Congreso Mundial
DE LA VIÑA
Y EL VINO
5 - 9 de Junio 2023
España
Cádiz / Jerez

44th World Congress of Vine and Wine

OIV
International Organisation
of Vine and Wine
Intergovernmental Organisation

The intelligent use of ethanol for direct determination of methanol in wines

4. SESSION FOOD SAFETY, CONSUMPTION, NUTRITION AND HEALTH:
Information Technology for Sustainability and Food Safety. (Blockchain. From production to consumer)”

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OIV, State and Interstate Standards for the determination of volatile compounds, including methyl alcohol, in Spirituous Beverages of Viti-Vinicultural Origin

GB/T 11858-2009
GB/T 15038-2008
GB 5009.266-2016
GB/T 10781-2021

BIS IS 3752:2005(R2009)

Commission Regulation (EC) No. 2870/2000

AOAC Official Methods 972.10/11, 2005

Norma Mexicana NMX-V-005-NORMEX-2018

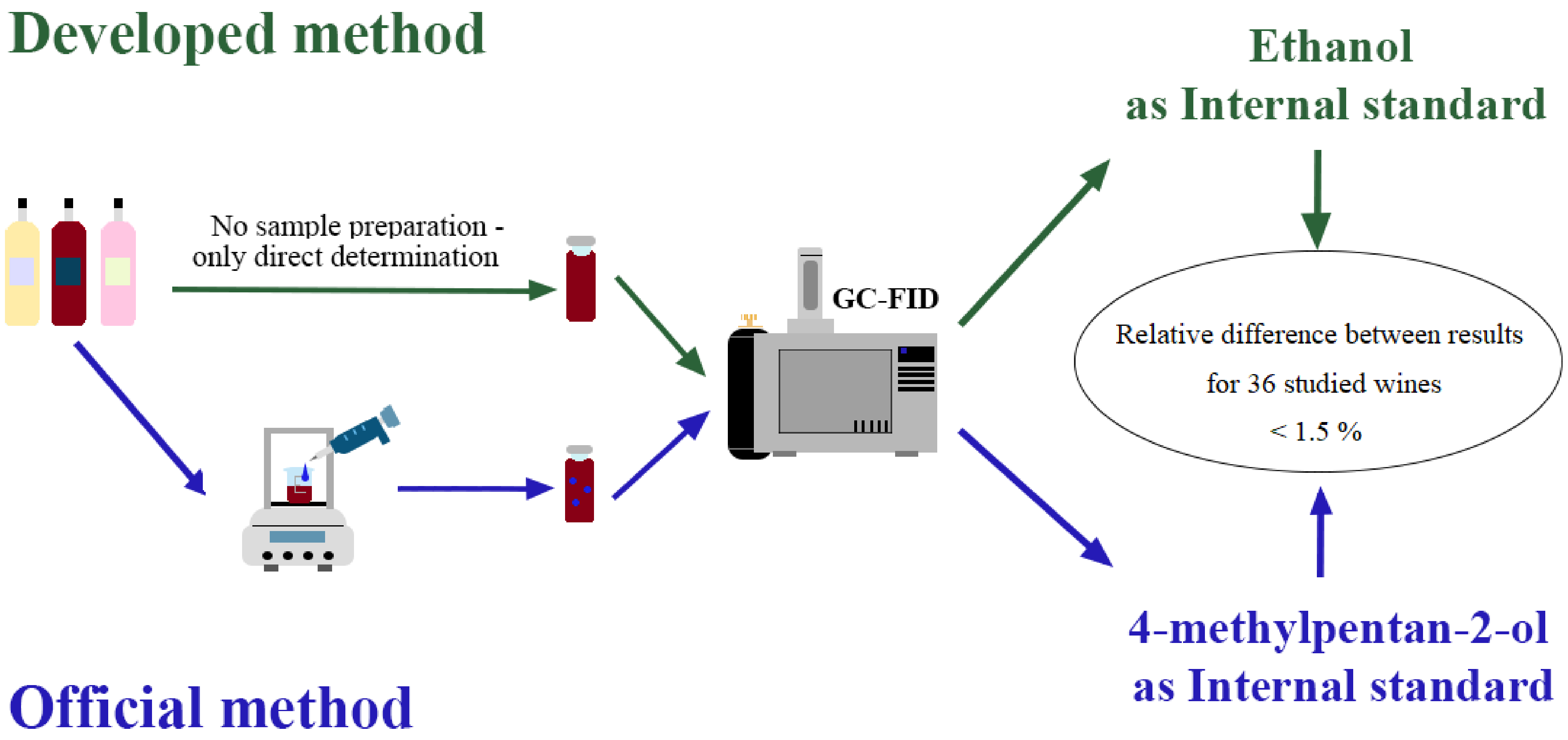
IV-MA-AS312-03A : R2015
IV-MA-BS-14 : R2009
IV-MA-AS315-27 : 2016

The traditional internal standard method is used in all above listed standards.

This method requires a manual procedure for adding an internal standard substance to the test sample.

In order to eliminate the manual procedure for addition an internal standard substance into the test sample, to increase the reliability of the experimental data obtained, to reduce the cost and reduce the analysis time, a modified internal standard method using ethyl alcohol, which is directly in the analyzed sample, is proposed as an internal standard.

The analysis of wines and other alcoholic beverages can be faster, simpler and more robust



Developed method

No sample preparation - only direct determination

GC-FID

Ethanol as Internal standard

Relative difference between results for 36 studied wines < 1.5 %

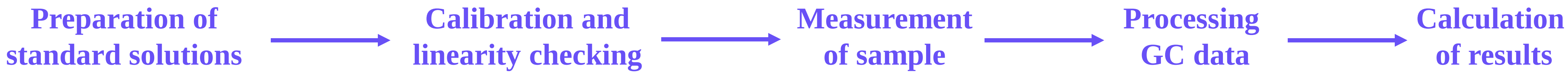
4-methylpentan-2-ol as Internal standard

Official method

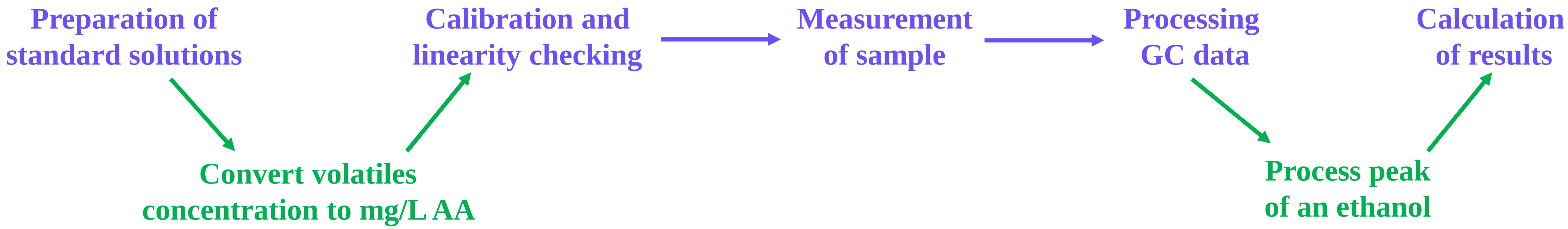
Method Validation Algorithms

In accordance with the requirements of **ISO/IEC 17025**, the method must be validated before its application in routine laboratory practice.

It is important to notice that the proposed method can be validated based on data obtained experimentally during validation of traditional method **EC2870/2000**. No more measurements except those indicated in **EC2870/2000**.

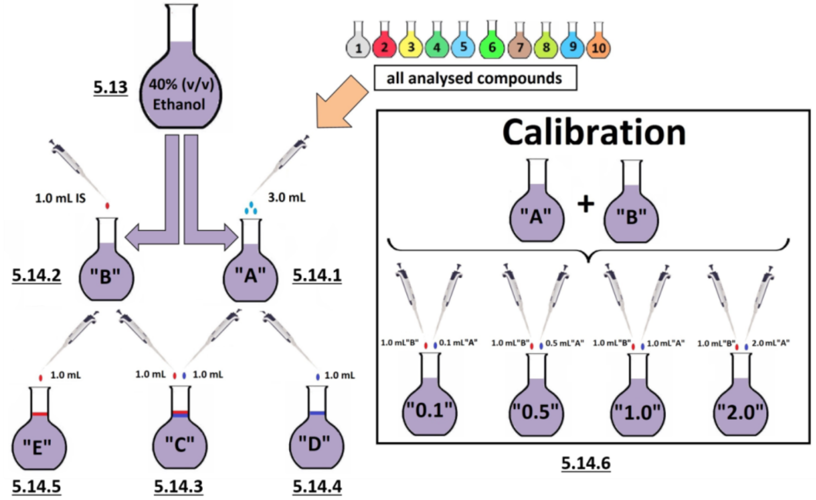


There is the following algorithm for the preparation of synchronous dual reports of GC measurements performed in accordance with the **EC 2870/2000** and according to the proposed method “**Ethanol is Internal Standard**”.



1. Prepare standard solutions of volatile compounds in accordance with EC 2870/2000

Compound	**Approximate concentration, mg/L AA**								
	"A"	**"B"**	**"C"**	**"D"**	**"E"**	**"0.1"**	**"0.5"**	**"1.0"**	**"2.0"**
Acetaldehyde	60000		600.0	600.0		60.0	300.0	600.0	1200.0
Ethyl acetate	60000		600.0	600.0		60.0	300.0	600.0	1200.0
Acetal	60000		600.0	600.0		60.0	300.0	600.0	1200.0
Methanol	60000		600.0	600.0		60.0	300.0	600.0	1200.0
2-butanol	60000		600.0	600.0		60.0	300.0	600.0	1200.0
ethanol	**789270**		**789270**	**789270**		**789270**	**789270**	**789270**	**789270**
1-propanol	60000		600.0	600.0		60.0	300.0	600.0	1200.0
2-methyl-1-propanol	60000		600.0	600.0		60.0	300.0	600.0	1200.0
1-butanol	60000		600.0	600.0		60.0	300.0	600.0	1200.0
2-methyl-1-butanol	60000		600.0	600.0		60.0	300.0	600.0	1200.0
3-methyl-1-butanol	60000		600.0	600.0		60.0	300.0	600.0	1200.0
3-pentanol (ISTD)		**60000**			**600.0**	**600.0**	**600.0**	**600.0**	**600.0**



! How to convert concentration to mg/L AA

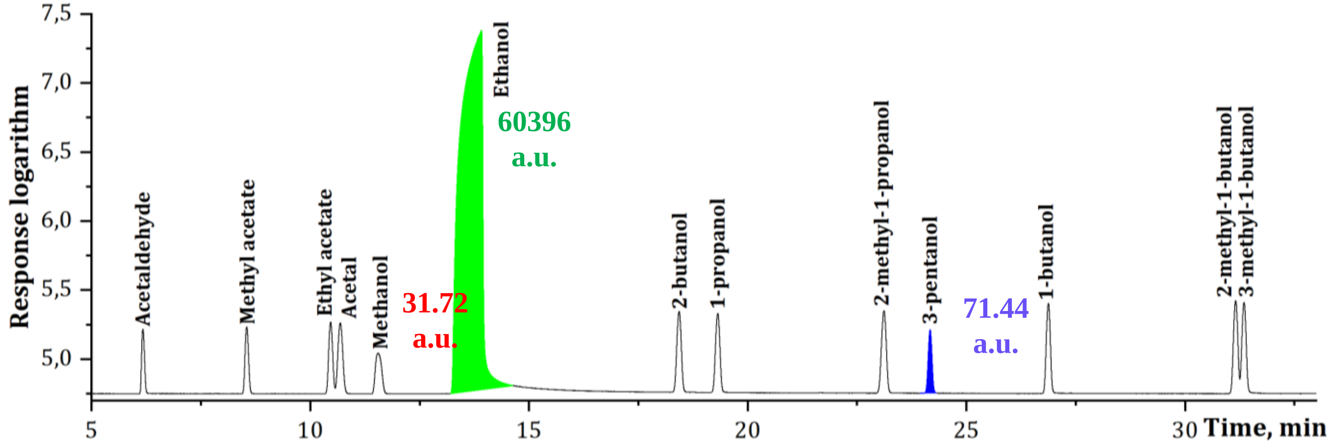
Use it for mg/kg

$$C_i\ (mg/L\ AA) = \frac{C_i\ (mg/kg) \cdot \rho_{sample} (kg/L)}{strength(\%\ v/v)} \cdot 100(\%)$$

Use it for mg/L

$$C_i\ (mg/L\ AA) = \frac{C_i\ (mg/L)}{strength(\%\ v/v)} \cdot 100(\%)$$

2. Measure the standard solution “C” to calculate factors $RRF_i^{\,3\text{-pentanol}}$ and $RRF_i^{\,Ethahol}$



Density of ethanol is constant

$$RRF_i^{Ethanol} = \frac{C_i^{st}\,(mg/L\ AA)}{A_i^{st}\,(a.\,u.)} \Big/ \frac{\rho_{Ethanol}\,(mg/L)}{A_{Ethanol}^{st}\,(a.\,u.)} = \frac{481.47}{31.72} \Big/ \frac{\mathbf{789270}}{60396} = \mathbf{1.161}$$

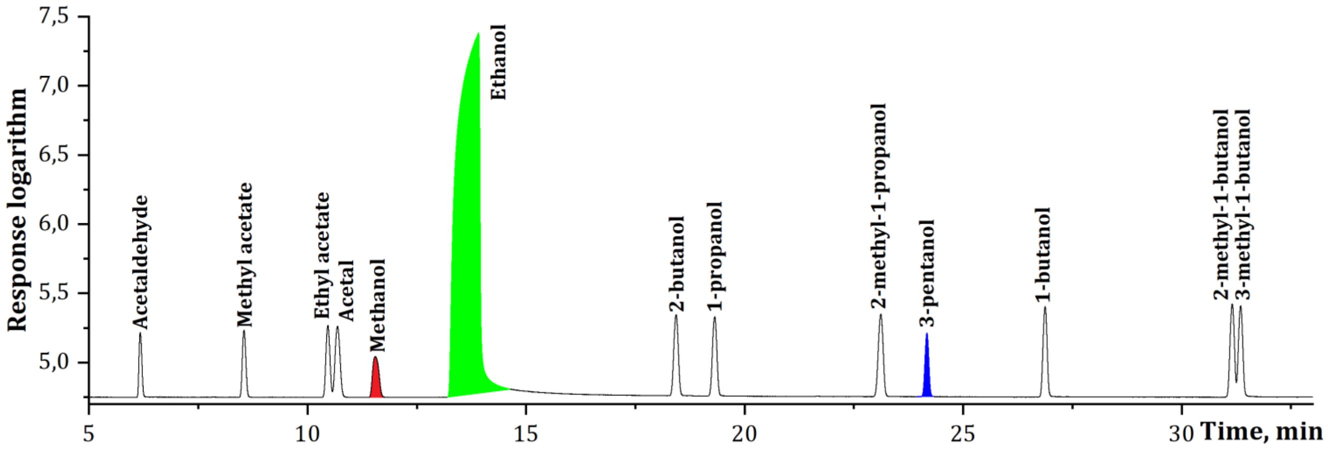
$$RRF_i^{3\text{-}pentanol} = \frac{C_i^{st}\,(\mu g/g)}{A_i^{st}\,(a.\,u.)} \Big/ \frac{C_{3\text{-}pentanol}^{st}\,(\mu g/g)}{A_{3\text{-}pentanol}^{st}\,(a.\,u.)} = \frac{243.32}{31.72} \Big/ \frac{261.28}{71.44} = 2.097$$

$$\left[\begin{array}{l} C_i^{st}\,(mg/L\ AA) = 481.47\ mg/L\ AA \\ \rho_{Ethanol}\,(mg/L\ AA) = 789270\ mg/L \\ A_i^{st}\,(a.\,u.) = 31.72\ a.\,u. \\ A_{Ethanol}^{st}\,(a.\,u.) = 60396\ a.\,u. \end{array}\right] \longleftarrow$$

Data from the preparation of
standard solution “C”

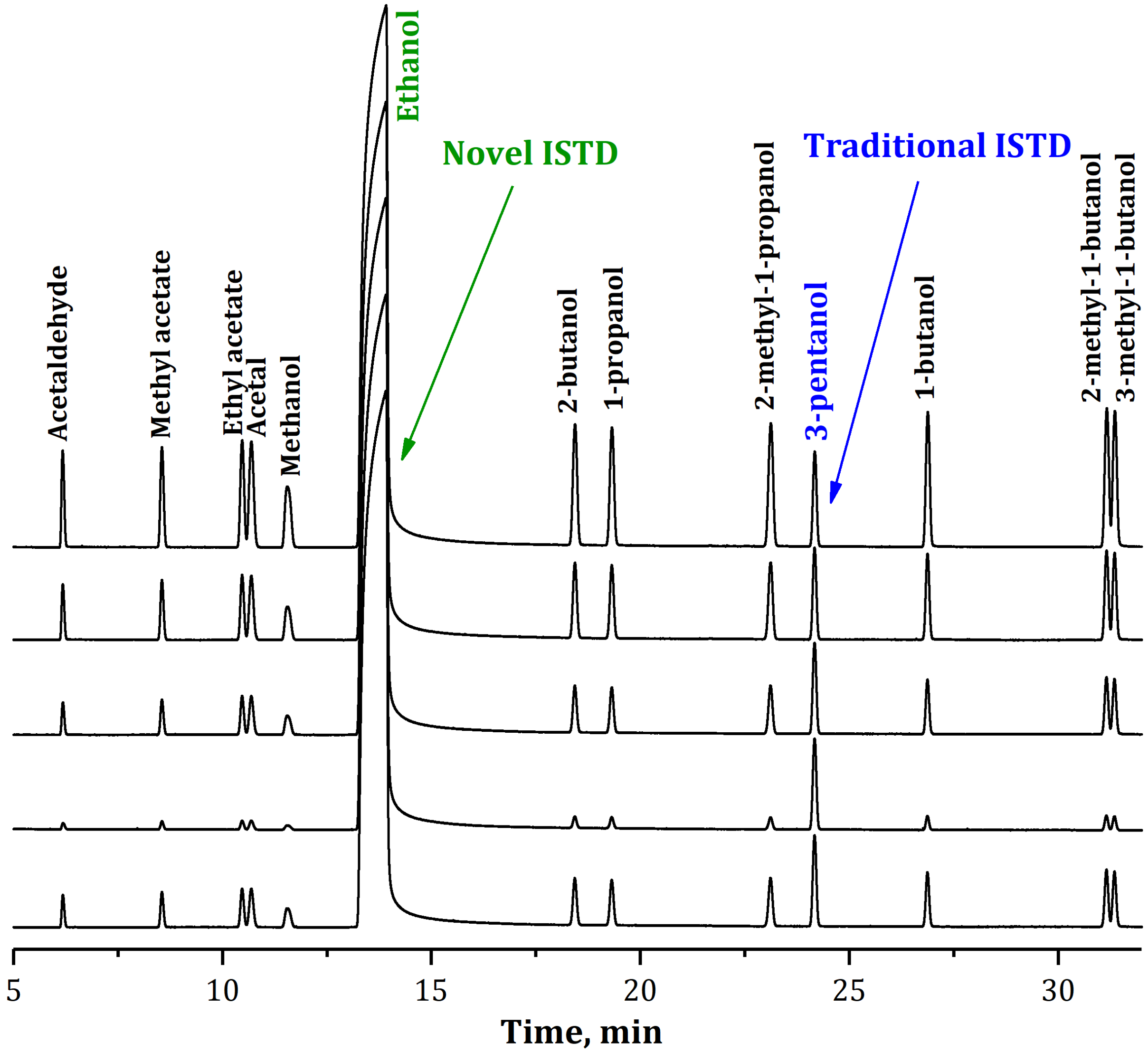
$$\longrightarrow \left[\begin{array}{l} C_i^{st}\,(\mu g/g) = 243.32\ \mu g/g \\ C_{3\text{-}pentanol}^{st}\,(\mu g/g) = 261.28\ \mu g/g \\ A_i^{st}\,(a.\,u.) = 31.72\ a.\,u. \\ A_{3\text{-}pentanol}^{st}\,(a.\,u.) = 71.44\ a.\,u. \end{array}\right]$$

