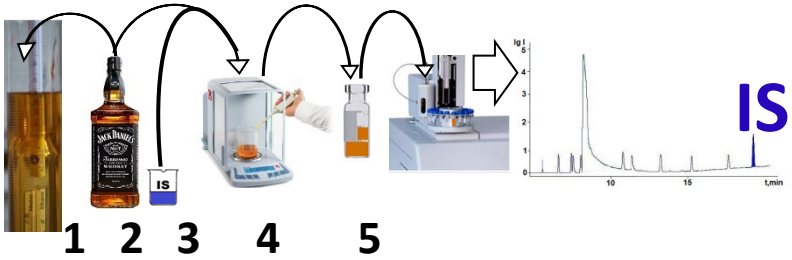
Intelligent use of ethanol for direct determination of volatile substances of spirit drinks of viti-vinicultural origin

It is possible to make the method easier, cheaper, trust and robust

Today: Method of Internal Standard. Traditional way. OIV, China, India, EC, USA, Mexico et. al.



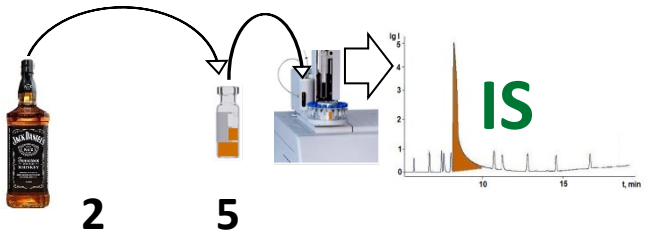
In accordance with the traditional internal standard method, the concentration of the *i*-th compound in terms of mg/kg is determined by the following formula:

$$C_i\ (\text{mg/ kg}) = RRF_i^{IS}\ \frac{A_i}{A_{IS}} C_{IS}\ (\text{mg/ kg})$$

To calculate the concentration of a compound, expressed in mg/l AA, (Absolute Alcohol – AA) it is necessary to measure the density of the sample and determine its strength (volumetric ethanol content):

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

Tomorrow: Ethanol is Internal Standard. Novel way. OIV, China, India, EC, USA, Mexico et. al.



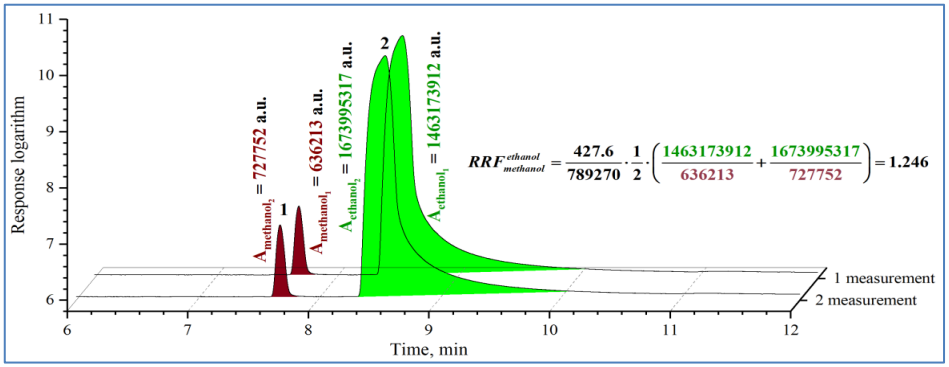
In accordance with the method "Ethanol as Internal Standard", the concentration of the *i*-th compound in terms of mg/l AA is determined by the following formula

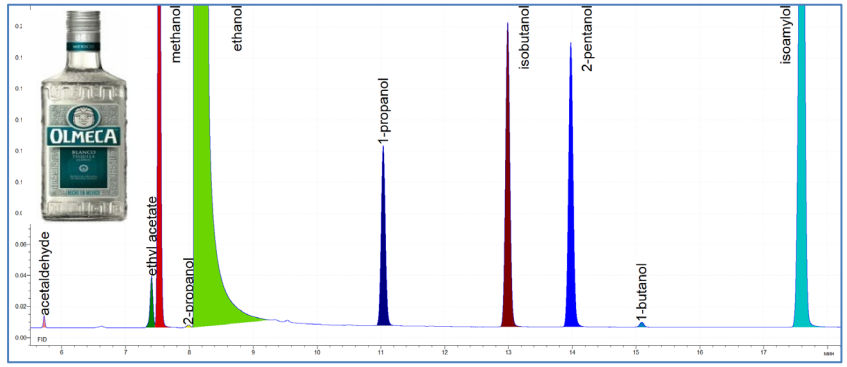
$$C_i\ (\text{mg/ l}\ AA) = RRF_i^{Eth} \cdot \frac{A_i}{A_{Eth}} \cdot \rho_{Eth}\,(\text{mg/ l})$$

1. It is not necessary to add any internal standard to the sample.

2. Ethanol is always present in alcoholic beverages and its concentration in mg/l AA is always known with a 100% guarantee and is equal to the density of ethanol $C_{ethanol} = 789300$ mg/l..

