MASS SPECTROMETRY

SG Isotech *EIM* (Ethanol Isotope Measurement) Module for IRMS – The missing link in Isotope Analysis

Online Continuous Flow Module for Non-exchangeable Hydrogen $(D/H)_n$ Ratio Isotope Analysis in Ethanol

Wine Authenticity Testing

Strong Spirit Authenticity Testing

Honey Authenticity Testing

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SG Isotech EIM-Module for Isotope Ratio MS

The missing link in Isotope Analysis

The EIM Module is a fully automated peripheral optimized for isotope analysis. It meets the increasing demand for isotope analysis for applications such as quality control; detection of adulteration of wine, alcoholic beverages, honey, and other consumables.

EIM Module applications for Isotope Ratio MS:

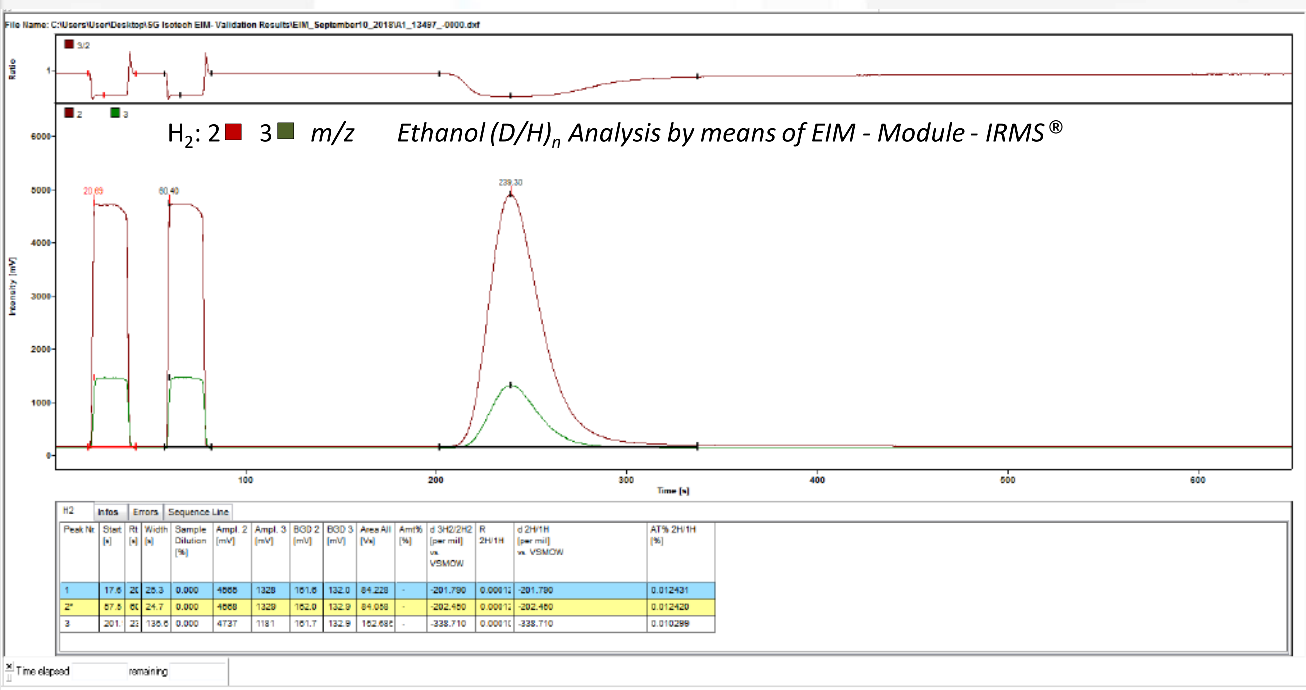
- Control of wine, strong spirits and honey
  - Determination of botanical origin by measuring addition of water and sugar to **known and unknown** samples
  - Verification of authenticity
- Forensic science
- Geographical origin of wine and honey

The EIM Module coupled to comonly used analitical instrumentation for isotopic analysis operates in true continuous flow mode for complete compatibility with principles of CF-IRMS.