3. Measure the standard solution “C” to calculate factors $RRF_i^{\text{3-pentanol}}$ and RRF_i^{Ethahol}

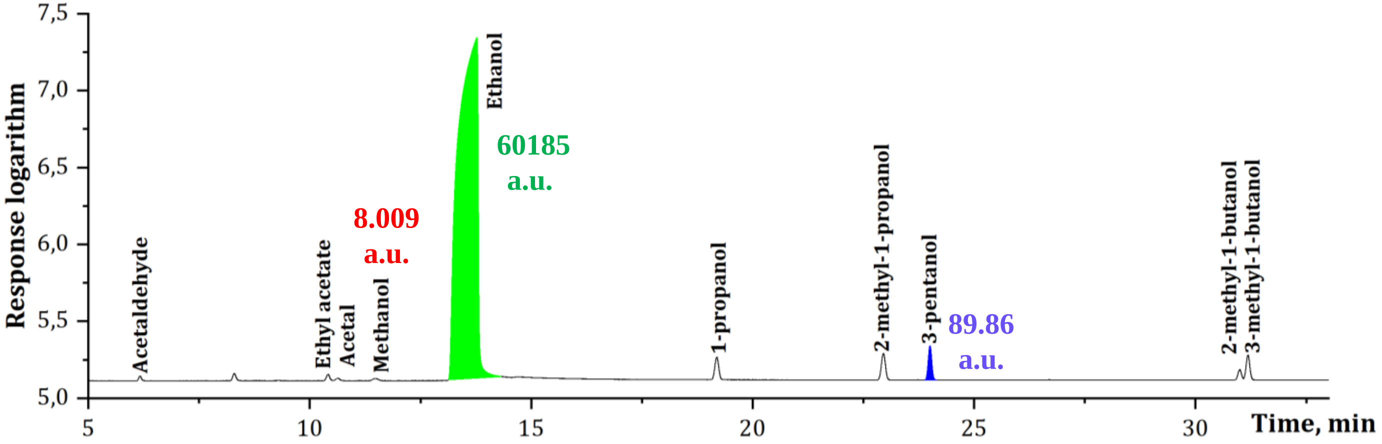


No	Compound	Time, min	Response, a.u.	C, mg/L AA	C, mg/L	C, mg/kg	$RRF^{\text{3-pentanol}}$	RRF^{Ethanol}
1	Acetaldehyde	6.177	26.548	428.25	171.30	219.05	**2.150**	**1.234**
2	Methyl acetate	8.552	34.498	682.51	273.00	292.51	**2.637**	**1.514**
3	Ethyl acetate	10.472	43.492	616.51	246.60	273.55	**1.889**	**1.085**
4	Acetal	10.692	52.283	552.98	221.19	265.98	**1.410**	**0.809**
5	Methanol	11.554	31.720	481.41	192.56	243.32	**2.097**	**1.161**
6	**Ethanol**	**13.915**	**60396.000**	**789270**			**1.742**	**1.000**
7	2-butanol	18.438	60.212	497.89	199.16	246.78	**1.102**	**0.633**
8	1-propanol	19.321	57.201	484.06	193.62	240.80	**1.128**	**0.648**
9	2-methyl-1-propanol	23.122	66.381	474.36	189.75	236.59	**0.952**	**0.547**
10	**3-pentanol**	**24.173**	**71.437**	**536.02**	**214.41**	**261.28**	**1.000**	**0.574**
11	1-butanol	26.878	65.238	496.77	198.71	245.20	**1.015**	**0.583**
12	2-methyl-1-butanol	31.161	73.344	514.01	205.60	251.17	**0.934**	**0.536**
13	3-methyl-1-butanol	31.354	69.622	492.71	197.08	243.64	**0.943**	**0.542**

4. Measure the standard solutions “0.1”, “0.5”, “1.0”, “2.0” for linearity checking



5. Measure the alcoholic beverage sample



$$C_i^{sample}(mg/L\ AA) = RRF_i^{Ethanol}\cdot\rho_{Ethanol}(mg/L)\cdot\frac{A_i^{sample}(a.u.)}{A_{Ethanol}^{sample}(a.u.)} = 1.161\times 789270\times\frac{8.009}{60185} = 121.9\ mg/L\ AA = 12.19\ g/hL\ AA$$

Done!

$$C_i^{sample}(\mu g/g) = RRF_i^{3\text{-}pentanol}\cdot\frac{M_{IS}(g)}{M_{Sample}(g)}\cdot C_{3\text{-}pentanol}\ (\mu g/g)\cdot\frac{A_i^{sample}(a.u.)}{A_{3\text{-}pentanol}^{sample}(a.u.)} = 2.097\times\frac{1.0069}{9.8812}\times 2736.9\times\frac{8.009}{89.86} = 52.12\ \underline{\mu g/g}$$

Need to be continued…

$$C_i^{sample}(g/L\ \mathrm{AA}) = C_i^{sample}(\mu g/g)\cdot\frac{\rho^{sample}(kg/m^3)\cdot 10}{strength(\%\ \mathrm{ABV})\cdot 1000} = 52.12\cdot\frac{948\cdot 10}{40.01\cdot 1000} = 12.35\ g/L\ \mathrm{AA}$$

$$\left[\begin{array}{l} M_{IS}(g) = 1.0069\ g \\ M_{Sample}(g) = 9.7512\ g \\ C_{3\text{-}pentanol}(\mu g/g) = 2736.9\ \mu g/g \\ strength(\%\ ABV) = 40\ \%\ ABV \\ \rho^{sample}\ (kg/m^3) = 948\ kg/m^3 \end{array}\right] \leftarrow$$

Data from the preparation of the alcoholic beverage sample

Relative difference

$$\frac{C_i^{sample}(g/hL\ AA) - C_i^{sample}(g/hL\ AA)}{C_i^{sample}(g/hL\ AA)}\cdot 100\ \% = \frac{12.35 - 12.19}{12.35}\cdot 100\ \% = 1.3\ \%$$

Experimental studies of 36 wine samples

Results of distillation

12 red wines

Type of wine	Sample No.	Strength of sample, % ABV	
		Declared*	Experimental**
dry	1	14.0	14.03 ± 0.03
	2	13.5	13.54 ± 0.03
	3	14.0	13.97 ± 0.03
semi-dry	4	13.5	13.58 ± 0.03
	5	13.5	13.53 ± 0.03
	6	14.5	14.48 ± 0.03
semi-sweet	7	11.0	10.96 ± 0.02
	8	13.0	12.97 ± 0.03
	9	14.0	13.96 ± 0.03
sweet	10	13.5	13.45 ± 0.03
	11	12.0	12.04 ± 0.02
	12	9.0	8.96 ± 0.02

12 white wines

Type of wine	Sample No.	Strength of sample, % ABV	
		Declared*	Experimental**
dry	13	12.5	12.53 ± 0.03
	14	12.0	12.04 ± 0.02
	15	13.5	13.47 ± 0.03
semi-dry	16	11.5	11.50 ± 0.02
	17	12.5	12.53 ± 0.03
	18	11.5	11.52 ± 0.02
semi-sweet	19	10.0	10.02 ± 0.02
	20	12.5	12.46 ± 0.02
	21	12.0	12.01 ± 0.02
sweet	22	13.0	13.03 ± 0.03
	23	8.2	8.19 ± 0.02
	24	12.5	12.43 ± 0.02

12 pink wines

Type of wine	Sample No.	Strength of sample, % ABV	
		Declared*	Experimental**
dry	25	11.5	11.54 ± 0.02
	26	13.0	13.04 ± 0.03
	27	13.5	13.53 ± 0.03
semi-dry	28	13.0	12.97 ± 0.03
	29	10.5	10.45 ± 0.02
	30	10.5	10.53 ± 0.02
semi-sweet	31	12.0	12.05 ± 0.02
	32	12.0	12.01 ± 0.02
	33	11.5	11.46 ± 0.02
sweet	34	9.5	9.53 ± 0.02
	35	11.5	11.52 ± 0.02
	36	11.0	10.95 ± 0.02

Declared – the value of the strength, declared by the manufacturer

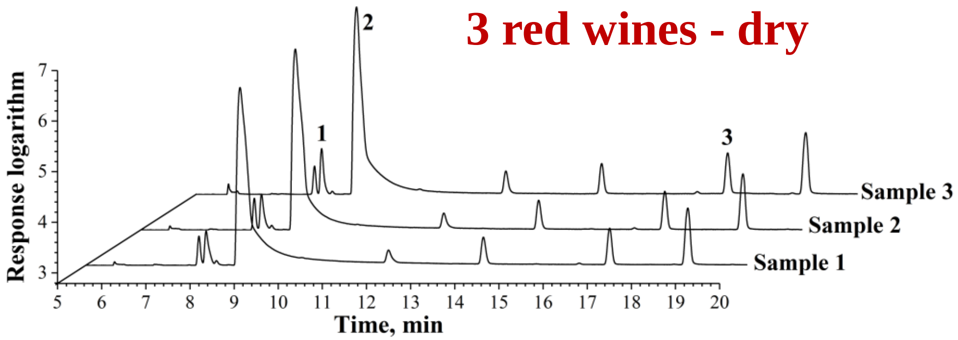
The 36 wine samples were distilled according to item 3 of OIV-MA-AS312-01A

Experimental – the value of the strength, obtained according to the item 4 of OIV-MA-AS312-01A “Alcoholic strength by volume”

The difference between obtained results for wine samples < 0.3 %

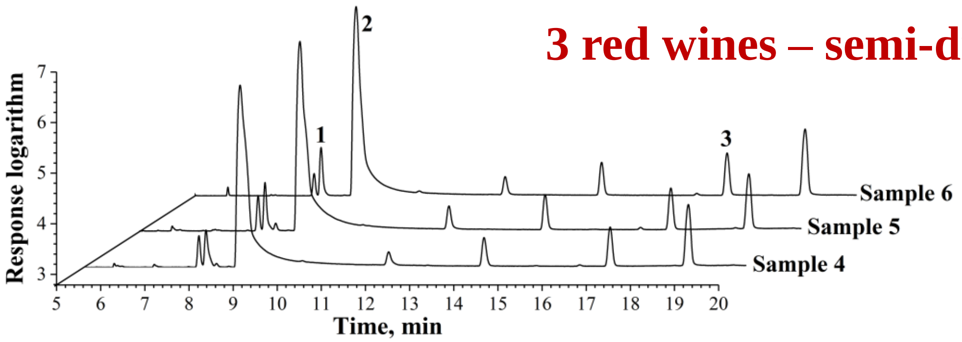
The difference between methods: 12 red wines

3 red wines - dry



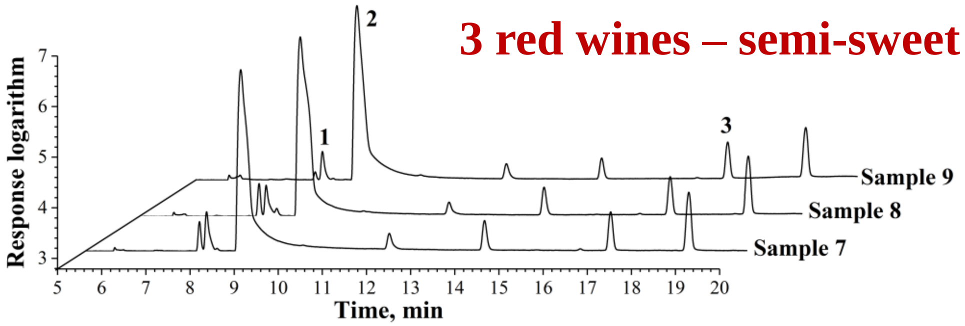
Sample no.	Concentration of methanol, *C** ± *SD*, mg/100 mL AA		Δ, %	Student's test		ANOVA	
	Developed method	**Official OIV method**		*p*	*t*	*p*	*F*
1	**120.2 ± 0.3**	**119.1 ± 1.6**	0.9	0.28	1.48	0.29	1.50
2	**99.4 ± 1.5**	**100.0 ± 1.1**	-0.6	0.72	0.42	0.59	0.35
3	**105.8 ± 0.1**	**105.7 ± 0.5**	0.1	0.70	0.44	0.65	0.24

3 red wines – semi-dry



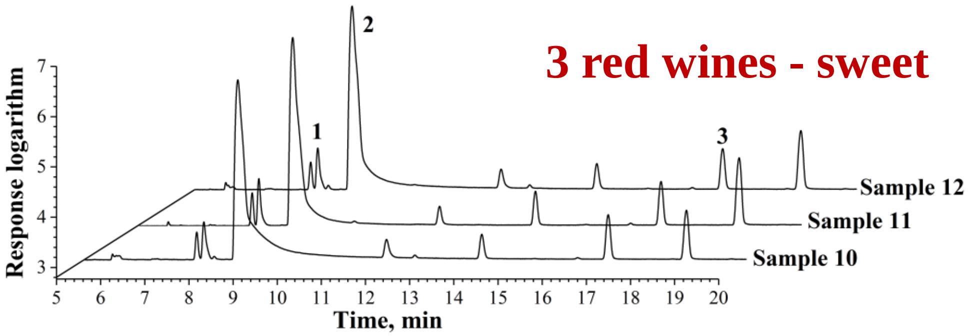
Sample no.	Concentration of methanol, *C** ± *SD*, mg/100 mL AA		Δ, %	Student's test		ANOVA	
	Developed method	**Official OIV method**		*p*	*t*	*p*	*F*
4	**120.7 ± 1.9**	**119.0 ± 2.4**	1.4	0.12	2.67	0.39	0.95
5	**119.9 ± 1.2**	**118.9 ± 1.8**	0.8	0.12	2.67	0.49	0.56
6	**100.4 ± 0.4**	**99.3 ± 0.8**	1.2	0.22	1.75	0.08	5.30

3 red wines – semi-sweet



Sample no.	Concentration of methanol, *C** ± *SD*, mg/100 mL AA		Δ, %	Student's test		ANOVA	
	Developed method	**Official OIV method**		*p*	*t*	*p*	*F*
7	**84.1 ± 0.2**	**84.5 ± 0.6**	-0.5	0.20	1.90	0.29	1.50
8	**82.5 ± 1.5**	**82.6 ± 1.2**	-0.2	0.82	0.26	0.87	0.03
9	**117.4 ± 0.4**	**117.4 ± 2.0**	-0.1	0.95	0.06	0.94	0.01

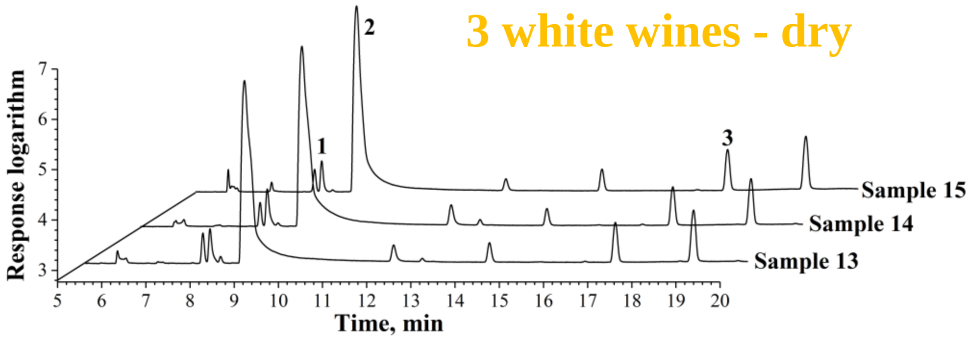
3 red wines - sweet



Sample no.	Concentration of methanol, *C** ± *SD*, mg/100 mL AA		Δ, %	Student's test		ANOVA	
	Developed method	**Official OIV method**		*p*	*t*	*p*	*F*
10	**113.1 ± 1.0**	113.7 ± 1.6	-0.5	0.38	1.11	0.61	0.30
11	**117.8 ± 1.4**	117.4 ± 1.7	0.4	0.63	0.56	0.74	0.12
12	**112.7 ± 0.2**	113.2 ± 1.1	-0.4	0.53	0.75	0.52	0.50

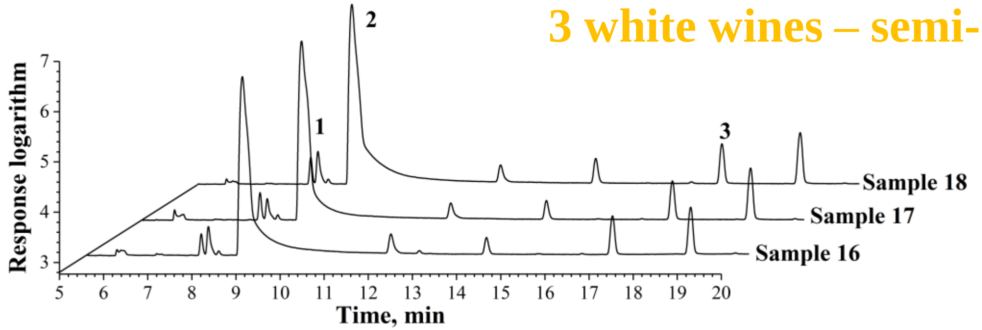
The difference between methods: 12 white wines

3 white wines - dry



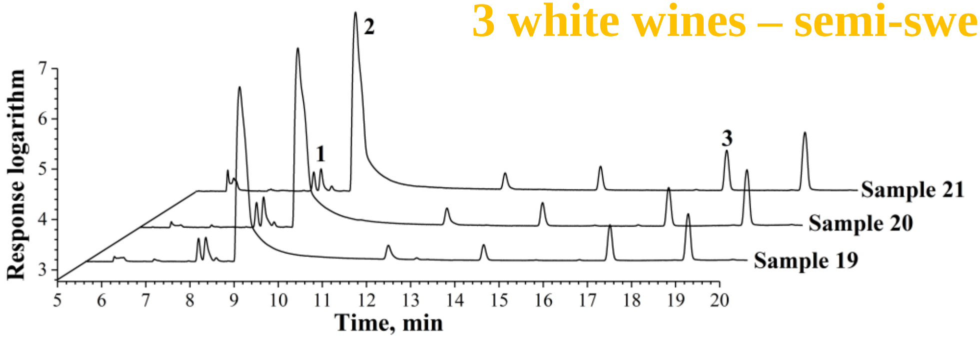
Sample no.	Concentration of methanol, $C^* \pm SD$, mg/100 mL AA		Δ, %	Student's test		ANOVA	
	Developed method	**Official OIV method**		*p*	*t*	*p*	*F*
13	**54.4 ± 0.7**	**53.9 ± 0.8**	0.9	0.10	2.87	0.45	0.71
14	**107.1 ± 0.4**	**107.3 ± 0.4**	-0.1	0.61	0.59	0.68	0.20
15	**80.0 ± 1.0**	**80.1 ± 0.9**	-0.2	0.84	0.22	0.86	0.04

3 white wines – semi-dry



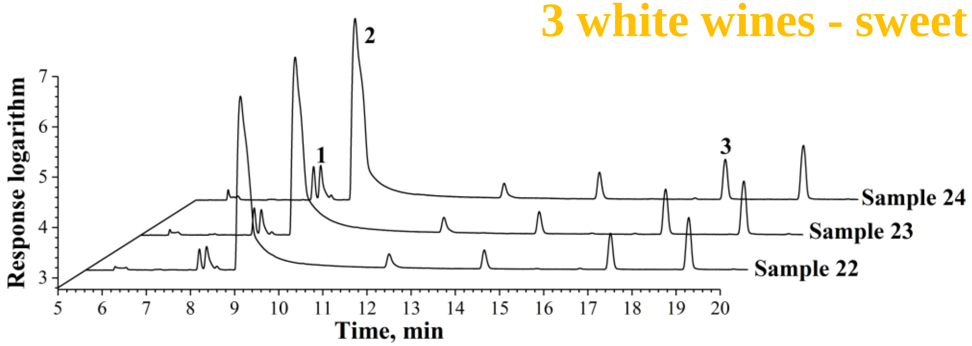
Sample no.	Concentration of methanol, $C^* \pm SD$, mg/100 mL AA		Δ, %	Student's test		ANOVA	
	Developed method	**Official OIV method**		*p*	*t*	*p*	*F*
16	**84.4 ± 1.5**	**84.5 ± 1.1**	-0.2	0.69	0.46	0.91	0.02
17	**42.3 ± 0.3**	**42.6 ± 0.3**	-0.7	0.46	0.91	0.29	1.45
18	**69.5 ± 0.1**	**69.9 ± 0.5**	-0.6	0.17	2.10	0.22	2.14

3 white wines – semi-sweet



Sample no.	Concentration of methanol, $C^* \pm SD$, mg/100 mL AA		Δ, %	Student's test		ANOVA	
	Developed method	**Official OIV method**		*p*	*t*	*p*	*F*
19	**44.6 ± 0.5**	**44.6 ± 0.7**	0.1	0.93	0.10	0.91	0.02
20	**74.3 ± 0.2**	**73.6 ± 1.1**	1.0	0.33	1.27	0.34	1.17
21	**63.2 ± 0.1**	**63.4 ± 0.9**	-0.3	0.73	0.40	0.75	0.12

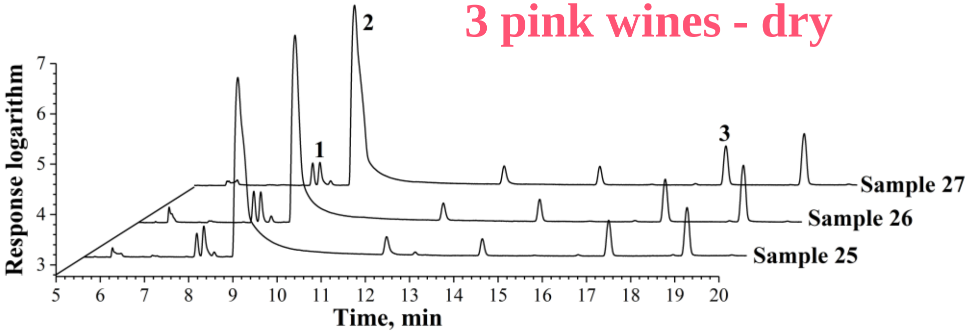
3 white wines - sweet



Sample no.	Concentration of methanol, $C^* \pm SD$, mg/100 mL AA		Δ, %	Student's test		ANOVA	
	Developed method	**Official OIV method**		*p*	*t*	*p*	*F*
22	**86.9 ± 0.8**	**87.2 ± 1.3**	-0.3	0.53	0.75	0.79	0.08
23	**58.5 ± 0.2**	**58.6 ± 0.4**	-0.1	0.76	0.36	0.83	0.05
24	**63.8 ± 0.2**	**64.6 ± 0.8**	-1.2	0.29	1.43	0.17	2.83

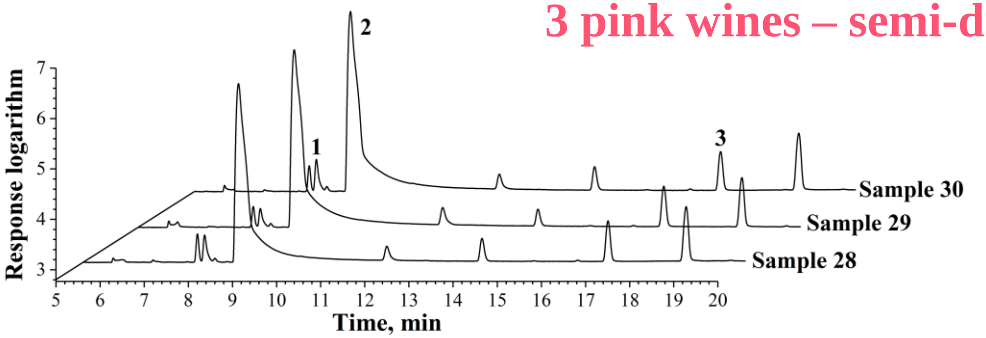
The difference between methods: 12 pink wines