Analysis of Volatile Compounds in Spirit Drinks has Never Been so Easy

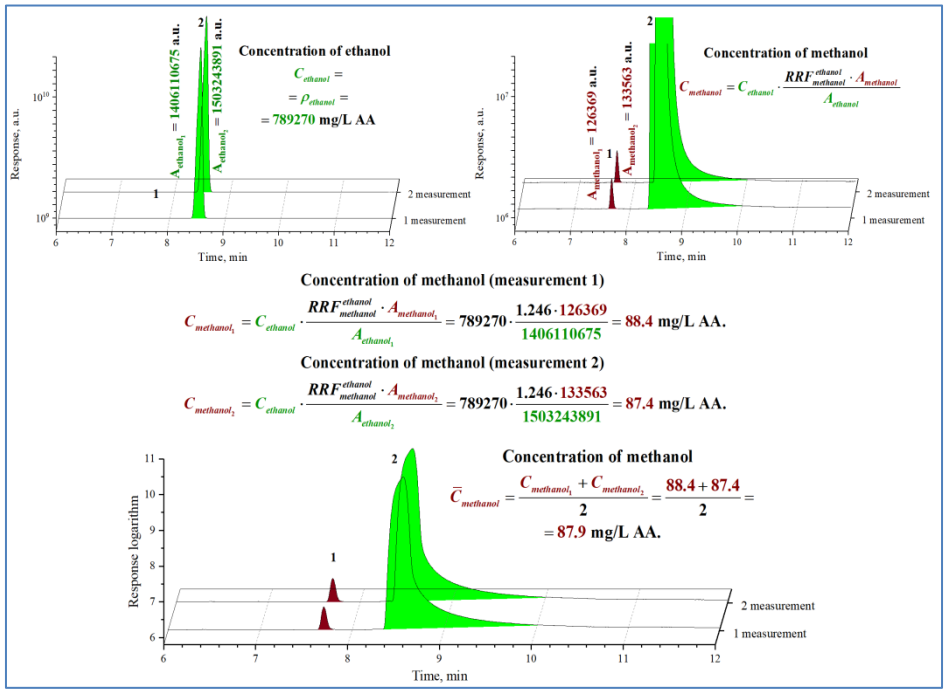




To determine the quantitative content of volatile compounds in ethanol-containing products, it is sufficient to take the areas under the peaks of analyzed compounds and ethanol from the measured chromatogram of the sample and substitute them into the following formula

$$C_i\,[\text{mg/ L } AA] = RRF_i^{ethanol} \cdot \frac{A_i}{A_{ethanol}} \cdot \rho_{ethanol}\,[\text{mg/ L}]$$

$RRF_i^{ethanol}$ – value of the relative response factor of the detector response to analyzed volatile compounds relative to the response to ethanol are tabular values for the modern GC; $\rho_{ethanol}$ = 789300 mg/L.



Compound	Peak area, a.u.	$RRF^{ethanol}$	Concentration, mg/L AA
acetaldehyde	0.23	1.391	20.6
methyl acetate	0.00	1.482	0.00
ethyl acetate	1.78	1.064	122.5
methanol	20.44	1.251	1649.9
2-propanol	0.13	0.794	6.60
ethanol	12235	1.000	789300
1-propanol	8.44	0.676	367.9
isobutanol	15.00	0.570	551.6
1-butanol	0.26	0.614	10.3
isoamilol	47.26	0.572	1743.8

The method OIV-MA-BS-BS-14 (EC2870/2000) is becoming simpler, faster, easier, cheaper, trust and robust

OIV-MA-BS-14

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

Type II 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

and are detected using a flame ionisation detector (FID). The concentration of each congener is determined with respect to the internal standard from response factors, which are obtained during calibration under the same chromatographic conditions as those of the spirit drink analysis.
Note: 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~~. It is not necessary to determine the alcohol strength of the product prior to analysis.

