Application note | 003702

GC-IRMS

GC-IRMS: Benefits of Static Headspace Sampling

for carbon isotope analysis of methanol and ethanol in water matrix; applicability to wine and spirits ethanol $\delta^{13}C$ determination

Authors

María de Castro[1], Ana I. Cabañero[2], Vanessa Peiro[3], Mario Tuthorn[1]

[1]Thermo Fisher Scientific

[2]Laboratorio Arbitral Agroalimentario, Ministerio de Agricultura, Pesca y Alimentación (MAPA), Spain

[3]SIdI, Universidad Autónoma de Madrid (UAM), Spain

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Introduction

Analyzing alcohols in water matrix using gas chromatography (GC) is challenging due to the large aqueous component content in the sample matrix. Water in the sample shortens the lifespan of GC columns and requires frequent maintenance of the injector and column. This can complicate the analysis of isotope signatures in wine and spirits by GC-C-IRMS, for example, which is crucial for verifying product authenticity and preventing fraud. In this case, the methodology focuses on $\delta^{13}C$ measurements of ethanol (EtOH), a useful indicator for detecting wine and spirits adulterations and determining its origin.[1,2]

Measuring $\delta^{13}C$ content can detect and quantify the addition of C4 plant sugars (such as sugar cane or corn isoglucose) to grape-derived products. The current official analytical procedures face challenges such as multiple steps required to extract EtOH from the wine matrix (e.g., distillation) and technical difficulties in ensuring the collected EtOH is free from isotope fractionation effects.

An optimized method for carbon isotope analysis of volatile alcohols in water matrix by GC-IRMS aims to streamline the process by eliminating the need for prior alcohol isolation, thus simplifying sample preparation and speeding up analysis. This technical note aims to demonstrate that Static Headspace Sampling (SHS) injection of methanol (MeOH) and EtOH in water matrix via a split/splitless injector simplifies not only sample preparation, but reduces column contamination, minimizes system maintenance, and produces robust and high-quality isotopic data. We report optimized methodology based on EtOH and MeOH standards, and EtOH $\delta^{13}C$ isotopic data for wine and spirits samples.

Analytical setup

All measurements are performed using a Thermo Scientific™ GC IsoLink™ II IRMS System with the 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 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 utilize the instrument capability.

EtOH and MeOH isotopically certified standards from the Indiana University were used for:

1. Developing chromatography method
2. Testing repeatability of the results

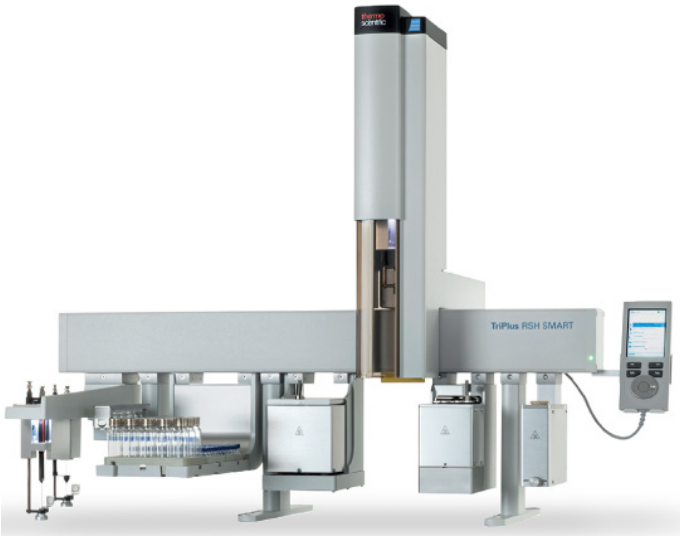

Figure 1. TriPlus RSH Series Autosampler equipped with a Static Headspace Sampling (SHS) injection option.

3. Confirming that the developed methodology is delivering accurate isotopic data
4. Defining LOD for each compound and evaluating possible linearity effects

The standards stock solutions were prepared as outlined in Table 1.

