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GC-IRMS

Benefits of Static Headspace Sampling for carbon isotope analysis of volatile organic compounds

Authors
María de Castro[1], Vanessa Peiro[2], Stefania Milano[1], Mario Tuthorn[1]

[1]Thermo Fisher Scientific
[2]SIdI, Universidad Autónoma de Madrid, Spain

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Introduction

Hazardous volatile organic compounds (VOCs), including BTEX (benzene, toluene, ethylbenzene, and xylene), pose significant health risks when released into the environment through industrial processes, fuel spills, and improper disposal practices. The isotopic composition of VOCs provides valuable information about origin and behavior in the environment, which can be used in the assessment of monitoring and remediation interventions. For instance, toluene maintains its isotopic signature even after transitioning from aqueous to gas phases, enabling the use of $\delta^{13}C$ to trace the source of contamination[1]. In the case of other BTEX and chlorinated VOCs, isotopic fractionation occurs during biotic and abiotic degradation. Therefore, accurate analysis of the compounds and their degradation products is crucial for monitoring attenuation in the environment, such as for the biodegradation of methyl tert-butyl ether (MTBE), often used in reformulated gasoline[2] and benzene, a highly soluble and carcinogenic compound[3].

Sensitivity is one of the major limitations for GC-IRMS analysis of environmental samples, resulting in the necessity to optimize sample introduction methods to achieve high accuracy and precision of isotope ratio results. The aim of this technical note is to demonstrate that Static Headspace Sampling (SHS) injection of VOCs via split/splitless injector eliminates the need for direct liquid sample injection, reducing column contamination and improving analyte separation and reproducibility of isotope data. By introducing the headspace vapor rather than the entire sample, the concentration of volatile compounds reaching the column is increased, resulting in improved sensitivity and lower detection limits.

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Analytical setup

All measurements can be performed using a Thermo Scientific™ GC IsoLink™ II IRMS System with a Thermo Scientific™ TriPlus™ RSH Series Autosampler equipped with a Static Headspace Sampling (SHS) injection option (Figure 1).

For the SHS technique, the samples are placed in tightly closed vials. Each vial is heated, and the volatile compounds are transferred from the solid or liquid sample into the gaseous phase above it (headspace) until a condition of thermodynamic equilibrium is reached. Afterwards, an aliquot of headspace is withdrawn and injected into the gas chromatograph. The trend of a volatile compound to transfer into the headspace is expressed by the partition coefficient K. The coefficient depends on the compound solubility in the matrix, and it is strongly affected by the temperature and the composition of the matrix itself.
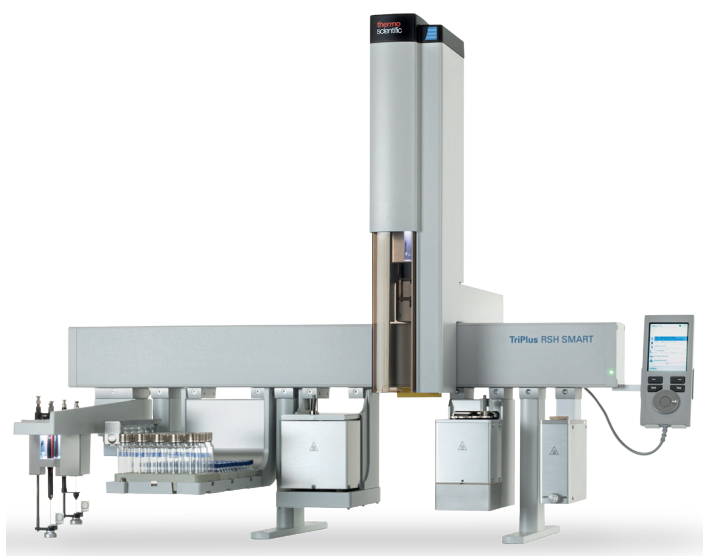
The analytical times are optimized according to the set parameters, such as equilibration time and analytical time, to fully exploit the instrument's capability. The methodology for GC-C-IRMS measurements of VOCs was developed and tested in three steps using the solutions prepared as outlined in Table 1; 1) BTEX Standard from Sigma Aldrich was used for developing chromatography method and testing reproducibility of the results, 2) BTX mix of isotopically certified standards from the Indiana University was used for confirming that the developed methodology is delivering accurate isotopic data, and finally, 3) testing of Limits Of Detection and possible linearity effects were performed using concentrations gradient (125/250/500/1000/1500 ppb) of BTX mix.