5. Reagents and Materials
Unless otherwise stated, use only reagents of a purity greater than 97 %, purchased from an ISO accredited supplier with a Certificate of Purity, free from other congeners at test dilution (this may be confirmed by injection of individual congener standards at the test dilution using GC conditions as in 6.4) and only water of at least grade 3 as defined in ISO 3696. Acetal and acetaldehyde must be stored in the dark at <5 °C, all other reagents should be stored according to the supplier's instructions.

5.1 Ethanol absolute (CAS 64-17-5)
5.2 Methanol (CAS 67-56-1)
5.3 Propan-1-ol (CAS 71-23-8)
5.4 2-methylpropan-1-ol (CAS 78-33-1)
~~5.5 Acceptable internal standards: pentan-3-ol (CAS 584-02-1), pentan-1-ol (CAS 71-41-0), 4-methylpentan-1-ol (CAS 626-89-1), 4-méthylpentan-2-ol (CAS 108-11-2), or methyl nonanoate (CAS 1731-84-6).~~
5.6 2-methylbutan-1-ol (CAS 137-32-6)
5.7 3-methylbutan-1-ol (CAS 123-51-3)
5.8 Ethyl acetate (CAS 141-78-6)
5.9 Butan-1-ol (CAS 71-36-3)
5.10 Butan-2-ol (CAS 78-92-2)
5.11 Acetaldehyde (CAS 75-07-0)
5.12 Acetal (CAS 105-57-7)
5.13 40% v/v ethanol solution
To prepare 400 ml/l ethanol solution pour 400 ml ethanol (5.1) into a 1 litre volumetric flask, make up to volume with distilled water and mix.
5.14 Preparation and storage of standard solutions (procedure suggested for the validated method: the calibration ranges should be adapted to the nature of the different types of products analysed by each laboratory).

All standard solutions must be stored at <5 °C and be prepared freshly on a monthly basis, if necessary. Masses of components and solutions should be recorded to the nearest 0.1 mg.

5.14.1 Standard solution - A
Pipette the following reagents into a 100 ml volumetric flask, containing approximately 60 ml ethanol solution (5.13) to minimise component evaporation, make up to volume with ethanol solution (5.13) and mix thoroughly. Record the weight of the flask, each component added and the total final weight of contents.

Component	Volume (ml)
Methanol (5.2)	3.0
Propan-1-ol (5.3)	3.0
2-methylpropan-1-ol (5.4)	3.0
2-methylbutan-1-ol (5.6)	3.0
3-methylbutan-1-ol (5.7)	3.0
Ethyl acetate (5.8)	3.0
Butan-1-ol (5.9)	3.0
Butan-2-ol (5.10)	3.0
Acetaldehyde (5.11)	3.0
Acetal (5.12)	3.0

NOTE - It is preferable to add acetal and acetaldehyde last in order to minimise losses through evaporation. The solutions may be prepared individually, and the final solution and dilutions prepared subsequently.

~~5.14.2 Standard solution - B~~
~~Pipette 3 ml of pentan-3-ol, or other suitable internal standard, (5.5) into a 100 ml volumetric flask, containing approximately 80 ml ethanol solution (5.13), make up to volume with ethanol solution (5.13) and mix thoroughly.~~
~~Record the weight of the flask, the weight of pentan-3-ol or other internal standard added and the total final weight of contents.~~

5.14.3 Standard solution - C
Pipette 1 ml solution A (5.14.1) ~~and 1 ml solution B (5.14.2)~~ into a 100 ml volumetric flask containing approximately 80 ml ethanol solution (5.13), make up to volume with ethanol solution (5.13) and mix thoroughly.
Record the weight of the flask, each component added and the total final weight of contents.

5.14.4 Standard solution - D
In order to maintain analytical continuity and an effective quality control, prepare a quality control standard using the previously prepared standard A (5.14.1) or, preferably, prepare a control standard as indicated for standard A, but using different batches or suppliers of reagents. Pipette 1 ml solution A (5.14.1) into a 100 ml volumetric flask containing approximately 80 ml ethanol solution (5.13), make up to volume with ethanol solution (5.13) and mix thoroughly.
Record the weight of the flask, each component added and the total final weight of contents.

