

Environmental

Dual injection for Hydrocarbon Oil Index (HOI) determination in water and soil with helium carrier gas conservation

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Keywords

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Goal

The aim of this application note is to demonstrate the performance of a high-throughput and cost-effective method for the determination of the Hydrocarbon Oil Index (HOI) also known as Total Petroleum Hydrocarbon (TPH), in water and soil samples by using a dual injection configuration and conserving helium gas.

Introduction

Petroleum products are complex mixtures of hundreds of hydrocarbon compounds, ranging from light, volatile, short-chained organic compounds to heavy, long-chained, branched compounds. Petroleum hydrocarbons can enter the environment through industrial accidents, spills, or leaks, and as by-products from commercial or private uses. Contamination of water and soil is a growing concern as lighter TPH fractions float in water and form thin surface films, whereas the heavier fractions accumulate in the sediment at the bottom of the water, affecting bottom-feeding fish and organisms. TPH released in soil can evaporate into the air, dissolve into the groundwater, and move away from the spillage area, contaminating the drinking water supplies, or remain bound to the soil particles for a long period, thus reducing the usability of land for development.

To protect public health from toxic effects due to TPH exposure, many European countries and the United States have made the determination of the content of mineral oils and petroleum products in water and soils a compulsory requirement for quality certifications. Because there are so many petroleum products, it is not practical to measure each one individually, therefore the total amount of all hydrocarbons found in a sample of soil, water, or air is usually monitored.¹

The United States Environmental Protection Agency (U.S. EPA), the International Organization for Standardization (ISO), as well as the Italian Institute of Environmental Protection and Research (ISPRA) have published several methods (U.S. EPA 8015 D,² ISO 16703:2011,³ ISO 14039:2004,⁴ ISO 9377:2000⁵, ISPRA Doc. N. 46/14,⁶ ISPRA Doc. n. 04/11⁷) that can be considered when analyzing TPH in water and soil using gas chromatography coupled to flame ionization detection.

The Hydrocarbon Oil Index (HOI) represents the total amount of compounds that can be extracted from the sample (potable water, surface water, and wastewater) with a non-polar solvent. The extracted compounds must not absorb on Florisil™ and must elute between *n*-decane (C10) and *n*-tetracontane (C40) when analyzed by GC on a non-polar column. Such fraction includes heating oils, diesel fuels, kerosene, lubricants, and transmission fluids.

In this work, the Thermo Scientific™ AI/AS 1610 autosampler in the Gemini configuration for simultaneous dual injections combined with the Thermo Scientific™ HeSaver-H₂Safer™ carrier gas saving technology was employed to increase the sample throughput and double the productivity while reducing helium consumption and cost per analysis. Method compliance in terms of sensitivity, recovery (C40/C20 ratio), linearity and precision were evaluated for quantitative assessment of the HOI in water and soil samples.

Experimental

In this study, two AS 1610 autosamplers set up in Gemini configuration and operating in high-throughput mode were installed onto a Thermo Scientific™ TRACE™ 1610 GC equipped with two Thermo Scientific™ iConnect™ split/splitless injectors (iConnect SSL) upgraded to work in Helium Saver/Hydrogen Safer mode and two Thermo Scientific™ iConnect™ flame ionization detectors (iConnect FID). The dual-tower AS 1610 Gemini configuration is shown in Figure 1. The two autosamplers (with a total capacity of 310 vials) are placed on the front and back injectors and work synchronized in such a way that the

sample injections take place simultaneously in two separated channels following two separate sample sequences, with data acquisition and processing achieved separately, doubling the throughput and hence the productivity.⁸



Figure 1. TRACE 1610 GC with AI/AS 1610 autosamplers operating in the Gemini configuration for dual injection

Helium is the most commonly used carrier gas for gas chromatography thanks to its high chromatographic efficiency and inertness. Recently, the helium price increase and supply issues caused by shortages led GC manufacturers, researchers, and analysts to investigate possible mitigation options that entail either switching to alternative carrier gases or reducing the helium consumption. The HeSaver-H₂Safer carrier gas saving technology⁹ offers an innovative and smart approach to dramatically reduce carrier gas consumption as the selected carrier gas (e.g., helium or hydrogen) is used only to supply the chromatographic column for the separation process, whereas a cheaper gas (e.g., nitrogen or argon) is used for inlet pressurization feeding split and purge flows, analyte vaporization, and transfer to the analytical column.

Chromatographic separation was achieved on a Thermo Scientific™ TraceGOLD™ TG-Mineral Oil (15 m × 0.32 mm × 0.15 μm, P/N 26069-5230) column. This column has a proprietary phase that guarantees long lifetime and stability up to 400 °C, ensuring reliable and reproducible results for fast mineral oil screening and TPH analysis. Detailed experimental conditions are reported in Table 1.

