimized to try to improve peak separation for samples with a heavy load of matrix and became: 40 °C (0 min), 1.5 °C/min–55 °C (25 min), 5 °C/min–65 °C (20 min), 30 °C/min–240 °C (5 min). Benzene was eluting during the first plateau at 55 °C and toluene during the second at 65 °C.

2.2.2. 2D-GC-C–IRMS

Different combinations of columns in the first and second dimensions were tested (Table S1, Supp. Info.). Usually, samples are analyzed at least in duplicates and several standards are injected in each sequence, which can take a long time. To address this, first attempts were made with short columns (15 m or 30 m) to minimize the run time (more details provided in supporting information). Both columns were in the same oven. Columns with different stationary phases (a polar and a non-polar phase) were chosen to increase chances of full separation of compounds of interest from the matrix by different mechanisms. The elution order generally follows the boiling points of the compounds in non-polar columns, because compounds are retained by dispersive interactions that increase with the size of the compound (van der Waals forces). BTEX are polarizable compounds as they contain several unsaturated bonds. They are thus more strongly retained on polar columns with polyethylene glycol based phases than non-polar paraffins that constitute most of the non-target compounds. BTEX are also weak hydrogen acceptors, allowing additional hydrogen bond interactions with the stationary phase of polar columns. Separation is then governed by the overall effect of those interactions. For each type of column tested, the thicker film commercially available was chosen to increase BTEX retention on the stationary phase.

After the preconcentration step via the purge-and-trap module, eluate from the first column was diverted either to a flame ionization detector (FID, Fig. 1, number 6) or to the second column via a microfluidic device (Deans Switch, Agilent, Fig. 1, number 5) in 2D-GC. Pressure was kept constant at the inlet (19.86 psi) and at the Deans Switch (12.23 psi) by a Pressure Control Module (PCM). Initial flow in the first column was 1.3 mL/min and 2.8 mL/min in the second column.

To determine the heart-cuts width, standards of BTEX were injected with the flow directed only to the FID to assess retention times in the first column. Based on these retention times, the heart-cuttings to divert the compounds to the second column and then to the IRMS were determined. The beginning and the duration of each Deans Switch cut could shift of a few seconds depending on the samples analyzed and after maintenance on the instrument. At the end of the second column, additional cuts can be made with the Heart Split valve (Fig. 1, number 8) to divert non-target compounds to the vent. Those cuts were determined following the same approach than for heart-cuts determination, i.e. through the injection of BTEX standards diverted to the second column by previously set heart-cuts. The Heart Split valve was kept closed, so the flow was directed to the IRMS. Based on the retention time of standards on the IRMS detector, closing and opening times for the Heart Split valve were then determined. By diverting non-target compounds to the vent, the oxidation capacity of the furnace tube is preserved and its lifespan is extended.

2.2.3. Standardization

CO_2 reference gas was calibrated against the international standard Vienna Pee Dee Belemnite (VPDB) by a Dual Inlet (DI) coupled to an IRMS. Accuracy of the analytical system was assessed daily by the injection of standards of benzene, toluene and *m*-xylene with known carbon isotope ratios, which were previously determined by an Elemental Analyzer (EA) coupled to an IRMS using three international reference materials (IAEA-CH-3, IAEA-CH-7 and NBS-22) with respect to the VPDB standard [24]. EA values for benzene, toluene and *m*-xylene standards were -25.6 ± 0.1‰, -27.7 ± 0.1‰ and -27.4 ± 0.02‰, respectively.

Concentration of these standards ranged from 5 to 20 μg/L and at least five of them were injected during each sequence. Samples were measured in duplicate. Isotope ratios were calculated with Eq. (1) as follows:

$$\delta 13C\,(‰) = \frac{R_{sample} - R_{standard}}{R_{standard}} .1000 \quad (1)$$

with R_{sample} being the $^{13}C/^{12}C$ ratio of the sample, and $R_{standard}$ the abundance ratio VPDB.

2.3. BTEX samples

BTEX samples in a complex matrix were collected from two different field sites. For each field site, a sample containing a high load of matrix was selected to assess the separation achieved by 2D-GC during method development. Once the method was validated, more samples from the two field sites were analyzed to assess the spatial heterogeneity of $\delta^{13}C$ values for benzene and toluene.

2.3.1. Field site X

Site X is a former refinery with a LNAPL source in the former tank area. This LNAPL gave rise to a BTEX plume that is undergoing biodegradation under reducing conditions. Gypsum ($CaSO_4{\cdot}2H_2O$) was applied on the surface to stimulate sulfate-reducing biodegradation. Groundwater samples were collected in 42 mL glass vials sealed without headspace with PTFE-lined silicone septa and screw caps. To make sure no degradation was occurring after sampling, samples from site X were acidified with hydrochloric acid immediately after collection. Sample A was selected for method development. This is a groundwater sample in the low range of concentrations encountered at this site (6 μg/L of benzene, 5 μg/L of toluene, 20 μg/L of ethylbenzene, 80 μg/L of *m,p*-xylene and 22 μg/L of *o*-xylene). Samples C, Da and Db were analyzed to assess the spatial heterogeneity of $\delta^{13}C$ values (see Section 3.2.4).

2.3.2. Field site Y

Site Y is a former refinery and a former crude oil pumping station. Soil vapor extraction has been implemented to remediate a historical BTEX plume. Gas-phase samples were taken in soil vapor extraction wells and in some monitoring wells where, after a dramatic drop of groundwater level, screened intervals were dry.

Gas-phase BTEX were sampled in methanol according to the method developed by Bouchard et al. [25]. The solvent-based gas-phase sampling device is shown in Fig. S1. Briefly, a 40 mL glass vial was filled with 30 mL of methanol and connected to a GilAir-5 pump (flow rate of 200 mL/min, Gilian, St. Petersburg, USA) via a metal adaptor designed to avoid contact of the sampling gas with the pump. The adaptor consisted of two metal tubes, one extended to the bottom of the vial and the other to the headspace only. When pumping air through the shorter tube, underpressure was created in the headspace of the vial, and the ambient air was pulled into the vial via the longer tube. Air migrated upward through the solvent as bubbles, and the gas-phase VOC dissolved in methanol. Purging time was determined based on BTEX concentrations measured using a portable GC. Once the sampling was completed, 20 mL of the methanol extract were kept for isotope analysis of benzene and toluene, while the remaining volume was used for concentration determination. Sample B was selected for method development. Samples E, F, and G were analyzed to assess the spatial heterogeneity of $\delta^{13}C$ values.

2.3.3. Sample preparation

Concentrations of BTEX in samples were measured by an accredited lab (Test America, USA) by GC–qMS (US EPA Method 8260 [26]). Based on these results, sample dilutions were carried out in 42 mL vials filled with Milli-Q water (Direct-Q UV, Millipore) to obtain a final concentration between 5 and 20 μg/L for each compound. For methanol extracts, the proportion of solvent after dilutions never

exceeded 1% (V:V) [27]. Indeed, it was observed that the baseline became noisy when higher proportions of methanol were injected. Methanol extracts, for which degradation can be excluded, were generally analyzed within 6 months. Repeated measurements of one extract confirmed that the isotope ratios do not change significantly (<0.5‰) over this period.