~~5.14.5 Standard solution - E~~
~~Pipette 10 ml solution B (5.14.2) into a 100 ml volumetric flask containing approximately 80 ml ethanol solution (5.13), make up to volume with ethanol solution (5.13) and mix thoroughly.~~
~~Record the weight of the flask, each component added and the total final weight of contents.~~

5.14.6 Standard solutions used to check the linearity of response of FID
Into separate 100 ml volumetric flasks, containing approximately 80 ml ethanol (5.13), pipette 0, 0.1, 0.5, 1.0, 2.0 ml solution A (5.14.1) ~~and 1 ml solution B (5.14.2)~~, make up to volume with ethanol solution (5.13) and mix thoroughly.
Record the weight of the flask, each component added and the total final weight of contents.

5.14.7 QC standard solution
Pipette 9 ml standard solution D (5.14.4) and 1 ml ~~of standard solution E (5.14.5)~~ ***ethanol solution (5.13)*** into a weighing vessel and mix thoroughly.
Record the weight of the flask, each component added and the total final weight of contents.

6. Apparatus and Equipment
6.1 Apparatus capable of measuring the density and alcoholic strength.
6.2 Analytical balance, capable of measuring to four decimal places.
6.3 A temperature programmed gas chromatograph fitted with a flame ionisation detector and integrator or other data handling system capable of measuring peak areas.
6.4 Gas chromatographic column(s), capable of separating the analytes such that the minimum resolution between the individual components (other than 2-methylbutan-1-ol and 3-methylbutan-1-ol) is, as a guide, at least 1.3, if a simple visual examination of the chromatogram is not sufficient.

NOTE - The following columns and GC conditions are given as suitable examples:

1 A retention gap 1 m x 0.32 mm i.d. connected to a CP-WAX 57 CB column 50 m x 0.32 mm i.d. 0.2 µm film thickness (stabilised polyethylene glycol) followed by a Carbowax 400 column 50 m x 0.32 mm i.d. 0.2 µm film thickness. (Columns are connected using press-fit connectors.)

Carrier gas and pressure:	Helium (135 kPa)
Column temperature:	35 °C for 17 min., 35 °C to 70 °C at 12 °C/min., hold at 70 C for 25 min.
Injector temperature:	150 °C
Detector temperature:	250 °C
Injection volume:	1 µl, split 20 to 100:1

2 A retention gap 1 m x 0.32 mm i.d. connected to a CP-WAX 57 CB column 50 m x 0.32 mm i.d. 0.2 µm film thickness (stabilised polyethylene glycol). (Retention gap is connected using a press-fit connector.)

Carrier gas and pressure:	Helium (65 kPa)
Column temperature:	35 °C for 10 min., 35 °C to 110 °C at 5 °C/min., 110 °C to 190 °C at 30 °C/min., hold at 190 °C for 2 min.
Injector temperature:	260 °C
Detector temperature:	300 °C
Injection volume:	1 µl, split 55:1

3 A packed column (5% CW 20M, Carbopak B), 2 m x 2 mm i.d.

Column temperature:	65 °C for 4 min., 65 °C to 140 °C at 10 °C/min., hold at 140 °C for 5 min., 140 °C to 150 °C at 5 °C/min., hold at 150 °C for 3 min.
Injector temperature:	65 °C
Detector temperature:	200 °C
Injection volume:	1 µl

7. Sampling and Samples.
7.1 Laboratory sample
On receipt, the alcoholic strength of each sample is measured (6.1).

8. Procedure (used for the validated method, and given as an example; the exact procedure, and in particular the calibration range, should be adapted to the nature of the spirit drinks analysed and to the procedures validated by each laboratory)
8.1 Test portion
~~8.1.1 Weigh an appropriate sealed weighing vessel and record the weight.~~
~~8.1.2 Pipette 9 ml laboratory sample into the vessel and record the weight (M_{SAMPLE}).~~
~~8.1.3 Add 1 ml of standard solution E (5.14.5) and record the weight (M_{IS}).~~
~~8.1.4 Shake the test material vigorously (at least 20 inversions). Samples must be stored at less than 5 °C prior to analysis in order to minimise any volatile losses.~~
~~***8.1.1 Put laboratory sample into 2 mL chromatographic vial for analysis.***~~
8.2 Blank test
~~8.2.1 Using a four decimal place balance (6.2), weigh an appropriate sealed weighing vessel and record the weight.~~
~~8.2.2 Pipette 9 ml 400 ml/l ethanol solution (5.13) into the vessel and record the weight.~~
~~8.2.3 Add 1 ml of standard solution E (5.14.5) and record the weight.~~
~~8.2.4 Shake the test material vigorously (at least 20 inversions). Samples must be stored at less than 5 °C prior to analysis in order to minimise any volatile losses.~~
~~***8.2.1 Put ethanol solution (5.13) into 2 mL chromatographic vial for analysis.***~~
8.3 Preliminary test

Inject standard solution C (5.14.3) to ensure that all of the analytes are separated with a minimum resolution of 1.3 (except 2-methylbutan-1-ol and 3-methylbutan-1-ol).