For the analysis, 10 mL of standard solution was transferred into 20 mL 18mm screw top headspace vials (PNC4020-18) with 18mm magnetic screw caps (PN C4020-47, 8mm hole). To lower partitioning coefficient of target compounds, 3g of NaCl were added to every vial before closure. This promotes higher concentration of VOCs in the gas phase and helps avoid fractionation due to lighter isotopic composition in the gas phase.

Detailed analytical setup for the TriPlus RSH Series Autosampler with the Static Headspace Sampling option, the Thermo Scientific™ iConnect™ Split/Splitless (SSL) Injector Module and the Thermo Scientific™ TRACE™ Series GC is listed in Table 2. The GC IsoLink II IRMS System operation is driven by the Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution (ISDS) Software.

Conditioning of the combustion reactor in the GC IsoLink II conversion interface was performed as outlined in Table 3.

Figure 1. TriPlus RSH Series Autosampler equipped with a Static Headspace Sampling (SHS) injection option

Table 1. Dilution of the BTEX/BTX stock solutions for carbon isotope analysis using GC-IRMS with Static Headspace Sampling injection

Name (Manufacturer)	Reference	Components	Original solution	Stock solution	Working standard	Standard in H$_2$O preparation
BTEX Standard (Sigma Aldrich)	CRM47993	Benzene, Ethylene, Toluene, m-p-Xylene, o-Xylene	2000 ng/µL (2000 ppm) in MeOH	150 µL Original solution + 450 µL MeOH = 0.6 ml, 500 ppm in MeOH		Serum bottle 100 mL Milli Q H$_2$O+ 87.5 µL Stock solution = 0.437 ng/µL (437 ppb)
BTX mix (Indiana University)	CAS# 71-43-2	Benzene #1, C6H6	99.8 %	4 µL Original solution + 2 mL MeOH = 2000 ppm	500 µL Benzene stock + 500 µL Toluene stock + 500 µL m-Xylene stock + 500 µL MeOH = BTX in MeOH 500 ppm each	Serum bottle 100 mL Milli Q H$_2$O + 100 µL Stock solution = 0.500 ng/µL (500 ppb)
	CAS# 108-88-3	Toluene #1, C7H8	99.5 %	4 µL Original solution + 2 mL MeOH = 2000 ppm		
	CAS# 108-38-3	m-Xylene #1, C8H10	≥99.0 %	4 µL Original solution + 2 mL MeOH = 2000 ppm		