3. Results and discussion

3.1. 1D-GC-C–IRMS analyses of field samples

First, an attempt was made to analyze samples A and B from the two field sites with the usual 1D-GC-C–IRMS set up. IRMS chromatograms are shown in Fig. 2. Sample A from site X was analyzed undiluted with the usual oven program described above and showed good separation for toluene (measured $\delta^{13}C$ of −21.2‰), ethylbenzene (−27.5‰) and *o*-xylene (−26.4‰, Fig. 2a). However, because benzene (measured $\delta^{13}C$ of −35.7‰) was coeluting with some unidentified matrix compounds, determination of its $\delta^{13}C$ value was not possible. *m,p*-xylenes were not analyzed because they were out of the linearity range of the detector.

Fig. 2b shows a chromatogram obtained from the injection of sample B from site Y (methanol extract diluted 1680 times in water). This sample was analyzed with an oven program designed to optimize peak separation while maintaining a good peak shape (see Section 2.2.1.). The Heart Split valve was closed for only 80 s for benzene, and 200 s for toluene, to send as much matrix as possible to the vent without cutting the peaks of interest. Despite these settings, both benzene and toluene (both 5 μg/L after dilution) showed severe coelutions with matrix compounds. Two other samples from two different sampling points of site Y were also injected with the same method. The chromatograms were similar to the one shown for sample B, with severe coelutions for both benzene and toluene (data not shown).

3.2. 2D-GC-C–IRMS

3.2.1. Determination of optimum combination of columns

Several combinations of columns in the first and second dimensions were tested to try and achieve full peak separation for BTEX (see Supporting Information for more details). A complete separation of both benzene and toluene from matrix compounds was achieved with a 60 m CP Wax 52 CB in the first position and a 30 m DB-5 column in the second position, with the following heart-cuttings: initially off, the Deans Switch valve was switched on around 24.5 min for typically 1 min (benzene) and around 32 min for 40–50 s (toluene). This combination of columns was also tested for the analysis of sample A from site X, for which additional cuts for ethylbenzene and xylenes were set as follows: around 39 min for 45 s (ethylbenzene), around 40.4 min for 35 s (*m,p*-xylenes), and around 42.8 min for 30 s (*o*-xylene). Total run time was about 48 min (see Supporting Information for oven program).

The performance of this third combination is illustrated in Fig. 3, with different chromatograms obtained for sample B from site Y. In the first column (polar), most of the non-polar matrix components as well as methanol were poorly retained and eluted early during the run while benzene and toluene were more strongly retained but still co-eluted with non-target compounds (Fig. 3a). After the second column (non-polar), benzene and toluene were fully resolved with a strikingly clean baseline before and after the peaks given the complexity of the sample (Fig. 3c). Cumulated areas of both benzene and toluene peaks represent only 2.3% of the area of the entire FID chromatogram taking into account non-target compounds (area of the FID chromatogram in Fig. 3b subtracted to the area of the FID chromatogram in Fig. 3a).

Table 1
$\delta^{13}C$ values of BTEX standards (20 μg/L) for different IRMS configurations ± the standard deviation. Standards were analyzed over several weeks.

	1D GC-C–IRMS $\delta^{13}C$ (‰)	2D GC-C–IRMS $\delta^{13}C$ (‰)
Benzene	−24.4 ± 0.1 (*n* = 27)	−24.3 ± 0.2 (*n* = 41)
Toluene	−26.5 ± 0.2 (*n* = 27)	−26.6 ± 0.2 (*n* = 41)
Ethylbenzene	−28.4 ± 0.2 (*n* = 26)	−28.4 ± 0.2 (*n* = 37)
m-xylene	−26.6 ± 0.2 (*n* = 27)	−26.6 ± 0.2 (*n* = 30)
o-xylene	−27.9 ± 0.2 (*n* = 27)	−27.9 ± 0.2 (*n* = 33)

Fig. 4 shows chromatograms after the first and the second separations for sample A from site X, the sample previously analyzed by 1D-GC (Fig. 2a). Benzene was fully resolved after the first column (Fig. 4a), which was not the case with 1D-GC-C–IRMS (Fig. 2a). However, *m,p*-xylenes were not completely baseline-separated from another compound and could only be properly resolved after the second dimension (Fig. 4c). Measured $\delta^{13}C$ values for toluene (−21.7‰), ethylbenzene (-26.9‰), and *o*-xylene (−26.1‰) were comparable to those obtained with 1D. However, due to complete baseline resolution in 2D, which was not achieved in 1D, a significant shift of the carbon isotope value was observed for benzene (−26.9‰) in 2D. Cumulated areas of BTEX peaks represent about 11% of the area of the entire FID chromatogram. The tests and analyses described in the following sections were performed with a 60 m CP Wax 52 CB column in the first dimension and a 30 m DB-5 column in the second dimension.

3.2.2. Analysis of BTEX standards

In order to determine metrics such as precision, accuracy, LOD, peak width, and the linearity range of the newly implemented 2D-GC-C–IRMS method and to make a comparison with classic 1D-GC-C–IRMS, BTEX standards usually injected in 1D were injected at different concentrations within the usual working range (5–20 μg/L). Precision of standards versus precision of samples will be discussed in more details in Section 3.2.3. For isotope measurements, a high linearity means that the measured isotope ratio is independent of the amount of compound injected and hence independent of the signal intensity [2]. Without a good linearity, isotope ratios of samples at different concentrations or sizes could not be compared.

Table 1 summarizes $\delta^{13}C$ values of standards at 20 μg/L compiled from several sequences for 1D-GC and 2D-GC. $\delta^{13}C$ values measured by 2D-GC-C–IRMS were in very good agreement with those measured by 1D-GC-C–IRMS for all the compounds. The precision, which was very good for 1D-GC with standards deviation around 0.1-0.2‰ for concentrations of 20 μg/L, did not deteriorate with the 2D-GC setup, and was high compared to the commonly accepted 0.5‰ for environmental samples [28,29]. The $\delta^{13}C$ values for 1D and 2D were not significantly different (Student's *t*-test, $p < 0.05$). Complex flow paths and heart-cuttings linked to 2D-GC did neither introduce systematic nor random offset in delta values nor higher variability. The $\delta^{13}C$ values of BTEX standards measured during different sequences, at variable concentrations (from 5 μg/L to 20 μg/L) and over an extended period of time (several months) did not show any difference for precision and accuracy between 1D and 2D-GC (Fig. S3 Supp. Info.).

For concentration analysis, a signal is quantified that increases as a function of concentration. For isotope analysis, the ratio between two isotopes is evaluated, which should remain constant independently of the analyte concentration. Hence, the method for quantifying LOD is different than for concentrations analyses. The LOD was determined with the method developed by Jochmann et al. [29]. According to this method, the LOD corresponds to the concentration for which either the average $\delta^{13}C$ deviates by more than 0.5‰ from the expected values, or if the standard deviation exceeds a given criterion, usually 0.5‰. In our case, the standard deviation