Table 1. Dilution of alcohol standards for carbon isotope analysis using GC-C-IRMS with Static Headspace Sampling injection

Name (Manufacturer)	Components	Original solution	Stock solution[1]	Standards preparation in salty water[2]
MetOH (Indiana University)	MeOH	≥ 99.9 wt. %	20 mL H_2O Milli-Q + 76 µL MeOH = 3000 ppm stock solution	20 mL vial: 9.5 mL Milli-Q H_2O with NaCl 5.1 M + 500 µL std solution = approx. 150 ppm MeOH
EtOH #3 *(Indiana University)	EtOH distillate from vodka	82 wt. % (rest water)	20 mL H_2O Milli-Q + 76 µL EtOH = 3000 ppm stock solution	20 mL vial: 9.5 mL Milli-Q H_2O with NaCl 5.1 M + 500 µL std solution = approx. 150 ppm EtOH #3
EtOH #4 *(Indiana University)	EtOH distillate from rum	80.7 wt. % (rest water)	20 mL H_2O Milli-Q + 76 µL EtOH = 3000 ppm stock solution	20 mL vial: 9.5 mL Milli-Q H_2O with NaCl 5.1 M + 500 µL std solution = approx. 150 ppm EtOH #4
MeOH + EtOH #3 (Indiana University)	MeOH + EtOH distillate from vodka	≥ 99.9 wt. % // 82 wt. % (rest water)	na	20 mL vial: 9 mL Milli-Q H_2O with NaCl 5.1 M + 500 µL stock MeOH + 500 µL stock EtOH #3 = approx. 150 ppm each
MeOH + EtOH #4 (Indiana University)	MeOH + EtOH distillate from rum	≥ 99.9 wt. % // 80.7 wt. % (rest water)	na	20 mL vial: 9 mL Milli-Q H_2O with NaCl 5.1 M + 500 µL stock MeOH + 500 µL stock EtOH #4 = approx. 150 ppm each

[1]MeOH density is 0.792Kg/L and EtOH density 0.790Kg/L, which is considered for concentration calculations of the standards solutions. Ethanol #3 is an azeotropic distillate from vodka (C3) with 82 wt. % (87.32 vol. %), and Ethanol #4 is a rum (C4) azeotropic distillate with 80.7 wt. %, but this is not considered for concentration calculations of the solutions.

[2]To lower partitioning coefficient of target compounds, it is recommended to add approximately 3 g of NaCl to every vial before closure. This promotes higher concentration of VOCs in the gas phase and helps avoiding fractionation due to lighter isotopic composition in the gas phase. To facilitate the standards and samples preparations, a solution of Milli Q water with NaCl 5.1M was prepared by adding 300 grams of NaCl to one liter of Milli Q water, which avoids the manual addition of NaCl to each vial.

*NOTE: There are two new and purer EtOH standards available from Indiana University: EtOH#1 from C3 plant (99.96 vol %) and Ethanol #2 from C4 plant (99.11 vol. %).

For the evaluation of the possible linearity effects, concentration gradient solutions were prepared using the 3000 ppm stock solution.

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 TRACE™ Series GC is listed in Table 2. The GC IsoLink II IRMS System operation is driven by Thermo Scientific™ Qtegra™ ISDS Software.

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

Table 2. Autosampler and gas chromatographer analytical setup for the isotopic analysis of MeOH and EtOH