EIM (Ethanol Isotope Measurement) Module for Isotope Ratio MS

EIM-IRMS® (Ethanol Isotope Measurement – Isotope Ratio Mass Spectrometry) revolutionized measurement of relative ratio of non-exchangeable Hydrogen stable isotopes (D/H)n (δDn) in ethanol previously quantitatively extracted from wine, strong spirit or fermented honey samples[1)].

This technology has become the integral part of EIM-Module for Isotope Ratio MS. EIM module provides rapid and quantitative intramolecular dehydration of ethanol sample over custom made EIM-catalyst, specifically designed by SG Isotech, prior to high precision isotope ratio measurement during a single analysis (see chromatogram below). Module provides precise values and quicker analysis times and precisions are attained on sub-microliter amounts of ethanol with analytical time of 10-15 minutes.



Peak Nr	Start [s]	Rt [s]	Width [s]	Sample Dilution [%]	Ampl. 2 [mV]	Ampl. 3 [mV]	BGD 2 [mV]	BGD 3 [mV]	Area All [Vs]	Amt% [%]	d 3H2/2H2 [per mil] vs. VSMOW	R 2H/1H	d 2H/1H [per mil] vs. VSMOW	AT% 2H/1H [%]
1	17.6	20	25.3	0.000	4666	1328	161.6	132.0	84.228	-	-201.790	0.00012	-201.790	0.012431
2*	57.5	60	24.7	0.000	4668	1329	162.0	132.9	84.058	-	-202.460	0.00012	-202.460	0.012420
3	201.	23	136.6	0.000	4737	1181	161.7	132.9	162.685	-	-338.710	0.00010	-338.710	0.010299

Figure 1

[1)] I. Smajlović, K.L. Sparks, J.P. Sparks, I. Leskošek Čukalović & S. Jović (2012): Ethanol isotope method (EIM) for uncovering illegal wine, *Natural Product Research: Formerly Natural Product Letters*, DOI: 10.1080/14786419.2012.673610
To link to this article: http://dx.doi.org/10.1080/14786419.2012.673610

EIM-Module-IRMS® results

Authentic grape samples were quantitatively fermented under controlled and standard conditions in order to achieve full conversion of sugar to ethanol (at least 98% of the theoretical yield). After fermentation, ethanol was extracted from wine samples by distillation to obtain 93 to 96%v/v with recovery of minimum 85% m/m. Prepared samples are injected into EIM-Module connected to Pyrolysis Conversion Elemental Analyzer coupled to Isotope Ratio MS for high precision isotope ratio monitoring of non-exchangeable $(D/H)_n$ in ethanol . Obtained δD_n results from EIM Module are in accordance with previously proposed range for authentic wine ethanol from -205 ‰ to -215 ‰ (Table 1).

No.	Sample	Number of analysis	Ethanol δD_n Mean Value (‰ vs. AAWES*)	St. Dev. (‰)
1.	Wine ethanol – Sample1	3	-213.84	1.77
2.	Wine ethanol – Sample2	3	-210.31	0.97
3.	Wine ethanol – Sample3	3	-213.96	1.67
4.	Wine ethanol – Sample4	3	-211.63	0.55
5.	Wine ethanol – Sample5	3	-214.10	1.14
6.	Wine ethanol – Sample6	3	-214.49	0.34
7.	Wine ethanol – Sample7	3	-213.17	0.44

*AAWES – Afusali Authentic Wine Ethanol Standard

Table 1

EIM-Module-IRMS® vs. Pyrolysis Conversion Elemental Analyzer IRMS results

Because of ethanol's hydroxyl group, which includes exchangeable Hydrogen atom, ethanol δD value is not a reliable and repeatable analytical parameter. EIM Module coupled to Pyrolysis Conversion Analyzer for Isotope Ratio MS solves this problem and gives high precision, repeatable and reproducible results by obtaining isotope ratio of non-exchangeable (D/H)n in ethanol sample during a single analysis.