3 pink wines - dry



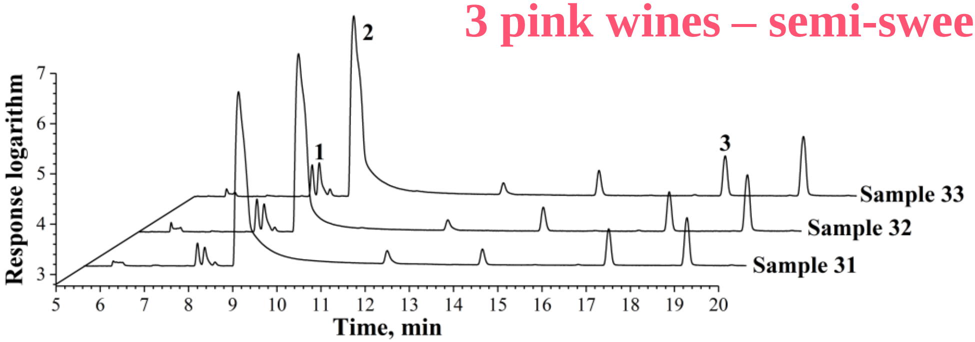
Sample no.	Concentration of methanol, $C^* \pm SD$, mg/100 mL AA		Δ, %	Student's test		ANOVA	
	Developed method	Official OIV method		*p*	*t*	*p*	*F*
25	44.8 ± 0.1	45.0 ± 0.4	-0.5	0.39	1.09	0.43	0.77
26	50.1 ± 0.3	50.3 ± 0.8	-0.5	0.55	0.72	0.66	0.23
27	76.2 ± 0.1	76.4 ± 0.6	-0.3	0.64	0.55	0.57	0.39

3 pink wines – semi-dry



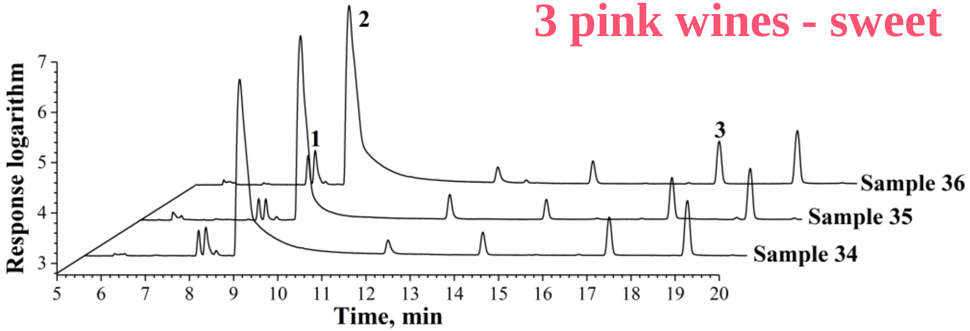
Sample no.	Concentration of methanol, $C^* \pm SD$, mg/100 mL AA		Δ, %	Student's test		ANOVA	
	Developed method	Official OIV method		*p*	*t*	*p*	*F*
28	81.9 ± 0.3	81.7 ± 0.7	0.3	0.49	0.85	0.66	0.22
29	37.0 ± 0.5	37.2 ± 0.7	-0.6	0.70	0.44	0.67	0.21
30	64.6 ± 0.3	64.5 ± 0.4	0.1	0.90	0.14	0.89	0.02

3 pink wines – semi-sweet



Sample no.	Concentration of methanol, $C^* \pm SD$, mg/100 mL AA		Δ, %	Student's test		ANOVA	
	Developed method	Official OIV method		*p*	*t*	*p*	*F*
31	86.1 ± 0.6	86.3 ± 1.3	-0.3	0.78	0.31	0.78	0.09
32	69.1 ± 0.5	69.6 ± 1.0	-0.6	0.26	1.56	0.53	0.47
33	42.6 ± 0.3	43.0 ± 0.4	-1.1	0.32	1.31	0.20	2.36

3 pink wines - sweet



Sample no.	Concentration of methanol, $C^* \pm SD$, mg/100 mL AA		Δ, %	Student's test		ANOVA	
	Developed method	Official OIV method		*p*	*t*	*p*	*F*
34	94.5 ± 0.1	94.1 ± 0.4	0.4	0.19	1.94	0.20	2.37
35	30.5 ± 0.5	30.7 ± 0.6	-0.6	0.64	0.54	0.71	0.15
36	77.4 ± 0.4	77.5 ± 0.2	-0.1	0.86	0.20	0.81	0.06

The difference between methods

Results of precision (intraday) and accuracy study ($n = 3$)

Concentration of methanol in standard solution, mg/L	**Developed method**		**Official OIV method**	
	***RSD*, %**	***Recovery*, %**	***RSD*, %**	***Recovery*, %**
50	**1.5**	**101.7**	**1.9**	**102.3**
100	**1.2**	**100.4**	**1.3**	**101.5**
150	**1.0**	**99.9**	**1.2**	**100.2**
200	**1.0**	**99.5**	**1.1**	**100.6**
250	**0.5**	**99.4**	**0.5**	**99.4**
500	**0.1**	**100.2**	**0.4**	**100.2**

Results of precision (interday) and accuracy study ($n = 9$)

Concentration of methanol in standard solution, mg/L	**Developed method**		**Official OIV method**	
	***RSD*, %**	***Recovery*, %**	***RSD*, %**	***Recovery*, %**
50	**1.6**	**101.6**	**2.1**	**102.3**
100	**1.4**	**100.7**	**1.7**	**101.3**
150	**1.3**	**100.2**	**1.4**	**100.3**
200	**1.1**	**99.8**	**1.1**	**100.4**
250	**0.7**	**99.4**	**0.9**	**99.9**
500	**0.4**	**100.5**	**0.5**	**100.6**

Results of values of *LOD* and *LOQ* study ($n = 10$)

Developed method		**Official OIV method**	
***LOD*, mg/L**	***LOQ*, mg/L**	***LOD*, mg/L**	***LOQ*, mg/L**
0.46	**1.39**	**0.65**	**1.96**

The relative difference between obtained results for wine samples < 1.5 %

Examples of
determination of methanol in 27 alcoholic beverages

Result for	 40 % ABV	 40 % ABV	 43 % ABV	 40 % ABV	 40 % ABV	 40 % ABV	 40 % ABV	 47 % ABV	 45 % ABV
	Rum	**Whiskey**	**Bourbon**	**Grain spirit**	**Brandy**	**Grappa**	**Calvados**	**Gin**	**Slivovice**
Official method, mg/L AA	**22.2±0.5**	**132±2**	**88.4±1.2**	**110±1.6**	**297±2**	**414±5**	**910±5**	**4.16±0.09**	**10546±97**
Developed method, mg/L AA	**22.3±0.6**	**130±1**	**88.9±0.5**	**111±0.7**	**297±1**	**412±2**	**913±2**	**4.19±0.16**	**10603±18**
Δ, %	**0.7**	**-0.9**	**0.6**	**0.9**	**-0.2**	**-0.6**	**0.3**	**0.8**	**0.5**
Result for	 38 % ABV	 14.5 % ABV	 38 % ABV	 15 % ABV	 18 % ABV	 8.5 % ABV	 70 % ABV	 27.5 % ABV	 40 % ABV
	Tsikoudia	**Sake**	**Tequila**	**Vermouth**	**Nalewka**	**Mulled wine**	**Rectified spirit**	**Cocktail**	**Vodka**
Official method, mg/L AA	**755±50**	**18.2±1.3**	**1456±35**	**17.5±0.1**	**168±5**	**25.3±3.0**	**6.05±0.39**	**77.3±0.7**	**21.8±0.2**
Developed method, mg/L AA	**761±20**	**18.1±1.4**	**1460±10**	**17.6±0.2**	**169±4**	**25.1±2.7**	**6.03±0.40**	**76.3±1.5**	**21.7±0.2**
Δ, %	**0.8**	**-1.0**	**0.3**	**0.6**	**0.9**	**-0.6**	**-0.4**	**-1.2**	**-0.7**
Result for	 38 % ABV	 17 % ABV	 35 % ABV	 25 % ABV	 16 % ABV	 16.5 % ABV	 35 % ABV	 40 % ABV	 56 % ABV
	Liqueurs							**Rakia**	**Baijiu**
	Sambuca	**Egg**	**Herbal**	**Limon**	**Cherry**	**Raspberry**	**Sloe gin**		
Official method, mg/L AA	**2.32±0.04**	**9.75±0.28**	**19.5±0.1**	**29.1±0.9**	**9.77±1.34**	**127±5**	**20.5±0.7**	**118623**	**115±5**
Developed method, mg/L AA	**2.34±0.05**	**9.81±0.14**	**19.6±0.1**	**29.4±1.0**	**9.82±1.27**	**126±4**	**20.7±0.4**	**11791**	**116±4**
Δ, %	**0.8**	**0.7**	**0.4**	**0.8**	**0.5**	**-1.1**	**0.5**	0.6	**0.6**

The relative difference between obtained values of concentrations (**Δ, %**) measured in accordance with the EC 2870/2000 according to the official internal standard method and in accordance with the developed internal standard method does not exceed **1.5 %.**

Examples of

Determination sums of aldehydes, esters and higher alcohols in 27 alcoholic beverages

Result for	 40 % ABV	 40 % ABV	 43 % ABV	 40 % ABV	 40 % ABV	 40 % ABV	 40 % ABV	 47 % ABV	 45 % ABV
	Rum	**Whiskey**	**Bourbon**	**Grain spirit**	**Brandy**	**Grappa**	**Calvados**	**Gin**	**Slivovice**
Official method, mg/L AA	**48.1 / 145 / 1043**	**162 / 589 / 6693**	**150 / 645 / 5546**	**44.0 / 84.7 / 4662**	**143 / 396 / 4801**	**191 / 289 / 2113**	**182 / 583 / 3690**	**1.70 / 0 / 1.54**	**210 / 907 / 6255**
Developed method, mg/L AA	**48.4 / 146 / 1051**	**160 / 584 / 6635**	**151 / 649 / 5580**	**44.4 / 85.4 / 4703**	**142 / 396 / 4794**	**190 / 288 / 2100**	**182 / 585 / 3702**	**1.72 / 0 / 1.55**	**211 / 912 / 6288**
Δ, %	**0.7 / 0.7 / 0.7**	**-0.9 / -0.9 / -0.9**	**0.6 / 0.6 / 0.6**	**0.9 / 0.9 / 0.9**	**-0.2 / -0.2 / -0.2**	**-0.6 / -0.6 / -0.6**	**0.3 / 0.3 / 0.3**	**0.8 / - / 0.9**	**0.5 / 0.5 / 0.5**
Result for	 38 % ABV	 14.5 % ABV	 38 % ABV	 15 % ABV	 18 % ABV	 8.5 % ABV	 70 % ABV	 27.5 % ABV	 40 % ABV
	Tsikoudia	**Sake**	**Tequila**	**Vermouth**	**Nalewka**	**Mulled wine**	**Rectified spirit**	**Cocktail**	**Vodka**
Official method, mg/L AA	**356 / 266 / 2297**	**37.6 / 47.0 / 1367**	**34.8 / 126 / 2895**	**30.5 / 0 / 5.94**	**47.4 / 74.4 / 10.3**	**22.7 / 55.9 / 871**	**4.83 / 25.2 / 0**	**61.9 / 84.0 / 728**	**0.504 / 0 / 0**
Developed method, mg/L AA	**359 / 268 / 2316**	**37.2 / 46.5 / 1352**	**34.9 / 127 / 2904**	**30.6 / 0 / 5.98**	**47.8 / 75.1 / 10.4**	**22.5 / 55.6 / 866**	**4.81 / 25.1 / 0**	**61.1 / 83.0 / 719**	**0.50 / 0 / 0**
Δ, %	**0.9 / 0.8 / 0.9**	**-1.1 / -1.1 / -1.1**	**0.4 / 0.3 / 0.3**	**0.6 / - / 0.6**	**0.9 / 0.9 / 0.9**	**-0.6 / -0.5 / -0.6**	**-0.4 / -0.4 / -**	**-1.3 / -1.2 / -1.2**	**-0.7 / - / -**
Result for	 38 % ABV	 17 % ABV	 35 % ABV	 25 % ABV	 16 % ABV	 16.5 % ABV	 35 % ABV	 40 % ABV	 56 % ABV
	Liqueurs							**Rakia**	**Baijiu**
	Sambuca	**Egg**	**Herbal**	**Limon**	**Cherry**	**Raspberry**	**Sloe gin**		
Official method, mg/L AA	**4.20 / 0 / 2.44**	**6.89 / 0 / 125**	**38.1 / 13.5 / 9.39**	**25.1 / 0 / 0**	**18.4 / 266 / 0**	**36.6 / 31.8 / 0**	**1.12 / 0 / 0**	**92.2/1334/6165**	**63.9 / 1072 / 2114**
Developed method, mg/L AA	**4.24 / 0 / 2.46**	**6.94 / 0 / 125**	**38.2 / 13.5 / 9.43**	**25.3 / 0 / 0**	**18.5 / 267 / 0**	**36.2 / 31.5 / 0**	**1.13 / 0 / 0**	**91.6/1325/6217**	**64.3 / 1079 / 2128**
Δ, %	**0.8 / - / 0.8**	**0.8 / - / 0.7**	**0.4 / 0.4 / 0.4**	**0.8 / - / -**	**0.5 / 0.6 / -**	**-1.0 / -1.1 / -**	**0.6 / - / -**	0.6/0.7/0.6	**0.6 / 0.6 / 0.6**

Aldehydes = acetaldehyde + acetal / Esters = ethyl acetate / Higher alcohols = butan-2-ol + propan-1-ol + 2-methylpropan-1-ol + butan-1-ol + 2-methylbutan-1-ol + 3-methylbutan-1-ol

The relative difference between obtained values of concentrations (**Δ, %**) measured in accordance with the EC 2870/2000 according to the official internal standard method and in accordance with the developed internal standard method does not exceed **1.5 %.**

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16. Simplified document OIV-MA-AS315-27 https://elab.bsu.by/download.php?id=316
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18. Simplified document Norma Mexicana NMX-V-005-NORMEX-2013 https://elab.bsu.by/download.php?id=311
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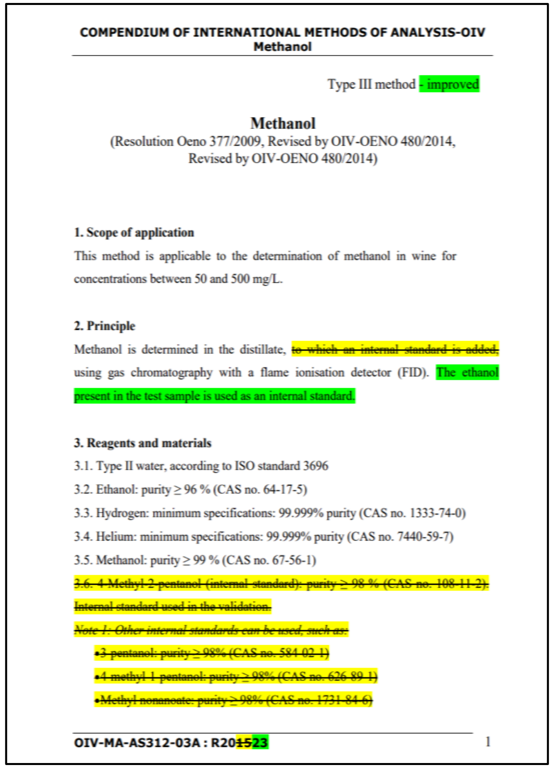
OIV-MA-AS312-03A - simplification
Methanol

The use of the proposed method ensures high reliability of the data obtained, significantly reduces time, labor, material and financial costs. Analysis of volatile compounds in spirit drinks has never been so easy. Here you can read modified text of official method, which allows to carry out analysis of alcoholic beverages using the developed method.

The places in the text document to be deleted are highlighted in yellow. Embedded parts of the test are highlighted in green.

You can read a full article about the improvements by following the link: **https://elab.bsu.by/article/360**



COMPENDIUM OF INTERNATIONAL METHODS OF ANALYSIS-OIV
Methanol

Type III method - improved

Methanol
(Resolution Oeno 377/2009, Revised by OIV-OENO 480/2014,
Revised by OIV-OENO 480/2014)

1. Scope of application

This method is applicable to the determination of methanol in wine for concentrations between 50 and 500 mg/L.

2. Principle

Methanol is determined in the distillate, ~~to which an internal standard is added,~~ using gas chromatography with a flame ionisation detector (FID). The ethanol present in the test sample is used as an internal standard.

3. Reagents and materials

3.1. Type II water, according to ISO standard 3696

3.2. Ethanol: purity ≥ 96 % (CAS no. 64-17-5)

3.3. Hydrogen: minimum specifications: 99.999% purity (CAS no. 1333-74-0)

3.4. Helium: minimum specifications: 99.999% purity (CAS no. 7440-59-7)

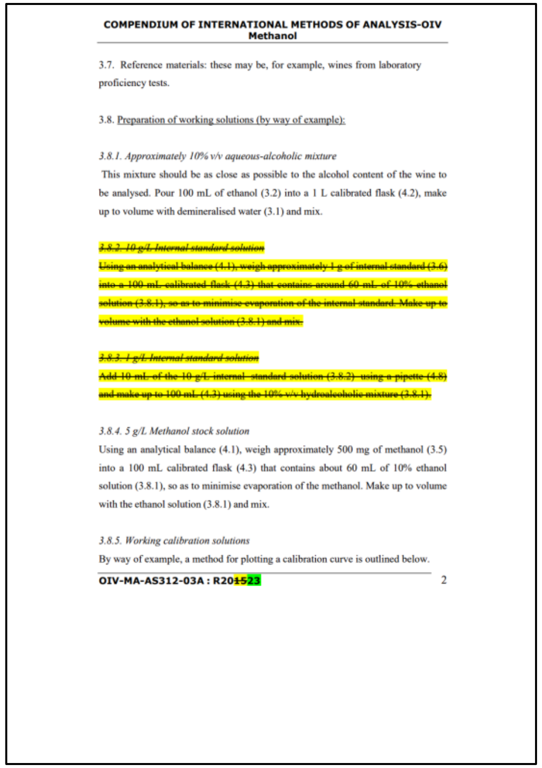
3.5. Methanol: purity ≥ 99 % (CAS no. 67-56-1)

~~3.6. 4-Methyl-2-pentanol (internal standard): purity ≥ 98 % (CAS no. 108-11-2). Internal standard used in the validation.~~

~~*Note 1: Other internal standards can be used, such as:*~~

- ~~3-pentanol: purity ≥ 98% (CAS no. 584-02-1)~~
- ~~4-methyl-1-pentanol: purity ≥ 98% (CAS no. 626-89-1)~~
- ~~Methyl nonanoate: purity ≥ 98% (CAS no. 1731-84-6)~~

OIV-MA-AS312-03A : R20~~15~~23 1



COMPENDIUM OF INTERNATIONAL METHODS OF ANALYSIS-OIV
Methanol

3.7. Reference materials: these may be, for example, wines from laboratory proficiency tests.

3.8. Preparation of working solutions (by way of example):

3.8.1. Approximately 10% v/v aqueous-alcoholic mixture

This mixture should be as close as possible to the alcohol content of the wine to be analysed. Pour 100 mL of ethanol (3.2) into a 1 L calibrated flask (4.2), make up to volume with demineralised water (3.1) and mix.

~~*3.8.2. 10 g/L Internal standard solution*~~

~~Using an analytical balance (4.1), weigh approximately 1 g of internal standard (3.6) into a 100 mL calibrated flask (4.3) that contains around 60 mL of 10% ethanol solution (3.8.1), so as to minimise evaporation of the internal standard. Make up to volume with the ethanol solution (3.8.1) and mix.~~

~~*3.8.3. 1 g/L Internal standard solution*~~

~~Add 10 mL of the 10 g/L internal standard solution (3.8.2) using a pipette (4.8) and make up to 100 mL (4.3) using the 10% v/v hydroalcoholic mixture (3.8.1).~~

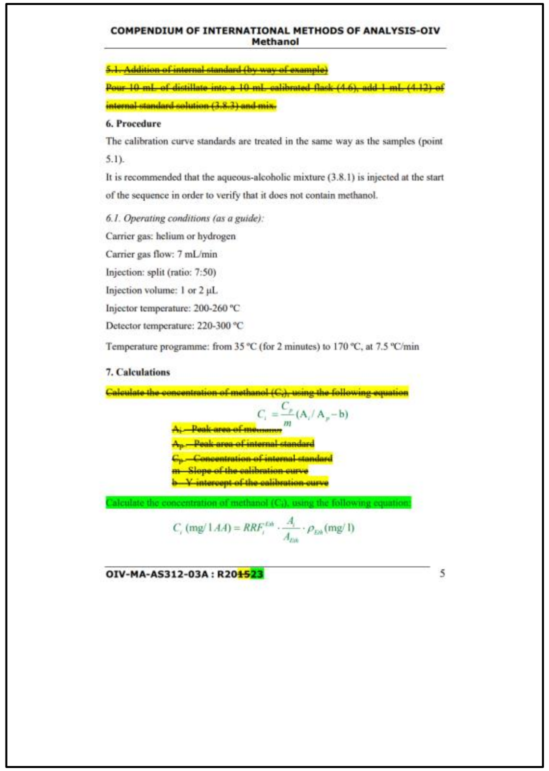
3.8.4. 5 g/L Methanol stock solution

Using an analytical balance (4.1), weigh approximately 500 mg of methanol (3.5) into a 100 mL calibrated flask (4.3) that contains about 60 mL of 10% ethanol solution (3.8.1), so as to minimise evaporation of the methanol. Make up to volume with the ethanol solution (3.8.1) and mix.