Table 1. Experimental conditions for analysis of TPH in water and soil

AI/AS 1610 autosampler parameters		TRACE 1610 GC parameters	
Injection volume (μL)	1–2	iConnect-SSL HeSaver-H₂Safer	
Injection type	Standard	Temperature (°C)	320
Sample mode	Standard	Liner	Thermo Scientific™ LinerGOLD™ Single Taper w/wool (P/N 453A1925-U)
Fill strokes	5	Inlet module and mode	SSL upgraded to HeSaver-H ₂ Safer, splitless
Air volume (μL)	1.0	Splitless time (min)	0.5
Sample depth	Bottom	Split flow (mL/min)	30
Injection mode	Fast	Septum purge flow (mL/min)	5
Wash program		Stop purge for (min)	0.5
Pre-injection wash cycles	1	Carrier gas, flow (mL/min)	He, 4.0
Pre-injection solvent wash volume (μL)	5	Oven temperature program	
Pre-injection wash vial 1	Solvent A (<i>n</i> -Hexane)	Temperature (°C)	50
Pre-injection wash vial 2	Solvent B (<i>n</i> -Hexane)	Hold time (min)	0.30
Sample wash cycles	2	Rate (°C/min)	100
Sample wash volume (μL)	6.0	Temperature 2 (°C)	100
Post-injection wash cycles	2	Rate (°C/min)	80
Post-injection solvent wash volume (μL)	4.0	Temperature 3 (°C)	175
Post-injection wash vial 1	Solvent C (<i>n</i> -Hexane)	Rate (°C/min)	50
Post-injection wash vial 2	Solvent D (<i>n</i> -Hexane)	Temperature 4 (°C)	300
Syringe	10 μL, Fixed needle (P/N 36500525)	Rate (°C/min)	40
		Temperature 5 (°C)	360
		Hold time (min)	1.5
		GC run time (min)	7.238
		Ready delay (min)	0.5
		FID	
		Temperature (°C)	360
		Air flow (mL/min)	350
		H ₂ flow (mL/min)	35
		N ₂ flow (mL/min)	40
		Aquisition rate (Hz)	10
		Analytical column	
		TraceGOLD TG-Mineral Oil	15 m × 0.32 mm × 0.15 μm (P/N 26069-5230)

Data acquisition, processing, and reporting

The dual tower Gemini configuration is fully controlled in the Thermo Scientific™ Chromeleon™ 7.3 Chromatography Data System (CDS), facilitating the acquisition and monitoring of multiple parallel sequences on the same GC as well as multiple systems within the same laboratory. The simultaneous injections in two channels for unattended analyses of up to 310 samples are achieved with easy-on / off set up. When working in high-throughput mode, it is possible to switch from dual to single mode operation simply by flagging the dedicated option in the Instrument pane (Figure 2): each autosampler, either front or back, can be employed in a single-autosampler operation to run sequences on a single channel without changing hardware settings or Chromeleon CDS configuration. Moreover, with the ever-evolving compliance requirements for data integrity and data security, Chromeleon CDS provides a secure platform for analytical laboratories to comply with modern regulatory guidelines including FDA 21 CFR Part 11 and European Commission (EU) Annex 11.

Integration of peaks for TPH calculation was performed by applying a baseline integration from C10 to C40 (water samples) and from C12 to C40 (soil samples). As per ISPRIA methods,^{7,8} integration started immediately after C10 (water samples) and C12 (soil samples) and ended immediately before C40. Figure 3 shows an example of overlaid chromatograms obtained for C10–C40 mix and STD mix Mineral Oil/Diesel. The Chromeleon CDS matrix correction feature allows for automated subtraction of the background, leading to a precise quantitation of the spiked samples.

Chromeleon CDS offers automated reporting capabilities, reducing manual intervention and the potential for human error. Users can create and customize report templates to meet their specific needs. Chromeleon CDS generates comprehensive

reports based on user-defined templates, ensuring consistency and efficiency in data presentation and compliance to both internal standards and external regulatory requirements.

Standard and sample preparation

Standard preparation

A Florida TPH standard mix (even *n*-alkanes in the range C8–C40, 500 µg/mL each in hexane, P/N 31266, Restek) was diluted in *n*-hexane (95%, for residue analysis, ECD tested halocarbons free grade, P/N 326710010, Thermo Scientific Chemicals) to a final concentration of 25 µg/mL each and used to verify the system performance in terms of chromatographic separation of even *n*-alkanes at baseline and recovery calculated based on C40/C20 ratio.

A standard mix stock solution Mineral Oil/Diesel in Cyclohexane, P/N 31630 Restek (5,000 µg/mL Diesel#2 additive-free + 5,000 µg/mL Mineral Oil, Total hydrocarbon concentration at 10,000 µg/mL) was diluted in *n*-hexane (95%, for residue analysis, ECD tested halocarbons free grade, P/N 326710010, Thermo Scientific Chemicals) and used to assess linearity by preparing a 9-point calibration curve ranging from 5 to 2,000 µg/mL (in-vial concentration for total hydrocarbons).

When dealing with TPH analysis it is critical to get a clean baseline, therefore the use of aluminum septa (Thermo Scientific™ AVCS Blue Cap, Alu Disk, P/N C5000-56AL, vial P/N 6ASV9-1P) is strongly recommended to reduce interfering peaks that can lead to inaccurate results.

Sample preparation

Water and soil samples were collected around the Milan metropolitan area. Samples were stored and prepared for analysis according to the official methods^{7,8} proposed by ISPRIA. A brief schematic of the sample preparation steps for water and soil samples is reported in Figure 4.