8.4 Calibration
The calibration should be checked using the following procedure. Ensure that the response is linear by successively analysing in triplicate each of the linearity standard solutions (5.14.6) ~~containing internal standard (IS)~~. From the integrator peak areas for each injection calculate the ratio R for each congener and plot a graph of R versus the concentration ratio of congener to internal standard (***ethanol***), C. A linear plot should be obtained, with a correlation coefficient of at least 0.99.

$$R = \frac{\text{Peak area of congener}}{\text{Peak area of IS}}$$

$$C = \frac{\text{Concentration of congener } (\mu g/g)}{\text{Concentration of IS } (\mu g/g)}$$

$$R = \frac{\text{Peak area of congener}}{\text{Peak area of ethanol}}$$

$$C = \frac{\text{Concentration of congener (g/100 L of anhydrous ethanol)}}{\text{Concentration of ethanol (78927 g/100 L)}}$$

8.5 Determination
Inject standard solution C (5.14.3) and 2 QC standard solutions (5.14.7). Follow with unknown samples (prepared according to 8.1 and 8.2) inserting one QC standard every 10 samples to ensure analytical stability. Inject one standard solution C (5.14.3) after every 5 samples.

9. Calculation
An automated system of data handling can be used, provided the data can be checked using the principles described in the method below and to good gas-chromatographic practice (calculation of response factors and/or establishment of calibration curves).
Measure peak areas for congener and internal standard ***(ethanol)*** peaks.

9.1 Response factor calculation.
From the chromatogram of the injection of standard solution C (5.14.3), calculate response factors for each congener using equation (1).

$$\text{(1) Response factor} = \frac{\text{Peak area of IS}}{\text{Peak area of congener}} \times \frac{\text{Conc. congener } (\mu g/g)}{\text{Conc. IS } (\mu g/g)}$$

~~where:~~
~~IS = internal Standard~~
~~Conc. congener = concentration of congener in solution C (5.14.3)~~
~~Conc. IS = concentration of internal standard in solution C (5.14.3).~~

$$\text{(1) Response factor} = \frac{\text{Peak area of ethanol}}{\text{Peak area of congener}} \times \frac{\text{Concentration of congener (g/100 L of anhydrous ethanol)}}{\text{Concentration of ethanol (78927 g/100 L)}}$$

9.2 Sample analysis
Using equation (2) below, calculate the concentration of each congener in the samples.

(2) Congener concentrations, ~~(µg/g)~~ ***(g/100 L)***=

$$\frac{\text{Peak area of congener}}{\text{Peak area of IS}} \times \frac{M_{IS}\,(g)}{M_{SAMPLE}\,(g)} \times \text{Conc. IS } (\mu g/g) \times RF$$

~~where:~~
~~M_{SAMPLE} = weight of sample (8.1.2);~~
~~M_{IS} = weight of internal standard (8.1.3);~~
~~Conc. IS = concentration of internal standard in solution E (5.14.5);~~
~~RF = response factor calculated using equation 1.~~

$$\frac{\text{Peak area of congener}}{\text{Peak area of ethanol}} \times \text{Concentration of ethanol (78927 g/100 L)} \times RF$$

9.3 Quality control standard solution analysis
Using equation (3) below, calculate the percentage recovery of the target value for each congener in the Quality Control standards (5.14.7):

The study of the matrix effect on the method in a wide range of alcoholic beverages

Matrix													
	Whiskey	Brandy	Rum	Gin	Vodka	Grappa	Tequila	Calvados	Sake	Bourbon	Rakia	Scotch	Ethanol 96% vol.
Compound	The relative difference between the measured concentrations,%												
acetaldehyde	-1.7	0.2	1.2	1.1	0.1	-1.7	1.8	0.1	-1.8	-1.2	-0.6	1.4	-1.6
ethyl acetate	-1.8	0.1	1.1	1.0	-	-1.7	1.8	0.1	-1.8	-1.3	-0.7	1.3	-
methanol	-1.7	0.2	1.2	1.1	0.1	-1.7	1.9	0.1	-1.8	-1.2	-0.6	1.4	-1.6
2-propanol	-1.7	0.1	1.2	1.1	0.1	-1.7	1.8	0.1	-	-1.3	-0.6	1.3	-1.6
1-propanol	-1.7	0.2	1.2	-	-	-1.7	1.8	-	-1.8	-1.2	-0.6	-	-
isobutanol	-1.7	0.1	1.2	-	-	-1.7	1.8	0.1	-1.8	-1.3	-0.6	1.4	-
1-butanol	-1.7	0.2	1.2	1.1	-	-1.7	1.9	0.1	-1.8	-1.3	-0.7	1.3	-
isoamylol	-1.7	0.2	1.2	1.1	-	-1.7	1.8	0.1	-1.8	-1.2	-0.6	1.3	-