3.8.5. Working calibration solutions

By way of example, a method for plotting a calibration curve is outlined below.

OIV-MA-AS312-03A : R20~~15~~23 2



COMPENDIUM OF INTERNATIONAL METHODS OF ANALYSIS-OIV
Methanol

~~5.1. Addition of internal standard (by way of example)~~

~~Pour 10 mL of distillate into a 10 mL calibrated flask (4.6), add 1 mL (4.12) of internal standard solution (3.8.3) and mix.~~

6. Procedure

The calibration curve standards are treated in the same way as the samples (point 5.1).

It is recommended that the aqueous-alcoholic mixture (3.8.1) is injected at the start of the sequence in order to verify that it does not contain methanol.

6.1. Operating conditions (as a guide):

Carrier gas: helium or hydrogen

Carrier gas flow: 7 mL/min

Injection: split (ratio: 7:50)

Injection volume: 1 or 2 μL

Injector temperature: 200-260 °C

Detector temperature: 220-300 °C

Temperature programme: from 35 °C (for 2 minutes) to 170 °C, at 7.5 °C/min

7. Calculations

~~Calculate the concentration of methanol (C_i), using the following equation~~

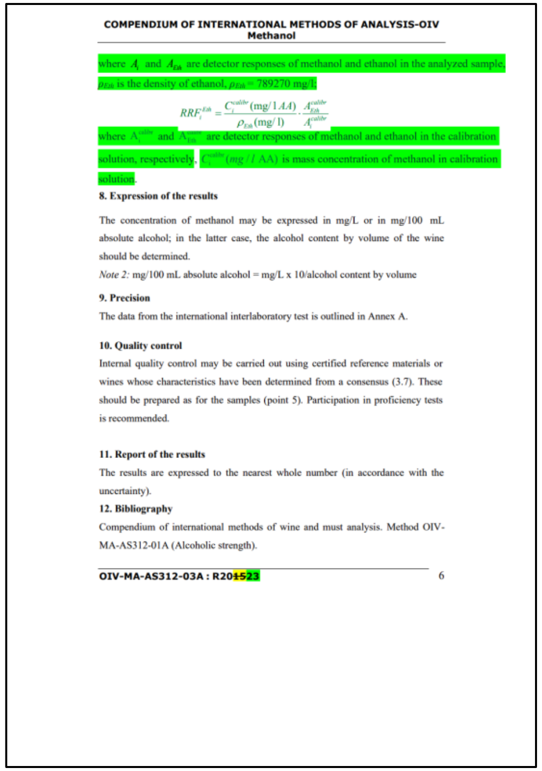
$$C_i = \frac{C_p}{m}(A_i / A_p - b)$$

~~A_i – Peak area of me~~
~~A_p – Peak area of internal standard~~
~~C_p – Concentration of internal standard~~
~~m – Slope of the calibration curve~~
~~b – Y intercept of the calibration curve~~

Calculate the concentration of methanol (C_i), using the following equation:

$$C_i\,(\mathrm{mg/l}\,AA) = RRF_i^{Eth} \cdot \frac{A_i}{A_{Eth}} \cdot \rho_{Eth}\,(\mathrm{mg/l})$$

OIV-MA-AS312-03A : R20~~15~~23 5



COMPENDIUM OF INTERNATIONAL METHODS OF ANALYSIS-OIV
Methanol

where A_i and A_{Eth} are detector responses of methanol and ethanol in the analyzed sample, ρ_{Eth} is the density of ethanol, ρ_{Eth} = 789270 mg/l;

$$RRF_i^{Eth} = \frac{C_i^{calibr}(\mathrm{mg/l}\,AA)}{\rho_{Eth}(\mathrm{mg/l})} \cdot \frac{A_{Eth}^{calibr}}{A_i^{calibr}}$$

where A_i^{calibr} and A_{Eth}^{calibr} are detector responses of methanol and ethanol in the calibration solution, respectively, $C_i^{calibr}(mg/l\ AA)$ is mass concentration of methanol in calibration solution.

8. Expression of the results

The concentration of methanol may be expressed in mg/L or in mg/100 mL absolute alcohol; in the latter case, the alcohol content by volume of the wine should be determined.

Note 2: mg/100 mL absolute alcohol = mg/L x 10/alcohol content by volume

9. Precision

The data from the international interlaboratory test is outlined in Annex A.

10. Quality control

Internal quality control may be carried out using certified reference materials or wines whose characteristics have been determined from a consensus (3.7). These should be prepared as for the samples (point 5). Participation in proficiency tests is recommended.

11. Report of the results

The results are expressed to the nearest whole number (in accordance with the uncertainty).

12. Bibliography

Compendium of international methods of wine and must analysis. Method OIV-MA-AS312-01A (Alcoholic strength).

OIV-MA-AS312-03A : R20~~15~~23 6

OIV-MA-BS-14 - simplification
Determination of the principal volatile substances of spirit drinks of viti-vinicultural origin

The use of the proposed method ensures high reliability of the data obtained, significantly reduces time, labor, material and financial costs. Analysis of volatile compounds in spirit drinks has never been so easy. Here you can read modified text of official method, which allows to carry out analysis of alcoholic beverages using the developed method.

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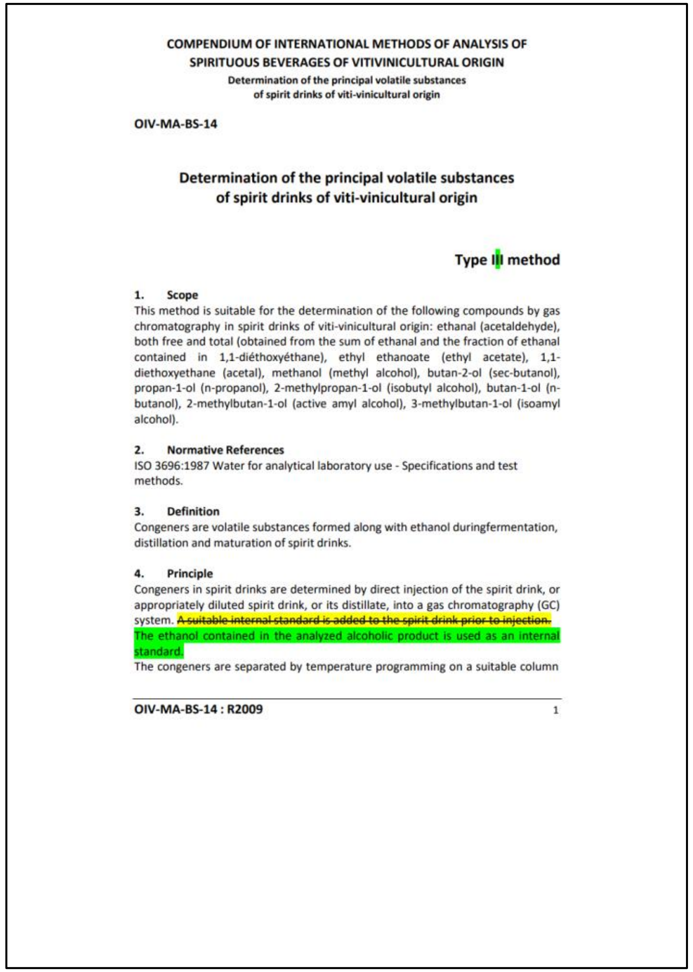


COMPENDIUM OF INTERNATIONAL METHODS OF ANALYSIS OF
SPIRITUOUS BEVERAGES OF VITIVINICULTURAL ORIGIN
Determination of the principal volatile substances
of spirit drinks of viti-vinicultural origin

OIV-MA-BS-14

Determination of the principal volatile substances of spirit drinks of viti-vinicultural origin

Type III method

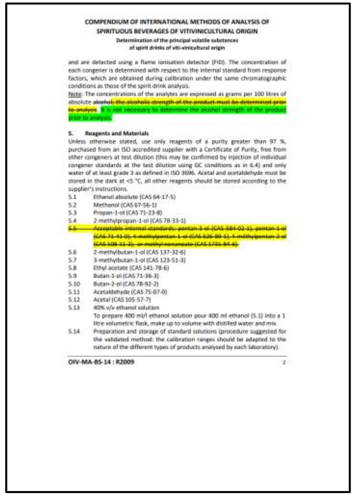
1. Scope
This method is suitable for the determination of the following compounds by gas chromatography in spirit drinks of viti-vinicultural origin: ethanal (acetaldehyde), both free and total (obtained from the sum of ethanal and the fraction of ethanal contained in 1,1-diéthoxyéthane), ethyl ethanoate (ethyl acetate), 1,1-diethoxyethane (acetal), methanol (methyl alcohol), butan-2-ol (sec-butanol), propan-1-ol (n-propanol), 2-methylpropan-1-ol (isobutyl alcohol), butan-1-ol (n-butanol), 2-methylbutan-1-ol (active amyl alcohol), 3-methylbutan-1-ol (isoamyl alcohol).

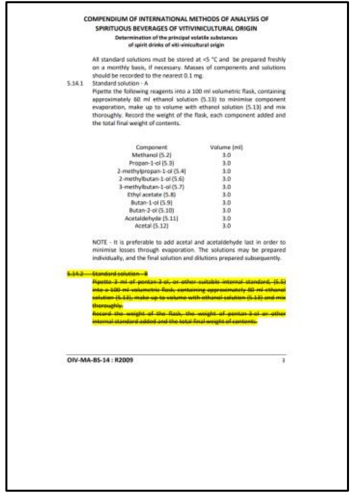
2. Normative References
ISO 3696:1987 Water for analytical laboratory use - Specifications and test methods.

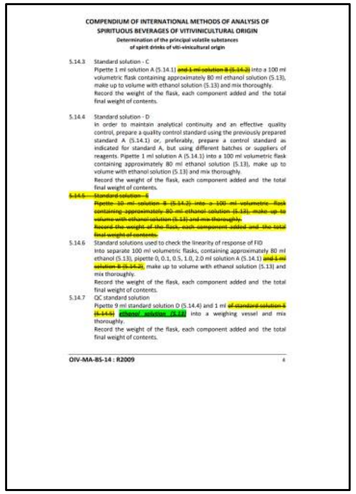
3. Definition
Congeners are volatile substances formed along with ethanol duringfermentation, distillation and maturation of spirit drinks.

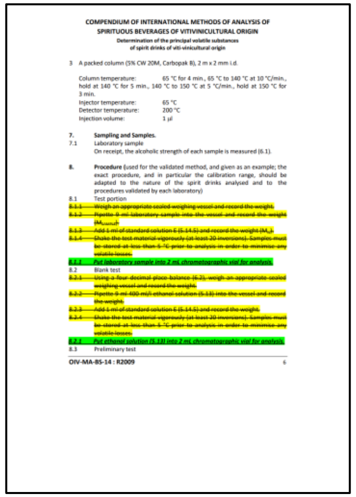
4. Principle
Congeners in spirit drinks are determined by direct injection of the spirit drink, or appropriately diluted spirit drink, or its distillate, into a gas chromatography (GC) system. ~~A suitable internal standard is added to the spirit drink prior to injection.~~ The ethanol contained in the analyzed alcoholic product is used as an internal standard.
The congeners are separated by temperature programming on a suitable column

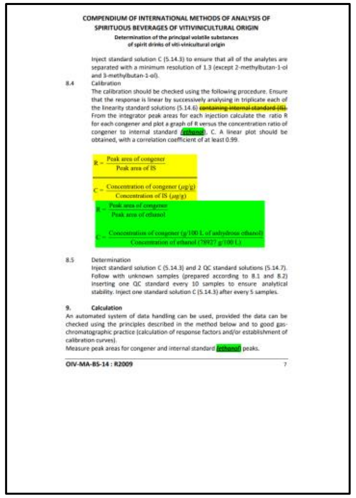
OIV-MA-BS-14 : R2009 1

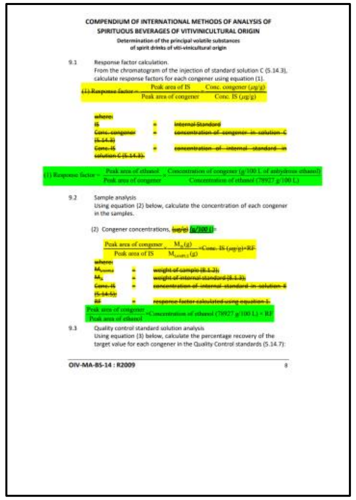


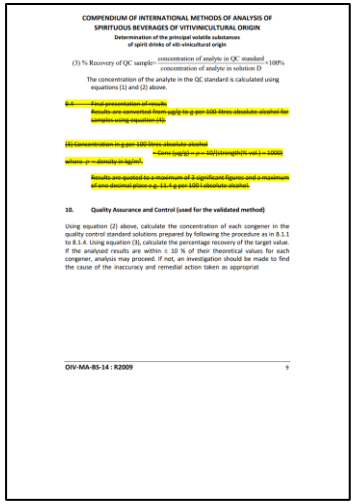










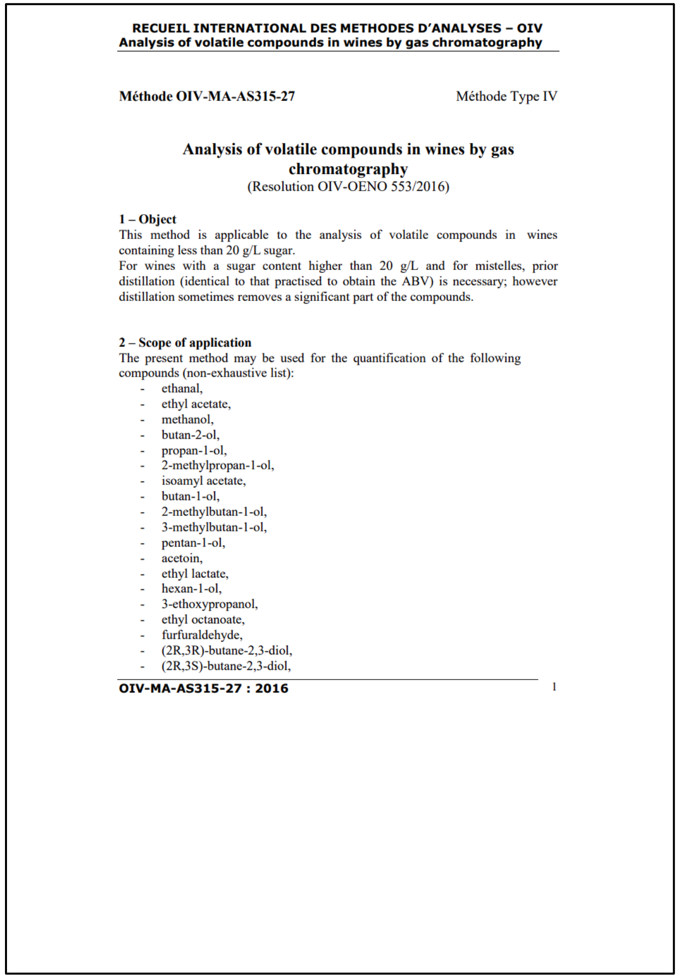


OIV-MA-AS315-27 – simplification
Analysis of volatile compounds in wines by gas chromatography

The use of the proposed method ensures high reliability of the data obtained, significantly reduces time, labor, material and financial costs. Analysis of volatile compounds in spirit drinks has never been so easy. Here you can read modified text of official method, which allows to carry out analysis of alcoholic beverages using the developed method.

The places in the text document to be deleted are highlighted in yellow. Embedded parts of the test are highlighted in green.

You can read a full article about the improvements by following the link: **https://elab.bsu.by/article/360**



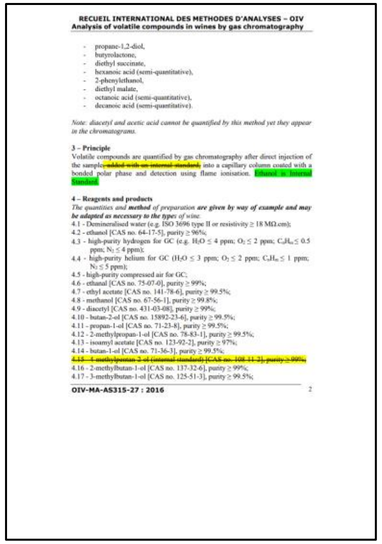
Méthode OIV-MA-AS315-27 Méthode Type IV

Analysis of volatile compounds in wines by gas chromatography
(Resolution OIV-OENO 553/2016)

1 – Object
This method is applicable to the analysis of volatile compounds in wines containing less than 20 g/L sugar.
For wines with a sugar content higher than 20 g/L and for mistelles, prior distillation (identical to that practised to obtain the ABV) is necessary; however distillation sometimes removes a significant part of the compounds.

2 – Scope of application
The present method may be used for the quantification of the following compounds (non-exhaustive list):

- ethanal,
- ethyl acetate,
- methanol,
- butan-2-ol,
- propan-1-ol,
- 2-methylpropan-1-ol,
- isoamyl acetate,
- butan-1-ol,
- 2-methylbutan-1-ol,
- 3-methylbutan-1-ol,
- pentan-1-ol,
- acetoin,
- ethyl lactate,
- hexan-1-ol,
- 3-ethoxypropanol,
- ethyl octanoate,
- furfuraldehyde,
- (2R,3R)-butane-2,3-diol,
- (2R,3S)-butane-2,3-diol,

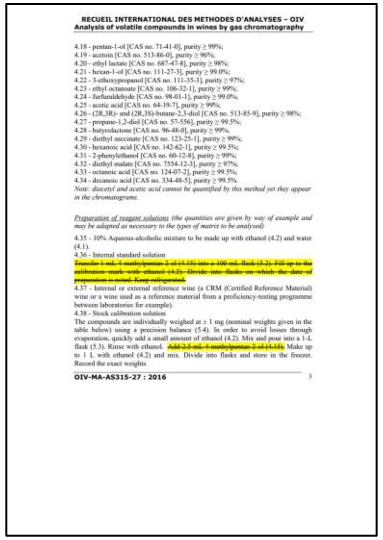


- propane-1,2-diol,
- butyrolactone,
- diethyl succinate,
- hexanoic acid (semi-quantitative),
- 2-phenylethanol,
- diethyl malate,
- octanoic acid (semi-quantitative),
- decanoic acid (semi-quantitative).

Note: diacetyl and acetic acid cannot be quantified by this method yet they appear in the chromatograms.

3 – Principle
Volatile compounds are quantified by gas chromatography after direct injection of the sample~~, added with an internal standard,~~ into a capillary column coated with a bonded polar phase and detection using flame ionisation. Ethanol is Internal Standard.

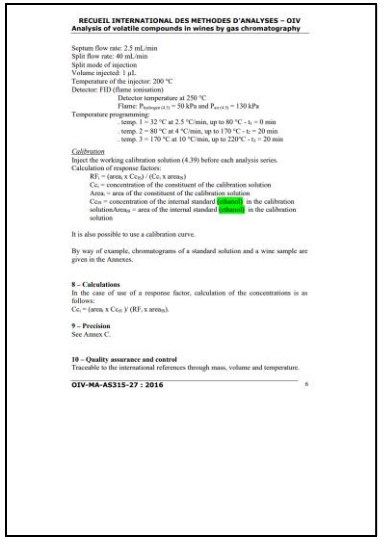
4 – Reagents and products
*The quantities and **method** of preparation **are given by way of example and may be adapted as necessary to the types** of wine.*
4.1 - Demineralised water (e.g. ISO 3696 type II or resistivity ≥ 18 MΩ.cm);
4.2 - ethanol [CAS no. 64-17-5], purity ≥ 96%;
4.3 - high-purity hydrogen for GC (e.g. $H_2O \leq 4$ ppm; $O_2 \leq 2$ ppm; $C_nH_m \leq 0.5$ ppm; $N_2 \leq 4$ ppm);
4.4 - high-purity helium for GC ($H_2O \leq 3$ ppm; $O_2 \leq 2$ ppm; $C_nH_m \leq 1$ ppm; $N_2 \leq 5$ ppm);
4.5 - high-purity compressed air for GC;
4.6 - ethanal [CAS no. 75-07-0], purity ≥ 99%;
4.7 - ethyl acetate [CAS no. 141-78-6], purity ≥ 99.5%;
4.8 - methanol [CAS no. 67-56-1], purity ≥ 99.8%;
4.9 - diacetyl [CAS no. 431-03-08], purity ≥ 99%;
4.10 - butan-2-ol [CAS no. 15892-23-6], purity ≥ 99.5%;
4.11 - propan-1-ol [CAS no. 71-23-8], purity ≥ 99.5%;
4.12 - 2-methylpropan-1-ol [CAS no. 78-83-1], purity ≥ 99.5%;
4.13 - isoamyl acetate [CAS no. 123-92-2], purity ≥ 97%;
4.14 - butan-1-ol [CAS no. 71-36-3], purity ≥ 99.5%;
~~4.15 - 4-methylpentan-2-ol (internal standard) [CAS no. 108-11-2], purity ≥ 99%;~~
4.16 - 2-methylbutan-1-ol [CAS no. 137-32-6], purity ≥ 99%;
4.17 - 3-methylbutan-1-ol [CAS no. 125-51-3], purity ≥ 99.5%;



4.18 - pentan-1-ol [CAS no. 71-41-0], purity ≥ 99%;
4.19 - acetoin [CAS no. 513-86-0], purity ≥ 96%;
4.20 - ethyl lactate [CAS no. 687-47-8], purity ≥ 98%;
4.21 - hexan-1-ol [CAS no. 111-27-3], purity ≥ 99.0%;
4.22 - 3-ethoxypropanol [CAS no. 111-35-3], purity ≥ 97%;
4.23 - ethyl octanoate [CAS no. 106-32-1], purity ≥ 99%;
4.24 - furfuraldehyde [CAS no. 98-01-1], purity ≥ 99.0%;
4.25 - acetic acid [CAS no. 64-19-7], purity ≥ 99%;
4.26 - (2R,3R)- and (2R,3S)-butane-2,3-diol [CAS no. 513-85-9], purity ≥ 98%;
4.27 - propane-1,2-diol [CAS no. 57-556], purity ≥ 99.5%;
4.28 - butyrolactone [CAS no. 96-48-0], purity ≥ 99%;
4.29 - diethyl succinate [CAS no. 123-25-1], purity ≥ 99%;
4.30 - hexanoic acid [CAS no. 142-62-1], purity ≥ 99.5%;
4.31 - 2-phenylethanol [CAS no. 60-12-8], purity ≥ 99%;
4.32 - diethyl malate [CAS no. 7554-12-3], purity ≥ 97%;
4.33 - octanoic acid [CAS no. 124-07-2], purity ≥ 99.5%;
4.34 - decanoic acid [CAS no. 334-48-5], purity ≥ 99.5%.
Note: diacetyl and acetic acid cannot be quantified by this method yet they appear in the chromatograms.