Table 2. Autosampler and gas chromatograph analytical setup

Triplus RSH Series autosampler parameters		iConnect SSL Injector Module parameters	
Thermo Scientific™ Fixed Needle Autosampler Syringe, Gas-tight, Headspace, 2.5 mL, 23 G, 65 mm (P/N 365Q2131)		Thermo Scientific™ LinerGOLD™ GC Liners, Direct Straight Liner (P/N 453A1335-UI)	
Injection type	Headspace	Injection temperature	230 °C
Sample draw	1 mL	Inlet module and mode	SSL, split
Sampling depth mode	Standard	Split flow	15 mL/min
Incubation mode	Constant	Split ratio	10:1
Agitator temperature	80 °C	Septum purge flow	2 mL/min, constant
Incubation time	25 min	Carrier gas, flow	Helium, 1.5 mL/min
Agitation speed	250 rpm	TRACE Series GC parameters	
Agitation on	15 s	Thermo Scientific™ TraceGOLD™ TG-624SilMS GC Columns, 30 m, 0.25 mm, 1.4 µm (P/N 26059-3320)	
Agitation off	5 s	Temperature	50 °C
Syringe temperature	90 °C	Hold time	4 min
Fill strokes volume	1.5 mL	Rate	40 °C/min
Fill strokes counts	3	Temperature 2	130 °C
Filling delay	1 s	Hold time	6 min
Pre-injection syringe flush	Enabled	GC run time	12 min
Post-injection syringe flush	120 s		
Filling speed	30 mL/min		
Injection speed	30 mL/min		
Injection depth	45 mm		
Penetration speed	25 mm/s		
Pre-injection delay	1 s		
Post-injection delay	3 s		
Wait for readiness flags	Active for syringe and agitator		
Agitator standby temp	80 °C		
Syringe standby temp	90 °C		
Synchro type	Normal		
Needle speed in vial	20 mm/sec		

Table 3. Combustion reactor conditioning parameters

Reactor conditioning type	Setup
Extended – used at the start of a sequence	45min O_2 + 60 min backflush + 0.3 min purge
Seed – used with each sample run	2 min O_2 + 0.2 min backflush + 0.1 min purge

Results

The BTEX Standard (Sigma Aldrich) was used to develop chromatography method and test reproducibility of the results. Chromatographic separation of the target compounds is shown in Figure 2. The BTEX Standard isotope data reported in Table 4 demonstrates excellent measurement precision for all compounds, as well as good RSD for total areas.
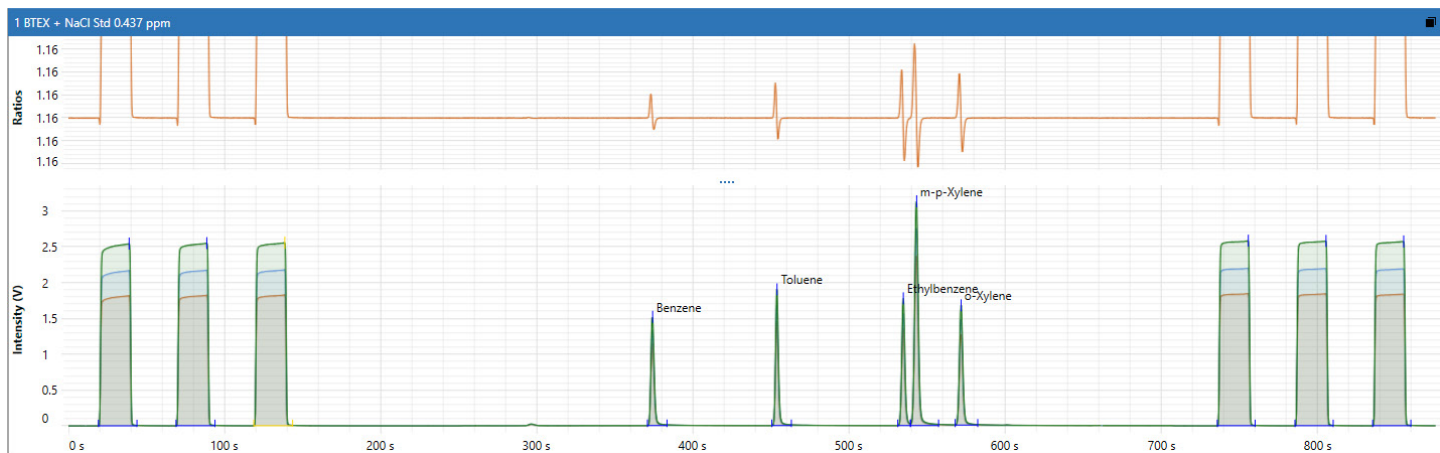
Figure 2. GC-IRMS chromatogram of the BTEX Standard (Sigma Aldrich) 0.437 ng/µL (437 ppb) in H_2O