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 (PN 365Q2131/PN 365L2321-SM)		Thermo Scientific™ LinerGOLD™ GC Liners, Direct Straight Liner (PN 453A1335-UI)	
Injection type	Static Headspace	Injection temperature	150 °C
Sample draw	0.5 mL	Inlet module and mode	SSL, split
Sampling depth mode	Standard	Split flow	24 mL/min
Agitator temperature	65 °C	Split ratio	20:1
Incubation time	15 min	Septum purge flow	3 mL/min, constant
Agitation speed	250 rpm	Carrier gas, flow	Helium, 1.2 mL/min
Agitator on	10 sec	**TRACE Series GC parameters**	
Agitator off	2 sec	Thermo Scientific™ TraceGOLD™ TR-WAX GC Column, 60 m, 0.25 mm, 0.25 µm (PN 260W154P)	
Syringe temperature	70 °C	Temperature	40 °C
Fill strokes volume	n.a.	Hold time	3.5 min
Fill strokes count	0	Rate	15 °C/m
Filling delay	3 sec	Temperature 2	95 °C
Pre-injection syringe flush	Enabled	Hold time	2 min
Post-injection syringe flush	60 s	Rate 2	25 °C/min
Filling speed	10 mL/min	Temperature 3	220 °C
Injection speed	30 mL/min	Hold time	4 min
Injection depth	45 mm	GC run time	18.2 min
Penetration speed	25 mm/s	Prep run timeout	120 min
Pre-injection delay	1 s		
Post-injection delay	3 s		
Needle speed in vial	20 mm/s		
Synchro type	Normal		

Table 3. Conditioning parameters for the combustion reactor of the GC IsoLink II Conversion Unit

Reactor conditioning type	Setup
Extended – used at the start of a sequence	45 min O_2 + 90 min Backflush + 0.1 min purge
Seed – used with each sample run	0.2-0.3 min O_2 + 0.2 min Backflush + 0.1 min purge

For optimization of the SHS injection of MeOH and EtOH via split/splitless injector the following analytical steps were followed:

1. Individual alcohols standards injections were performed for developing the chromatography method, testing repeatability and accuracy of the results
2. A certified standards mix (MeOH + EtOH #3, from Indiana University) was prepared to evaluate its chromatographic separation, as well as to ensure that the precision and accuracy of the data were not compromised by injecting both alcohols together, since you could find both of them at the same time in real samples
3. Concentration gradients were analyzed for the evaluation of possible linearity effects using isotopically certified standards (Indiana University)

Results

For the analysis, 9.5 mL of Milli-Q H_2O with NaCl 5.1 M and 500 µL of stock solution were transferred into 20 mL, 18 mm screw top, headspace vials (PN 6ASV20-1) with 18mm magnetic screw caps (PN 6PMSC18-STH, 8 mm hole). Chromatographic separation of the target compounds is shown in Figure 2.

