

EIM-IRMS VALIDATION REPORT

Place of installation:	CHINA NATIONAL RESEARCH INSTITUTE OF FOOD AND FERMENTATION INDUSTRIES CORPORATION LIMITED, Building 6, No.24, Jiuxianqiao Middle Road, Chaoyang District, Beijing, China
Period of installation:	11.10.2018. – 03.11.2018.
Analytical equipment on which EIM Module was installed:	1300 Liquid Autosampler – EIM – TC EA – ConFloIV – Delta _{plus} XP IRMS (Isotope Ratio Mass Spectrometry)
Installation Engineer:	Ivan Smajlović, MSc
Persons representing the Client:	Zhong Qiding, PhD, Director Daobing Wang, PhD, Researcher Mrs. Hong Wei Yue

EIM-Module – Flash 2000 -ConFloIV - IRMS method parameters:	1.	
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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)		

Ethanol samples from fermented orange juice samples previously prepared at C.N.R.I.F.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

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

Ethanol δD_n values (‰ vs. AAWES at SGI Scale)													
	Tuesday		Thursday		Monday		Wednesday		Thursday		Saturday		Reference δD_n values (‰ vs. AAWES at SGI Scale)
	21.07.2018.		11.10.2018.		15.10.2018.		17.10.2018.		18.10.2018.		20.10.2018.		
	Mean	St.Dev.*	Mean	St.Dev.*	Mean	St.Dev.*	Mean	St.Dev.*	Mean	St.Dev.*	Mean	St.Dev.*	
DB73-01	-214.50	1.84	-215.00	0.66	-214.07	0.77	-214.68	1.13	-213.32	0.62	-215.78	0.20	-205 - (-215) ¹⁾
DB73-02 (DB73-08)**	-218.50	0.47	-216.60**	0.66	-215.51**	0.74	-215.37**	0.68	-214.77**	0.50	-216.57**	0.15	-205 - (-215) ¹⁾
DB73-03	-225.60	0.42	-218.87	0.41	-217.16	0.40	-217.28	1.03	-215.64	0.19	-217.81	1.57	-205 - (-215) ¹⁾
DB73-04	-212.40	0.70	-215.13	1.75	-212.07	0.44	-213.26	0.46	-213.11	0.62	-212.09	0.48	-205 - (-215) ¹⁾
DB73-05	-223.20	1.33	-222.49	0.72	-219.67	0.46	-220.38	0.53	-219.70	0.26	-219.36	0.15	-205 - (-215) ¹⁾
DB73-06	-225.30	0.80	-218.36	0.12	-215.68	0.47	-216.12	0.49	-216.37	0.35	-217.06	0.32	-205 - (-215) ¹⁾
DB73-07	-216.40	1.71	-219.03	1.08	-217.51	1.36	-216.05	0.79	-217.80	0.50	-217.83	0.57	-205 - (-215) ¹⁾
DB73-08 (DB73-02)**	-215.40	0.70	-221.64**	0.93	-220.64**	0.89	-220.89**	0.86	-220.69**	0.52	-220.27**	0.79	-205 - (-215) ¹⁾
DB73-09	-223.10	0.01	-220.87	0.97	-220.81	0.58	-221.07	0.60	-220.62	0.55	-220.71	0.42	-205 - (-215) ¹⁾
DB73-10	-220.20	1.23	-220.81	0.23	-221.08	0.53	-221.12	0.48	-220.62	0.77	-220.48	0.61	-205 - (-215) ¹⁾
DB73-11					-206.65	0.33							-205 - (-215) ¹⁾
DB73-12					-212.02	0.62							-205 - (-215) ¹⁾
DB73-13					-208.38	0.46							-205 - (-215) ¹⁾
DB73-14					-217.86	0.47							-205 - (-215) ¹⁾
DB73-15					-215.47	0.33							-205 - (-215) ¹⁾
DB73-16					-211.45	0.59							-205 - (-215) ¹⁾
DB73-17					-228.22	0.49							-205 - (-215) ¹⁾
DB73-18					-210.77	0.65							-205 - (-215) ¹⁾
DB73-19					-222.36	0.40							-205 - (-215) ¹⁾