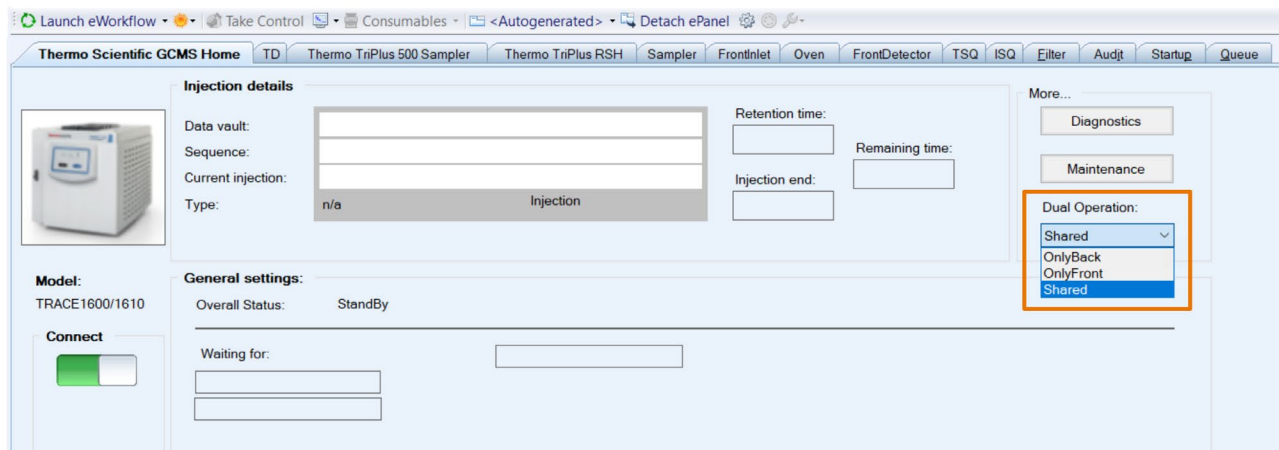


Figure 2. Chromeleon 7.3 CDS browser showing the Instrument pane to switch between dual and single autosampler configuration in one simple click

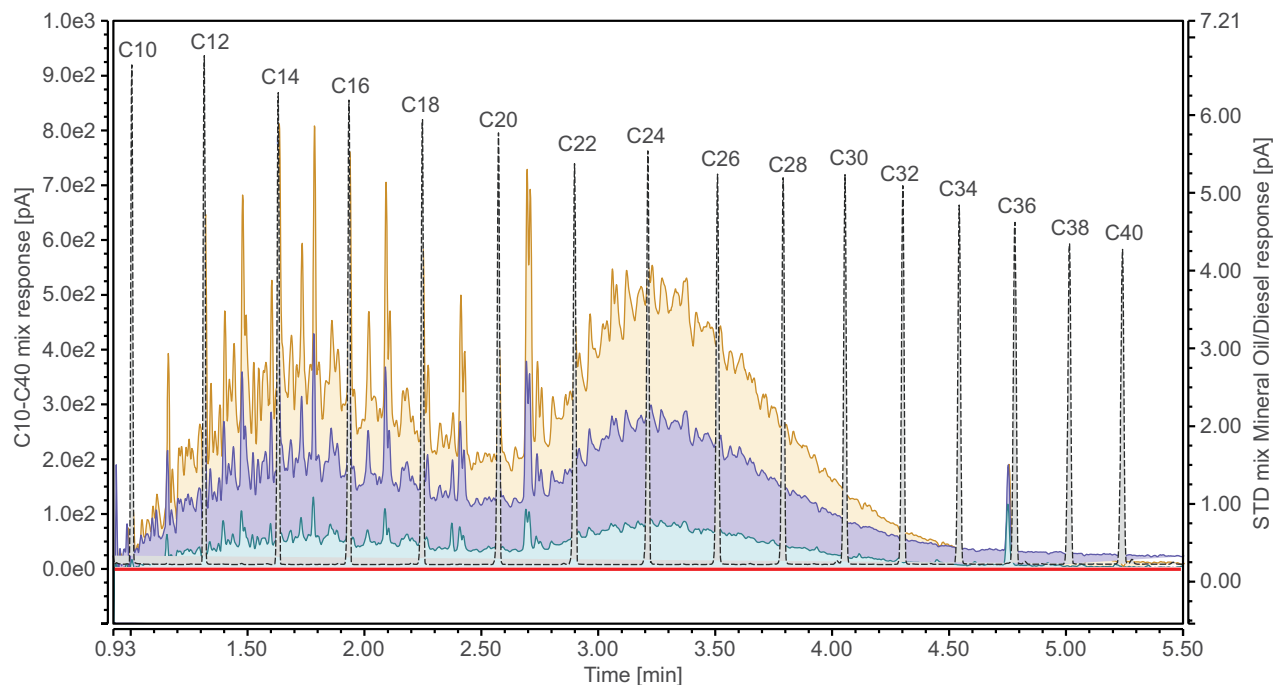


Figure 3. Overlaid chromatograms of Florida mix and standard mix solution Mineral Oil/Diesel showing the baseline integration

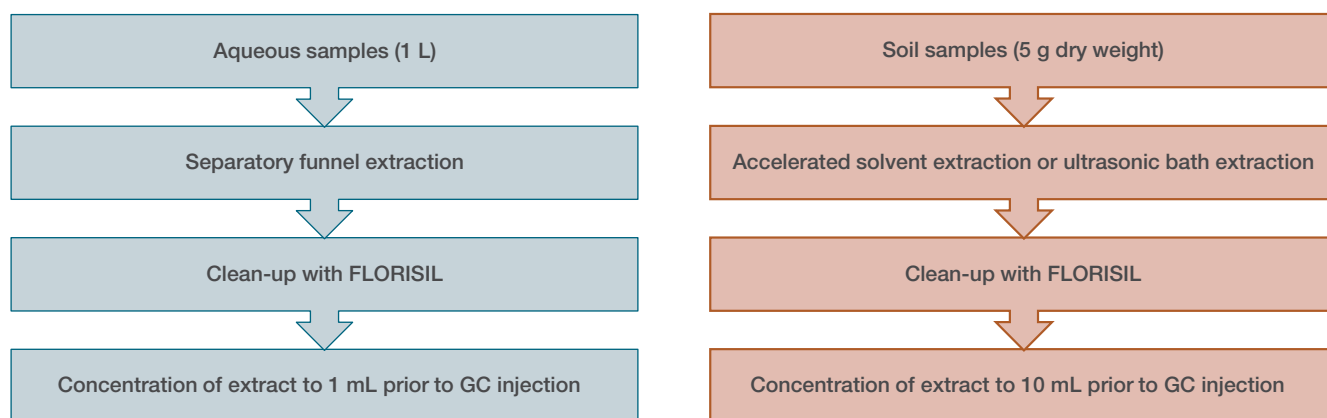


Figure 4. Brief schematic of the sample preparation procedure for aqueous and soil samples