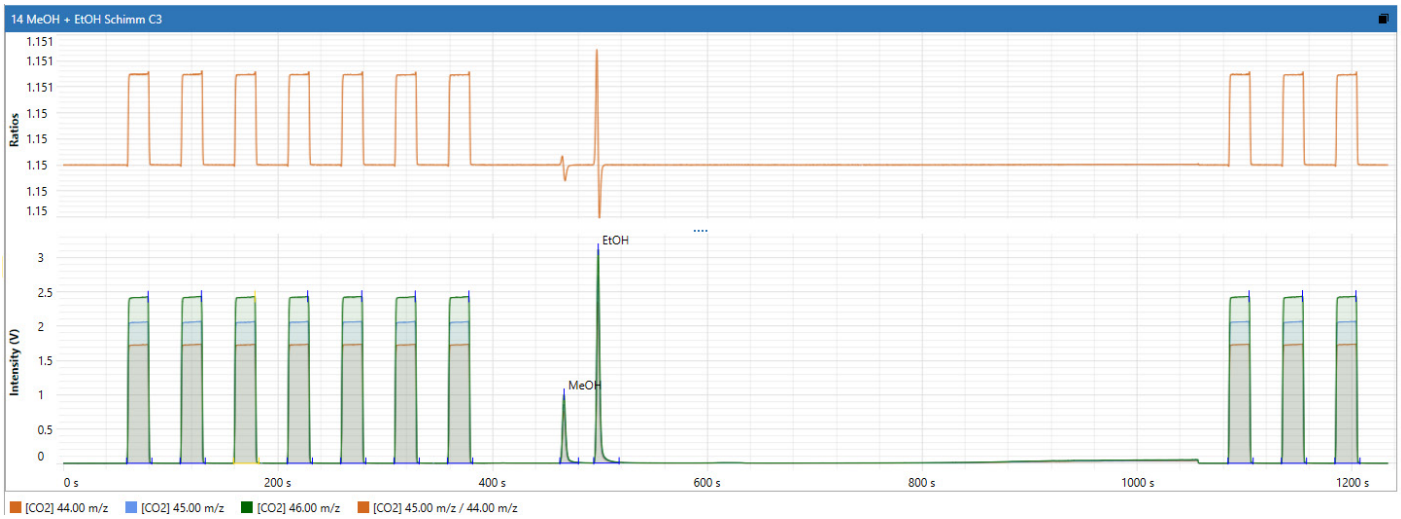

Figure 2. GC-IRMS chromatogram of the MeOH and EtOH #3 certified standards mix; 150 ng/uL (150ppm) each in H_2O

Data evaluation was performed using Qtegra ISDS Software tools for data normalization, where first isotopically certified standards injections were treated as Compound Specific Isotope Analysis (CSIA) δ Standard and the following 10 repetitions were treated as unknown samples (see Sample list in Figure 3). The resulting in-software evaluation shows high correlation between the measured and known isotope ratios values (Figure 4).

Sample List

	Label	Status	Comment	Evaluate	Sample Type	Reference	Vial	Inject Volume [µl]	Inject
1	Blank		45mins O2 + 2hs BF_18s seed	☑	Unknown		164	500	☑
2	MeOH + EtOH Schimm C3		5ml in 10ml vial_1.5g NaCl	☑	Delta Standard (CSIA)	Alcohols Schimm #3	171	500	☑
3	EtOH Schimm C4		5ml in 10ml vial_1.5g NaCl	☑	Delta Standard (CSIA)	EtOH Schimm #4	172	500	☑
4	MeOH + EtOH Schimm C3		5ml in 10ml vial_1.5g NaCl	☑	Unknown		173	500	☑
5	EtOH Schimm C4		5ml in 10ml vial_1.5g NaCl	☑	Unknown		174	500	☑
6	MeOH + EtOH Schimm C3		5ml in 10ml vial_1.5g NaCl	☑	Unknown		175	500	☑
7	EtOH Schimm C4		5ml in 10ml vial_1.5g NaCl	☑	Unknown		176	500	☑
8	MeOH + EtOH Schimm C3		5ml in 10ml vial_1.5g NaCl	☑	Unknown		177	500	☑
9	EtOH Schimm C4		5ml in 10ml vial_1.5g NaCl	☑	Unknown		178	500	☑
10	MeOH + EtOH Schimm C3		5ml in 10ml vial_1.5g NaCl	☑	Unknown		179	500	☑
11	EtOH Schimm C4		5ml in 10ml vial_1.5g NaCl	☑	Unknown		180	500	☑
12	MeOH + EtOH Schimm C3		5ml in 10ml vial_1.5g NaCl	☑	Unknown		181	500	☑
13	EtOH Schimm C4		5ml in 10ml vial_1.5g NaCl	☑	Unknown		182	500	☑
14	MeOH + EtOH Schimm C3		5ml in 10ml vial_1.5g NaCl	☑	Unknown		183	500	☑
15	EtOH Schimm C4		5ml in 10ml vial_1.5g NaCl	☑	Unknown		184	500	☑
16	MeOH + EtOH Schimm C3		5ml in 10ml vial_1.5g NaCl	☑	Unknown		185	500	☑
17	EtOH Schimm C4		5ml in 10ml vial_1.5g NaCl	☑	Unknown		186	500	☑
18	MeOH + EtOH Schimm C3		5ml in 10ml vial_1.5g NaCl	☑	Unknown		187	500	☑
19	EtOH Schimm C4		5ml in 10ml vial_1.5g NaCl	☑	Unknown		188	500	☑
20	MeOH + EtOH Schimm C3		5ml in 10ml vial_1.5g NaCl	☑	Unknown		189	500	☑
21	EtOH Schimm C4		5ml in 10ml vial_1.5g NaCl	☑	Unknown		190	500	☑
22	MeOH + EtOH Schimm C3		5ml in 10ml vial_1.5g NaCl	☑	Unknown		191	500	☑
23	EtOH Schimm C4		5ml in 10ml vial_1.5g NaCl	☑	Unknown		192	500	☑
24	Blank		5ml in 10ml vial_1.5g NaCl	☑	Unknown		165	500	☑