Table 4. Isotope data acquired for the BTEX Standard (Sigma Aldrich) 0.437 ng/µL (437 ppb) in H_2O using an SHS injection option

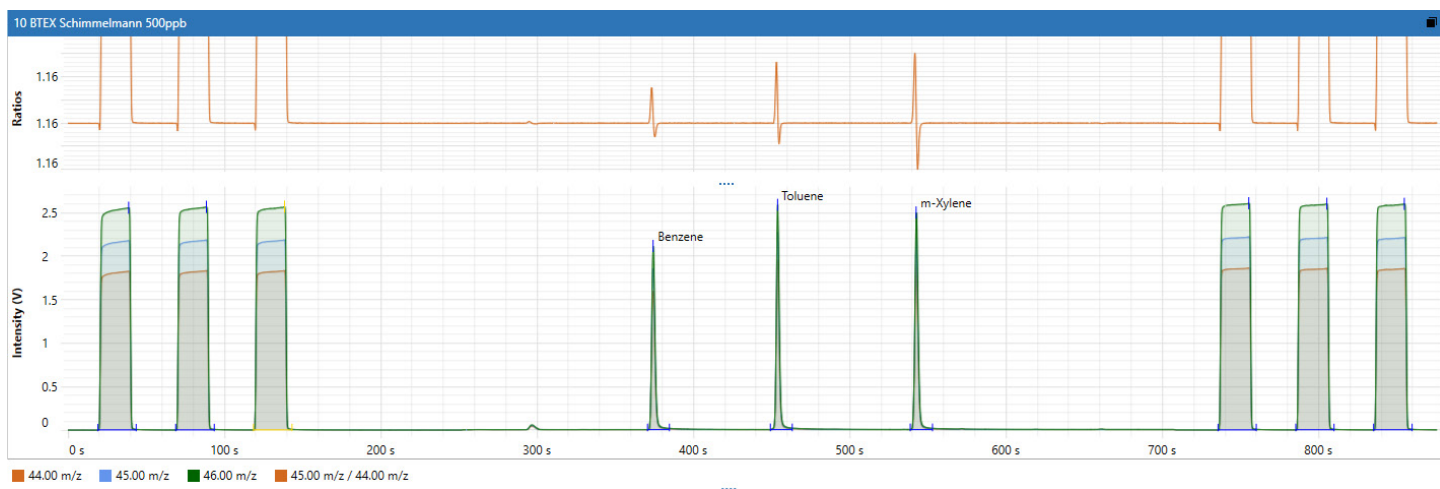|	$\delta^{13}C$ (‰)		Total area (V/s)		
Component	Average (n=10)	SD (n=10)	Average (n=10)	SD (n=10)	RSD (%)
Benzene	-28.76	0.08	3.61	0.09	2.52
Toluene	-28.78	0.12	3.91	0.10	2.47
Ethylbenzene	-30.70	0.08	3.94	0.10	2.64
m-p-Xylene	-27.64	0.05	8.49	0.21	2.50
o-Xylene	-29.30	0.13	4.06	0.10	2.51

Figure 3. GC-IRMS chromatogram of the BTX mix (Indiana University) 0.500ng/µL (500 ppb) in H_2O

Figure 3 shows BTX mix (Indiana University) measurement using the same methodology. The analysis was done to evaluate isotope data accuracy by using isotopically certified standards.

Data evaluation was performed using Qtegra ISDS Software tools for data normalization where sample 1 was treated as conditioning, sample 2 as Compound Specific Isotope Analysis (CSIA) δ Standard, and the following 9 repetitions were treated as unknown samples (see Sample list in Figure 4). The resulting in-software evaluation shows high correlation between the measured and known isotope ratios values (Figure 5).