*Repeatability standard deviation of minimum 4 injection runs of the same sample

**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



Ethanol δD_n values (‰ vs. AAWES at SGI Scale)									
	Sunday		Monday		Tuesday		Wednesday		Reference δD_n values (‰ vs. AAWES at SGI Scale)
	21.10.2018.		22.10.2018.		23.07.2018.		24.07.2018.		
	Mean	St.Dev.*	Mean	St.Dev.*	Mean	St.Dev.*	Mean	St.Dev.*	
DB73-01					-215.16	0.14			-205 – (-215) ¹⁾
DB73-02 (DB73-08)**					-216.77**	0.52			-205 – (-215) ¹⁾
DB73-03					-217.05	0.16			-205 – (-215) ¹⁾
DB73-04					-212.72	0.81			-205 – (-215) ¹⁾
DB73-05					-220.30	0.83			-205 – (-215) ¹⁾
DB73-06					-217.14	0.37			-205 – (-215) ¹⁾
DB73-07					-217.57	0.48			-205 – (-215) ¹⁾
DB73-08 (DB73-02)**					-220.64**	0.41			-205 – (-215) ¹⁾
DB73-09					-220.83	0.42			-205 – (-215) ¹⁾
DB73-10					-221.00	1.26			-205 – (-215) ¹⁾
DB73-11	-204.57	0.87	-208.21	0.76			-204.12	1.43	-205 – (-215) ¹⁾
DB73-12	-208.80	0.92	-212.43	0.67			-209.59	0.42	-205 – (-215) ¹⁾
DB73-13	-206.87	0.91	-209.80	0.94			-207.74	1.02	-205 – (-215) ¹⁾
DB73-14	-216.72	0.63	-217.62	0.80			-218.66	0.54	-205 – (-215) ¹⁾
DB73-15	-211.65	0.26	-213.80	0.76			-214.50	0.42	-205 – (-215) ¹⁾
DB73-16	-210.22	0.90	-209.45	0.30			-210.95	0.18	-205 – (-215) ¹⁾
DB73-17	-229.08	0.65	-229.58	0.64			-229.59	0.64	-205 – (-215) ¹⁾
DB73-18	-208.72	0.90	-209.03	0.76			-209.05	0.75	-205 – (-215) ¹⁾
DB73-19	-222.98	0.32	-222.42	0.40			-222.46	0.40	-205 – (-215) ¹⁾

*Repeatability standard deviation of minimum 4 injection runs of the same sample

**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	Ethanol δD_n mean value (% vs. AAWES at SGI Scale)	Intralaboratory Reproducibility standard deviation sR=(STDEV of all Measurements of the same sample)	Intralaboratory Repeatability stanard deviation sr=(Average STDEV for the same sample)
DB73-01	-214.64	0.79	0.76
DB73-02 (DB73-08)*	-216.30	1.23	0.53
DB73-03	-218.49	3.28	0.60
DB73-04	-212.97	1.06	0.75
DB73-05	-220.73	1.50	0.61
DB73-06	-218.01	3.33	0.43
DB73-07	-217.46	0.99	0.92
DB73-08 (DB73-02)*	-220.02	2.08	0.73
DB73-09	-221.14	0.87	0.63
DB73-10	-220.76	0.34	0.73
DB73-11	-205.89	1.90	0.85
DB73-12	-210.71	1.79	0.66
DB73-13	-208.20	1.23	0.83
DB73-14	-217.72	0.80	0.61
DB73-15	-213.85	1.62	0.44
DB73-16	-210.52	0.87	0.49
DB73-17	-229.12	0.64	0.60
DB73-18	-209.39	0.93	0.76
DB73-19	-222.55	0.29	0.38
Average		1.35	0.65

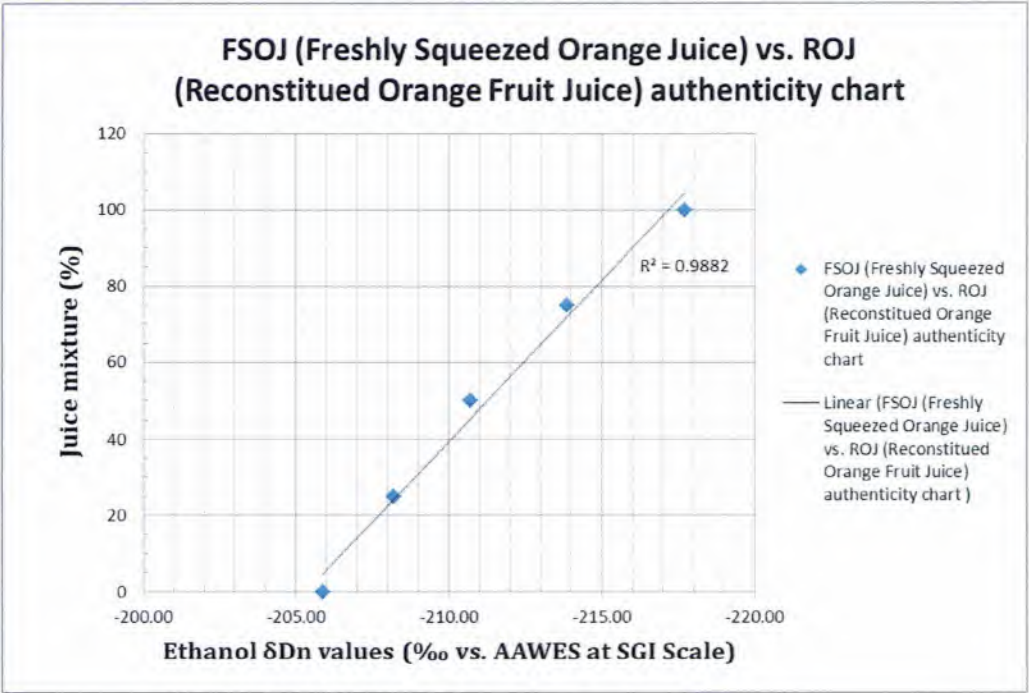
*Sample replacement error

FSOJ (Freshly squeezed orange juice) vs. ROJ (Reconstructed orange fruit juice) authenticity chart