Figure 3. Qtegra ISDS Software sample list for in-software data normalization using a CSIA standard

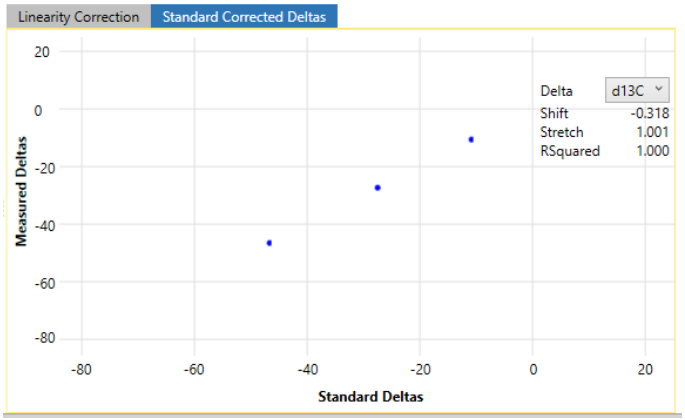

Figure 4. Qtegra ISDS Software data evaluation of measured isotope values vs. known isotope values of a CSIA standard

The certified standards isotope data reported in Table 4 demonstrate excellent measurement precision and accuracy for all compounds, as well as good RSD for total areas.

The Figure 5 shows data from three different analysis sequences done during the method development process, showing excellent intra and inter-sequence repeatability, and confirming that the developed methodology is delivering accurate isotopic data.

Table 4. Isotope data for the MeOH and EtOH standards; 150 ng/µL (150 ppm) in H_2O acquired using an SHS injection option

Component	δ¹³C (‰)				Total Area (V/s)		
	Certified δ¹³C (‰)	Average (‰) (n=10)	SD (n=10)	Offset vs Certified (‰)	Average (n=10)	SD (n=10)	RSD (%)
MeOH	-46.77	-46.75	0.10	0.02	6.76	0.20	2.97
EtOH #3	-27.53	-27.43	0.07	0.10	15.63	0.39	2.48
EtOH #4	-10.98	-11.05	0.08	-0.07	13.68	0.41	3.01