<u>Preparation of reagent solutions</u> (the quantities are given by way of example and may be adapted as necessary to the types of matrix to be analysed)

4.35 - 10% Aqueous-alcoholic mixture to be made up with ethanol (4.2) and water (4.1).
4.36 - Internal standard solution
~~Transfer 1 mL 4-methylpentan-2-ol (4.15) into a 100 mL flask (5.2). Fill up to the calibration mark with ethanol (4.2). Divide into flasks on which the date of preparation is noted. Keep refrigerated.~~
4.37 - Internal or external reference wine (a CRM (Certified Reference Material) wine or a wine used as a reference material from a proficiency-testing programme between laboratories for example).
4.38 - Stock calibration solution
The compounds are individually weighed at ± 1 mg (nominal weights given in the table below) using a precision balance (5.4). In order to avoid losses through evaporation, quickly add a small amount of ethanol (4.2). Mix and pour into a 1-L flask (5.3). Rinse with ethanol. ~~Add 2.5 mL 4-methylpentan-2-ol (4.15).~~ Make up to 1 L with ethanol (4.2) and mix. Divide into flasks and store in the freezer. Record the exact weights.



Septum flow rate: 2.5 mL/min
Split flow rate: 40 mL/min
Split mode of injection
Volume injected: 1 µL
Temperature of the injector: 200 °C
Detector: FID (flame ionisation)
Detector temperature at 250 °C
Flame: $P_{hydrogen (4.3)} = 50$ kPa and $P_{air (4.5)} = 130$ kPa
Temperature programming:
. temp. 1 = 32 °C at 2.5 °C/min, up to 80 °C - t_1 = 0 min
. temp. 2 = 80 °C at 4 °C/min, up to 170 °C - t_2 = 20 min
. temp. 3 = 170 °C at 10 °C/min, up to 220°C - t_3 = 20 min

<u>Calibration</u>
Inject the working calibration solution (4.39) before each analysis series.
Calculation of response factors:
$RF_i = (area_i \times Cc_{IS}) / (Cc_i \times area_{IS})$
Cc_i = concentration of the constituent of the calibration solution
$Area_i$ = area of the constituent of the calibration solution
Cc_{IS} = concentration of the internal standard (ethanol) in the calibration solution$Area_{IS}$ = area of the internal standard (ethanol) in the calibration solution

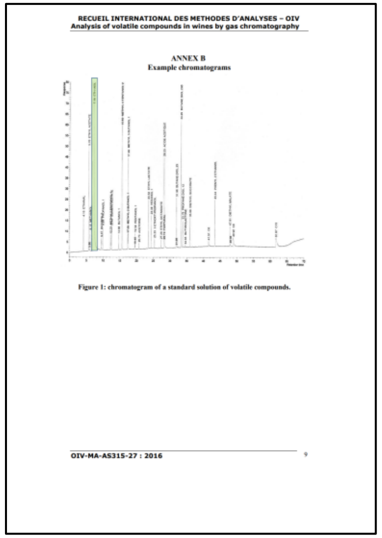
It is also possible to use a calibration curve.

By way of example, chromatograms of a standard solution and a wine sample are given in the Annexes.

8 – Calculations
In the case of use of a response factor, calculation of the concentrations is as follows:
$Cc_i = (area_i \times Cc_{IS}) / (RF_i \times area_{IS})$.

9 – Precision
See Annex C.

10 – Quality assurance and control
Traceable to the international references through mass, volume and temperature.



ANNEX B
Example chromatograms

Figure 1: chromatogram of a standard solution of volatile compounds.

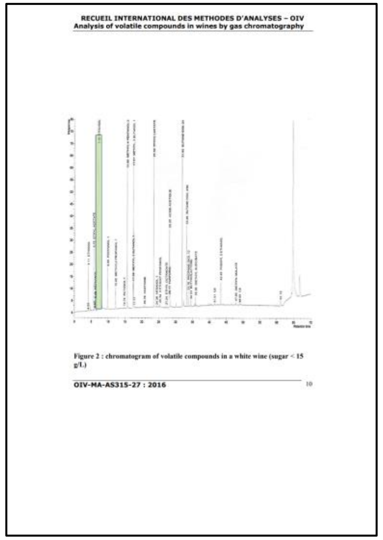


Figure 2 : chromatogram of volatile compounds in a white wine (sugar < 15 g/L)

EC 2870/2000 – simplification
Determination of Volatile Substances and Methanol of Spirit

The use of the proposed method ensures high reliability of the data obtained, significantly reduces time, labor, material and financial costs. Analysis of volatile compounds in spirit drinks has never been so easy. Here you can read modified text of official method, which allows to carry out analysis of alcoholic beverages using the developed method. The places in the text document to be deleted are highlighted in yellow. Embedded parts of the test are highlighted in green.
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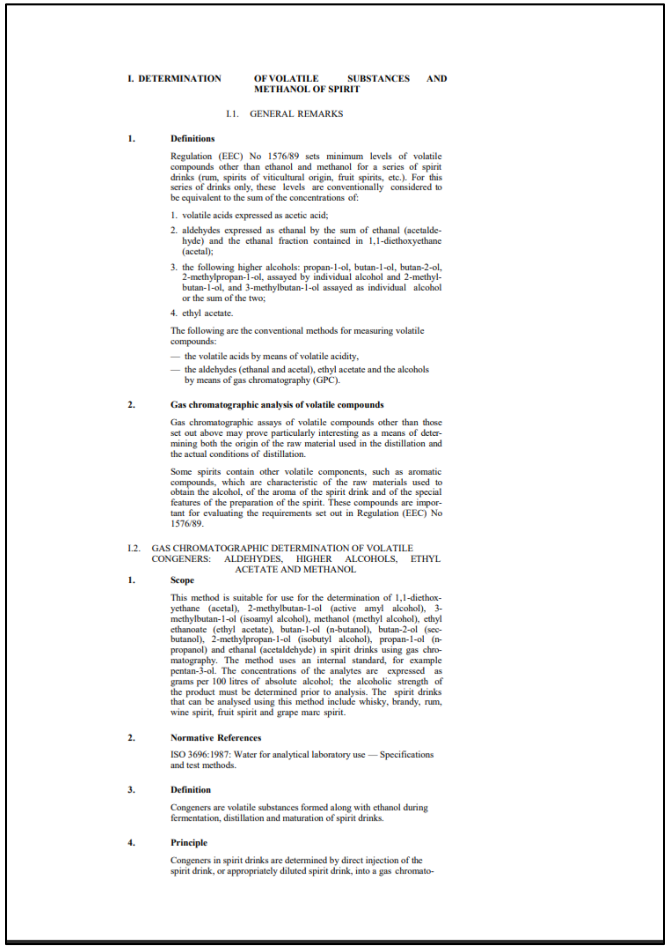


I. DETERMINATION OF VOLATILE SUBSTANCES AND METHANOL OF SPIRIT

I.1. GENERAL REMARKS

1. **Definitions**

Regulation (EEC) No 1576/89 sets minimum levels of volatile compounds other than ethanol and methanol for a series of spirit drinks (rum, spirits of viticultural origin, fruit spirits, etc.). For this series of drinks only, these levels are conventionally considered to be equivalent to the sum of the concentrations of:

1. volatile acids expressed as acetic acid;
2. aldehydes expressed as ethanal by the sum of ethanal (acetaldehyde) and the ethanal fraction contained in 1,1-diethoxyethane (acetal);
3. the following higher alcohols: propan-1-ol, butan-1-ol, butan-2-ol, 2-methylpropan-1-ol, assayed by individual alcohol and 2-methylbutan-1-ol, and 3-methylbutan-1-ol assayed as individual alcohol or the sum of the two;
4. ethyl acetate.

The following are the conventional methods for measuring volatile compounds:

— the volatile acids by means of volatile acidity,
— the aldehydes (ethanal and acetal), ethyl acetate and the alcohols by means of gas chromatography (GPC).

2. **Gas chromatographic analysis of volatile compounds**

Gas chromatographic assays of volatile compounds other than those set out above may prove particularly interesting as a means of determining both the origin of the raw material used in the distillation and the actual conditions of distillation.

Some spirits contain other volatile components, such as aromatic compounds, which are characteristic of the raw materials used to obtain the alcohol, of the aroma of the spirit drink and of the special features of the preparation of the spirit. These compounds are important for evaluating the requirements set out in Regulation (EEC) No 1576/89.

I.2. GAS CHROMATOGRAPHIC DETERMINATION OF VOLATILE CONGENERS: ALDEHYDES, HIGHER ALCOHOLS, ETHYL ACETATE AND METHANOL

1. **Scope**

This method is suitable for use for the determination of 1,1-diethoxyethane (acetal), 2-methylbutan-1-ol (active amyl alcohol), 3-methylbutan-1-ol (isoamyl alcohol), methanol (methyl alcohol), ethyl ethanoate (ethyl acetate), butan-1-ol (n-butanol), butan-2-ol (sec-butanol), 2-methylpropan-1-ol (isobutyl alcohol), propan-1-ol (n-propanol) and ethanal (acetaldehyde) in spirit drinks using gas chromatography. The method uses an internal standard, for example pentan-3-ol. The concentrations of the analytes are expressed as grams per 100 litres of absolute alcohol; the alcoholic strength of the product must be determined prior to analysis. The spirit drinks that can be analysed using this method include whisky, brandy, rum, wine spirit, fruit spirit and grape marc spirit.

2. **Normative References**

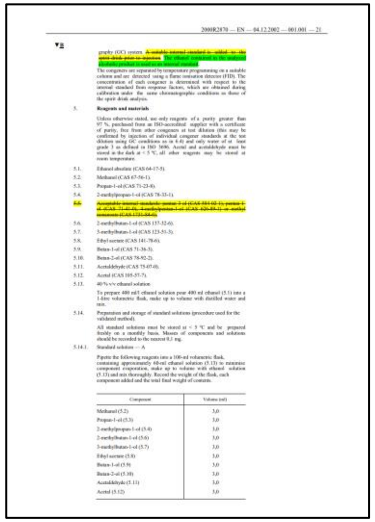
ISO 3696:1987: Water for analytical laboratory use — Specifications and test methods.

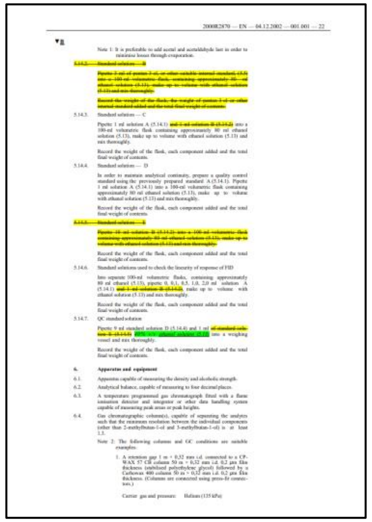
3. **Definition**

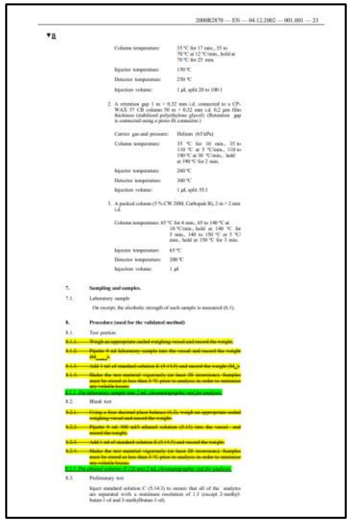
Congeners are volatile substances formed along with ethanol during fermentation, distillation and maturation of spirit drinks.

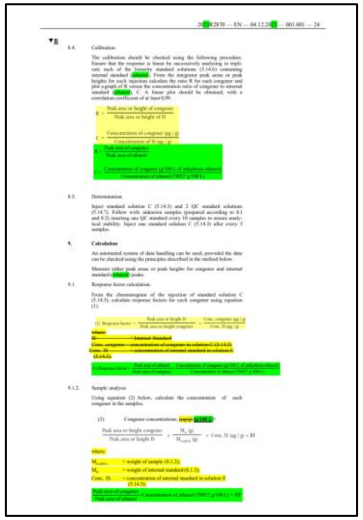
4. **Principle**

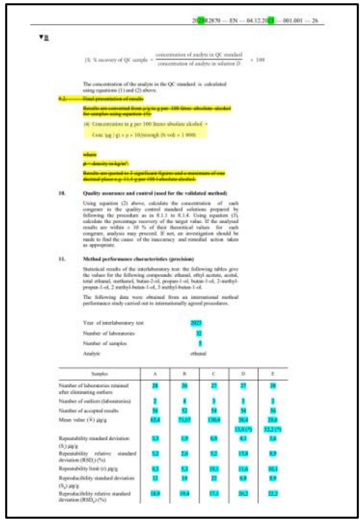
Congeners in spirit drinks are determined by direct injection of the spirit drink, or appropriately diluted spirit drink, into a gas chromato-











National Standard of People’s Republic of China GB/T 11858-2008 – simplification

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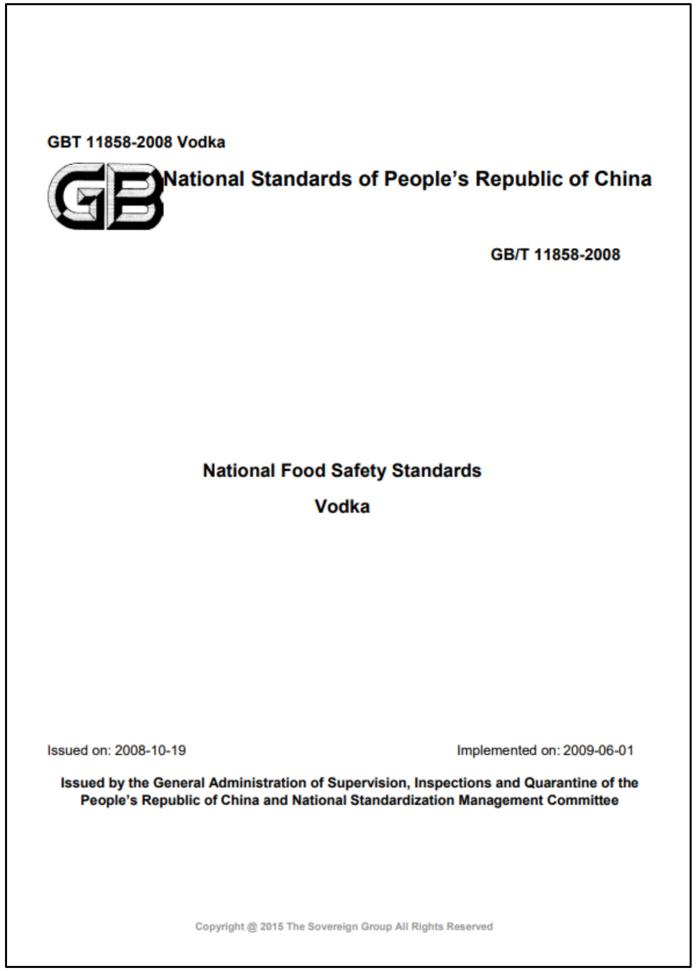


GBT 11858-2008 Vodka

National Standards of People’s Republic of China

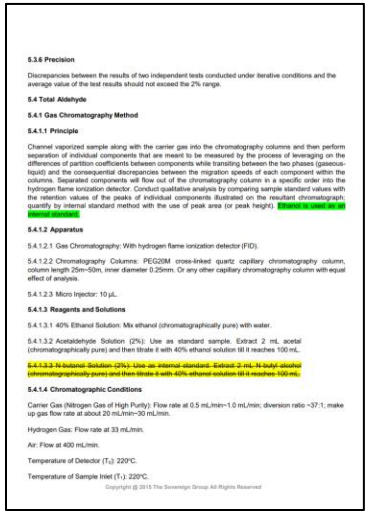
GB/T 11858-2008

National Food Safety Standards

Vodka

Issued on: 2008-10-19
Implemented on: 2009-06-01

Issued by the General Administration of Supervision, Inspections and Quarantine of the People’s Republic of China and National Standardization Management Committee



5.3.6 Precision

Discrepancies between the results of two independent tests conducted under iterative conditions and the average value of the test results should not exceed the 2% range.

5.4 Total Aldehyde

5.4.1 Gas Chromatography Method

5.4.1.1 Principle

Channel vaporized sample along with the carrier gas into the chromatography columns and then perform separation of individual components that are meant to be measured by the process of leveraging on the differences of partition coefficients between components while transiting between the two phases (gaseous-liquid) and the consequential discrepancies between the migration speeds of each component within the columns. Separated components will flow out of the chromatography column in a specific order into the hydrogen flame ionization detector. Conduct qualitative analysis by comparing sample standard values with the retention values of the peaks of individual components illustrated on the resultant chromatograph; quantify by internal standard method with the use of peak area (or peak height). Ethanol is used as an internal standard.

5.4.1.2 Apparatus

5.4.1.2.1 Gas Chromatography: With hydrogen flame ionization detector (FID).

5.4.1.2.2 Chromatography Columns: PEG20M cross-linked quartz capillary chromatography column, column length 25m~50m, inner diameter 0.25mm. Or any other capillary chromatography column with equal effect of analysis.

5.4.1.2.3 Micro Injector: 10 μL.

5.4.1.3 Reagents and Solutions

5.4.1.3.1 40% Ethanol Solution: Mix ethanol (chromatographically pure) with water.

5.4.1.3.2 Acetaldehyde Solution (2%): Use as standard sample. Extract 2 mL acetal (chromatographically pure) and then titrate it with 40% ethanol solution till it reaches 100 mL.

~~5.4.1.3.3 N-butanol Solution (2%): Use as internal standard. Extract 2 mL N-butyl alcohol (chromatographically pure) and then titrate it with 40% ethanol solution till it reaches 100 mL.~~

5.4.1.4 Chromatographic Conditions

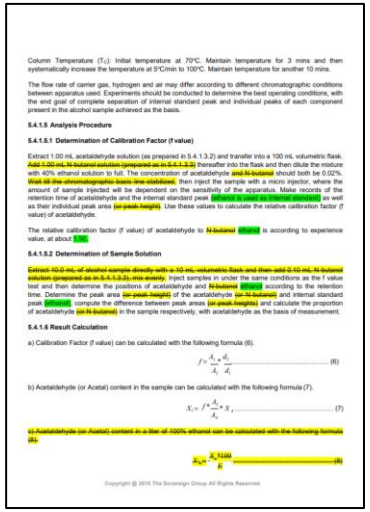
Carrier Gas (Nitrogen Gas of High Purity): Flow rate at 0.5 mL/min~1.0 mL/min; diversion ratio ~37:1; make up gas flow rate at about 20 mL/min~30 mL/min.

Hydrogen Gas: Flow rate at 33 mL/min.

Air: Flow at 400 mL/min.

Temperature of Detector (T_d): 220°C.

Temperature of Sample Inlet (T_1): 220°C.



Column Temperature (T_c): Initial temperature at 70°C. Maintain temperature for 3 mins and then systematically increase the temperature at 5°C/min to 100°C. Maintain temperature for another 10 mins.

The flow rate of carrier gas, hydrogen and air may differ according to different chromatographic conditions between apparatus used. Experiments should be conducted to determine the best operating conditions, with the end goal of complete separation of internal standard peak and individual peaks of each component present in the alcohol sample achieved as the basis.

5.4.1.5 Analysis Procedure

5.4.1.5.1 Determination of Calibration Factor (f value)

Extract 1.00 mL acetaldehyde solution (as prepared in 5.4.1.3.2) and transfer into a 100 mL volumetric flask. ~~Add 1.00 mL N-butanol solution (prepared as in 5.4.1.3.3)~~ thereafter into the flask and then dilute the mixture with 40% ethanol solution to full. The concentration of acetaldehyde ~~and N-butanol~~ should both be 0.02%. ~~Wait till the chromatographic basic line stabilized,~~ then inject the sample with a micro injector, where the amount of sample injected will be dependent on the sensitivity of the apparatus. Make records of the retention time of acetaldehyde and the internal standard peak (ethanol is used as internal standard) as well as their individual peak area ~~(or peak height)~~. Use these values to calculate the relative calibration factor (f value) of acetaldehyde.

The relative calibration factor (f value) of acetaldehyde to ~~N-butanol~~ ethanol is according to experience value, at about 1.50.

5.4.1.5.2 Determination of Sample Solution

~~Extract 10.0 mL of alcohol sample directly with a 10 mL volumetric flask and then add 0.10 mL N-butanol solution (prepared as in 5.4.1.3.3), mix evenly.~~ Inject samples in under the same conditions as the f value test and then determine the positions of acetaldehyde and ~~N-butanol~~ ethanol according to the retention time. Determine the peak area ~~(or peak height)~~ of the acetaldehyde ~~(or N-butanol)~~ and internal standard peak (ethanol), compute the difference between peak areas ~~(or peak heights)~~ and calculate the proportion of acetaldehyde ~~(or N-butanol)~~ in the sample respectively, with acetaldehyde as the basis of measurement.

5.4.1.6 Result Calculation

a) Calibration Factor (f value) can be calculated with the following formula (6).