In order to examine the effect and influence of the surrounding medium (water) on ethanol's hydroxyl group, agricultural and wine ethanol were prepared and tested.

Five refined ethanol samples from beet sugar with an alcoholic strength of 96 % vol. were prepared and split in two parts. One part was used as a reference sample without additional modification, and the second part was diluted with water to 50% vol and then again distilled to alcoholic strength of 96 % v/v.

All prepared Ethanol samples were initially analyzed directly on Pyrolysis Conversion Analyzer coupled to IRMS (Table 2 and Chart 1) and then using EIM method with EIM Module (Table 3 and Chart 2).

Sample	First Part (no dilution)		Second Part (50% dilution)	
	Average δD values (‰ vs. V-SMOW)		Average δD values (‰ vs. V-SMOW)	
Refined	1	-279.62	1'	-266.92
Refined	2	-286.24	2'	-270.74
Refined	3	-295.66	3'	-282.12
Refined	4	-285.67	4'	-271.74
Refined	5	-292.48	5'	-279.45
Average value	-287.93		-274.19	
Total Average value	-281.06			
Standard Deviation with reference to V-SMOW	9.37			

Table 2: Ethanol δD values from Pyrolysis Conversion Elemental Analyzer coupled to IRMS

Sample	First Part (no dilution)		Second Part (50% dilution)	
	Ethylene		Ethylene	
	Average δDn values (‰ vs. AAWES*)		Average δDn values (‰ vs. AAWES*)	
Refined	1	-281.63	1'	-279.86
Refined	2	-284.57	2'	-283.42
Refined	3	-283.86	3'	-282.05
Refined	4	-286.61	4'	-284.98
Refined	5	-276.08	5'	-274.09
Average value	-282.55		-280.88	
Total Average value	**-281.72**			
Standard Deviation with reference to AAWES*	**3.99**			

*AAWES – Afusali Authentic Wine Ethanol Standard

Table 3: Ethanol δD_n values from EIM Module connected to Pyrolysis Conversion Elemental Analyzer coupled to IRMS

Wine samples were distilled and wine ethanol was analyzed in the same manner as previously described, using Pyrolysis Conversion Elemental Analyzer IRMS and EIM-IRMS® with EIM Module. As noted in Table 4, accuracy, repeatability and standard deviation is significantly better using EIM-IRMS® method with EIM Module.

Sample	Wine Ethanol	
	Ethanol	Ethylene
	Average δD values (‰ vs. V-SMOW)	Average δDn values (‰ vs. AAWES*)
Wine – sample 1	-209.08	-211.94
Wine – sample 2	-219.02	-209.86
Wine – sample 3	-221.91	-209.40
Wine – sample 4	-223.93	-208.31
Wine – sample 5	-219.86	-208.08
Wine – sample 6	-198.23	-207.14
Wine – sample 7	-196.35	-206.75
Wine – sample 8	-207.16	-207.16
Wine – sample 9	-201.61	-205.51
Wine – sample 10	-209.76	-213.83
Wine – sample 11	-205.96	-213.10
Average value	-210.26	**-209.19**
Standard Deviation with reference to V-SMOW (ethanol δD values) and AAWES* (ethanol δDn values)	9.67	**2.73**

**AAWES – Afusali Authentic Wine Ethanol Standard*

Table 4: Wine ethanol δD and δD_n values

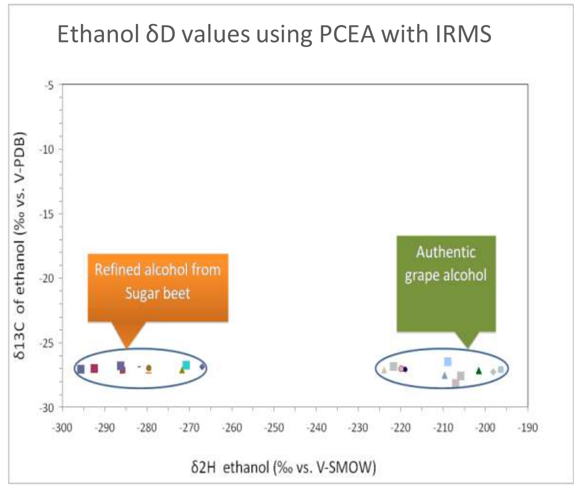


Chart 1: Graphical preview of ethanol δD values from Pyrolysis Conversion Elemental Analyzer coupled to IRMS