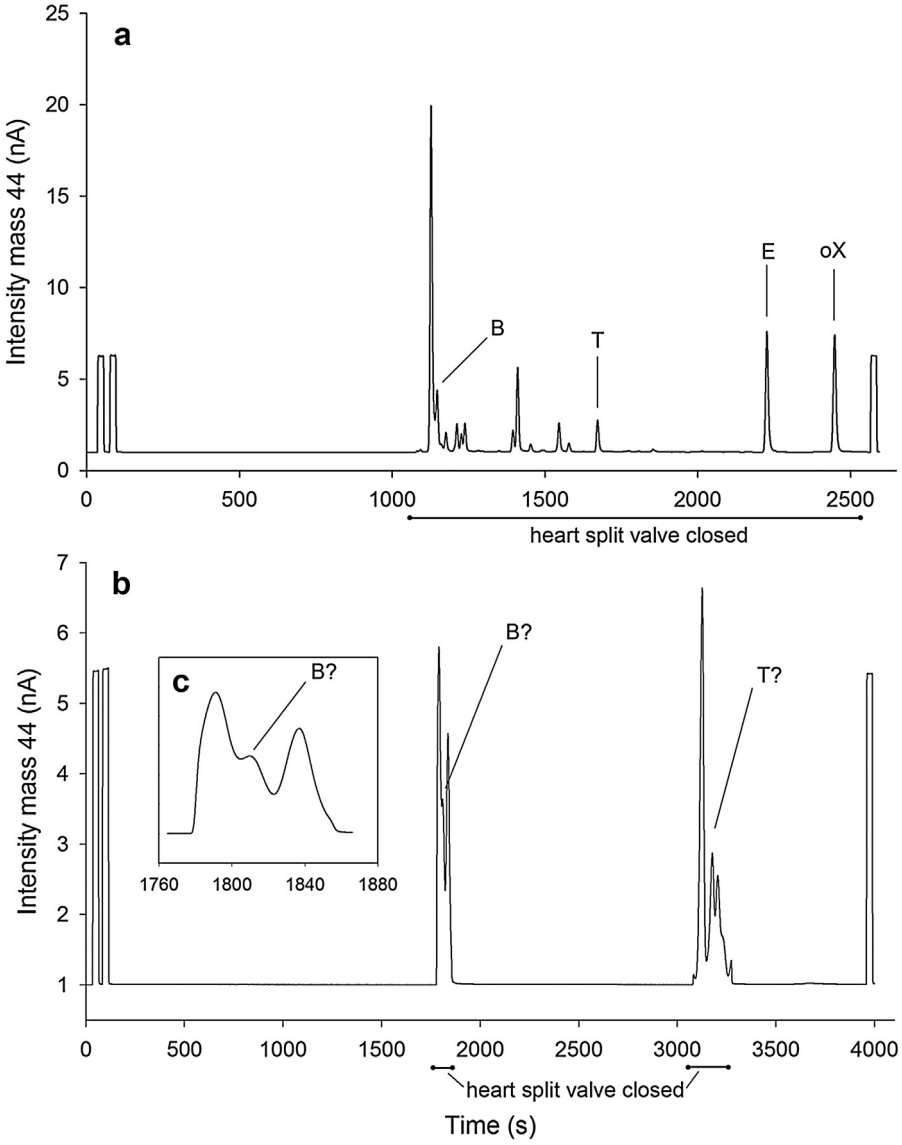

Fig. 2. IRMS chromatograms obtained with 1D-GC–IRMS. a) is an undiluted groundwater sample (sample A) from site X. The Heart Split valve was closed from 1050 s. This sample was analyzed with the oven program before optimization. b) is a sample (sample B) in methanol from site Y diluted 1680 times in water. The Heart Split valve was closed only from 1750 s to 1830s for benzene and from 3050s to 3250s for toluene to send most of the matrix to the vent. c) is an expanded view showing the possible benzene peak.

was higher than 0.5‰ for 2.5 μg/L for all BTEX (data not shown). The lowest concentration fulfilling above-mentioned criteria was 5 μg/L and is thus the LOD of the method. This LOD can change depending on the desired precision for isotope measurements.

$\delta^{13}C$ values measured by 2D-GC-C–IRMS showed an enrichment in $\delta^{13}C$ for benzene, toluene, and m-xylene compared to values measured by EA-IRMS (see Section 2.2.3). Purge-and-trap devices are known to cause an offset toward enriched values due to air-water partitioning. This has already been observed by Zwank et *al.* for benzene and toluene [30]. During this study, the benchmark for method development was 1D-GC-C–IRMS, and there were no significant differences in $\delta^{13}C$ values obtained with 1D or 2D-GC. Measurements by both setups were done with the same purge-and-trap device. Therefore, the systematic effect caused by this device was not corrected, because the correction would have been the same for both 1D and 2D-GC-C–IRMS.

$\delta^{13}C$ values and intensities as a function of concentration for BTEX standards are shown in Fig. 5 for both 1D and 2D-GC. Within the tested concentration range, the peak intensity correlated very well with concentration. There is no significant difference in accuracy between 1D and 2D for the 5 tested compounds. Taking into account the three concentration levels, standard deviations were better in 2D (from 0.05 to 0.31‰, $n = 3$ for each point) than in 1D-GC–IRMS (from 0.03 to 0.65‰, $n = 3$ for each point). Previous studies about $\delta^{13}C$ values of BTEX measured by 1D-GC-C–IRMS showed a standard deviation comprised between 0.2 and 1.5‰ [31] and between 0.2 and 0.35‰ [30] for water samples. For Polycyclic Aromatic Hydrocarbons (PAH), O'Malley et al. obtained standard deviations comprised between 0.17 and 0.31‰ for standards measured by 1D-GC-C–IRMS [32]. Standard deviations in the current study were as good as or better than in these previous studies, although they were performed on low matrix samples.

In previous studies on 2D-GC hyphenated to an IRMS detector, precision and accuracy with a 2D setup were similar to those obtained with 1D, with precision in the range of 0.1–0.2‰ for the analysis of flavor components [15], or 0.05–0.2‰ for the analysis of steroids in urine [16]. Other studies showed a significant increase in precision and accuracy, which was linked to an enhancement of