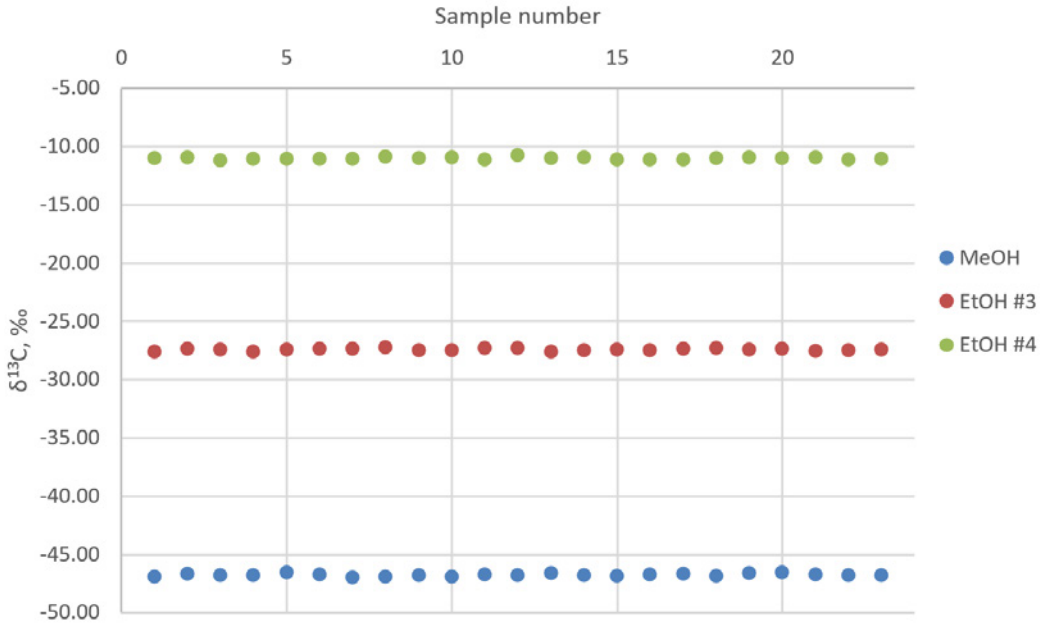

Figure 5. Isotope data acquired in different sequences for the MeOH and EtOH certified standards; 150 ng/µL (150 ppm) in H_2O using an SHS injection option

Finally, concentration gradients of the MeOH + EtOH #4 certified standard mix were analyzed with results showing that the isotope ratio of MeOH and EtOH are independent of the amount of material analyzed within the range from 120 ppm to 1500 ppm (Table 5).

Table 5. Isotope ratio data of MeOH and EtOH #4 mix in the concentration range from 120 ppm to 1500 ppm

Compound	MeOH					EtOH #4				
Certified $\delta^{13}C$ (‰)	-46.77					-10.98				
ng/µL (ppm)	Ampl. 44 (mV)		$\delta^{13}C$ (‰)			Ampl. 44 (mV)		$\delta^{13}C$ (‰)		
	Avg. (n=3)	SD (n=3)	Avg. (n=3)	SD (n=3)	Offset vs Cert.	Avg. (n=3)	SD (n=3)	Avg. (n=3)	SD (n=3)	Offset vs Cert.
120	0.32	0.02	-46.95	0.03	-0.18	1.03	0.06	-10.78	0.28	0.20
240	0.66	0.01	-46.75	0.05	0.02	2.08	0.02	-11.19	0.13	-0.21
480	1.34	0.08	-46.51	0.14	0.26	4.15	0.23	-10.68	0.21	0.30
1000	2.58	0.18	-46.66	0.13	0.11	7.71	0.47	-10.75	0.07	0.23
1500	3.88	0.13	-46.57	0.12	0.20	10.82	0.26	-10.79	0.01	0.19

Analysis of EtOH in wine and spirits samples

The Laboratorio Arbitral Agroalimentario – MAPA, Spain, supplied an in-house wine standard (Quality Control, QC) and wine and spirits samples that had been previously analyzed using the OIV official method (ethanol collection from wine with Cadiot columns for EA-IRMS analysis). These were provided along with each sample distillate for comparison with the SHS GC-IRMS methodology.

Some of the wine samples may still contain active bacteria. To prevent potential bacterial activity, two valid procedures can be used: filtration (0.22 µm) or sterilization. For this study, sterilization with benzoic acid was chosen. To determine the required amount and effectiveness of benzoic acid, two vials of each wine type (white and red) were prepared. One vial, without benzoic acid, was analyzed immediately after preparation. The other vial, containing approximately 25 mg of benzoic acid (a spatula tip), was stored at room temperature for 24 hours before analysis. The results (peak size and $\delta^{13}C$) were identical, demonstrating 100% efficacy.

Note: the total sample volume can be filtered when transferring it to the 2 mL storage vial, or the entire sample volume can be sterilized directly in the storage vial. This eliminates the need to add benzoic acid to each individual vial.