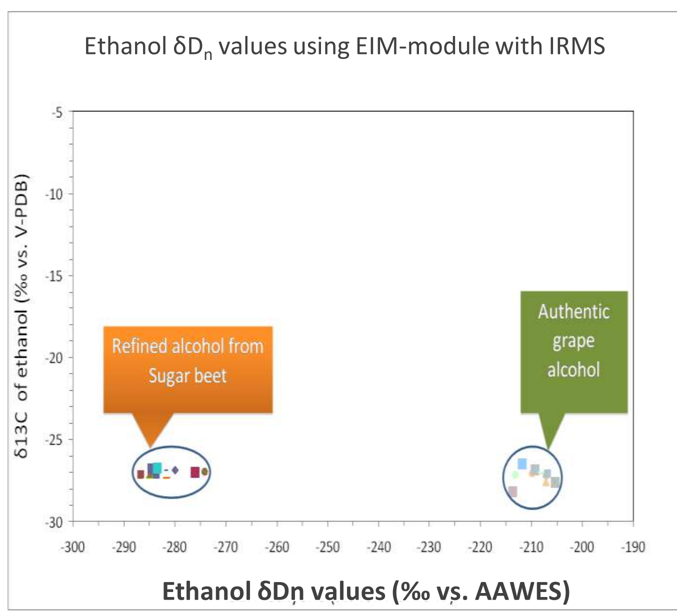


Chart 2: Graphical preview of ethanol δD_n values from EIM Module connected to Pyrolysis Conversion Elemental Analyzer coupled to IRMS

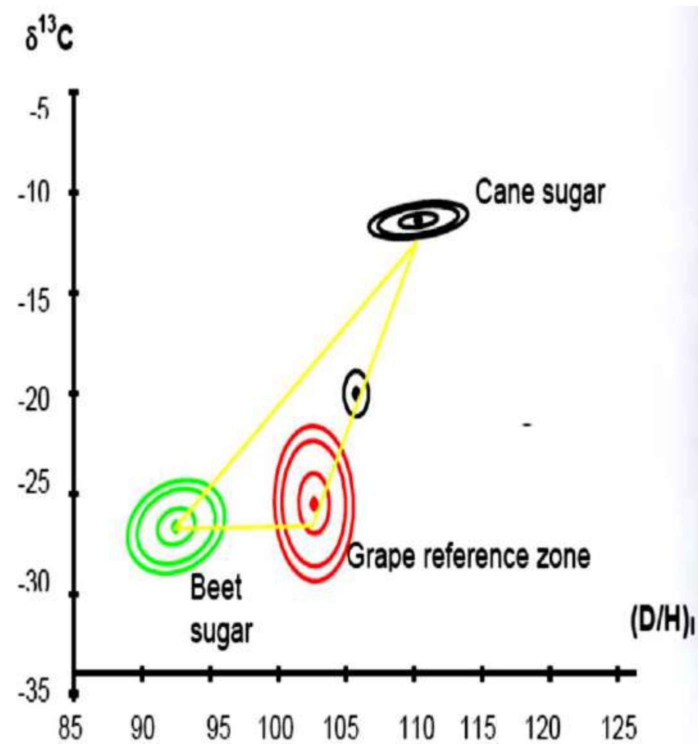
EIM-Module for Isotope Ratio MS – The missing link in Isotope Analysis

Detection of Adulteration in Known and Unknown Wine Samples

Comparison between Official OIV[1] Method OIV-MA-AS311-05:R2011 and EIM-Module-IRMS® method

Untill now IRMS equipment could not give the complete information on wine authenticity (presence of ethanol origining from agricultural C3 plants like beet sugar or addition of water), so NMR method (Chart 3), which is very demanding and time consuming, has been used.

