APPLICATION NOTE

EA-IRMS: ^{13}C and Simultaneous ^{18}O and ^{2}H Isotope Analysis in Ethanol with Thermo Scientific Delta V Isotope Ratio Mass Spectrometers

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Key Words
Continuous Flow, $\delta^{13}C$, $\delta^{2}H$, $\delta^{18}O$, Isotope Ratio MS, Wine

Introduction

Isotopic analyses of foods have become a widespread tool to evaluate the quality, authenticity and origin of labeled products. According to the standard procedure, wines, alcoholic beverages and fermented fruit juices are distilled to gain ethanol. Ethanol can be analyzed for oxygen and carbon isotope composition by elemental analyzers coupled to isotope ratio MS (Calderone et al. 2006).

The analyses allow the quantification of exogenous sugar added during the fermentation to increase the alcohol grade of the wine. The control of spirit drinks is needed also for the detection of frauds, such as mislabeling regarding both ingredients and origin. The $^{18}O/^{16}O$ ratio of water can also be used for the assessment of fruit juices. In a recent study, the applicability of high temperature carbon reduction techniques, such as in theThermo Scientific™ FlashEA™ 1112 HT, has been tested for the determination of $^{2}H/^{1}H$ ratios (Bréas et al. 2007).

The FlashEA 1112 HT combines the two techniques needed for the evaluation of C, H and O isotopes in ethanol: Dumas combustion for ^{13}C analysis, and high temperature conversion for simultaneous ^{2}H and ^{18}O analysis. These techniques are also combined in the latest EA-IRMS developed, the Thermo Scientific™ EA IsoLink™ IRMS System. Two preparation techniques can be used for sample introduction: placing the ethanol in a capsule, or injecting the ethanol directly into the reactor.

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This application note shows the ability and performance of the analysis of ethanol with combustion and with a high temperature carbon reduction technique in combination with a Thermo Scientific™ Delta™ V Isotope Ratio Mass Spectrometer (IRMS).

Technique

$\delta^{13}C$ determination: 1 µL of purified ethanol was injected with a 1.2 µL syringe into a small tin container for liquids. The container was closed using a pair of wire cut pliers with a smooth and sharp edge. Excess tin was removed. The capsules were placed in a solids autosampler, Thermo Scientific™ MAS 200R. The long-term test (Figure 2) shows that evaporation and accompanied fractionation can be excluded. The elemental analyzer used was a FlashEA 1112 HT with a single reactor system combining combustion and reduction in one reactor (see schematic, Figure 1). The settings are given in Table 1.

The Thermo Scientific™ ConFlo™ Universal Interface diluted the CO_2 sample peak with 1 bar helium resulting in a split of about 1:12.

Table 1. FlashEA 1112 HT settings for C analysis.

FlashEA 1112 HT Parameters	
Reactor Temperature	1020 °C
GC Temperature	45 °C
Carrier Flow	90 mL/min
O_2 Flow	250 mL/min
O_2 Injection Time	1 s
Autosampler delay	23 s
Autosampler Type	MAS 200R

$\delta^{2}H$ and $\delta^{18}O$ determination: 0.1 µL of pure ethanol was injected with a 0.5 µL syringe into a glassy carbon reactor in a high temperature carbon reduction system of a FlashEA 1112 HT. The system settings are given in Tables 2 and 3. The autosampler used was a Thermo Scientific AS 3000 (Thermo Fisher Scientific, Italy). Samples were stored in 2 ml vials with standard caps and septa.

Table 2. FlashEA 1112 HT settings for H and O analysis.

FlashEA 1112 HT Parameters	
Reactor Temperature	1400 °C
GC Temperature	90 °C
Carrier Flow	100 mL/min
Autosampler Type	AS 3000
Syringe Size	0.5 µL

Table 3. AS 3000 Autosampler parameters.

AS 3000 Parameters	
Injection volume	0.1 µL
Plunger strokes	7
Rinses	0
Pre-injection dwell time	0 s
Post-injection dwell time	10 s
Solvent wash cycle	0

Calculation

The raw $\delta^{13}C$ values were blank corrected and subsequently corrected versus the internal ethanol standard (see blue rows in Table 4) using a spreadsheet template from Werner & Brand (2001). The standard was measured before and after the run, and a mean value was calculated using the standards' raw values as indicated with the star (* in Table 4). The obtained average (-26.51‰, N=8) was corrected for the specified value (-26.93‰) resulting in a correction factor (corrfac) using the formula

$$\text{corrfac} = \frac{\delta_{real} - \delta_{meas}}{\frac{\delta_{meas}}{1000} - 1}$$

where δ_{real} is the specified delta value of the standard versus V-PDB and δ_{meas} is the measured delta value (raw delta value) of the standard. This correction factor was then applied to the raw delta values (δ_{raw}) of the unknown samples to calculate a corrected delta value (δ_{corr}) using this formula:

$$\delta_{corr} = \delta_{raw} + \text{corrfac} + \frac{\delta_{raw} \bullet \text{corrfac}}{1000}$$