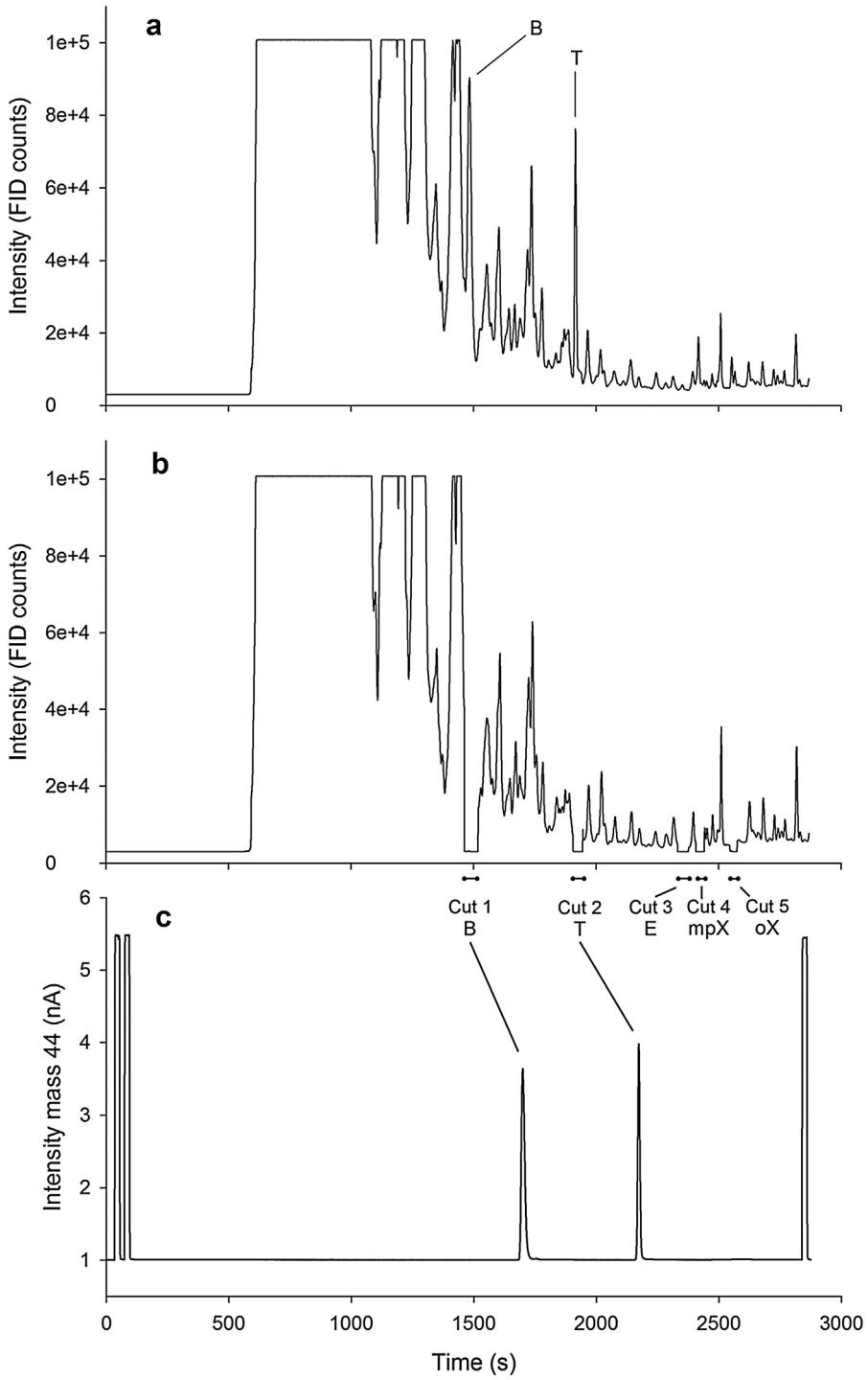

Fig. 3. 2D-GC–IRMS analysis of sample B from site Y with CP Wax 52 CB column (first dimension) and the DB-5 (second dimension). a) FID chromatogram without cuts (sample diluted 700 times), b) FID chromatogram with cuts for BTEX (sample diluted 700 times) and c), the corresponding IRMS chromatogram. Only benzene (Heart Split valve closed from 1650 to 1745s) and toluene (from 2130 to 2220s) were sent to the IRMS.

baseline separation for the compounds of interest in 2D compared to 1D-GC. For example, in one of the first studies concerning 2D-GC-C–IRMS, Nitz et al. were able to significantly improve both the accuracy and the precision of the measurements for flavor compounds in a reference mixture with 2D-GC (0.2–0.3‰) compared to 1D (0.3–0.6‰), by achieving better baseline resolution for the compounds of interest in 2D [33]. Nara et *al.* also showed that 2D-GC successfully improved both accuracy and precision for isotopic analysis of pentanes in biomass burning smoke by removing the tailing from acetylene or ethylene peak [19]. However, standard deviations remained high (0.7–0.9‰) even with 2D-GC, because the amount injected for the compounds of interest was low (0.4 nmolC).

With the 2D setup, benzene peak intensities decreased by half for a given concentration (Fig. 5a) and peak width at half height doubled in 2D compared to 1D-GC (data not shown). A longer total length column (90 m) in 2D compared to 1D (60 m) may be responsible for wider peaks. However, despite the lower peak intensity for 2D versus 1D, even for the standard with the lowest concentra-

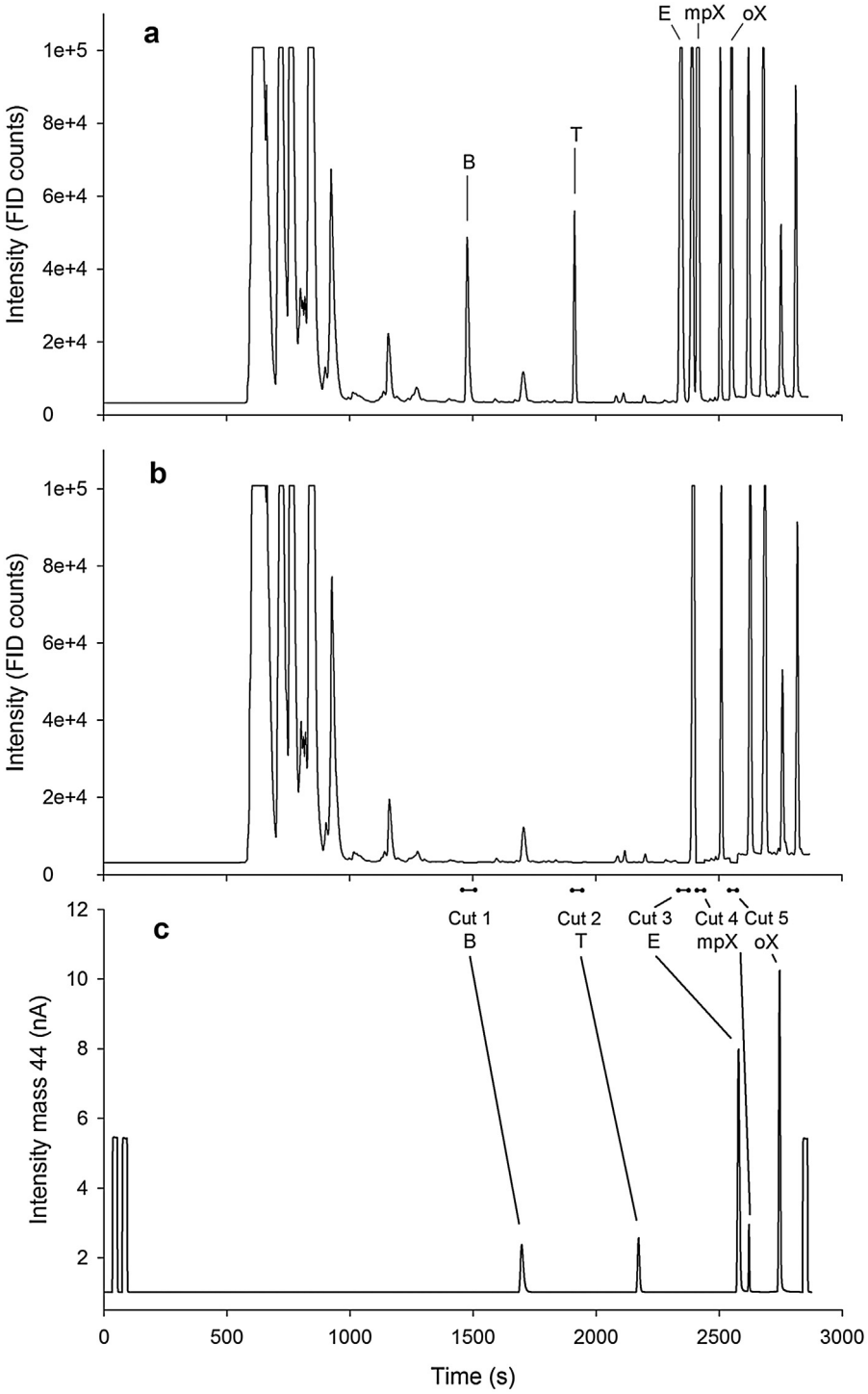

Fig. 4. 2D-GC–IRMS analysis of sample A from site X with CP Wax 52 CB column (first dimension) and the DB-5 (second dimension). a) FID chromatogram without cuts (undiluted sample), b) FID chromatogram with cuts for BTEX (undiluted sample) and c), the corresponding IRMS chromatogram. The small peak after ethylbenzene is *m,p*-xylene partially cut by the Heart Split valve (too intense to be analyzed in the same run).

tion (5 ug/L), the precision was comparable for the two approaches (σ = 0.15‰ and 0.16‰ for 10 μg/L in 1D and 2D-GC, respectively) or better with 2D compared to 1D (σ = 0.29‰ and 0.19‰ for 5 μg/L in 1D and 2D-GC, respectively). The same observations can be made for toluene (Fig. 5b). Intensities decreased by 30% but precision is still very high for toluene (standard deviation <0.2‰) in both setup. For benzene and toluene, the working linearity range in 2D was unchanged compared to 1D despite decreased amplitudes of the peaks.