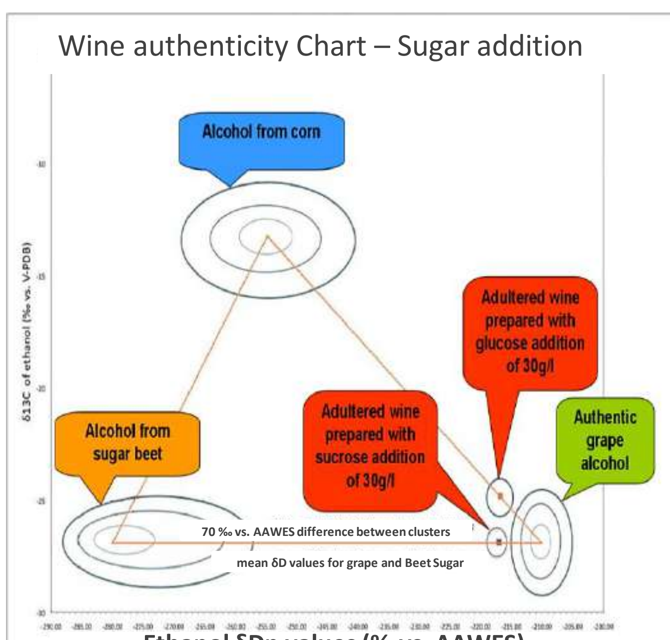
Chart 3



Now, compared to Official OIV NMR Method: OIV-MA-AS311-05:R2011 results (Chart 3), analytical results gained with new EIM-IRMS® technology expresses a much larger free space between clusters for beet sugar and grape (Chart 4 and 5), making for more accurate detection.

The difference between mean δD_n values for grape and beet sugar is ~70‰ vs. AAWES, and the distance between clusters is ~40‰ vs. AAWES (Afusali Authentic Wine Ethanol Standard)…

Chart 4



[1] OIV – International Organization of Vine and Wine

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Detection of Adulteration in Known and Unknown Wine Samples

…this enables for a much better comparison of samples, easier and more accurate detection of illegal practices in wine production (Chart 5).