Results and discussion

System performance

A clean blank run (Figure 5) is critically important for the determination of the HOI. This baseline is crucial for distinguishing between the actual hydrocarbons present in the water sample and any potential contaminants or background noise originating from the instrument or solvents used. Without a clean blank, it becomes difficult to accurately quantify the hydrocarbon concentration, leading to potential false positives or inflated results. This is particularly important when dealing with low-level detections where even minor contamination can significantly affect the results. For this reason, a regular check of the cleanness of the GC system, the use of high purity solvent, and the use of aluminum septa for the vials are mandatory for correctly quantifying TPH, especially at levels below 10 µg/mL.

The blank run is automatically subtracted from each standard and sample chromatogram to remove any baseline drift and ensure a reliable integration.

Recovery

One critical aspect of the Hydrocarbon Oil Index analysis is the possible discrimination of the oil fraction in the injector. This may occur during the analysis of compounds with a wide range of boiling points. The recovery of high boiling compounds compared to ones with lower boiling points may not be good enough, especially when working with standard split/splitless injectors. For this reason, the ISO method specifically indicates that the relative response (peak area) of *n*-tetracontane (C40) compared with *n*-eicosane (C20) must be not less than 0.8. This recovery must be verified regularly on the system in use.

The performance of the TRACE 1610 GC and the iConnect SSL injector working in the HeSaver configuration showed minimal discrimination and an optimal recovery for the high boiling hydrocarbons. A series of six replicates of the Florida standard mixture (even *n*-paraffins from C10 to C40) shows a recovery of the C40 compared to C20 higher than 90%, proving the full compliance of the system (Figure 6).

Repeatability and robustness

Regulatory compliance in environmental monitoring implies the verification of reliability, accuracy, and precision of the results. Additionally, the robustness of the overall analytical system is key for testing laboratories to provide consistent results over time with minimum downtime and high productivity.

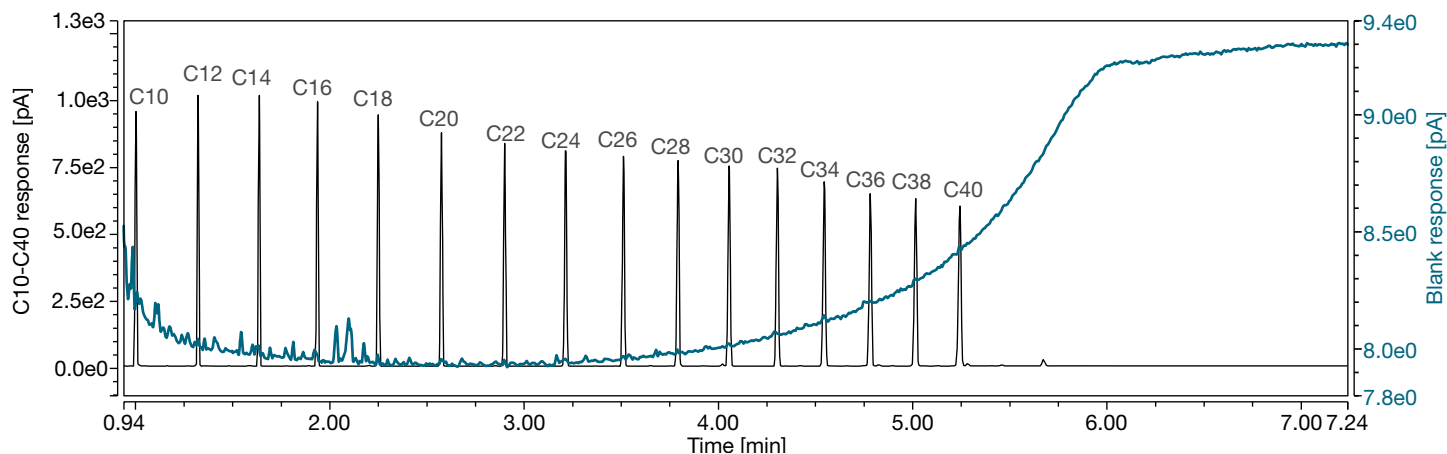


Figure 5. Solvent blank run (*n*-hexane, blue trace, fully zoomed signal intensity on the right axis), injected immediately after *n*=6 repeated injections of Florida mix at 25 µg/mL each (black trace)