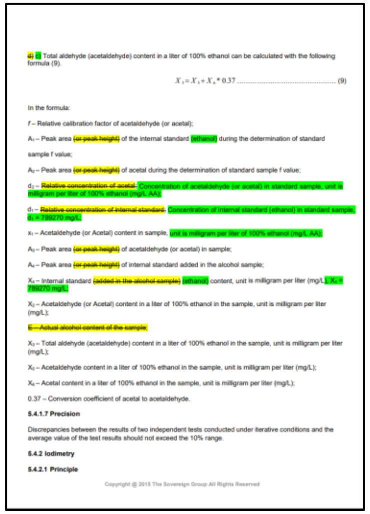
$$f = \frac{A_1}{A_2} * \frac{d_2}{d_1} \quad (6)$$

b) Acetaldehyde (or Acetal) content in the sample can be calculated with the following formula (7).

$$X_1 = f * \frac{A_3}{A_4} * X_4 \quad (7)$$

~~c) Acetaldehyde (or Acetal) content in a liter of 100% ethanol can be calculated with the following formula (8).~~

$$\cancel{X_2 = \frac{X_1 * 100}{E} \quad (8)}$$



~~d)~~ c) Total aldehyde (acetaldehyde) content in a liter of 100% ethanol can be calculated with the following formula (9).

$$X_3 = X_5 + X_6 * 0.37 \quad (9)$$

In the formula:

f – Relative calibration factor of acetaldehyde (or acetal);

A_1 – Peak area ~~(or peak height)~~ of the internal standard (ethanol) during the determination of standard sample f value;

A_2 – Peak area ~~(or peak height)~~ of acetal during the determination of standard sample f value;

d_2 – ~~Relative concentration of acetal;~~ Concentration of acetaldehyde (or acetal) in standard sample, unit is milligram per liter of 100% ethanol (mg/L AA);

d_1 – ~~Relative concentration of internal standard.~~ Concentration of internal standard (ethanol) in standard sample, d_1 = 789270 mg/L;

x_1 – Acetaldehyde (or Acetal) content in sample, unit is milligram per liter of 100% ethanol (mg/L AA);

A_3 – Peak area ~~(or peak height)~~ of acetaldehyde (or acetal) in sample;

A_4 – Peak area ~~(or peak height)~~ of internal standard added in the alcohol sample;

X_4 – Internal standard ~~(added in the alcohol sample)~~ (ethanol) content, unit is milligram per liter (mg/L), X_4 = 789270 mg/L;

X_2 – Acetaldehyde (or Acetal) content in a liter of 100% ethanol in the sample, unit is milligram per liter (mg/L);

~~E – Actual alcohol content of the sample;~~

X_3 – Total aldehyde (acetaldehyde) content in a liter of 100% ethanol in the sample, unit is milligram per liter (mg/L);

X_5 – Acetaldehyde content in a liter of 100% ethanol in the sample, unit is milligram per liter (mg/L);

X_6 – Acetal content in a liter of 100% ethanol in the sample, unit is milligram per liter (mg/L);

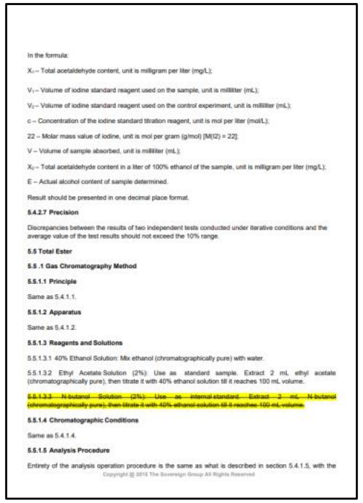
0.37 – Conversion coefficient of acetal to acetaldehyde.

5.4.1.7 Precision

Discrepancies between the results of two independent tests conducted under iterative conditions and the average value of the test results should not exceed the 10% range.

5.4.2 Iodimetry

5.4.2.1 Principle



In the formula:

X_1 – Total acetaldehyde content, unit is milligram per liter (mg/L);

V_1 – Volume of iodine standard reagent used on the sample, unit is milliliter (mL);

V_2 – Volume of iodine standard reagent used on the control experiment, unit is milliliter (mL);

c – Concentration of the iodine standard titration reagent, unit is mol per liter (mol/L);

22 – Molar mass value of iodine, unit is mol per gram (g/mol) [M(I2) = 22];

V – Volume of sample absorbed, unit is milliliter (mL);

X_2 – Total acetaldehyde content in a liter of 100% ethanol of the sample, unit is milligram per liter (mg/L);

E – Actual alcohol content of sample determined.

Result should be presented in one decimal place format.

5.4.2.7 Precision

Discrepancies between the results of two independent tests conducted under iterative conditions and the average value of the test results should not exceed the 10% range.

5.5 Total Ester

5.5 .1 Gas Chromatography Method

5.5.1.1 Principle

Same as 5.4.1.1.

5.5.1.2 Apparatus

Same as 5.4.1.2.

5.5.1.3 Reagents and Solutions

5.5.1.3.1 40% Ethanol Solution: Mix ethanol (chromatographically pure) with water.

5.5.1.3.2 Ethyl Acetate Solution (2%): Use as standard sample. Extract 2 mL ethyl acetate (chromatographically pure), then titrate it with 40% ethanol solution till it reaches 100 mL volume.

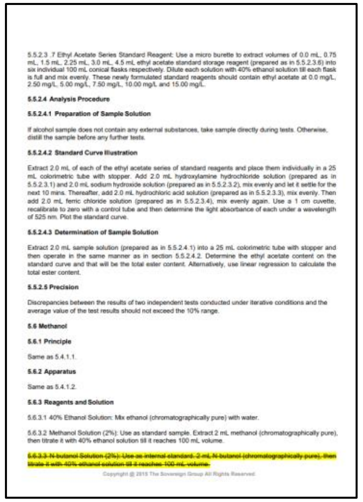
~~5.5.1.3.3 N-butanol Solution (2%): Use as internal standard. Extract 2 mL N-butanol (chromatographically pure), then titrate it with 40% ethanol solution till it reaches 100 mL volume.~~

5.5.1.4 Chromatographic Conditions

Same as 5.4.1.4.

5.5.1.5 Analysis Procedure

Entirety of the analysis operation procedure is the same as what is described in section 5.4.1.5, with the



5.5.2.3 .7 Ethyl Acetate Series Standard Reagent: Use a micro burette to extract volumes of 0.0 mL, 0.75 mL, 1.5 mL, 2.25 mL, 3.0 mL, 4.5 mL ethyl acetate standard storage reagent (prepared as in 5.5.2.3.6) into six individual 100 mL conical flasks respectively. Dilute each solution with 40% ethanol solution till each flask is full and mix evenly. These newly formulated standard reagents should contain ethyl acetate at 0.0 mg/L, 2.50 mg/L, 5.00 mg/L, 7.50 mg/L, 10.00 mg/L and 15.00 mg/L.

5.5.2.4 Analysis Procedure

5.5.2.4.1 Preparation of Sample Solution

If alcohol sample does not contain any external substances, take sample directly during tests. Otherwise, distill the sample before any further tests.

5.5.2.4.2 Standard Curve Illustration

Extract 2.0 mL of each of the ethyl acetate series of standard reagents and place them individually in a 25 mL colorimetric tube with stopper. Add 2.0 mL hydroxylamine hydrochloride solution (prepared as in 5.5.2.3.1) and 2.0 mL sodium hydroxide solution (prepared as in 5.5.2.3.2), mix evenly and let it settle for the next 10 mins. Thereafter, add 2.0 mL hydrochloric acid solution (prepared as in 5.5.2.3.3), mix evenly. Then add 2.0 mL ferric chloride solution (prepared as in 5.5.2.3.4), mix evenly again. Use a 1 cm cuvette, recalibrate to zero with a control tube and then determine the light absorbance of each under a wavelength of 525 nm. Plot the standard curve.

5.5.2.4.3 Determination of Sample Solution

Extract 2.0 mL sample solution (prepared as in 5.5.2.4.1) into a 25 mL colorimetric tube with stopper and then operate in the same manner as in section 5.5.2.4.2. Determine the ethyl acetate content on the standard curve and that will be the total ester content. Alternatively, use linear regression to calculate the total ester content.

5.5.2.5 Precision

Discrepancies between the results of two independent tests conducted under iterative conditions and the average value of the test results should not exceed the 10% range.

5.6 Methanol

5.6.1 Principle

Same as 5.4.1.1.

5.6.2 Apparatus

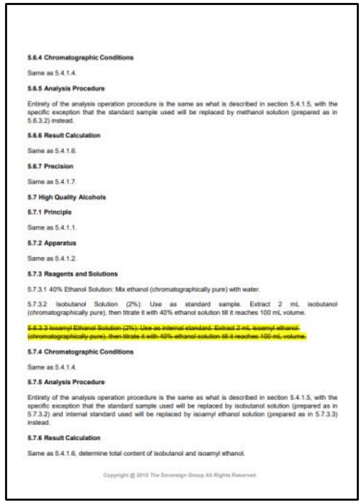
Same as 5.4.1.2.

5.6.3 Reagents and Solution

5.6.3.1 40% Ethanol Solution: Mix ethanol (chromatographically pure) with water.

5.6.3.2 Methanol Solution (2%): Use as standard sample. Extract 2 mL methanol (chromatographically pure), then titrate it with 40% ethanol solution till it reaches 100 mL volume.

~~5.6.3.3 N-butanol Solution (2%): Use as internal standard. 2 mL N-butanol (chromatographically pure), then titrate it with 40% ethanol solution till it reaches 100 mL volume.~~



5.6.4 Chromatographic Conditions

Same as 5.4.1.4.

5.6.5 Analysis Procedure

Entirety of the analysis operation procedure is the same as what is described in section 5.4.1.5, with the specific exception that the standard sample used will be replaced by methanol solution (prepared as in 5.6.3.2) instead.

5.6.6 Result Calculation

Same as 5.4.1.6.

5.6.7 Precision

Same as 5.4.1.7.

5.7 High Quality Alcohols

5.7.1 Principle

Same as 5.4.1.1.

5.7.2 Apparatus

Same as 5.4.1.2.

5.7.3 Reagents and Solutions

5.7.3.1 40% Ethanol Solution: Mix ethanol (chromatographically pure) with water.

5.7.3.2 Isobutanol Solution (2%): Use as standard sample. Extract 2 mL isobutanol (chromatographically pure), then titrate it with 40% ethanol solution till it reaches 100 mL volume.

~~5.6.3.3 Isoamyl Ethanol Solution (2%): Use as internal standard. Extract 2 mL isoamyl ethanol (chromatographically pure), then titrate it with 40% ethanol solution till it reaches 100 mL volume.~~

5.7.4 Chromatographic Conditions

Same as 5.4.1.4.

5.7.5 Analysis Procedure

Entirety of the analysis operation procedure is the same as what is described in section 5.4.1.5, with the specific exception that the standard sample used will be replaced by isobutanol solution (prepared as in 5.7.3.2) and internal standard used will be replaced by isoamyl ethanol solution (prepared as in 5.7.3.3) instead.

5.7.6 Result Calculation

Same as 5.4.1.6, determine total content of isobutanol and isoamyl ethanol.

BIS IS 3752:2005(R2009) - simplification

Alcoholic Drinks - Methods of Test

The use of the proposed method ensures high reliability of the data obtained, significantly reduces time, labor, material and financial costs. Analysis of volatile compounds in spirit drinks has never been so easy. Here you can read modified text of official method, which allows to carry out analysis of alcoholic beverages using the developed method.

The places in the text document to be deleted are highlighted in yellow. Embedded parts of the test are highlighted in green.

You can read a full article about the improvements by following the link: https://elab.bsu.by/article/359

इंटरनेट मानक

Disclosure to Promote the Right To Information

Whereas the Parliament of India has set out to provide a practical regime of right to information for citizens to secure access to information under the control of public authorities, in order to promote transparency and accountability in the working of every public authority, and whereas the attached publication of the Bureau of Indian Standards is of particular interest to the public, particularly disadvantaged communities and those engaged in the pursuit of education and knowledge, the attached public safety standard is made available to promote the timely dissemination of this information in an accurate manner to the public.

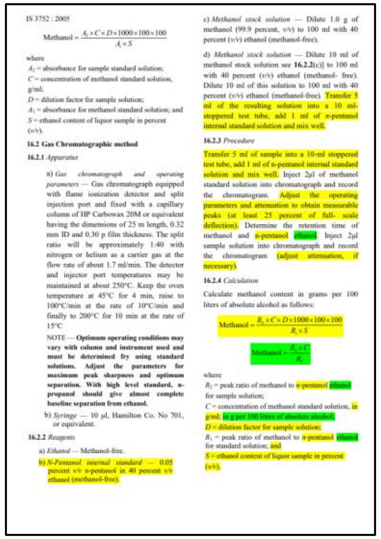
"जानने का अधिकार, जीने का अधिकार"
Mazdoor Kisan Shakti Sangathan
"The Right to Information, The Right to Live"

"पुराने को छोड नये के तरफ"
Jawaharlal Nehru
"Step Out From the Old to the New"

IS 3752 (2005): Alcoholic drinks - Methods of test [FAD 14: Drinks and Carbonated Beverages]

"ज्ञान से एक नये भारत का निर्माण"
Satyanarayan Gangaram Pitroda
"Invent a New India Using Knowledge"

"ज्ञान एक ऐसा खजाना है जो कभी चुराया नहीं जा सकता है"
Bhartṛhari—Nītiśatakam
"Knowledge is such a treasure which cannot be stolen"



IS 3752 : 2005

$$\text{Methanol} = \frac{A_2 \times C \times D \times 1000 \times 100 \times 100}{A_1 \times S}$$

where

A_2 = absorbance for sample standard solution;
C = concentration of methanol standard solution, g/ml;
D = dilution factor for sample solution;
A_1 = absorbance for methanol standard solution; and
S = ethanol content of liquor sample in percent (v/v).

16.2 Gas Chromatographic method

16.2.1 *Apparatus*

a) *Gas chromatograph and operating parameters* — Gas chromatograph equipped with flame ionization detector and split injection port and fixed with a capillary column of HP Carbowax 20M or equivalent having the dimensions of 25 m length, 0.32 mm ID and 0.30 p film thickness. The split ratio will be approximately 1:40 with nitrogen or helium as a carrier gas at the flow rate of about 1.7 ml/min. The detector and injector port temperatures may be maintained at about 250°C. Keep the oven temperature at 45°C for 4 min, raise to 100°C/min at the rate of 10°C/min and finally to 200°C for 10 min at the rate of 15°C

NOTE — **Optimum operating conditions may vary with column and instrument used and must be determined fry using standard solutions. Adjust the parameters for maximum peak sharpness and optimum separation. With high level standard, n-propanol should give almost complete baseline separation from ethanol.**

b) *Syringe* — 10 µl, Hamilton Co. No 701, or equivalent.

16.2.2 *Reagents*

a) *Ethanol* — Methanol-free.

b) *N-Pentanol internal standard* — 0.05 percent v/v n-pentanol in 40 percent v/v ethanol (methanol-free).

c) *Methanol stock solution* — Dilute 1.0 g of methanol (99.9 percent, v/v) to 100 ml with 40 percent (v/v) ethanol (methanol-free).

d) *Methanol stock solution* — Dilute 10 ml of methanol stock solution see **16.2.2**(c)] to 100 ml with 40 percent (v/v) ethanol (methanol- free). Dilute 10 ml of this solution to 100 ml with 40 percent (v/v) ethanol (methanol-free). Transfer 5 ml of the resulting solution into a 10 ml-stoppered test tube, add 1 ml of *n*-pentanol internal standard solution and mix well.

16.2.3 *Procedure*

Transfer 5 ml of sample into a 10-ml stoppered test tube, add 1 ml of n-pentanol internal standard solution and mix well. Inject 2µl of methanol standard solution into chromatograph and record the chromatogram. Adjust the operating parameters and attenuation to obtain measurable peaks (at least 25 percent of full- scale deflection). Determine the retention time of methanol and n-pentanol ethanol. Inject 2µl sample solution into chromatograph and record the chromatogram (adjust attenuation, if necessary).

16.2.4 *Calculation*

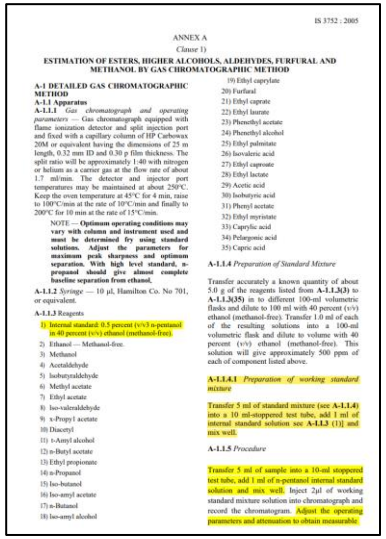
Calculate methanol content in grams per 100 liters of absolute alcohol as follows:

$$\text{Methanol} = \frac{R_2 \times C \times D \times 1000 \times 100 \times 100}{R_1 \times S}$$

$$\text{Methanol} = \frac{R_2 \times C}{R_1}$$

where

R_2 = peak ratio of methanol to n-pentanol ethanol for sample solution;
C = concentration of methanol standard solution, in g/ml; in g per 100 liters of absolute alcohol;
D = dilution factor for sample solution;
R_1 = peak ratio of methanol to *n*-pentanol ethanol for standard solution; and
S = ethanol content of liquor sample in percent (v/v).



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ANNEX A

Clause 1)

ESTIMATION OF ESTERS, HIGHER ALCOHOLS, ALDEHYDES, FURFURAL AND METHANOL BY GAS CHROMATOGRAPHIC METHOD

A-1 DETAILED GAS CHROMATOGRAPHIC METHOD

A-1.1 Apparatus

A-1.1.1 *Gas chromatograph and operating parameters* — Gas chromatograph equipped with flame ionization detector and split injection port and fixed with a capillary column of HP Carbowax 20M or equivalent having the dimensions of 25 m length, 0.32 mm ID and 0.30 p film thickness. The split ratio will be approximately 1:40 with nitrogen or helium as a carrier gas at the flow rate of about 1.7 ml/min. The detector and injector port temperatures may be maintained at about 250°C. Keep the oven temperature at 45°C for 4 min, raise to 100°C/min at the rate of 10°C/min and finally to 200°C for 10 min at the rate of 15°C/min.

NOTE — **Optimum operating conditions may vary with column and instrument used and must be determined fry using standard solutions. Adjust the parameters for maximum peak sharpness and optimum separation. With high level standard, n-propanol should give almost complete baseline separation from ethanol.**

A-1.1.2 *Syringe* — 10 µl, Hamilton Co. No 701, or equivalent.

A-1.1.3 Reagents

1) Internal standard: 0.5 percent (v/v3 n-pentanol in 40 percent (v/v) ethanol (methanol-free).
2) Ethanol — Methanol-free.
3) Methanol
4) Acetaldehyde
5) Isobutyraldehyde
6) Methyl acetate
7) Ethyl acetate
8) Iso-valeraldehyde
9) x-Propyl acetate
10) Diacetyl
11) t-Amyl alcohol
12) n-Butyl acetate
13) Ethyl propionate
14) n-Propanol
15) Iso-butanol
16) Iso-amyl acetate
17) n-Butanol
18) Iso-amyl alcohol
19) Ethyl caprylate
20) Furfural
21) Ethyl caprate
22) Ethyl laurate
23) Phenethyl acetate
24) Phenethyl alcohol
25) Ethyl palmitate
26) Isovaleric acid
27) Ethyl caproate
28) Ethyl lactate
29) Acetic acid
30) Isobutyric acid
31) Phenyl acetate
32) Ethyl myristate
33) Caprylic acid
34) Pelargonic acid
35) Capric acid

A-1.1.4 *Preparation of Standard Mixture*

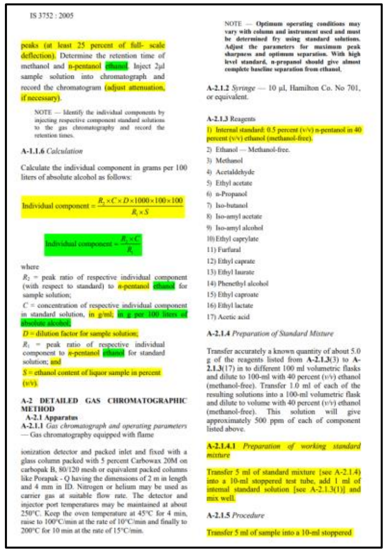
Transfer accurately a known quantity of about 5.0 g of the reagents listed from **A-1.1.3(3)** to **A-1.1.3(35)** in to different 100-ml volumetric flasks and dilute to 100 ml with 40 percent (v/v) ethanol (methanol-free). Transfer 1.0 ml of each of the resulting solutions into a 100-ml volumetric flask and dilute to volume with 40 percent (v/v) ethanol (methanol-free). This solution will give approximately 500 ppm of each of component listed above.

A-1.1.4.1 *Preparation of working standard mixture*

Transfer 5 ml of standard mixture (see **A-1.1.4**) into a 10 ml-stoppered test tube, add 1 ml of internal standard solution see **A-1.1.3** (1)] and mix well.

A-1.1.5 *Procedure*

Transfer 5 ml of sample into a 10-ml stoppered test tube, add 1 ml of n-pentanol internal standard solution and mix well. Inject 2µl of working standard mixture solution into chromatograph and record the chromatogram. Adjust the operating parameters and attenuation to obtain measurable



IS 3752 : 2005

peaks (at least 25 percent of full- scale deflection). Determine the retention time of methanol and n-pentanol ethanol. Inject 2µl sample solution into chromatograph and record the chromatogram (adjust attenuation, if necessary).

NOTE — Identify the individual components by injecting respective component standard solutions to the gas chromatography and record the retention times.