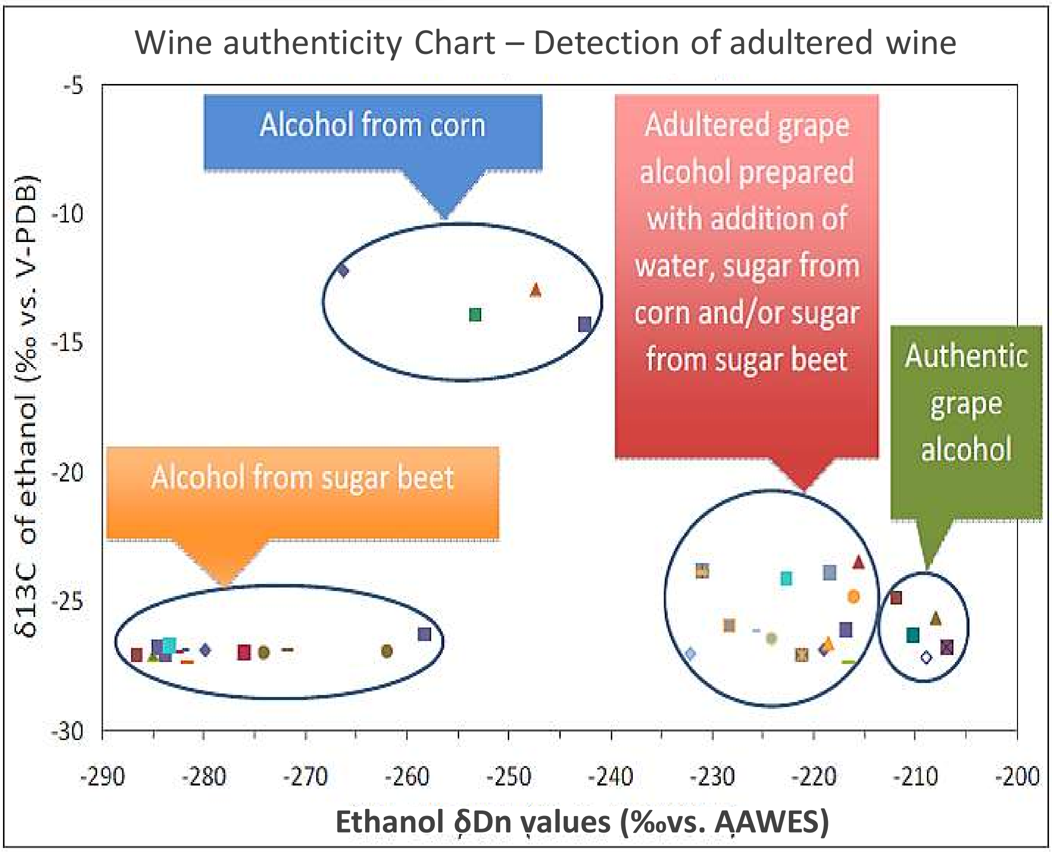


Chart 5

EIM module connected to Pyrolysis Conversion Elemental Analyzer coupled to Isotope Ratio MS gives repeatable and reliable ethanol δD_n values.

EIM-Module for Isotope Ratio MS - The missing link in Isotope Analysis

Detection of water addition to grape must prior to alcoholic fermentation

Currently, water detection is done by analyzing Oxygen isotope via Gas Bench directly from a wine sample. This method requires information about an authentic sample from a database in order to determine water addition with confidence (Figure 3).

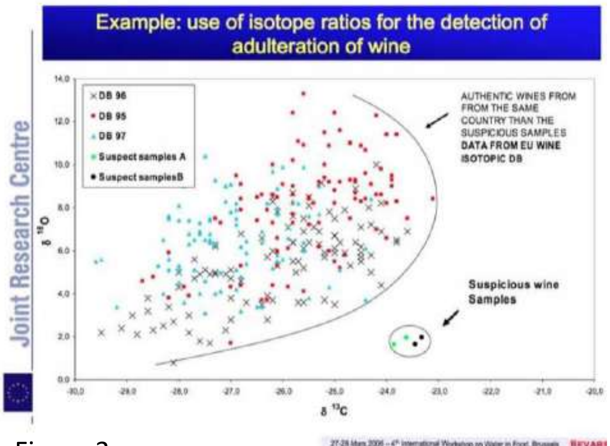


Figure 3

For determining addition of water in wine samples EIM-IRMS® utilizes information obtained from wine ethanol as it carries both information about addition of sugar **and** addition of water prior and during alcoholic fermentation (Chart 6) .

If water was added prior to alcoholic fermentation not only that $\delta^{18}O$ values of grape and wine water will be changed, but also Ethanol δD_n value would be changed, and become more negative (Chart 7).

For unknown samples, addition of water will be determined as illegal production practice (addition of water or addition of sugar).

For known wine samples, addition of water will be determined using Gas Bench .