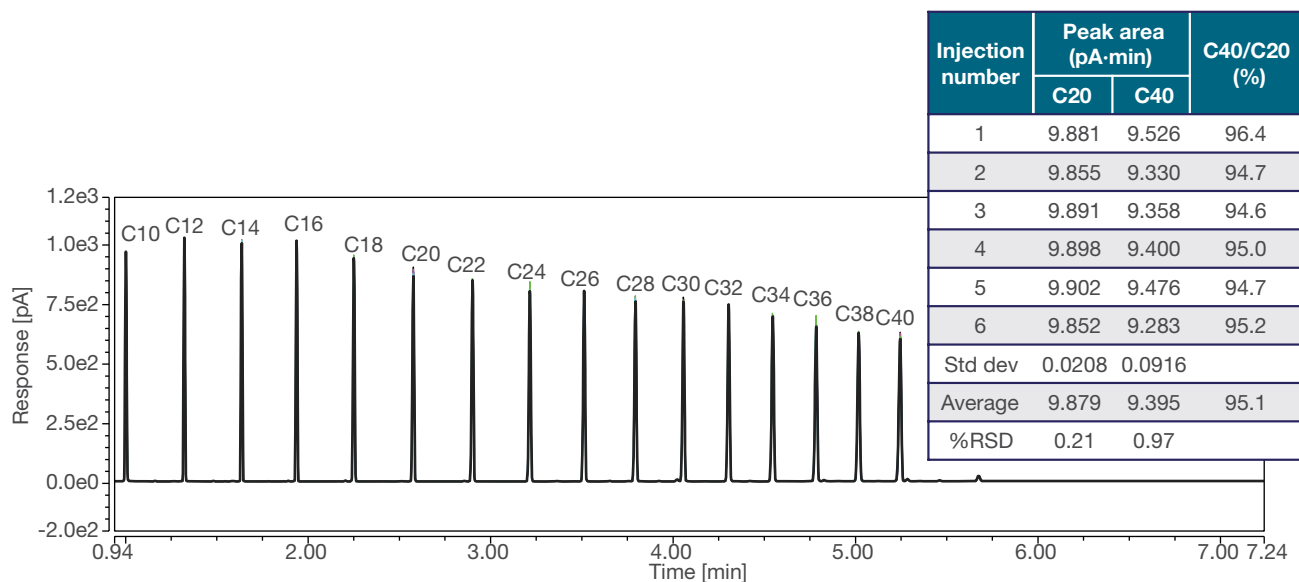


Figure 6. Overlaid chromatograms (*n*=6) of Florida mix at 25 µg/mL, each showing a recovery of C40 vs C20 consistently >90%

A series of six consecutive injections of the Florida standard mixture at 25 µg/mL each showed a very good peak area repeatability for both absolute peak areas (<1% RSD) and retention time (RT) (0.02% for C40), as detailed in Table 2.

The robustness over time was evaluated with an analytical sequence including replicates for checking the recovery, a 9-level calibration curve, QC standards (at 50 and 400 µg/mL), samples (soil extracts), as described in Table 3, for a total of 278 injections. The %RSD of the QC calculated amount across the sequence were ≤3.0% (Figure 7), and the recovery of C40 was always >89%. No maintenance was performed during the sequence, demonstrating high robustness and consistent results over time.

Linearity and LOQ

To calibrate the system, a standard mixture in cyclohexane, including a diesel fraction and a mineral oil fraction, was used to prepare nine calibration levels from 5 to 2,000 µg/mL, as described in the above section.

As reported in Figure 8, the linear response was very good with a coefficient of determination (R^2) close to 1. Also, the relative amount deviation (that is, the error on the measured concentration) at each calibration point was below 2.6%.

According to the sample preparation protocol, a concentration ratio of ca 1,000:1 is achieved after the clean-up step. This allows the instrument LOQ (considered as the lowest level of the calibration curve) to stay 10 times lower or even less than the limit of acceptance in the sample (i.e., 0.1 mg/L in water) and provide confident quantification level in compliance with official regulations.

Table 2. Peak area and retention time repeatability (n=6) for Florida mix at 25 µg/mL each

	Retention time (min)															
	C10	C12	C14	C16	C18	C20	C22	C24	C26	C28	C30	C32	C34	C36	C38	C40
Florida rep 1	1.002	1.322	1.637	1.938	2.252	2.577	2.902	3.215	3.513	3.793	4.057	4.307	4.547	4.785	5.018	5.245
Florida rep 2	1.002	1.322	1.637	1.938	2.252	2.577	2.902	3.217	3.513	3.795	4.058	4.307	4.548	4.787	5.018	5.247
Florida rep 3	1.002	1.322	1.637	1.938	2.252	2.577	2.902	3.215	3.512	3.793	4.057	4.305	4.547	4.783	5.017	5.243
Florida rep 4	1.002	1.322	1.637	1.937	2.25	2.577	2.902	3.215	3.513	3.795	4.057	4.305	4.547	4.785	5.017	5.245
Florida rep 5	1.002	1.322	1.635	1.937	2.25	2.577	2.9	3.215	3.513	3.793	4.057	4.305	4.545	4.783	5.017	5.245
Florida rep 6	1.002	1.322	1.637	1.937	2.25	2.575	2.9	3.213	3.512	3.793	4.055	4.303	4.545	4.782	5.015	5.243
Average	1.002	1.322	1.636	1.938	2.251	2.576	2.901	3.215	3.513	3.794	4.057	4.305	4.546	4.784	5.017	5.245
Std dev	0	0	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001
RSD%	0.00%	0.00%	0.04%	0.05%	0.04%	0.03%	0.03%	0.03%	0.02%	0.02%	0.03%	0.03%	0.03%	0.04%	0.03%	0.02%