Results

Carbon isotope analysis was performed at the Chemical Institute of the Hungarian Customs and Finance Guard in Budapest in January 2007. The $\delta^{13}C$ values obtained from the ethanol samples introduced by a liquid tin container show a precision (S.D.) of better than 0.1‰ (Table 4). Two adjacent data were discarded as they showed obvious contamination by deviating $\delta^{13}C$ values (# in Table 4). Sample extracted from wine ("Ethanol Wine") reached the target value as given by another lab. Sample "Ethanol Pineapple" is in an expected range taking into account that pineapple is a CAM plant and correlates well with the reference value of the sample resulted from a proficiency test (-14.07‰). A long-term test with the encapsulated ethanol in the tin containers showed that there is no drifting due to fractionation by evaporation (Figure 4). Sample preparation is simple, low priced, and reproducible.

$\delta^{2}H$ and $\delta^{18}O$ values obtained from high temperature conversion in a FlashEA 1112 HT give precisions (S.D.) of 0.12‰ and 0.08‰, respectively (Table 5). The data was corrected against VSMOW and rescaled using the GISP standard. The lab standard was injected before and after the sequence to exclude drift effects. Standards VSMOW and GISP were injected ten times, the ethanol sample was injected five times.

First injection of all samples was discarded to exclude memory effects. The analysis time for both isotopes with one injection was 325 s allowing for more than 200 analyses per 24 hrs including peak center routines and magnet field changes.

The data presented in Figure 2, Table 4 and Table 5 are not warranted because they exceed product specifications. The warranted product specifications for $\delta^{2}H$ is ±2‰ (1 sd) measured on 0.5 µl of water and for $\delta^{18}O$ is ±0.2‰ (1 sd) measured on 0.5 µl of water and for $\delta^{13}C$ is ±0.1‰ (1 sd) measured on Acetanilide or Urea.

Conclusion

C isotopes of ethanol can be measured easily and with low cost using standard equipment and tin capsules for liquids (Figure 2). Precision is better than ±0.1‰. H and O isotopes of ethanol can be measured simultaneously with high precision and with a high throughput of up to 200 samples/day (Figure 4). The combination of the two required techniques – high temperature carbon reduction and Dumas combustion – makes the FlashEA 1112 HT a universal instrument for multi-element isotope ratio analysis in food authentication analysis in combination with any Thermo Scientific™ Isotope Ratio Mass Spectrometer.

The EA IsoLink IRMS System, the latest Thermo Scientific™ development in EA-IRMS, can deliver the same performance as reported here.

