

EIM-IRMS VALIDATION REPORT

Place of installation:	Imprint Analytics GmbH, Technologiezentrum Mittelburgenland Bauteil A, 1. OG, Werner von Siemens Straße 1, A-7343 Neutal, Austria
Period of installation:	10.09.2018. – 20.09.2018.
Analytical equipment on which EIM Module was installed:	AI 1310 Liquid Autosampler – EIM Module – Flash 2000 – ConFloIV – Delta V Advantage IRMS (Isotope Ratio Mass Spectrometry)
Installation Engineer:	Ivan Smajlović, MSc
Persons representing the Client:	Dr. Bernd Bodiselitsch, General Manager Dr. David Psomiadis, Head of the Laboratory Dr. Balázs Horváth, Technical Director

EIM-Module – Flash 2000 -ConFloIV - IRMS method parameters:	1. [REDACTED]	
	2. [REDACTED]	
	3. [REDACTED]	
	4. [REDACTED]	
	5. [REDACTED]	
	6. [REDACTED]	

Ethanol wine samples previously prepared at C.N.R.I.F.F.I. (China National Research Institute for Food and Fermentation Industries Ltd.):

Sample	Sample number	Authentic	Dilution with water prior to alcoholic fermentation (20%v/v)	Addition of Beet sugar to grape must prior to alcoholic fermentation (30 g/L)	Addition of Beet sugar to grape must prior to alcoholic fermentation (20g/L)
Grape no.1 Sugar concentration: 125 g/L	DB73 - 01	✓			
	DB73 - 02			✓ (21 g of Beet sugar into 700mL of grape must)	
	DB73 - 03		✓ (140 mL of tap water + 560 mL of grape must)		
Grape no.2 Sugar concentration: 160 g/L	DB73 - 04	✓			
	DB73 - 05			✓ (29.4 g of Beet sugar into 700mL of grape must)	
	DB73 - 06		✓ (140 mL of tap water + 560 mL of grape must)		
	DB73 - 07				✓ (14 g of Beet sugar into 700mL of grape must)
Grape no.3 Sugar concentration: 120 g/L	DB73 - 08	✓			
	DB73 - 09			✓ (21 g into 700mL of Beet sugar grape must)	

	DB73 - 10		✓ (140 mL of tap water + 560 mL of grape must)		
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Ethanol samples from fermented orange juice samples previously prepared at C.N.R.I.F.I. (China National Research Institute for Food and Fermentation Industries Ltd.):

Sample	Sample number	FSOJ* (11.5°Bx)	Mixture of FSOJ* and ROJ** (25% - 75%)	Mixture of FSOJ* and ROJ** (50% - 50%)	Mixture of FSOJ* and ROJ** (75% - 25%)	ROJ** (11.5°Bx)
Orange juice	DB73 - 11	✓				
	DB73 - 12			✓		
	DB73 - 13				✓	
	DB73 - 14					✓
	DB73 - 15		✓			
	DB73 - 16	✓ (17.5g of Beet sugar to 700g FSJ*) Sugar concentration : 140 g/L				
	DB73 - 17					✓ (17.5 g of Beet sugar in 700g ROJ**) Sugar concentration: 140g/L
	DB73 - 18	✓				
	DB73 - 19	✓ (120g sugar syrup conc. 500g/L + 580g FSOJ*)				

* FSOJ - Freshly Squeezed Orange Juice

** ROJ - Reconstituted Orange Juice for the orange concentrate 65°Bx diluted with water up to 11.5°Bx

Ethanol wine samples prepared during method validation at Imprint Analytics GmbH, Neutal, Austria

183199 ; 12,0 %vol, White wine, dry, Welschriesling, Austria			
Sample	Distillation volume (mL)	Ethanol distillate volume (mL)	Alc. Strength (%vol)
A1	200	22	83,8
A2	200	22,5	93,2
A3	200	23	91,4
A4	200	17	91,952
183200 ; 10,5 %vol, Red wine, Cuvee, Hungary			
Sample	Distillation volume (mL)	Ethanol distillate volume (mL)	Alc. Strength (%vol)
A1	200	20	91,738
A2	200	18	92,864
A3	200	18	92,110
A4	200	17	90,697
183201 ; 12,5 %vol, Red wine, Portugieser, Hungary			
Sample	Distillation volume (mL)	Ethanol distillate volume (mL)	Alc. Strength (%vol)
A1	200	20	90,171
A2	200	18	93,592
A3	200	18	93,155

All prepared and fermented samples were distilled using distillation apparatus which was provided by SG Isotech and with possibility to quantitatively extract ethanol from fermented samples with recovery rate of more than 85% and alcoholic strength of more than 91% vol. and for the needs of isotopic testing using the EIM-IRMS method.