	Peak area (pA·min)															
	C10	C12	C14	C16	C18	C20	C22	C24	C26	C28	C30	C32	C34	C36	C38	C40
Florida rep 1	9.656	9.956	9.888	10.027	10.103	9.881	9.862	9.926	9.919	9.817	9.903	9.821	9.763	9.613	9.458	9.526
Florida rep 2	9.616	9.874	9.853	10.023	10.028	9.855	9.795	9.847	9.848	9.733	9.871	9.738	9.678	9.465	9.287	9.331
Florida rep 3	9.663	9.936	9.898	10.051	10.028	9.891	9.856	9.883	9.876	9.757	9.921	9.817	9.708	9.505	9.321	9.358
Florida rep 4	9.694	9.962	9.900	10.086	10.088	9.893	9.838	9.903	9.890	9.781	9.880	9.814	9.734	9.546	9.328	9.400
Florida rep 5	9.700	9.977	9.915	10.115	10.134	9.902	9.876	9.945	9.936	9.848	9.995	9.908	9.795	9.607	9.428	9.476
Florida rep 6	9.642	9.933	9.917	10.036	10.027	9.852	9.816	9.856	9.832	9.757	9.795	9.724	9.645	9.422	9.195	9.283
Average	9.662	9.940	9.895	10.056	10.068	9.879	9.840	9.893	9.883	9.782	9.894	9.804	9.721	9.526	9.336	9.395
Std dev	0.032	0.036	0.023	0.037	0.047	0.021	0.031	0.039	0.040	0.043	0.066	0.067	0.055	0.077	0.096	0.092
RSD%	0.33%	0.37%	0.24%	0.37%	0.46%	0.21%	0.31%	0.39%	0.41%	0.44%	0.66%	0.68%	0.57%	0.81%	1.03%	0.97%

Table 3. Sequence structure applied to evaluate instrument robustness by injecting n=200 soil samples, bracketed by QCs and blanks (total number of injections = 278)

Number of injections	Injection type	Number of injections	Injection type
	Blanks	3 x	QC 50 ppm in vial
6 x	Florida mix 400 µg/mL total hydrocarbons	3 x	QC 400 ppm in vial
	Blanks	3 x	Florida mix 400 µg/mL total hydrocarbons
27 x	Calibration curve. 9 levels, 3 inj x level		Blanks
	Blanks	50 x	Real samples (soil)
3 x	QC 50 ppm in vial		Blanks
3 x	QC 400 ppm in vial	3 x	QC 50 ppm in vial
3 x	Florida mix 400 µg/mL total hydrocarbons	3 x	QC 400 ppm in vial
	Blanks	3 x	Florida mix 400 µg/mL total hydrocarbons
50 x	Real samples (soil)		Blanks
	Blanks	50 x	Real samples (soil)
3 x	QC 50 ppm in vial		Blanks
3 x	QC 400 ppm in vial	3 x	QC 50 ppm in vial
3 x	Florida mix 400 µg/mL total hydrocarbons	3 x	QC 400 ppm in vial
	Blanks	3 x	Florida mix 400 µg/mL total hydrocarbons
50 x	Real samples (soil)		Blanks
	Blanks		

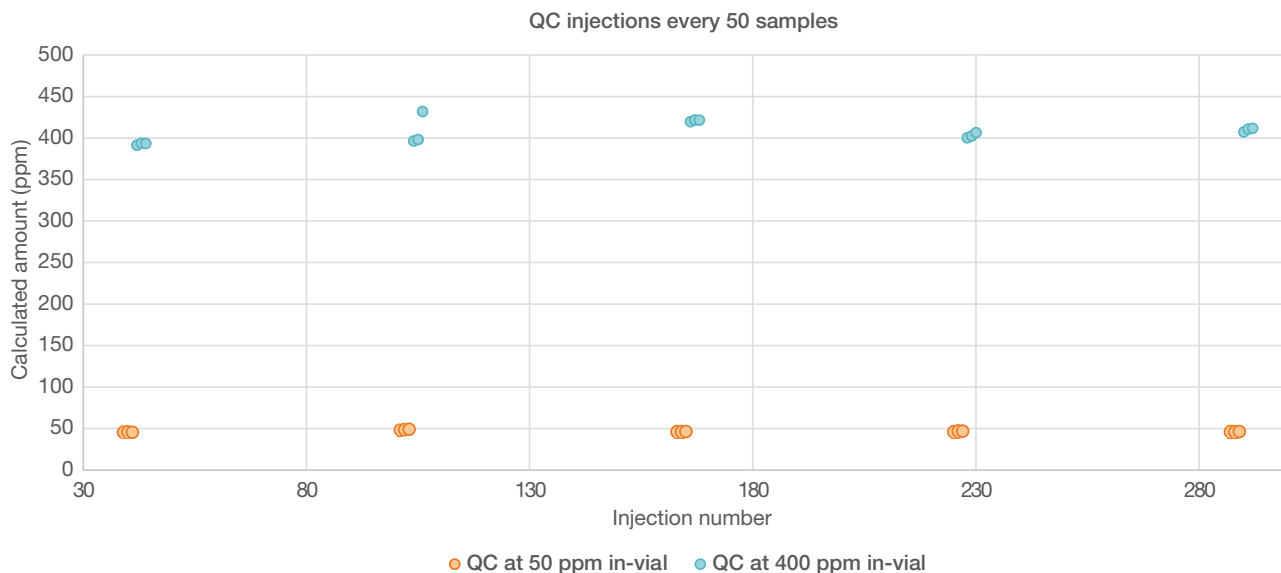


Figure 7. QC injections bracketing four series of n=50 samples and blank runs. Relative standard deviations of the QC calculated amounts were 2.5% for QC at 50 µg/mL and 3.0% for QC at 400 µg/mL.

The ability to lower the limit of quantitation of the GC system is important to possibly reduce the reconcentration step during the sample preparation or possibly start from less sample volume.