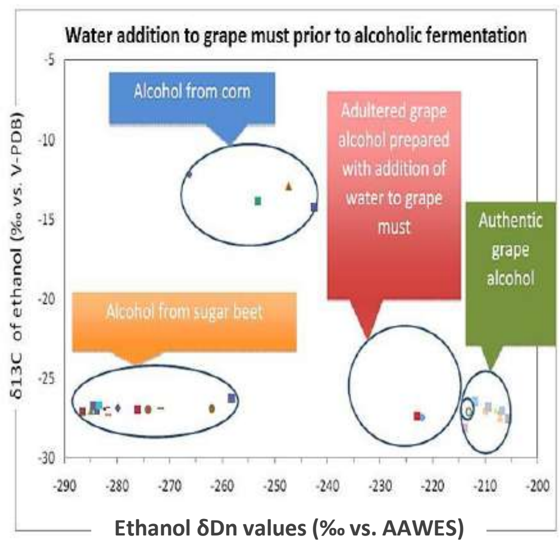


Chart 6

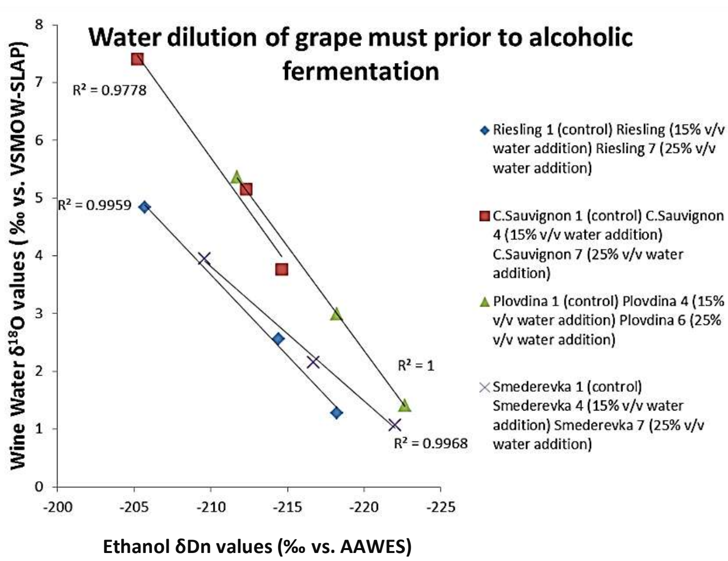


Chart 7

Acknowledgements

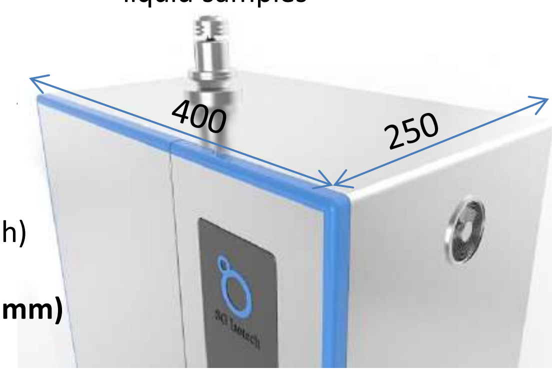
EIM-Module and EIM-IRMS® method has been validated through inter-laboratory testing between three laboratories: Imprint Analytics, Neutal (Austria), Izotoptech Zrt. at MTA Atomki Institute for Nuclear Research of the HAS - Hungarian Academy of Science, Debrecen (Hungary) and C.N.R.I.F.F.I. - China National Research Institute of Food and Fermentation Industries Corporation Limited, Beijing (People's Republic of China).
SG Isotech also thanks Prof. Jed P. Sparks, Kimberly L. Sparks and Cornell University Stable Isotope Laboratory (COIL), NY, United States, for cooperation and providing more online EIM – Module - IRMS® results.

The EIM Module can be connected to any current Pyrolysis Conversion Analyzer equipped for continuous flow application with Open Split universal interface coupled to Isotope Ratio MS.

Instrument Description

- ✓ Base unit EIM Module (one furnace) with temperature control display
- ✓ Water removal trap
- ✓ Software regulation of temperature and Helium flow
- ✓ Can be connected to AS3000, AI 1310 Liquid Autosampler or GC Pal Autosamplers for liquid samples

Gases
Helium: 99.999% purity
Power supply
230 V, 50/60 Hz, 1400 VA
Dimensions and Weight
400 x 250 x 500 mm (w x d x h)
15 kg (net value)
EIM Module Dimensions (in mm)



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