For isotope analysis, previous knowledge of concentrations is necessary to adjust peak intensity by dilution. Different dilution ratios are necessary if target analyte concentrations differ substantially, which is often the case for BTEX. Additional tests were performed to investigate whether $\delta^{13}C$ values were still linear for higher concentrations, while keeping reference gas peaks at the same intensity. These tests showed that delta values were stable and reproducible from 5 to 65 μg/L for benzene and toluene (data not shown). However, it would be preferable to work in the low

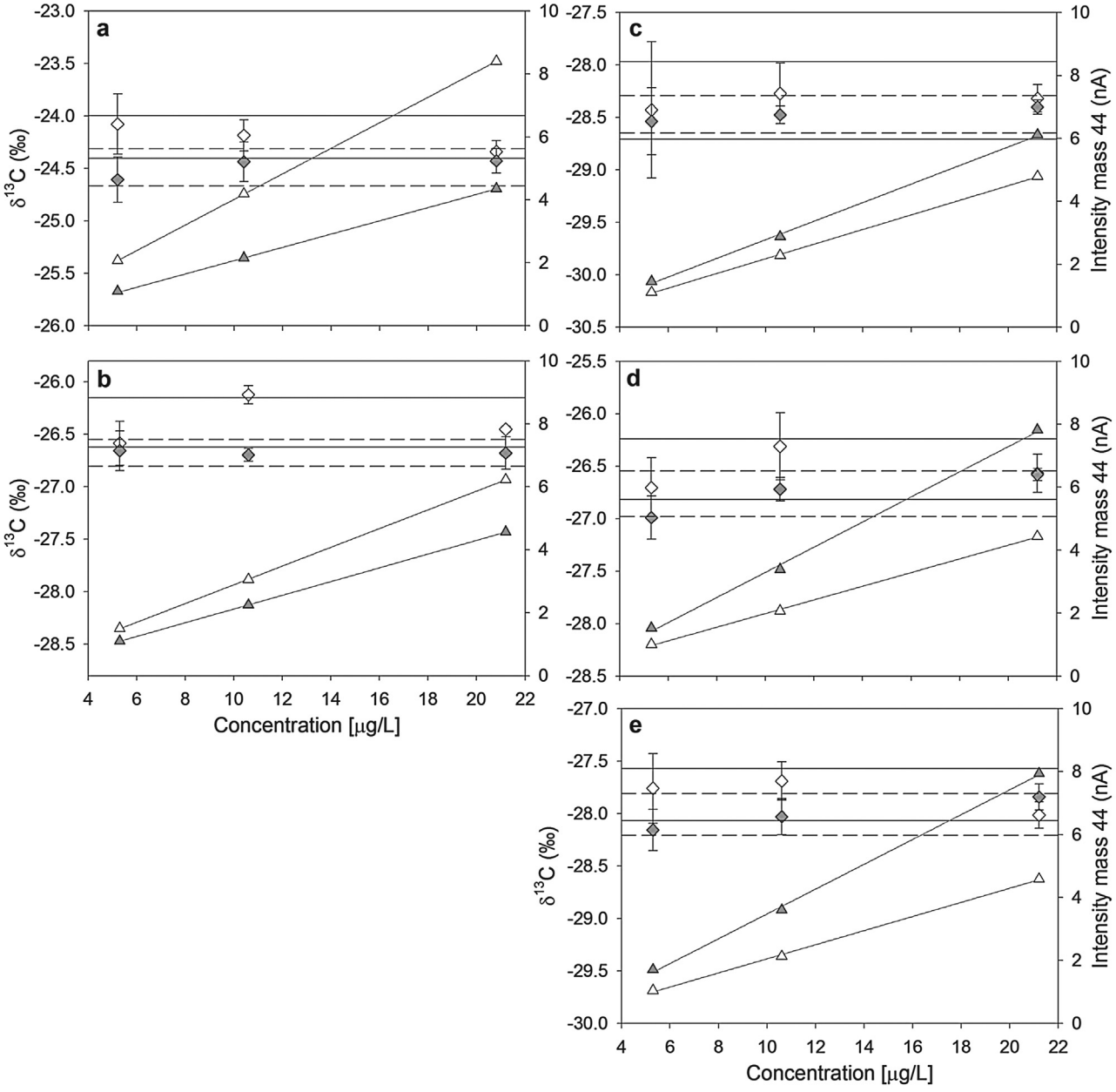

Fig. 5. Comparison of $\delta^{13}C$ values and intensities between 1D and 2D-GC–IRMS for a) benzene, b) toluene, c) ethylbenzene, d) *m*-xylene and e) *o*-xylene standards analyzed at different concentrations (5, 10 and 20 µg/L). White and gray diamonds represent $\delta^{13}C$ values for 1D and 2D-GC respectively. White and gray triangles show the amplitude of mass 44 for 1D and 2D-GC respectively. Triplicate measurements were made for each point. Solid black lines and dash lines stand for standard deviation around the mean taking into account all standards injected for 1D and 2D-GC respectively. Gray solid lines stand for the linear curve fit of peak amplitudes vs. concentrations.

range of concentrations (5–20 µg/L) to avoid a high load of target and non-target compounds in both GC columns and also in the interface.

3.2.3. *Spiking tests*

In order to assess whether the separation achieved in 2D-GC–IRMS was still sufficient when the ratio between matrix to target compound was increased, a sample from site Y that did not contain benzene was spiked with a benzene standard of known isotopic composition. This spiking test also aimed to assess the influence of the matrix load on retention times and $\delta^{13}C$ values. It focused on benzene because it is eluting among numerous matrix compounds after the first column (Fig. 3a), and thus might be particularly sensitive to changes in the ratio between target and matrix compounds. Furthermore, benzene is the regulatory driver for contaminated site remediation in many countries [34,35]. To make sure that it did not contain benzene or that benzene was below the detection limit, the sample to be spiked was injected once prior to the spiking test at a dilution corresponding to highest matrix load in the test. The chromatogram is shown in Fig. 6b. Benzene concentration was very low and the peak could not be integrated by the software (intensity below 0.2 nA).