To simplify the preparation of standards and samples, a solution of Milli-Q water with 5.1 M NaCl was prepared by adding 300 grams of NaCl to one liter of Milli-Q water. This avoids the need to manually add NaCl to each vial. Stock solutions of the isotopically certified standards and samples distillates were prepared as shown in Table 6, with concentrations similar to those expected in the samples.

According to the principle of identical treatment, benzoic acid should be added to the standards and samples distillates vials as well, just as it is added to the wine and spirits samples. However, a test was conducted to evaluate any possible effects of benzoic acid addition by analyzing standards prepared with and without benzoic acid. The results showed no effect, therefore it is not necessary to add benzoic acid to the standards and samples distillates vials.

Table 6. Stock solutions of the isotopically certified standards and samples distillates to be analysed along with the wine and spirits samples

Name (Manufacturer)	Components	Original solution	Stock solution in H_2O with NaCl 5.1 M
EtOH #4 (Indiana University)	EtOH distillate from rum	80.7 wt. % (rest water)	2 mL vial: 360 µL EtOH + 1640 µL Milli-Q H_2O = 150000 ppm (15 vol. %)
MeOH + EtOH #3 (Indiana University)	MeOH + EtOH distillate from vodka	≥ 99.9 % // 82 wt. % (rest water)	2 mL vial: MeOH 300 µL + EtOH 360 µL + 1340 Milli-Q H_2O = 150000 ppm (15 vol. %) each
Samples distillates	EtOH distillate from samples	92 wt. % (rest water)	2 mL vial: 360 µL EtOH + 1640 µL Milli-Q H_2O = 150000 ppm (15 vol. %)

For the analysis, 10 mL of Milli-Q H_2O with 5.1 M NaCl were transferred into 20 mL, 18 mm screw top, headspace vials with 18 mm magnetic screw caps. Approximately 25 mg of benzoic acid was added to each vial that will be containing wine or spirits samples. Then, 50 µL of wine or distillate, and stock standard solutions were added to the vials. For the spirits samples, a volume between 25-40 µL was added to the vial (depending on the EtOH vol. %).

Figure 6 shows clean separation of EtOH from other components in a wine sample analyzed by SHS GC-IRMS method.