A-1.1.6 *Calculation*

Calculate the individual component in grams per 100 liters of absolute alcohol as follows:

$$\text{Individual component} = \frac{R_2 \times C \times D \times 1000 \times 100 \times 100}{R_1 \times S}$$

$$\text{Individual component} = \frac{R_2 \times C}{R_1}$$

where

R_2 = peak ratio of respective individual component (with respect to standard) to *n*-pentanol ethanol for sample solution;
C = concentration of respective individual component in standard solution, in g/ml; in g per 100 liters of absolute alcohol;
D = dilution factor for sample solution;
R_1 = peak ratio of respective individual component to *n*-pentanol ethanol for standard solution; and
S = ethanol content of liquor sample in percent (v/v).

A-2 DETAILED GAS CHROMATOGRAPHIC METHOD

A-2.1 Apparatus

A-2.1.1 *Gas chromatograph and operating parameters* — Gas chromatography equipped with flame ionization detector and packed inlet and fixed with a glass column packed with 5 percent Carbowax 20M on carbopak B, 80/120 mesh or equivalent packed columns like Porapak - Q having the dimensions of 2 m in length and 4 mm in ID. Nitrogen or helium may be used as carrier gas at suitable flow rate. The detector and injector port temperatures may be maintained at about 250°C. Keep the oven temperature at 45°C for 4 min, raise to 100°C/min at the rate of 10°C/min and finally to 200°C for 10 min at the rate of 15°C/min.

NOTE — **Optimum operating conditions may vary with column and instrument used and must be determined fry using standard solutions. Adjust the parameters for maximum peak sharpness and optimum separation. With high level standard, n-propanol should give almost complete baseline separation from ethanol,**

A-2.1.2 *Syringe* — 10 µl, Hamilton Co. No 701, or equivalent.

A-2.1.3 Reagents

1) Internal standard: 0.5 percent (v/v) n-pentanol in 40 percent (v/v) ethanol (methanol-free).
2) Ethanol — Methanol-free.
3) Methanol
4) Acetaldehyde
5) Ethyl acetate
6) n-Propanol
7) Iso-butanol
8) Iso-amyl acetate
9) Iso-amyl alcohol
10) Ethyl caprylate
11) Furfural
12) Ethyl caprate
13) Ethyl laurate
14) Phenethyl alcohol
15) Ethyl caproate
16) Ethyl lactate
17) Acetic acid

A-2.1.4 *Preparation of Standard Mixture*

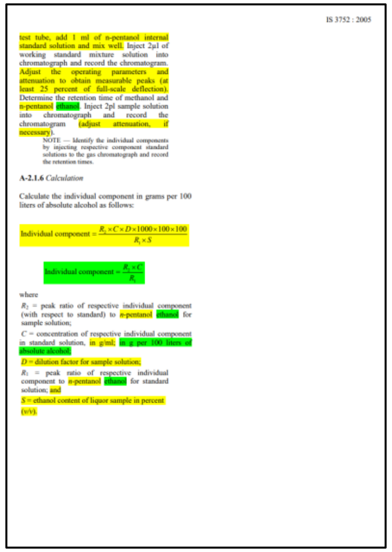
Transfer accurately a known quantity of about 5.0 g of the reagents listed from **A-2.1.3**(3) to **A-2.1.3**(17) in to different 100 ml volumetric flasks and dilute to 100-ml with 40 percent (v/v) ethanol (methanol-free). Transfer 1.0 ml of each of the resulting solutions into a 100-ml volumetric flask and dilute to volume with 40 percent (v/v) ethanol (methanol-free). This solution will give approximately 500 ppm of each of component listed above.

A-2.1.4.1 *Preparation of working standard mixture*

Transfer 5 ml of standard mixture [see A-2.1.4) into a 10-ml stoppered test tube, add 1 ml of internal standard solution [see A-2.1.3(1)] and mix well.

A-2.1.5 *Procedure*

Transfer 5 ml of sample into a 10-ml stoppered



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test tube, add 1 ml of n-pentanol internal standard solution and mix well. Inject 2µl of working standard mixture solution into chromatograph and record the chromatogram. Adjust the operating parameters and attenuation to obtain measurable peaks (at least 25 percent of full-scale deflection). Determine the retention time of methanol and n-pentanol ethanol. Inject 2pl sample solution into chromatograph and record the chromatogram (adjust attenuation, if necessary).

NOTE — Identify the individual components by injecting respective component standard solutions to the gas chromatograph and record the retention times.

A-2.1.6 *Calculation*

Calculate the individual component in grams per 100 liters of absolute alcohol as follows:

$$\text{Individual component} = \frac{R_2 \times C \times D \times 1000 \times 100 \times 100}{R_1 \times S}$$

$$\text{Individual component} = \frac{R_2 \times C}{R_1}$$

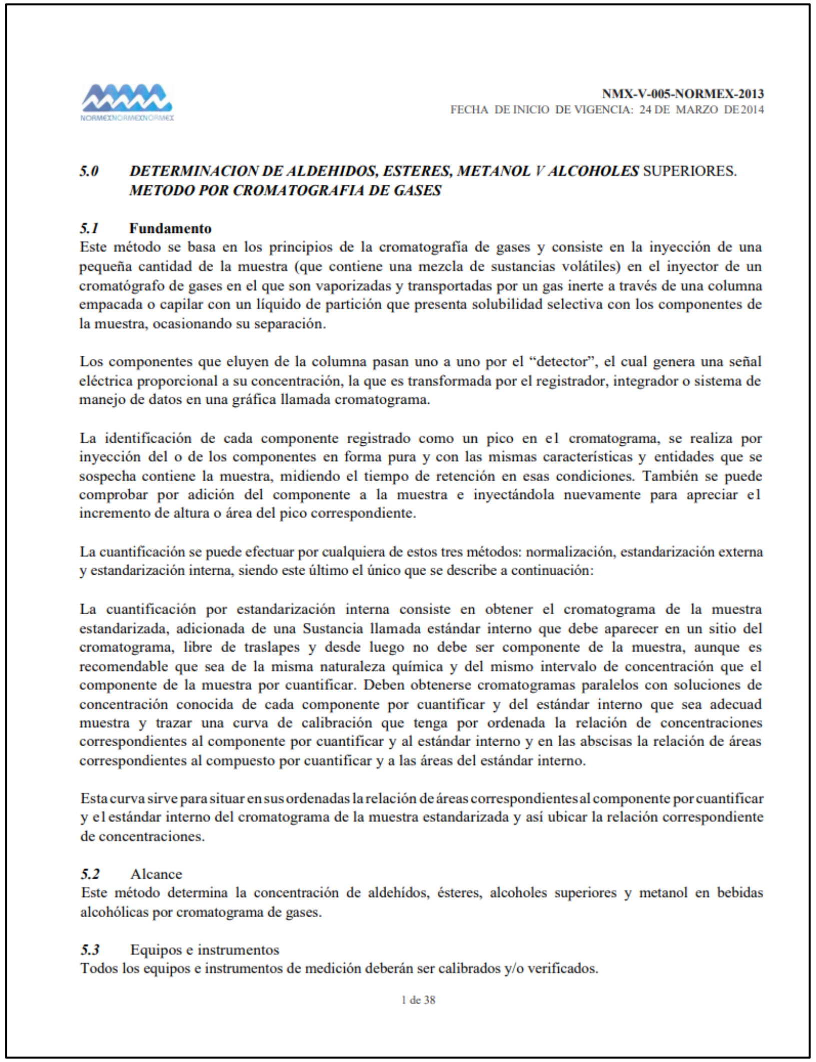
where

R_2 = peak ratio of respective individual component (with respect to standard) to *n*-pentanol ethanol for sample solution;
C = concentration of respective individual component in standard solution, in g/ml; in g per 100 liters of absolute alcohol;
D = dilution factor for sample solution;
R_1 = peak ratio of respective individual component to *n*-pentanol ethanol for standard solution; and
S = ethanol content of liquor sample in percent (v/v).

Norma Mexicana NMX-V-005-NORMEX-2013 – simplification

Determination de Aldehidos, Esters, Methanol y Alcoholes Superiores. Metodo por cromatografia de Gases

The use of the proposed method ensures high reliability of the data obtained, significantly reduces time, labor, material and financial costs. Analysis of volatile compounds in spirit drinks has never been so easy. Here you can read modified text of official method, which allows to carry out analysis of alcoholic beverages using the developed method.
The places in the text document to be deleted are highlighted in yellow. Embedded parts of the test are highlighted in green.
You can read a full article about the improvements by following the link: https://elab.bsu.by/article/358



5.0 *DETERMINACION DE ALDEHIDOS, ESTERES, METANOL V ALCOHOLES* SUPERIORES. *METODO POR CROMATOGRAFIA DE GASES*

5.1 Fundamento

Este método se basa en los principios de la cromatografía de gases y consiste en la inyección de una pequeña cantidad de la muestra (que contiene una mezcla de sustancias volátiles) en el inyector de un cromatógrafo de gases en el que son vaporizadas y transportadas por un gas inerte a través de una columna empacada o capilar con un líquido de partición que presenta solubilidad selectiva con los componentes de la muestra, ocasionando su separación.

Los componentes que eluyen de la columna pasan uno a uno por el "detector", el cual genera una señal eléctrica proporcional a su concentración, la que es transformada por el registrador, integrador o sistema de manejo de datos en una gráfica llamada cromatograma.

La identificación de cada componente registrado como un pico en el cromatograma, se realiza por inyección del o de los componentes en forma pura y con las mismas características y entidades que se sospecha contiene la muestra, midiendo el tiempo de retención en esas condiciones. También se puede comprobar por adición del componente a la muestra e inyectándola nuevamente para apreciar el incremento de altura o área del pico correspondiente.

La cuantificación se puede efectuar por cualquiera de estos tres métodos: normalización, estandarización externa y estandarización interna, siendo este último el único que se describe a continuación:

La cuantificación por estandarización interna consiste en obtener el cromatograma de la muestra estandarizada, adicionada de una Sustancia llamada estándar interno que debe aparecer en un sitio del cromatograma, libre de traslapes y desde luego no debe ser componente de la muestra, aunque es recomendable que sea de la misma naturaleza química y del mismo intervalo de concentración que el componente de la muestra por cuantificar. Deben obtenerse cromatogramas paralelos con soluciones de concentración conocida de cada componente por cuantificar y del estándar interno que sea adecuad muestra y trazar una curva de calibración que tenga por ordenada la relación de concentraciones correspondientes al componente por cuantificar y al estándar interno y en las abscisas la relación de áreas correspondientes al compuesto por cuantificar y a las áreas del estándar interno.

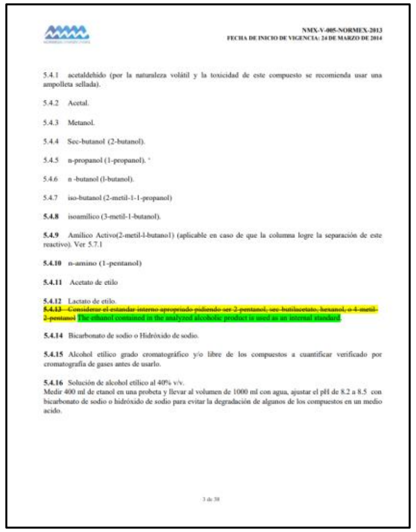
Esta curva sirve para situar en sus ordenadas la relación de áreas correspondientes al componente por cuantificar y el estándar interno del cromatograma de la muestra estandarizada y así ubicar la relación correspondiente de concentraciones.

5.2 Alcance

Este método determina la concentración de aldehídos, ésteres, alcoholes superiores y metanol en bebidas alcohólicas por cromatograma de gases.

5.3 Equipos e instrumentos

Todos los equipos e instrumentos de medición deberán ser calibrados y/o verificados.



5.4.1 acetaldehido (por la naturaleza volátil y la toxicidad de este compuesto se recomienda usar una ampolleta sellada).

5.4.2 Acetal.

5.4.3 Metanol.

5.4.4 Sec-butanol (2-butanol).

5.4.5 n-propanol (1-propanol).

5.4.6 n -butanol (l-butanol).

5.4.7 iso-butanol (2-metil-1-1-propanol)

5.4.8 isoamilico (3-metil-1-butanol).

5.4.9 Amilico Activo(2-metil-l-butano1) (aplicable en caso de que la columna logre la separación de este reactivo). Ver 5.7.1

5.4.10 n-amino (1-pentanol)

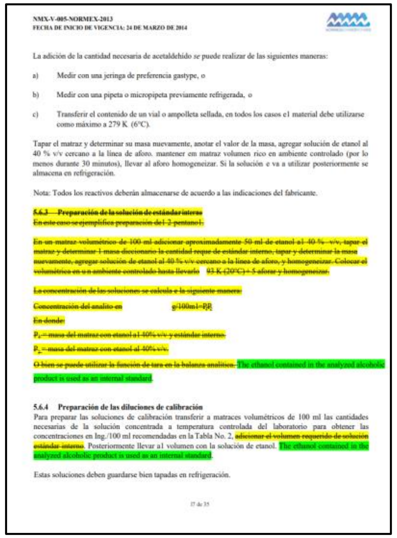
5.4.11 Acetato de etilo

5.4.12 Lactato de etilo.
~~**5.4.13** Considerar el estandar interno apropriado pidiendo ser 2-pentanol, sec-butilacetato, hexanol, o 4-metil-2-pentanol~~ The ethanol contained in the analyzed alcoholic product is used as an internal standard.

5.4.14 Bicarbonato de sodio o Hidróxido de sodio.

5.4.15 Alcohol etilico grado cromatográfico y/o libre de los compuestos a cuantificar verificado por cromatografía de gases antes de usarlo.

5.4.16 Solución de alcohol etilico al 40% v/v.
Medir 400 ml de etanol en una probeta y llevar al volumen de 1000 ml con agua, ajustar el pH de 8.2 a 8.5 con bicarbonato de sodio o hidróxido de sodio para evitar la degradación de algunos de los compuestos en un medio acido.



La adición de la cantidad necesaria de acetaldehido *se* puede realizar de las siguientes maneras:

a) Medir con una jeringa de preferencia gastype, o

b) Medir con una pipeta o micropipeta previamente refrigerada, o

c) Transferir el contenido de un vial o ampolleta sellada, en todos los casos el material debe utilizarse como máximo a 279 K (6°C).

Tapar el matraz y determinar su masa nuevamente, anotar el valor de la masa, agregar solución de etanol al 40 % v/v cercano a la linea de aforo. mantener em matraz volumen rico en ambiente controlado (por lo menos durante 30 minutos), llevar al aforo homogeneizar. Si la solución e va a utilizar posteriormente se almacena en refrigeración.

Nota: Todos los reactivos deberán almacenarse de acuerdo a las indicaciones del fabricante.

~~**5.6.3 Preparación de la solución de estándar interno**~~
~~En este caso se ejemplifica preparación del 2-pentanol.~~

~~En un matraz volumétrico de 100 ml adicionar aproximadamente 50 ml de etanol al 40 % v/v, tapar el matraz y determinar 1 masa diccionario la cantidad reque de estándar interno, tapar y determinar la masa nuevamente, agregar solución de etanol al 40 % v/v cercano a la linea de aforo, y homogeneizar. Colocar el volumétrico en u n ambiente controlado hasta llevarlo 93 K (20°C) ± 5 aforar y homogeneizar.~~

~~La concentración de las soluciones se calcula e la siguiente manera:~~

~~Concentración del analito en g/100ml=P1P2~~

~~En donde:~~

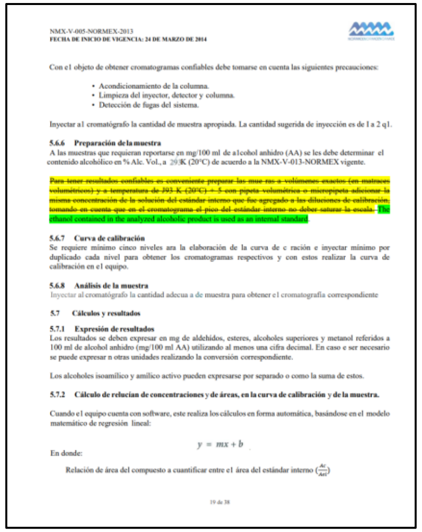
~~P_1 = masa del matraz con etanol al 40% v/v y estándar interno.~~

~~P_2 = masa del matraz con etanol al 40% v/v.~~

~~O bien se puede utilizar la función de tara en la balanza analitica.~~ The ethanol contained in the analyzed alcoholic product is used as an internal standard.

5.6.4 Preparación de las diluciones de calibración
Para preparar las soluciones de calibración transferir a matraces volumétricos de 100 ml las cantidades necesarias de la solución concentrada a temperatura controlada del laboratorio para obtener las concentraciones en lng./100 ml recomendadas en la Tabla No. 2, ~~adicionar el volumen requerido de solución estándar interno~~. Posteriormente llevar al volumen con la solución de etanol. The ethanol contained in the analyzed alcoholic product is used as an internal standard.

Estas soluciones deben guardarse bien tapadas en refrigeración.



Con el objeto de obtener cromatogramas confiables debe tomarse en cuenta las siguientes precauciones:

- Acondicionamiento de la columna.
- Limpieza del inyector, detector y columna.
- Detección de fugas del sistema.

Inyectar al cromatógrafo la cantidad de muestra apropiada. La cantidad sugerida de inyección es de l a 2 ql.

5.6.6 Preparación de la muestra
A las muestras que requieran reportarse en mg/100 ml de alcohol anhidro (AA) se les debe determinar el contenido alcohólico en % Alc. Vol., a 293K (20°C) de acuerdo a la NMX-V-013-NORMEX vigente.

~~Para tener resultados confiables es conveniente preparar las mue ras a volúmenes exactos (en matraces volumétricos) y a temperatura de J93 K (20°C) ± 5 con pipeta volumétrica o micropipeta adicionar la misma concentración de la solución del estándar interno que fue agregado a las diluciones de calibración, tomando en cuenta que en el cromatograma el pico del estándar interno no deber saturar la escala.~~ The ethanol contained in the analyzed alcoholic product is used as an internal standard.

5.6.7 Curva de calibración
Se requiere mínimo cinco niveles ara la elaboración de la curva de c ración e inyectar mínimo por duplicado cada nivel para obtener los cromatogramas respectivos y con estos realizar la curva de calibración en el equipo.

5.6.8 Análisis de la muestra
Inyectar al cromatógrafo la cantidad adecua a de muestra para obtener el cromatografía correspondiente

5.7 Cálculos y resultados

5.7.1 Expresión de resultados
Los resultados se deben expresar en mg de aldehídos, esteres, alcoholes superiores y metanol referidos a 100 ml de alcohol anhidro (mg/100 ml AA) utilizando al menos una cifra decimal. En caso e ser necesario se puede expresar n otras unidades realizando la conversión correspondiente.

Los alcoholes isoamílico y amílico activo pueden expresarse por separado o como la suma de estos.

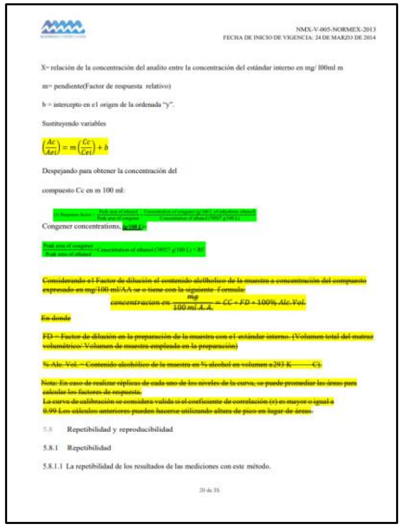
5.7.2 Cálculo de relucían de concentraciones y de áreas, en la curva de calibración y de la muestra.

Cuando el equipo cuenta con software, este realiza los cálculos en forma automática, basándose en el modelo matemático de regresión lineal:

$$y = mx + b$$

En donde:

Relación de área del compuesto a cuantificar entre el área del estándar interno $\left(\frac{Ac}{Aei}\right)$



X= relación de la concentración del analito entre la concentración del estándar interno en mg/ l00ml m

m= pendiente(Factor de respuesta relativo)

b = intercepto en el origen de la ordenada "y".