Sample number	Mixture	Ethanol δD_n values (% vs. AAWES at SGI Scale)
DB73-11	100% FSOJ	-205.89
DB73-12	50% FSOJ + 50% ROJ	-210.71
DB73-13	75% FSOJ + 25% ROJ	-208.20
DB73-14	100% ROJ	-217.72
DB73-15	25% FSOJ + 75% ROJ	-213.85

* FSOJ - Freshly Squeezed Orange Juice

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



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).

$$r = 2.8 * sr = 1.82 \text{ ‰}$$

$$R = 2.8 * sR = 3.78 \text{ ‰}$$

The accuracy of the measurement can be summarized as:

Measurement accuracy δD_n value in wine ethanol (‰ vs. AAWES)

- Average intralaboratory repeatability (r) (as repeatability of measurements): 1.82 ‰
- Average intralaboratory reproducibility (R) (as reproducibility of measurements): 3.78‰

Since the repeatability limit (r) of measurement is 1.82 ‰, this means that for the lower authenticity range limit value of -215 ‰ vs. AAWES the authenticity limit value (A.L.) is -216.82 ‰ vs. AAWES. This means that if sample ethanol δD_n value falls in the range from -205 to -215‰ vs. AAWES we can consider it to be authentic and if its ethanol δD_n value is more negative than -216.82‰ vs. AAWES, we can consider that the sample was tempered with by addition of sugar and/or water prior to alcoholic fermentation.

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 with support and in the presence and assistance of Dr Daobing Wang and Mrs. Hong Wei Yue. Preparation of all samples including alcoholic fermentation was done previously at C.N.R.I.F.F.I. in the presence and assistance of Dr. Daobing Wang and Mr. Guanghao Wang from C.N.R.I.F.F.I. 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.

Statement: In accordance with observed results and calculated measurement uncertainty it is concluded that by using EIM-Module-IRMS we have successfully detected previously prepared adulterated samples:

- DB73-02 – Addition of Beet sugar (30g/L) to grape must prior to alcoholic fermentation;
- DB73-03 – Dilution of grape must with water (20%v/v) prior to alcoholic fermentation;
- DB73-05 – Addition of Beet sugar (30g/L) to grape must prior to alcoholic fermentation;
- DB73-06 – Dilution of grape must with water (20%v/v) prior to alcoholic fermentation;
- DB73-07 – Addition of Beet sugar (20g/L) to grape must prior to alcoholic fermentation;
- DB73-09 – Addition of Beet sugar (30g/L) to grape must prior to alcoholic fermentation;
- DB73-10 – Dilution of grape must with water (20%v/v) prior to alcoholic fermentation;
- DB73-14 – ROJ – Reconstituted Orange Juice for the orange concentrate 65°Bx diluted with water up to 11.5°Bx;
- DB73-17 – ROJ – Reconstituted Orange Juice for the orange concentrate 65°Bx diluted with water up to 11.5°Bx + 25g/L of Beet Sugar;
- DB73-19 - Freshly Squeezed Orange Juice with addition of beet sugar syrup


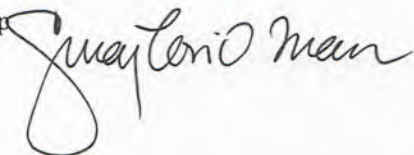
We hereby confirm the successful validation of EIM-IRMS analytical method at the C.N.R.I.F.F.I. - China National Research Institute of Food And Fermentation Industries Corporation Limited, Location: Building 6, No.24, Jiuxianqiao Middle Road, Chaoyang District, Beijing, China

In Beijing,

SG Isotech DOO Pančevo,
Maksima Gorkog 58/C, 26000 Pančevo,
Republic of Serbia

Representative signature

Institutional stamp
Date: 04.11.2018.




C.N.R.I.F.F.I. - China National Research Institute of Food
And Fermentation Industries Corporation Limited,
National Standardization Center of Food&Fermentation
Industry

Location: Building 6, No.24, Jiuxianqiao Middle Road,
Chaoyang District, Beijing, China

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Institutional stamp

Date: 04.11.2018



Handwritten signature of Zhong Qiding