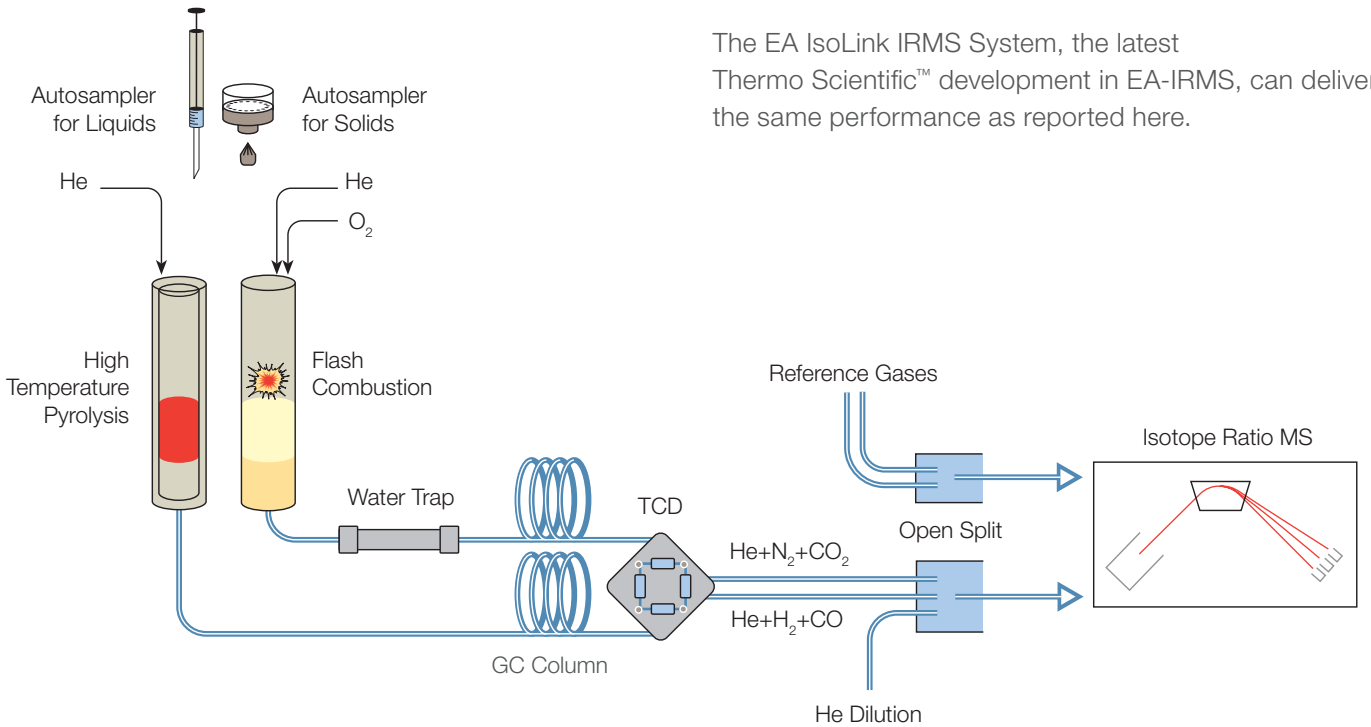

Figure 1. Schematic of Thermo Scientific FlashEA 1112 HT with ConFlo interface and Delta V Isotope Ratio MS.
Left furnace with reactor for high temperature pyrolysis and right furnace for dynamic flash combustion and reduction in a single reactor.

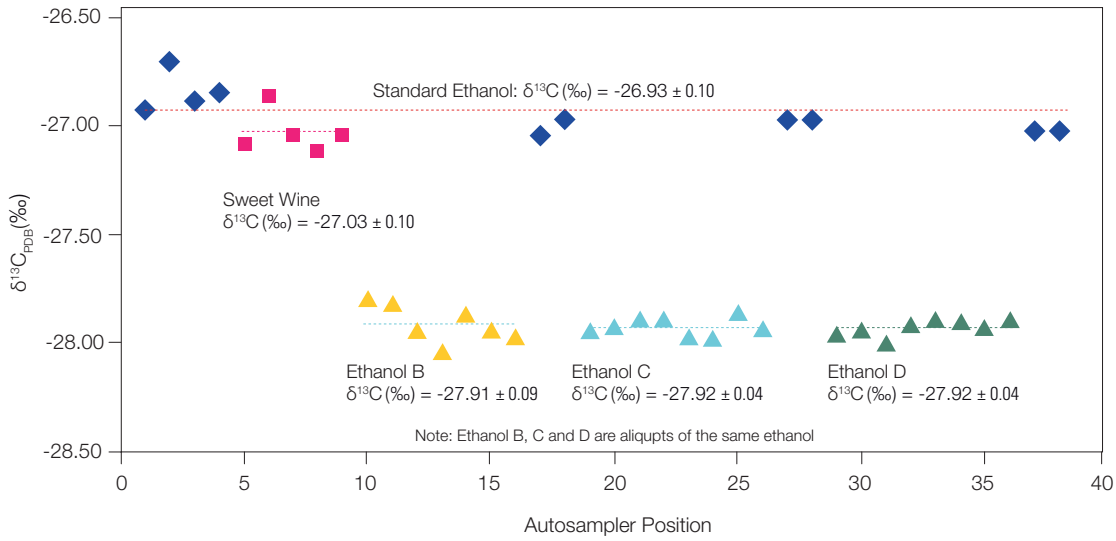

Figure 2. Long-term stability of $\delta^{13}C$ value. Ethanol B, C and D are identical. Run time 6 hours. Values corrected vs. internal ethanol standard (see calculation).

Table 4. $\delta^{13}C$ values (‰) of ethanol extracted from wine and pineapple juice. Values are corrected vs. internal ethanol standard.