A simple way to get a lower LOQ is to inject more into the GC system. By injecting 2 μL instead of 1 μL , it was possible to add one point in the lower part of the curve and start from 2 $\mu\text{g/mL}$ of hydrocarbon concentration in the vial. The R^2 was still higher than 0.999 and the relative amount deviation at 2 $\mu\text{g/mL}$ remained below 10%, as shown in Figure 9.

Helium carrier gas savings

The AS 1610 autosampler in the Gemini configuration provided simultaneous injection on two channels. Having the ability to use the iConnect SSL injector in the HeSaver mode for both channels allowed a significant savings in helium carrier gas consumption.⁹ The gas savings can be easily calculated with the [Helium Saver Calculator](#).¹⁰ The helium cylinder lifetime is increased 4.5 times for a 24/7/365 working system, with a significant cost savings for the laboratory (Figure 10).

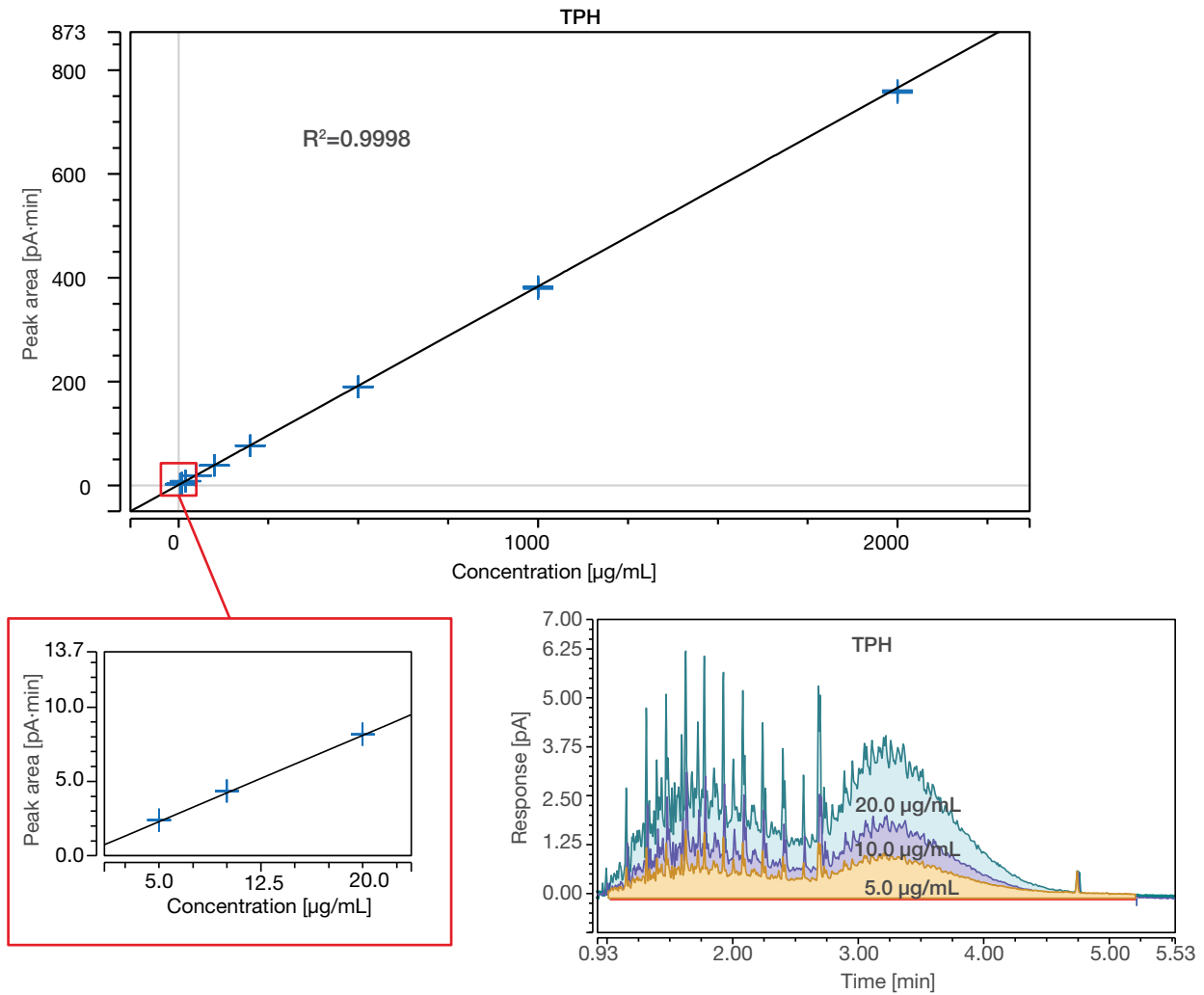


Figure 8. Linear 9-level calibration curve in the range 5 to 2,000 $\mu\text{g/mL}$. The insets report chromatographic traces of the lowest three calibration levels at 5, 10, 20 $\mu\text{g/mL}$.