For this test, the benzene concentration was held constant (15 µg/L) while an increasing amount of matrix was added in the form of the methanol extract mentioned above (see Section 2.3.2). The amount of methanol extract was varied from 50 µL to 400 µL to match the range of dilutions made for sample measurements. FID chromatograms corresponding to 50 µL and 400 µL of methanol extract are shown on Fig. 7a and b. respectively. With 50 µL, benzene peak area represented about 6% of the total area of

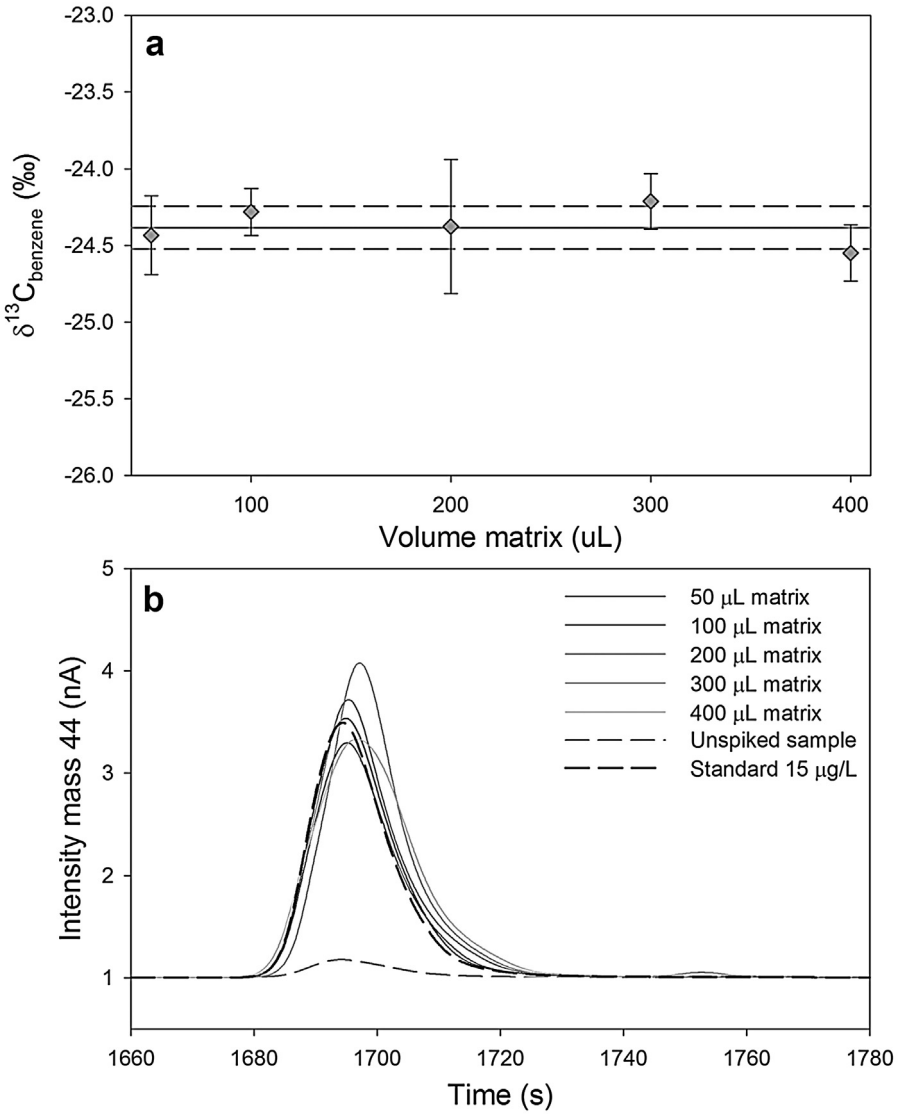

Fig. 6. a) Influence of the volume of matrix from sample C on $\delta^{13}C$ value for spiked benzene at 15 µg/L. Gray diamonds represent the mean $\delta^{13}C$ value for standard in vials that received different volumes of matrix ($n = 3$ for each volume). The solid gray line shows the mean value obtained for the same standard analyzed the same day in vials with MilliQ water only ($n = 10$). The dash lines represent the standard deviation measured in this sequence (1σ). b) superimposed 2D-GC–IRMS chromatograms showing benzene peak in a standard at 15 µg/L (black dashes), in vials spiked with increasing volumes of sample C and in the unspiked sample (420 µL of matrix, gray dashes).

the chromatogram (estimated by comparing the area of a standard at 15 µg/L to the chromatogram showed in Fig. 7a to which the area of the standard was added). With 400 µL of methanol extract, the FID detector was saturated by matrix compounds until toluene eluted.

Spiking test results are shown in Fig. 6. Baseline separation was always possible independently of the volume of methanol extract added. $\delta^{13}C$ values of the spiked sample were consistent regardless of the volume of matrix added (Fig. 6a). Standard deviations were not dependent on the amount of methanol extract added and ranged from 0.15‰ (100 µL of methanol extract) to 0.44‰ (200 µL) for triplicates. The two highest amounts of matrix (300 and 400 µL) both exhibited a standard deviation of 0.18‰. Overall, the measurement precision was not degraded by the presence of high loads of non-target compounds. In a similar spiking test made for toluene, standard deviations for triplicate injections ranged from 0.09‰ to 0.32‰ for volumes of methanol extract varying between 50 and 300 µL (data not shown). With increasing amounts of matrix, a minor shift in retention time was observed for benzene (maximum 2s, heart-cut width of 57s). Peak broadening occurred for only the highest volume of matrix (peak at half height 20% wider than for standards, Fig. 6b). No tailing was observed. Retention times tend to increase when the GC column is loaded with a larger mass of sample. For example, Dietz showed that compounds eluting right after or in a region containing a large unresolved band of peaks exhibited some shifts in retention times of up to 1.5 min depending on the solvent used for the analysis [36]. The observed slight peak broadening could be caused by an alteration of the stationary phase by the high load of compounds. In the case of isotope analyses, a relatively high amount of target compound is required which can lead to column overload, especially when the ratio of target compounds to non-target compounds is very low. The shift in retention times seemed to be negligible compared to the width of the heart-cut since isotope values do not change with higher load of matrix. Usually cut width is determined based on retention time in standard mixtures. However, for samples with heavy load of matrix the heart-cuts should be widened, while keeping in mind that broader cuts could send more non-target compounds to the second column and compromise a proper baseline separation of the target compounds in this second column.

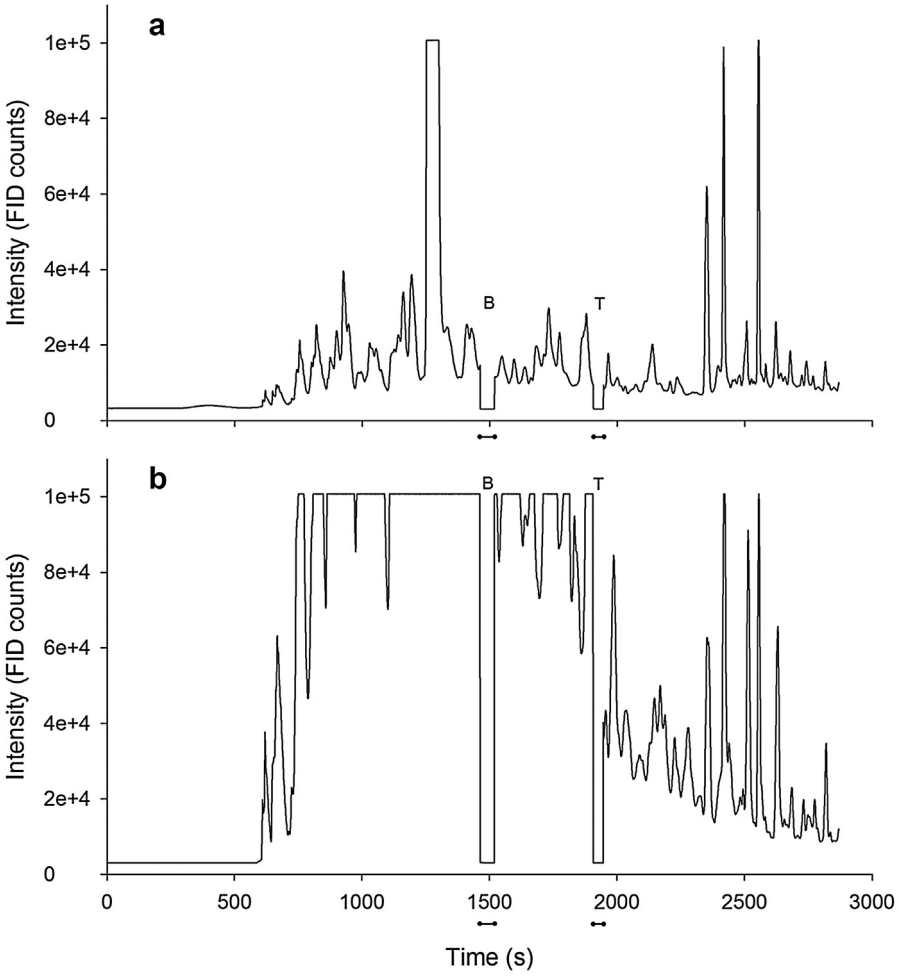

Fig. 7. a) FID chromatogram of the vial spiked with 50 μL of sample C from site Y with cuts for benzene and toluene (dashes stand for cuts), b) FID chromatogram of the vial spiked with 400 μL of matrix from sample C with cuts for benzene and toluene.