Measured isotope data for the BTX mix (Indiana University), including the offset against the certified isotope values, are reported in Table 5, showing excellent data reproducibility, and confirming that the developed methodology is delivering accurate isotopic data.

Finally, concentration gradients of the BTX mix (Indiana University) were analyzed with results showing that the isotope ratio of Benzene, Toluene and m-Xylene is independent of the amount of material analyzed within the range from 125 ppb to 1500 ppb (Table 6).

Figure 4. Qtegra ISDS Software sample list for determination of in-software data normalization using a CSIA standard
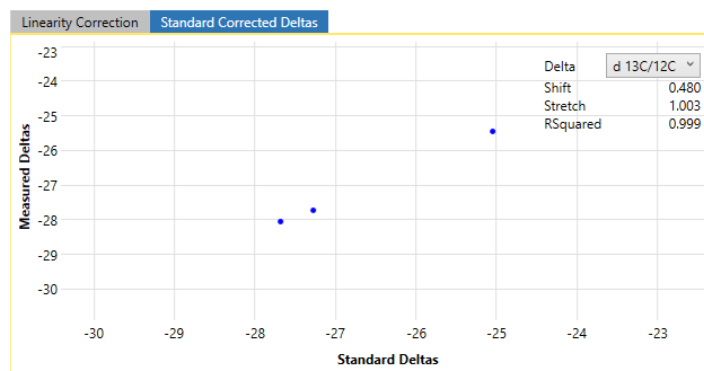

Figure 5. Qtegra ISDS Software data evaluation of measured isotope values vs. known isotope values of a CSIA standard

Table 5. Isotope data acquired for the BTX mix (Indiana University) 0.500ng/μl (500 ppb) in H$_2$O using an SHS injection option.

	δ^{13}C (‰)				Total Area (V/s)		
Component	Certified	Average (n=9)	SD (n=9)	Offset vs. certified	Average (n=9)	SD (n=9)	RSD (%)
Benzene	-27.68	-27.46	0.06	0.22	3.91	0.12	3.19
Toluene	-25.02	-25.03	0.04	-0.01	4.22	0.13	3.17
m-Xylene	-27.27	-27.24	0.04	0.03	4.51	0.15	3.37

Table 6. Isotope ratio data of individual compounds in the BTX mix in the concentration range from 125 ppb to 1500 ppb

Compound	Benzene			Toluene			m-Xylene		
Certified δ^{13}C (‰)	**-27.68**			**-25.02**			**-27.27**		
Concentration (ppb)	δ^{13}C (‰) (Peaks amplitude range 0.28 - 3.56 mV)			δ^{13}C (‰) (Peaks amplitude range 0.35 - 4.28 mV)			δ^{13}C (‰) (Peaks amplitude range 0.29 - 3.48 mV)		
	Avg	SD (n=3)	Offset vs certified	Avg	SD (n=3)	Offset vs certified	Avg	SD (n=3)	Offset vs certified
125	-27.48	0.28	0.2	-25.46	0.05	-0.44	-27.3	0.23	-0.03
250	-27.52	0.07	0.16	-25.31	0.12	-0.29	-27.51	0.17	-0.24
500	-27.37	0.06	0.31	-24.89	0.10	0.13	-27.29	0.02	-0.02
1000	-27.21	0.06	0.47	-24.75	0.09	0.27	-27.18	0.09	0.09
1500	-27.35	0.07	0.33	-24.81	0.06	0.21	-27.24	0.03	0.03

Conclusions

A method for Static Headspace Sampling GC-IRMS was optimized and applied for carbon isotope analysis of volatile organic compounds. The isotopic data obtained by SHS analysis of the BTX mix demonstrate excellent precision and accuracy for GC-C-IRMS analysis of VOCs, including improved sensitivity and lower detection limits. Additionally, this technique offers the advantage of quick and minimal sample preparation combined with lower consumption of reagents and solvents, and a longer lifetime of chromatographic columns since only the volatile fraction is injected in the column.

Literature

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