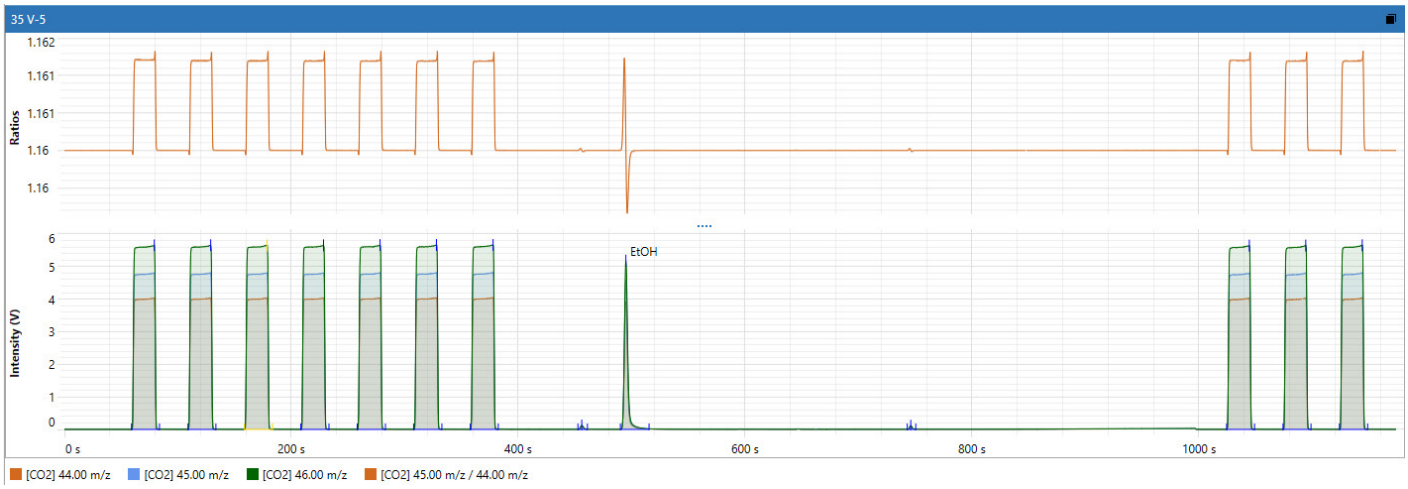

Figure 6. GC-IRMS chromatogram of the EtOH in a wine sample using SHS methodology

Table 7 shows the results obtained by SHS GC-IRMS and comparison with the data obtained from the Laboratorio Arbitral Agroalimentario – MAPA, Spain, for the samples distillates analysis by EA-IRMS using the official method. We report excellent correlation of the SHS GC-IRMS and EA-IRMS data.

Table 7. Carbon isotope data of wine and spirits samples and samples distillates acquired by SHS GC-IRMS methodology and comparison to EA-IRMS data

Sample	Sample	Volume added to vial (µL)	Average SHS GC-IRMS $\delta^{13}C$ (‰) (n=3)	SD (‰) (n=3)	EA-IRMS MAPA $\delta^{13}C$ (‰)	Offset GC-IRMS vs EA-IRMS (‰)
Orujo (~40 vol. %)	Distillate stock (EtOH 15 vol. %)	50	-26.52	0.08	-26.56	-0.04
	Raw sample	25	-26.51	0.10		-0.05
Vermut (~15 vol. %)	Distillate stock (EtOH 15 vol. %)	50	-26.69	0.08	-26.70	-0.01
	Raw sample	40	-26.82	0.11		0.12
Brandy (~40 vol. %)	Distillate stock (EtOH 15 vol. %)	50	-26.48	0.08	-26.26	0.22
	Raw sample	25	-26.54	0.10		0.27
Red wine (~14 vol. %)	Distillate stock (EtOH 15 vol. %)	50	-25.69	0.08	-25.62	0.07
	Raw sample	50	-25.71	0.07		0.09
Wine QC (~14 vol. %)	Distillate stock (EtOH 15 vol. %)	50	-25.52	0.07	-25.42	0.10
	Raw sample	50	-25.60	0.02		0.18

Conclusions

A method for Static Headspace Sampling GC-IRMS was optimized and applied for carbon isotope analysis of methanol and ethanol in aqueous matrix. The isotopic data obtained by SHS GC-IRMS analysis of the alcohols standard mixes demonstrate excellent precision and accuracy, including high sensitivity and low detection limits.

The lifespan of the chromatographic column is extended since only the volatile fraction is injected. During this study, a total of 851 injections (including alcohol standards and real samples) were performed on the TR-WAX chromatographic column. By the end of the study, the column showed no signs of damage, no degradation in chromatographic separation performance, no background issues, and there was no evidence of bleeding. Preventively, 5 cm of the column head was cut three times, and no other maintenance or conditioning was necessary.

The SHS GC-IRMS technique eliminates both the need for time-consuming sample preparation, and the use of expensive specific distillation devices. The newly developed methodology substantially reduces the total sample preparation time from more than 5 hours to few minutes. This results in analytical setup simplification and higher automation, making routine SHS GC-IRMS analysis of ethanol in wines and spirits feasible.

References

1. Yamada K, Yoshida N, Calderone G, et al. (2007) Determination of hydrogen, carbon and oxygen isotope ratios of ethanol in aqueous solution at millimole levels, *Rapid Commun Mass Spectrom*, 21:1431–1437.
2. Cabañero A, Recio JL, Rupérez M (2008) Isotope ratio mass spectrometry coupled to liquid and gas chromatography for wine ethanol characterization, *Rapid Commun Mass Spectrom*, 22:3111–3118.

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