3.2.4. Analysis of samples

Different samples from site X and site Y were analyzed with the newly implemented 2D-GC-C–IRMS configuration to test whether baseline separation and high precision were achieved for all samples. The study also aimed to assess whether $\delta^{13}C$ values for benzene and toluene exhibited any spatial variability.

Results for both sites are compiled in Table 2. Baseline separation was achieved for all the samples. Each sample was analyzed in triplicate and the standard deviation of these triplicates was always ≤0.2‰. For site X, $\delta^{13}C$ values range from −28.7 to −28.0‰ for benzene (Δδ 0.7‰) and from −27.0 to −21.2‰ (Δδ 5.8‰) for toluene. In an anaerobic aquifer, toluene is usually degraded more readily than benzene [37–39], which is consistent with the wider range of $\delta^{13}C$ values and the substantial enrichment of ^{13}C in some samples.

For site Y, $\delta^{13}C$ values range from −26.4 to −26.1‰ for benzene (Δδ 0.3‰) and from −18.9 to −17.8‰ (Δδ 1.1‰) for toluene. $\delta^{13}C$ values for petroleum hydrocarbons usually lie between −35‰ and −25‰ [2]. Toluene is enriched in ^{13}C compared to this expected range of values. The site had been contaminated decades ago. It is very likely that biodegradation of toluene occurred, explaining the enrichment currently measured.

4. Conclusions and future research needs

A 2D-GC-C–IRMS method to analyze complex environmental samples such as gas-phase samples has been successfully developed for BTEX, even when the ratio of aromatics to non-targeted compounds is very low. Accuracy remained comparable to 1D-GC–IRMS whilst precision was still very high (standard deviations ≤0.2‰ for working concentrations). For late eluting compounds, precision was even better in 2D than in 1D. 2D-GC was successfully applied to groundwater and gas-phase samples that could not be analyzed by classic 1D-GC–IRMS, giving new insights into attenuation processes of BTEX in contaminated sites by broadening the spectrum of environmental samples suitable for isotope ratio measurements.

One of the criticisms of 2D-GC is the duration of the run that can easily exceed 60 min. The final 2D-GC oven program implemented in this study is shorter than one of the program implemented for 1D by 1000 s (about 16.7 min) for a much better resolving capacity (Fig. 2b. versus Figs. 4 and 5). Moreover, there is no need for an additional oven for the second column and both columns lie within a GC oven.

When developing and implementing 2D-GC hyphenated to an IRMS detector, one should carefully determine the width of the heart-cuts, especially because they are usually defined through the injection of standards; a high mass of matrix in samples can lead to a shift in retention time and to a partial peak recovery from the first dimension. This latter can then be responsible for erroneous $\delta^{13}C$ measurements.

Because of the small shifts in retention time due to matrix load that were observed, doubts can remain about peak identification without a mass spectrometer. For practical application, it might be useful to include such a detector in the setup by splitting the

Table 2
Range of $\delta^{13}C$ values and concentrations measured for benzene and toluene for different samples from sites X and Y. Concentrations (accredited laboratory) are given before dilution for water samples (site X) and methanol extracts (site Y). All samples are from the same sampling campaign but from different sampling points. For site X, samples Da and Db correspond to different depths from a same multilevel piezometer (Da being the shallowest point). Values are the average of triplicates ± the standard deviation.

Field Site	Sample number	Benzene		Toluene	
		Conc. (μg/L)	$\delta^{13}C$ (‰)	Conc. (μg/L)	$\delta^{13}C$ (‰)
Site X					
	C	2900	−28.0 ± 0.1	20,000	−27.0 ± 0.1
	Da	900	−28.7 ± 0.1	890	−24.7 ± 0.1
	Db	12,000	−28.3 ± 0.1	350	−21.2 ± 0.1
Site Y					
	E	2978	−26.4 ± 0.2	1561	−18.6 ± 0.1
	F	8672	−26.1 ± 0.1	8237	−17.8 ± 0.1
	G	604	−26.1 ± 0.1	1994	−18.9 ± 0.1

flow after the second column between the IRMS and a quadrupole mass spectrometer to confirm compound identification and proper chromatographic separation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2017.02.060.