EIM-IRMS Results:

Reference material: AFUSALI - Afusali Authentic Wine Ethanol Standard (AAWES) with value -211.89‰ at SGI Scale

	Thursday		Friday		Saturday		Monday		Wednesday		Reference δD_n values (‰ at SGI Scale)
	13.09.2018.		14.09.2018.		15.09.2018.		17.09.2018.		19.09.2018.		
	Mean	St.Dev.	Mean	St.Dev.	Mean	St.Dev.	Mean	St.Dev.	Mean	St.Dev.	
DB73-01	-214.16	1.41	-214.96	0.76					-213.54	0.48	-205 - (-215) ¹⁾
DB73-02	-216.11	0.82	-215.56	1.76					-216.08	1.06	-205 - (-215) ¹⁾
DB73-03	-211.71*	1.28*	-218.71	1.28	-218.15	2.62			-218.05	1.20	-205 - (-215) ¹⁾
DB73-04	-211.71	0.50	-212.25	3.51	-211.64	1.62			-213.43	0.40	-205 - (-215) ¹⁾
DB73-05	-220.39	2.38	-220.41	0.80	-220.45	1.13			-219.85	1.44	-205 - (-215) ¹⁾
DB73-06	-215.60*	0.59*	-220.74	1.38	-219.78	2.44			-217.85	0.34	-205 - (-215) ¹⁾
DB73-07	-214.79	1.25	-214.37	0.43	-216.06	0.09			-216.21	1.12	-205 - (-215) ¹⁾
DB73-08**	-220.34	0.95	-221.14	0.52	-223.85	1.34			-224.32	0.90	-205 - (-215) ¹⁾
DB73-09	-220.27	0.55	-220.13	0.83	-221.89	2.55			-221.30	0.51	-205 - (-215) ¹⁾
DB73-10	-222.85	0.34	-223.71	0.75	-223.54	0.98			-224.58	0.87	-205 - (-215) ¹⁾
DB73-11	-206.69	1.35	-207.59	0.81			-206.63	0.80	-210.16	1.51	-205 - (-215) ¹⁾
DB73-12	-212.93	1.09	-211.27	0.60			-212.38	1.03	-212.20	0.49	-205 - (-215) ¹⁾
DB73-13	-209.99	2.54	-209.47	0.49			-209.62	1.28	-211.37	1.23	-205 - (-215) ¹⁾
DB73-14	-220.19	2.11	-221.07	0.74			-220.42	1.42	-221.58	1.70	-205 - (-215) ¹⁾
DB73-15	-213.08	1.53	-213.74	1.68			-213.10	0.48	-213.41	0.82	-205 - (-215) ¹⁾
DB73-16	-210.95	1.18	-211.80	0.74			-209.23	0.71	-212.37	0.98	-205 - (-215) ¹⁾
DB73-17	-230.54	0.74	-231.14	1.26			-231.58	1.18	-230.68	1.61	-205 - (-215) ¹⁾
DB73-18	-209.91	2.06	-209.43	0.55			-210.94	1.68	-212.85	2.52	-205 - (-215) ¹⁾
DB73-19	-221.33	0.52	-221.81	2.44			-220.24	1.08	-223.31	0.26	-205 - (-215) ¹⁾

* Not included in calculation (bad sampling)

**Sample replacement error

¹⁾Scientific paper: 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

Intralaboratory Repeatability and Reproducibility calculation:

Sample Number	Mean	Intralaboratory Reproducibility sR =(STDEV of all measurements of the same sample)	Repeatability sr = (STDEV I day + STDEV II day + STDEV III day + STDEV IV day)
DB73-01	-214.22	0.71	0.88
DB73-02	-215.92	0.31	1.21
DB73-03	-218.30	0.35	1.70
DB73-04	-212.26	0.83	1.51
DB73-05	-220.27	0.28	1.44
DB73-06	-219.46	1.47	1.39
DB73-07	-215.36	0.92	0.72
DB73-08	-222.41	1.97	0.93
DB73-09	-220.90	0.84	1.11
DB73-10	-223.67	0.71	0.73
DB73-11	-207.77	1.66	1.12
DB73-12	-212.19	0.69	0.80
DB73-13	-210.11	0.86	1.38
DB73-14	-220.81	0.63	1.49
DB73-15	-213.33	0.31	1.13
DB73-16	-211.09	1.37	0.90
DB73-17	-230.99	0.47	1.20
DB73-18	-210.78	1.52	1.70
DB73-19	-221.67	1.27	1.08
Average		0.90	1.18

Ethanol wine samples gained from wine distilled at Imprint Analytics, Neutal, Austria

	183199				183200				183201		
	A1	A2	A3	A4	A1	A2	A3	A4	A1	A2	A3
1	-211.56	-213.45	-213.39	-210.25	-208.80	-211.73	-209.58	-212.11	-207.33	-208.09	-208.73
2	-211.32	-214.79	-212.08	-209.91	-208.59	-209.53	-212.35	-208.57	-212.22	-210.00	-212.92
3	-210.33	-215.81	-212.94			-210.67	-212.33	-210.83	-210.47	-210.41	-213.48
4	-212.62		-213.75	-209.65	-210.86		-210.16			-208.28	
Average value (%o at SGI Scale)	-211.46	-214.68	-213.04	-209.93	-209.42	-210.64	-211.10	-210.50	-210.00	-209.20	-211.71
St.Dev.	0.94	1.18	0.72	0.30	1.25	1.10	1.45	1.80	2.48	1.18	2.60
Mean	-212.28				-210.42				-210.30		
Repeatability (sr)	0.79				1.40				2.08		
Reproducibility (sR)	1.88				1.39				2.13		
Average Repeatability (sr)					1.42						
Average Reproducibility (sR)					1.80						

* The accuracy of measurement is expressed by the precision limits of the repeatability and reproducibility which can be calculated from the sample standard deviation of the test results. The repeatability precision limit (r) and the reproducibility precision limit (R) are calculated as a second fold repeatability standard deviation (sr) or the second fold reproducibility standard deviation (sR).

$$r = 2 * 1.18 = 2.36$$

$$R = 2 * 0.90 = 1.80$$

The accuracy of the measurement can be summarized as:
Measurement accuracy δDn value in wine ethanol (‰ vs. AAWES)

- Average intralaboratory repeatability (sr) (as repeatability of measurements): 1.18 ‰
- Average intralaboratory reproducibility (sR) (as reproducibility of measurements): 0.90 ‰

Since the repeatability limit (r) of measurement is 2.36 ‰, this means that for the lower authenticity range limit value of -215 ‰ vs. AAWES the authenticity limit value (A.L.) is -217.36 ‰ vs. AAWES.

Note: The values listed in the table only apply to the results obtained during validation of EIM-IRMS method, operated by the SG Isotech engineer Ivan Smajlović. Preparation of all samples including alcoholic fermentation was done in the presence and assistance of Dr. Daobing Wang and Mr. Guanghao Wang from CNRIFFI in China.

Distillation of all samples from C.N.R.I.F.F.I. was done by Mr. Guanghao Wang and in accordance with the instructions from the SG Isotech engineer Ivan Smajlović.

Distillation of wine samples 183199, 183200 and 183201 at Imprint Analytics was done by Julia Zolles and in accordance with instruction from the SG Isotech engineer Ivan Smajlović.

Statement: We hereby confirm the successful validation of EIM-IRMS analytical method at the Imprint Analytics Laboratory, Neutal, Austria.

In Neutal,

SG Isotech DOO Pančevo,
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Republic of Serbia

Representative signature


Institutional stamp


Date: 21.09.2018.

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Date: 21.09.2018

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