Sustituyendo variables

$$\left(\frac{Ac}{Aei}\right) = m\left(\frac{Cc}{Cei}\right) + b$$

Despejando para obtener la concentración del

compuesto Cc en m 100 ml:

Congener concentrations, ~~g/100 L~~:

~~Considerando el Factor de dilución el contenido alcohólico de la muestra a concentración del compuesto expresado en mg/100 ml/AA se o tiene con la siguiente formula:~~

~~$$concentracion\ en\ \frac{mg}{100\ ml\ A.A.} = CC * FD * 100\%\ Alc.Vol.$$~~

~~En donde~~

~~FD = Factor de dilución en la preparación de la muestra con el estándar interno. (Volumen total del matraz volumétrico/ Volumen de muestra empleada en la preparación)~~

~~% Alc. Vol. = Contenido alcohólico de la muestra en % alcohol en volumen a293 K C).~~

~~Nota: En caso de realizar réplicas de cada uno de los niveles de la curva, se puede promediar las áreas para calcular los factores de respuesta.~~
~~La curva de calibración se considera valida si el coeficiente de correlación (r) es mayor o igual a 0.99 Los cálculos anteriores pueden hacerse utilizando altura de pico en lugar de áreas.~~

5.8 Repetibilidad y reproducibilidad

5.8.1 Repetibilidad

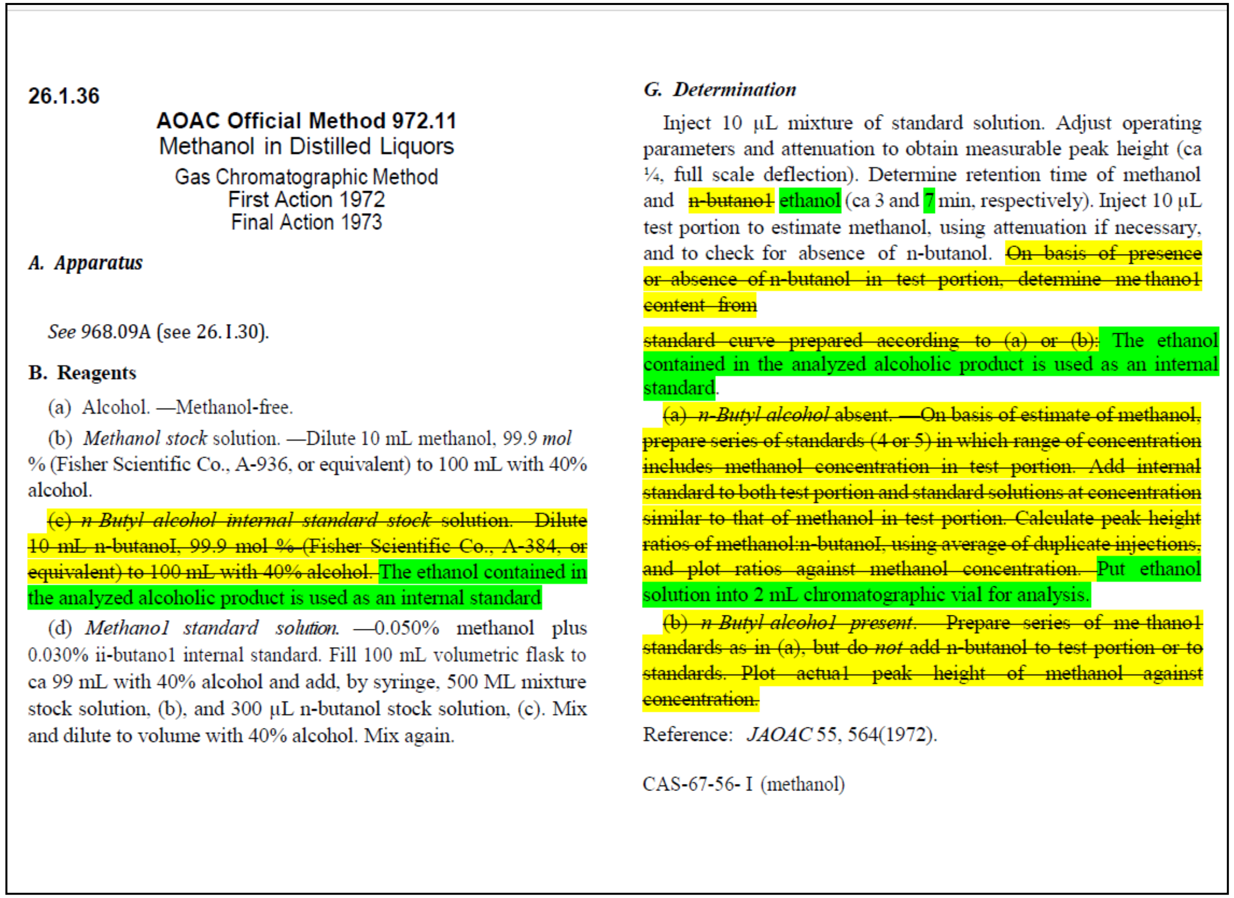
5.8.1.1 La repetibilidad de los resultados de las mediciones con este método.

AOAC Official Method 972.11 - simplification

Methanol in Distilled Liquors. Gas Chromatographic Method

The use of the proposed method ensures high reliability of the data obtained, significantly reduces time, labor, material and financial costs. Analysis of volatile compounds in spirit drinks has never been so easy. Here you can read modified text of official method, which allows to carry out analysis of alcoholic beverages using the developed method.
The places in the text document to be deleted are highlighted in yellow. Embedded parts of the test are highlighted in green.
You can read a full article about the improvements by following the link: **https://elab.bsu.by/article/355**

Determination of methanol has never been so easy !



26.1.36

AOAC Official Method 972.11
Methanol in Distilled Liquors
Gas Chromatographic Method
First Action 1972
Final Action 1973

A. Apparatus

See 968.09A (see 26.I.30).

B. Reagents

(a) Alcohol. —Methanol-free.

(b) *Methanol stock* solution. —Dilute 10 mL methanol, 99.9 *mol* % (Fisher Scientific Co., A-936, or equivalent) to 100 mL with 40% alcohol.

~~(c) *n Butyl alcohol internal standard stock* solution. Dilute 10 mL n-butanol, 99.9 mol % (Fisher Scientific Co., A-384, or equivalent) to 100 mL with 40% alcohol.~~ The ethanol contained in the analyzed alcoholic product is used as an internal standard

(d) *Methano1 standard solution.* —0.050% methanol plus 0.030% ii-butano1 internal standard. Fill 100 mL volumetric flask to ca 99 mL with 40% alcohol and add, by syringe, 500 ML mixture stock solution, (b), and 300 µL n-butanol stock solution, (c). Mix and dilute to volume with 40% alcohol. Mix again.

G. Determination

Inject 10 µL mixture of standard solution. Adjust operating parameters and attenuation to obtain measurable peak height (ca ¼, full scale deflection). Determine retention time of methanol and ~~n-butano1~~ ethanol (ca 3 and 7 min, respectively). Inject 10 µL test portion to estimate methanol, using attenuation if necessary, and to check for absence of n-butanol. ~~On basis of presence or absence of n-butanol in test portion, determine methano1 content from~~

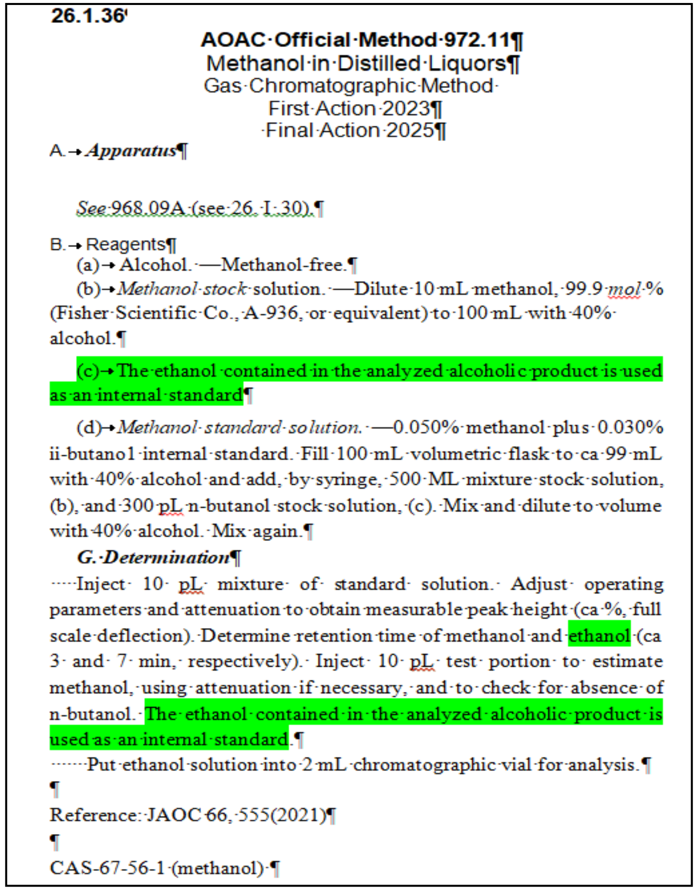
~~standard curve prepared according to (a) or (b):~~ The ethanol contained in the analyzed alcoholic product is used as an internal standard.

~~(a) *n-Butyl alcohol* absent. —On basis of estimate of methanol, prepare series of standards (4 or 5) in which range of concentration includes methanol concentration in test portion. Add internal standard to both test portion and standard solutions at concentration similar to that of methanol in test portion. Calculate peak height ratios of methanol:n-butanol, using average of duplicate injections, and plot ratios against methanol concentration.~~ Put ethanol solution into 2 mL chromatographic vial for analysis.

~~(b) *n Butyl alcohol present.* Prepare series of methanol standards as in (a), but do *not* add n-butanol to test portion or to standards. Plot actual peak height of methanol against concentration.~~

Reference: *JAOAC* 55, 564(1972).

CAS-67-56- I (methanol)



26.1.36

AOAC Official Method 972.11¶
Methanol in Distilled Liquors¶
Gas Chromatographic Method
First Action 2023¶
Final Action 2025¶

A.→ ***Apparatus***¶

See 968.09A (see 26. I.30).¶

B.→ Reagents¶
(a)→ Alcohol. —Methanol-free.¶
(b)→ *Methanol stock* solution. —Dilute 10 mL methanol, 99.9 *mol* % (Fisher Scientific Co., A-936, or equivalent) to 100 mL with 40% alcohol.¶

(c)→ The ethanol contained in the analyzed alcoholic product is used as an internal standard¶

(d)→ *Methanol standard solution.* —0.050% methanol plus 0.030% ii-butano1 internal standard. Fill 100 mL volumetric flask to ca 99 mL with 40% alcohol and add, by syringe, 500 ML mixture stock solution, (b), and 300 pL n-butanol stock solution, (c). Mix and dilute to volume with 40% alcohol. Mix again.¶

G. Determination¶

Inject 10 pL mixture of standard solution. Adjust operating parameters and attenuation to obtain measurable peak height (ca %, full scale deflection). Determine retention time of methanol and ethanol (ca 3 and 7 min, respectively). Inject 10 pL test portion to estimate methanol, using attenuation if necessary, and to check for absence of n-butanol. The ethanol contained in the analyzed alcoholic product is used as an internal standard.¶

Put ethanol solution into 2 mL chromatographic vial for analysis.¶

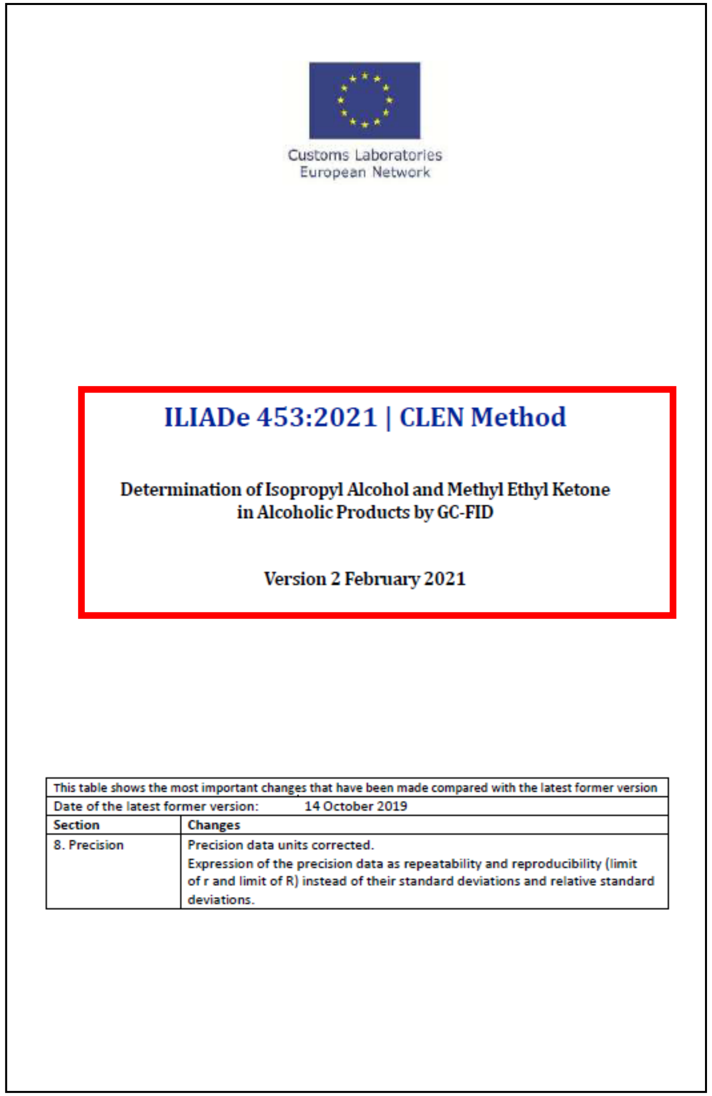
¶

Reference: JAOC 66, 555(2021)¶

¶

CAS-67-56-1 (methanol)¶

After the Interlaboratory Study under the CLEN auspices the new method was accepted for regulatory purposes



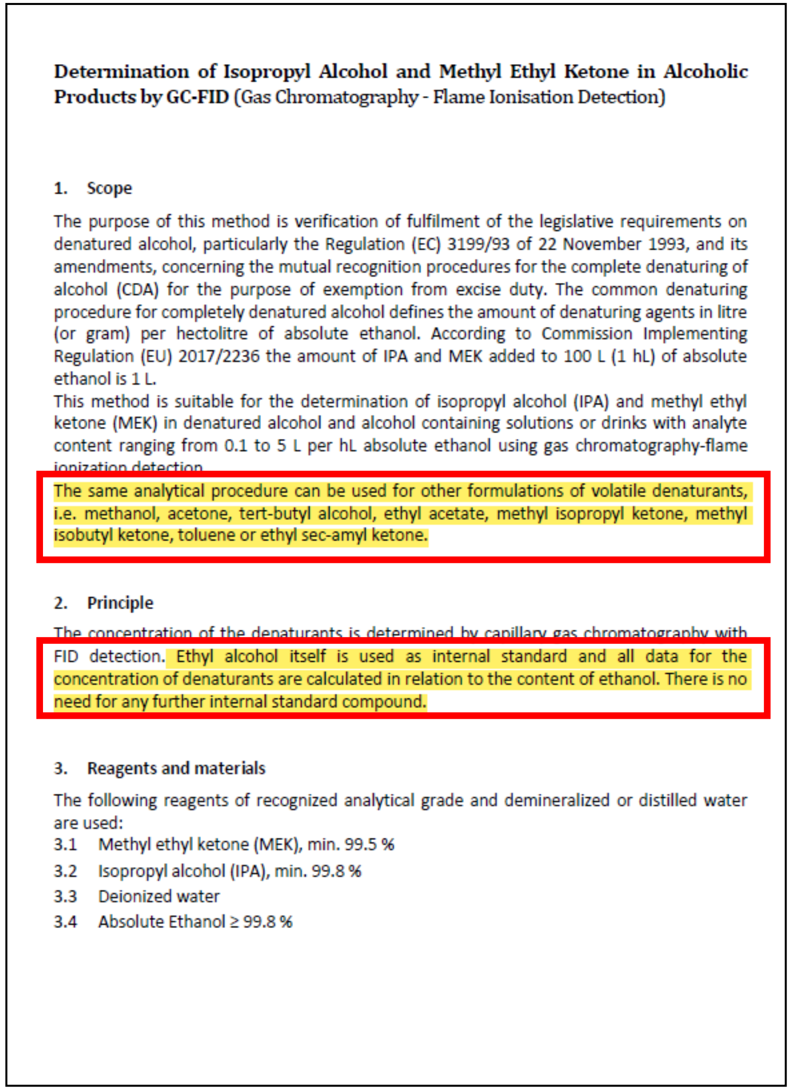
Customs Laboratories
European Network

ILIADe 453:2021 | CLEN Method

Determination of Isopropyl Alcohol and Methyl Ethyl Ketone
in Alcoholic Products by GC-FID

Version 2 February 2021

This table shows the most important changes that have been made compared with the latest former version	
Date of the latest former version: 14 October 2019	
Section	**Changes**
8. Precision	Precision data units corrected. Expression of the precision data as repeatability and reproducibility (limit of r and limit of R) instead of their standard deviations and relative standard deviations.



Determination of Isopropyl Alcohol and Methyl Ethyl Ketone in Alcoholic Products by GC-FID (Gas Chromatography - Flame Ionisation Detection)

1. Scope

The purpose of this method is verification of fulfilment of the legislative requirements on denatured alcohol, particularly the Regulation (EC) 3199/93 of 22 November 1993, and its amendments, concerning the mutual recognition procedures for the complete denaturing of alcohol (CDA) for the purpose of exemption from excise duty. The common denaturing procedure for completely denatured alcohol defines the amount of denaturing agents in litre (or gram) per hectolitre of absolute ethanol. According to Commission Implementing Regulation (EU) 2017/2236 the amount of IPA and MEK added to 100 L (1 hL) of absolute ethanol is 1 L.
This method is suitable for the determination of isopropyl alcohol (IPA) and methyl ethyl ketone (MEK) in denatured alcohol and alcohol containing solutions or drinks with analyte content ranging from 0.1 to 5 L per hL absolute ethanol using gas chromatography-flame ionization detection.
The same analytical procedure can be used for other formulations of volatile denaturants, i.e. methanol, acetone, tert-butyl alcohol, ethyl acetate, methyl isopropyl ketone, methyl isobutyl ketone, toluene or ethyl sec-amyl ketone.

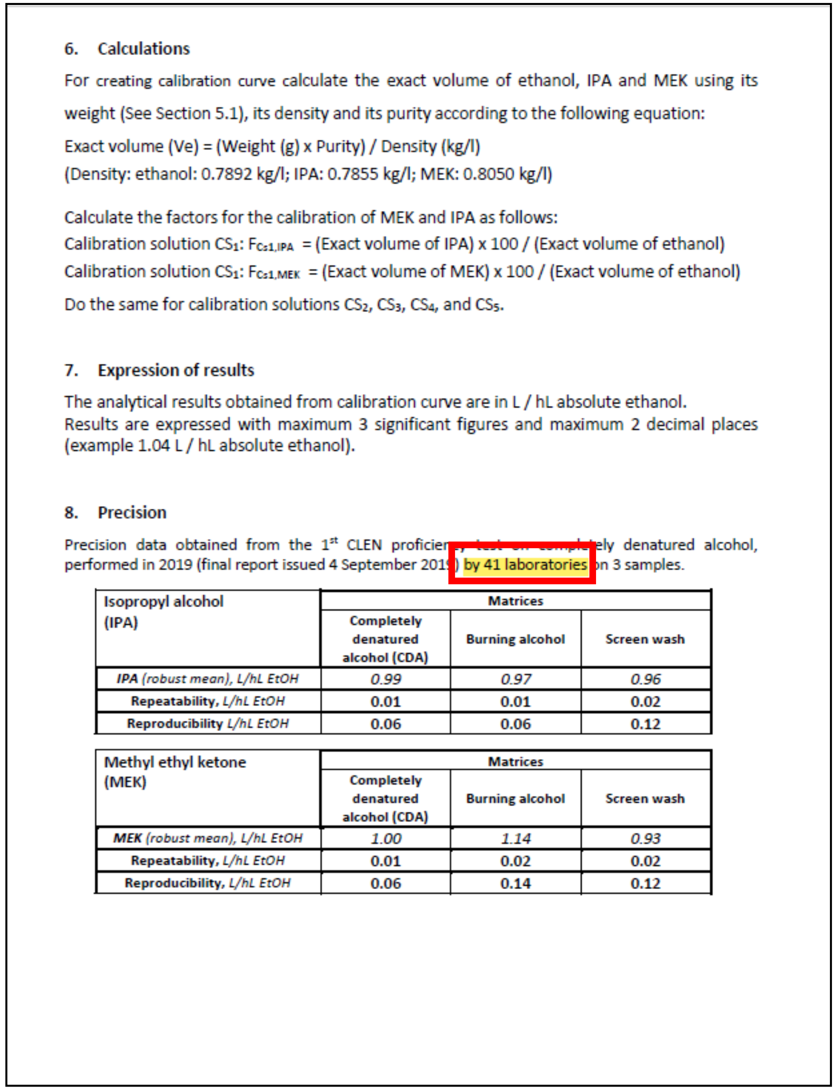
2. Principle

The concentration of the denaturants is determined by capillary gas chromatography with FID detection. Ethyl alcohol itself is used as internal standard and all data for the concentration of denaturants are calculated in relation to the content of ethanol. There is no need for any further internal standard compound.

3. Reagents and materials

The following reagents of recognized analytical grade and demineralized or distilled water are used:

3.1 Methyl ethyl ketone (MEK), min. 99.5 %
3.2 Isopropyl alcohol (IPA), min. 99.8 %
3.3 Deionized water
3.4 Absolute Ethanol ≥ 99.8 %



6. Calculations

For creating calibration curve calculate the exact volume of ethanol, IPA and MEK using its weight (See Section 5.1), its density and its purity according to the following equation:

Exact volume (Ve) = (Weight (g) x Purity) / Density (kg/l)
(Density: ethanol: 0.7892 kg/l; IPA: 0.7855 kg/l; MEK: 0.8050 kg/l)

Calculate the factors for the calibration of MEK and IPA as follows:
Calibration solution CS_1: $F_{CS1,IPA}$ = (Exact volume of IPA) x 100 / (Exact volume of ethanol)
Calibration solution CS_1: $F_{CS1,MEK}$ = (Exact volume of MEK) x 100 / (Exact volume of ethanol)

Do the same for calibration solutions CS_2, CS_3, CS_4, and CS_5.

7. Expression of results

The analytical results obtained from calibration curve are in L / hL absolute ethanol.
Results are expressed with maximum 3 significant figures and maximum 2 decimal places (example 1.04 L / hL absolute ethanol).

8. Precision

Precision data obtained from the 1st CLEN proficien[illegible]ely denatured alcohol, performed in 2019 (final report issued 4 September 201[illegible]) by 41 laboratories on 3 samples.

Isopropyl alcohol (IPA)	Matrices		
	Completely denatured alcohol (CDA)	**Burning alcohol**	**Screen wash**
***IPA** (robust mean), L/hL EtOH*	*0.99*	*0.97*	*0.96*
Repeatability, *L/hL EtOH*	0.01	0.01	0.02
Reproducibility *L/hL EtOH*	0.06	0.06	0.12

Methyl ethyl ketone (MEK)	Matrices		
	Completely denatured alcohol (CDA)	**Burning alcohol**	**Screen wash**
***MEK** (robust mean), L/hL EtOH*	*1.00*	*1.14*	*0.93*
Repeatability, *L/hL EtOH*	0.01	0.02	0.02
Reproducibility, *L/hL EtOH*	0.06	0.14	0.12

The time has come
for an Interlaboratory Study under the OIV auspices
to have the new method
accepted for regulatory purposes

Siarhei.Charapitsa@gmail.com

Thank you for your attention!