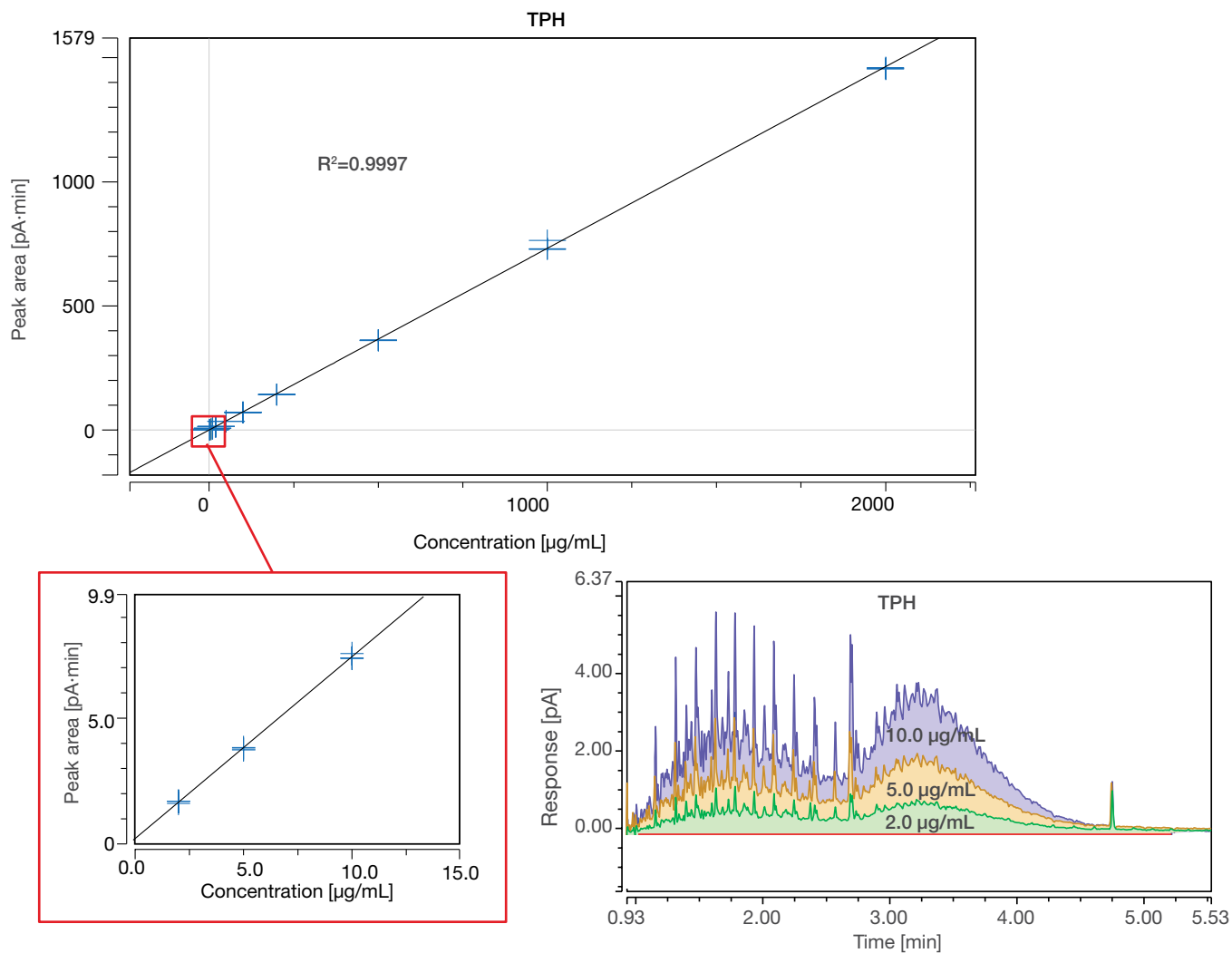


Figure 9. Linear 10-level calibration curve in the range 2 to 2,000 µg/mL. The insets report chromatographic traces of the lowest three calibration levels at 2, 5, 10 µg/mL.

	Helium usage featuring Helium Saver Technology	Standard helium usage
He volume used per sample:	0.06 Liters	0.25 Liters
N ₂ volume used per sample:	0.19 Liters	0
Estimated lifetime of helium cylinder (if using 24/7/365):	1.7914 Years	0.4003 Years
Estimated lifetime of helium cylinder (if using 8 hrs x 5 days/wk for 365):	7.3598 Years	1.6775 Years
Annual cost savings (if using 24/7/365):	\$581.50	\$0.00
Lifetime cost savings (assuming 14 years of GC-MS instrument life time):	\$8,140.99	\$0.00

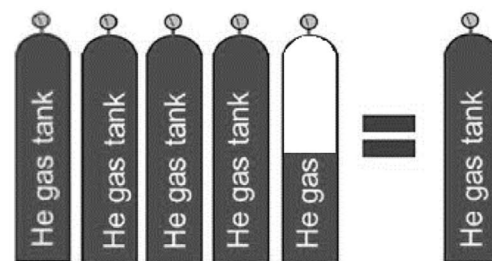


Figure 10. Gas Saver Calculator reporting the helium savings for TPH analysis when using the HeSaver-H₂Safer mode compared to a standard SSL injector. The cylinder cost is indicative and country dependent.

Conclusions

The Hydrocarbon Oil Index analysis in water and soil is a routine test for environmental laboratories. Robust solutions offering time- and cost-saving opportunities are key to maintain laboratory efficiency and face increasing workloads.

- The TRACE 1610 GC with a dual channel SSL-FID and the AS 1610 autosampler in the Gemini high-throughput configuration can double the number of samples through simultaneous injections, while Chromeleon CDS allows easy management of instrument control, data processing, and reporting for both channels.
- The system performance exceeds the regulatory requirements in terms of recovery, repeatability, linearity, and limit of quantitation, well below the minimum acceptable hydrocarbon contamination.
- The system robustness helps minimize maintenance for extended instrument uptime with consistent results over longer sequences.
- The innovative HeSaver-H₂Safer technology used on both channels provides a significant reduction of the helium gas consumption, also during sample analysis, extending 4.5-fold the cylinder lifetime and providing a significant cost savings to the laboratory.

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10. Helium Saver Calculator | Thermo Fisher Scientific - NL

 Learn more at thermofisher.com/wateranalysis