Identifier	Analysis #	Ampl 44 mV	Area 44 Vs	$\delta^{13}C$ Raw data	$\delta^{13}C$ Raw data blank corr	*corr	$\delta^{13}C$ avg Std	$\delta^{13}C$ Std vs VPDB	Diff-Std = corrfac	$\delta^{13}C$ corr vs Std	$\delta^{13}C$ corr avg Std	S.D.
Ethanol Standard	3602	9960	143.177	-26.573	-26.571	*				-26.99		
Ethanol Standard	3603	9034	127.262	-26.589	-26.586	*				-27.01		
Ethanol Standard	3604	9468	134.92	-26.555	-26.552	*				-26.97		
Ethanol Standard	3605	10559	154.367	-26.537	-26.535	*				-26.97		
Ethanol Standard	3606	9275	131.629	-26.518	-26.516	*				-26.94		
~~Ethanol Standard~~	~~3607~~	~~6334~~	~~85.281~~	~~-25.758~~	~~-25.753~~	*				~~-26.17#~~		
~~Ethanol Wine~~	~~3608~~	~~6644~~	~~89.712~~	~~-17.551~~	~~-17.538~~					~~-17.96#~~		
Ethanol wine	3609	6227	83.467	-18.738	-18.725					-19.15		
Ethanol wine	3610	3254	42.191	-18.821	-18.795					-19.22		
Ethanol wine	3611	9375	133.524	-18.63	-18.622					-19.05	-19.14	0.09
Ethanol pineapple	3612	8622	120.118	-13.929	-13.915					-14.34		
Ethanol pineapple	3613	9662	137.765	-13.932	-13.921					-14.35		
Ethanol pineapple	3614	7215	97.926	-14.04	-14.024					-14.45		
Ethanol pineapple	3615	9883	141.773	-13.855	-13.843					-14.27		
Ethanol pineapple	3616	10927	160.475	-13.828	-13.818					-14.25		
Ethanol pineapple	3617	8558	119.371	-13.867	-13.854					-14.28	-14.32	0.07
Ethanol Standard	3618	8724	121.718	-26.513	-26.51	*				-26.93		
Ethanol Standard	3619	11192	164.83	-26.4	-26.398	*				-26.82		
Ethanol Standard	3620	10225	147.945	-26.401	-26.399	*	-26.51	-26.93	-0.43	-26.82	-26.93	0.07

#Discarded due to obvious contamination.
*Internal ethanol standards used for mean value.

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Table 5. δ^2H and $\delta^{18}O$ values (‰) and precision (S.D.) of ethanol, SMOW, GISP and a lab standard injected in a high temperature conversion reactor.

Sample	Mean δ^2H	S.D.	Mean $\delta^{18}O$	S.D.
Lab Standard	-53.69	0.20	-7.84	0.04
VSMOW	0.00	0.34	0.00	0.08
GISP	-189.50	0.40	-24.80	0.05
Ethanol	-234.80	0.12	-24.18	0.08
Lab Standard	-53.76	0.39	-7.85	0.03

Standards: N=10; ethanol: N=5; first data skipped

EU Commission Regulations Concerning Analysis of Wines

- Commission Regulation (EEC) No 2676/90 of 17 September 1990 determining Community methods for the analysis of wines
- Commission Regulation (EC) No 822/97 of 6 May 1997 amending Regulation (EEC) No 2676/90 determining Community methods for the analysis of wines
- Commission Regulation (EC) No 440/2003 of 10 March 2003 amending Regulation (EEC) No 2676/90 determining Community methods for the analysis of wines

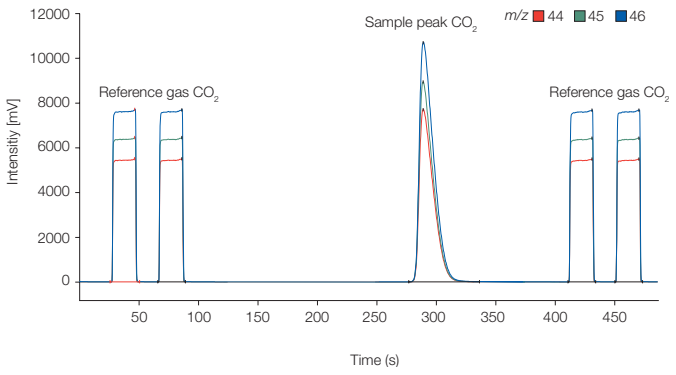

Figure 3. Example chromatogram of an ethanol analysis showing the mass traces 44, 45 and 46 for evaluation of $^{13}C/^{12}C$ ratio. Sample drop was 170 s before the sample peak, just after the first two reference gas injections.

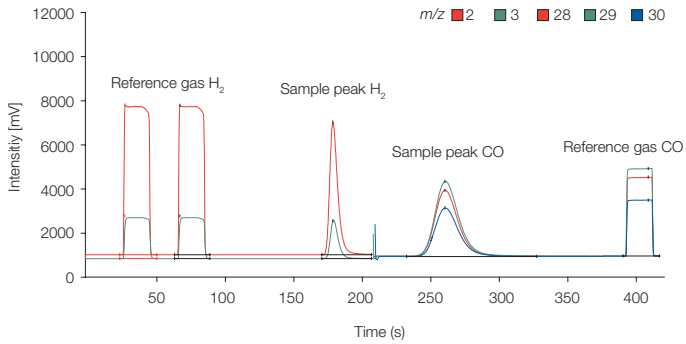

Figure 4: Example chromatogram of ethanol for simultaneous H and O isotope analysis showing the mass traces 2 and 3 and 28/29/30 for evaluation of $^1H/^2H$ and $^{18}O/^{16}O$ ratio, respectively. Sample injection was 90 s before the H_2 sample peak, just after the first two reference gas injections. Automatic fast magnet jump at 210 s allowed for subsequent monitoring of CO traces.

References

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