References

[1] T.C. Schmidt, L. Zwank, M. Elsner, M. Berg, R.U. Meckenstock, S.B. Haderlein, Compound-specific stable isotope analysis of organic contaminants in natural environments: a critical review of the state of the art, prospects, and future challenges, Anal. Bioanal. Chem. 378 (2003) 283–300.
[2] C.M. Aelion, P. Höhener, D. Hunkeler, R. Aravena, Environmental Isotopes in Biodegradation and Bioremediation, CRC Press (Taylor and Francis), Boca Raton, 2010.
[3] L. Zwank, M. Berg, M. Elsner, T.C. Schmidt, R.P. Schwarzenbach, S.B. Haderlein, New evaluation scheme for two-dimensional isotope analysis to decipher biodegradation processes: application to groundwater contamination by MTBE, Environ. Sci. Technol. 39 (2005) 1018–1029.
[4] A. Badin, G. Buttet, J. Maillard, C. Holliger, D. Hunkeler, Multiple dual CCl isotope patterns associated with reductive dechlorination of tetrachloroethene, Environ. Sci. Technol. 48 (2014) 9179–9186.
[5] M. Blessing, T.C. Schmidt, R. Dinkel, S.B. Haderlein, Delineation of multiple chlorinated ethene sources in an industrialized area—a forensic field study using compound-Specific isotope analysis, Environ. Sci. Technol. 43 (2009) 2701–2707.
[6] D. Hunkeler, N. Chollet, X. Pittet, R. Aravena, J.A. Cherry, B.L. Parker, Effect of source variability and transport processes on carbon isotope ratios of TCE and PCE in two sandy aquifers, J. Contam. Hydrol. 74 (2004) 265–282.
[7] S. Bashir, K.L. Hitzfeld, M. Gehre, H.H. Richnow, A. Fischer, Evaluating degradation of hexachlorcyclohexane (HCH) isomers within a contaminated aquifer using compound-specific stable carbon isotope analysis (CSIA), Water Res. 71 (2015) 187–196.
[8] T.B. Hofstetter, M. Berg, Assessing transformation processes of organic contaminants by compound-specific stable isotope analysis, Trends Anal. Chem. 30 (2011) 618–627.
[9] M. Braeckevelt, A. Fischer, M. Kästner, Field applicability of compound-specific isotope analysis (CSIA) for characterization and quantification of in situ contaminant degradation in aquifers, Appl. Microbiol. Biotechnol. 94 (2012) 1401–1421.
[10] R.U. Meckenstock, B. Morasch, C. Griebler, H.H. Richnow, Stable isotope fractionation analysis as a tool to monitor biodegradation in contaminated acquifers, J. Contam. Hydrol. 75 (2004) 215–255.
[11] D. Hunkeler, R.U. Meckenstock, B. Sherwood Lollar, T.C. Schmidt, J.T. Wilson, A Guide for Assessing Biodegradation and Source Identification of Organic Ground Water Contaminants Using Compound Specific Isotope Analysis (CSIA), Office of Research and Development, US EPA, Oklahoma, USA, 2008.
[12] C.L. Yaws, Chemical Properties Handbook, McGraw-Hill, 1999.
[13] S. Herrmann, C. Vogt, A. Fischer, A. Kuppardt, H.-H. Richnow, Characterization of anaerobic xylene biodegradation by two-dimensional isotope fractionation analysis, Environ. Microbiol. Rep. 1 (2009) 535–544.
[14] H. Kawashima, M. Murakami, Measurement of the stable carbon isotope ratio of atmospheric volatile organic compounds using chromatography, combustion, and isotope ratio mass spectrometry coupled with thermal desorption, Atmos. Environ. 89 (2014) 140–147.
[15] D. Juchelka, T. Beck, U. Hener, F. Dettmar, A. Mosandl, Multidimensional gas chromatography coupled on-line with isotope ratio mass spectrometry (MDGC-IRMS): progress in the analytical authentication of genuine flavor components, J. High Resolut. Chromatogr. 21 (1998) 145–151.
[16] A.D. Brailsford, I. Gavrilović, R.J. Ansell, D.A. Cowan, A.T. Kicman, Two-dimensional gas chromatography with heart-cutting for isotope ratio mass spectrometry analysis of steroids in doping control, Drug Test Anal. 4 (2012) 962–969.
[17] E. Dumont, B. Tienpont, N. Higashi, K. Mitsui, N. Ochiai, H. Kanda, F. David, P. Sandra, Heart-cutting two-dimensional gas chromatography in combination with isotope ratio mass spectrometry for the characterization of the wax fraction in plant material, J. Chromatogr. A 1317 (2013) 230–238.
[18] Y. Horii, K. Kannan, G. Petrick, T. Gamo, J. Falandysz, N. Yamashita, Congener-specific carbon isotopic analysis of technical PCB and PCN mixtures using two-Dimensional gas chromatography-Isotope ratio mass spectrometry, Environ. Sci. Technol. 39 (2005) 4206–4212.
[19] H. Nara, F. Nakagawa, N. Yoshida, Development of two-dimensional gas chromatography/isotope ratio mass spectrometry for the stable carbon isotopic analysis of C2–C5 non-methane hydrocarbons emitted from biomass burning, RCM 20 (2006) 241–247.
[20] S. Sewenig, D. Bullinger, U. Hener, A. Mosandl, Comprehensive authentication of (E)-α(β)-ionone from raspberries, using constant flow MDGC-C/P-IRMS and enantio-MDGC–MS, J. Agric. Food Chem. 53 (2005) 838–844.
[21] W. Bertsch, Two-Dimensional gas chromatography. concepts, instrumentation, and applications–part 1: fundamentals conventional two-dimensional gas chromatography, selected applications, J. High Resolut. Chromatogr. 22 (1999) 647–665.
[22] H.J.W. Henderickx, J.J.M. Ramaekers, Analysis of a C9-C10 aromatic hydrocarbon pyrolysis distillate by multidimensional capillary GC and multidimensional capillary GC–MS, J. High Resolut. Chromatogr. 17 (1994) 407–410.
[23] D. Sciarrone, P.Q. Tranchida, C. Ragonese, L. Schipilliti, L. Mondello, Multidimensional GC coupled to MS for the simultaneous determination of oxygenate compounds and BTEX in gasoline, J. Sep. Sci. 33 (2010) 594–599.
[24] T.B. Coplen, W.A. Brand, M. Gehre, M. Gröning, H.A.J. Meijer, B. Toman, R.M. Verkouteren, New guidelines for δ13C measurements, Anal. Chem. 78 (2006) 2439–2441.
[25] D. Bouchard, P.W. McLoughlin, D. Hunkeler, R.J. Pirkle, δ13C and δ37Cl on gas-phase TCE for source identification investigation – innovative solvent-based sampling method, 2014 INEF Conference (2014).
[26] US-EPA, Method 8260 B: Volatile Organic Compounds by Gas Chromatography/mass Spectrometry (GC/MS), United States Environmental Protection Agency, 1996.
[27] D. Bouchard, D. Hunkeler, Solvent-based dissolution method to sample gas-phase volatile organic compounds for compound-specific isotope analysis, J. Chromatogr. A 1325 (2014) 16–22.
[28] B. Sherwood Lollar, S.K. Hirschorn, M.M.G. Chartrand, G. Lacrampe-Couloume, An approach for assessing total instrumental uncertainty in compound-Specific carbon isotope analysis: implications for environmental remediation studies, Anal. Chem. 79 (2007) 3469–3475.
[29] M.A. Jochmann, M. Blessing, S.B. Haderlein, T.C. Schmidt, A new approach to determine method detection limits for compound-specific isotope analysis of volatile organic compounds, RCM 20 (2006) 3639–3648.
[30] L. Zwank, M. Berg, T.C. Schmidt, S.B. Haderlein, Compound-Specific carbon isotope analysis of volatile organic compounds in the low-microgram per liter range, Anal. Chem. 75 (2003) 5575–5583.

[31] C.A. Kelley, B.T. Hammer, R.B. Coffin, Concentrations and stable isotope values of BTEX in gasoline-contaminated groundwater, Environ. Sci. Technol. 31 (1997) 2469–2472.
[32] V.P. O'Malley, T.A. Abrajano, J. Hellou, Determination of the 13C12c ratios of individual PAH from environmental samples: can PAH sources be apportioned? Org. Geochem. 21 (1994) 809–822.
[33] S. Nitz, B. Weinreich, F. Drawert, Multidimensional gas chromatography–isotope ratio mass spectrometry (MDGC-IRMS). Part A: system description and technical requirements, J. High Resolut. Chromatogr. 15 (1992) 387–391.
[34] US-EPA, National Primary Drinking Water Regulations, 2009 (EPA 816-F-09-004).
[35] Swiss-Federal-Council, Ordinance on the Remediation of Polluted Sites, Swiss Federal Council, 1998.
[36] E.A. Dietz, Shifting of gas chromatographic retention times due to solvent effects — a study using sulfur chemiluminescence detection, J. High Resolut. Chromatogr. 19 (1996) 485–491.
[37] D. Aronson, P.H. Howard, Anaerobic Biodegradation of Organic Chemicals in Groundwater: A Summary of Field and Laboratory Studies, American Petroleum Institute, 1998.
[38] H.I. Essaid, I.M. Cozzarelli, R.P. Eganhouse, W.N. Herkelrath, B.A. Bekins, G.N. Delin, Inverse modeling of BTEX dissolution and biodegradation at the Bemidji, MN crude-oil spill site, J. Contam. Hydrol. 67 (2003) 269–299.
[39] L.R. Krumholz, L.E. Caldwell, J.M. Suflita, Biodegradation of 'BTEX' hydrocarbons under anaerobic conditions, in: Bioremediation: Principles and Applications, Cambridge University Press, 1996, pp. 61–99.