The relative difference between the test results obtained according to the Commission Regulation (EC) 2870/2000 and according to the method “Ethanol – IS” does not exceed ± 2%. ***Food Control***, **2021**, doi.org/10.1016/j.foodcont.2020.107528

It is time for initiating Interlaboratory Study under patronage of OIV

SHORT COMMUNICATION

Single-Laboratory Validation of a Gas Chromatographic Method of Direct Determination of Volatile Compounds in Spirit Drinks: Need for an Improved Interlaboratory Study

SIARHEI V. CHARAPITSA and SVETLANA N. SYTOVA
Belarusian State University, Institute for Nuclear Problems, Bobruyskaya St, 11, Minsk, Belarus
ANTON L. KORBAN and LIDIA N. SOBOLENKO
Belarusian State University, Institute for Nuclear Problems, Bobruyskaya St, 11, Minsk, Belarus; Belarusian State University, Nezavisimosti Ave, 4, Minsk, Belarus

BIO Web of Conferences **15**, 02030 (2019) https://doi.org/10.1051/bioconf/20191502030
42nd World Congress of Vine and Wine

Interlaboratory study of ethanol usage as an internal standard in direct determination of volatile compounds in alcoholic products

S. Charapitsa[1], S. Sytova[1], A. Korban[1,2], L. Sobolenko[1,2], V. Egorov[2], S. Leschev[2], M. Zakharov[3], R. Čabala[4], R. Busarova[5], I. Shestakovich[6], A. Tolstouhova[6], S. Ondroušek[7], J. Vávra[7], M. Yilmaztekin[8], and T. Cabaroglu[9]

[1] Institute for Nuclear Problems of Belarusian State University, 220030, Bobruyskaya Str., 11, Minsk, Belarus
[2] Chemistry Faculty, Department of Analytical Chemistry, Belarusian State University, Leningradskaya Str., 14, 220050, Minsk, Belarus
[3] All-Russian Scientific Research Institute of the Brewing, Non-Alcoholic and Wine Industry – Branch of the V.M. Gorbatov Federal Scientific Center of Food Systems of RAS, 119021, Rossolimo Str., 17, Moscow, Russia
[4] Charles University, Faculty of Science, Department of Analytical Chemistry, Hlavova Str., 2030/8, 128 40, Prague 2, Czech Republic
[5] JS "Mosazervinzavod", 115088, Yuzhnoportovaya Str., 30, Moscow, Russia
[6] Republican Centre for Hygiene, Epidemiology and Public Health, 220099, Kazintsa Str., 50, Minsk, Belarus
[7] General Directorate of Customs, Customs Technical Laboratory, Budějovická Str., 7, 140 96, Prague 4, Czech Republic
[8] Inonu University, Faculty of Engineering, Department of Food Engineering, 44280, Malatya, Turkey
[9] Cukurova University, Faculty of Agriculture, Department of Food Engineering, 01330, Adana, Turkey

References

1. *Journal of AOAC International, v.82, No.6, 1999*, https://www.researchgate.net/publication/12710841_Gas_Chromatographic_Determination_of_Volatile_Congeners_in_Spirit_Drinks_Interlaboratory_Study
2. *Journal of Agricultural and Food Chemistry,* **2013**, *61*, 2950-2956. https://pubs.acs.org/doi/10.1021/jf3044956
3. *Journal of Chemical Metrology,* **2018**, 12, 59-69. https://www.acgpubs.org/JCM/2018/Volume%2012/Issue%201/5-14-JCM-1802-063.pdf
4. *Journal of AOAC International,* **2019**, 102, 2, 669-672. https://academic.oup.com/jaoac/article/102/2/669/5658221
5. *42nd World Congress of Vine and Wine,* **2019**, https://www.bio-conferences.org/articles/bioconf/full_html/2019/04/bioconf-oiv2019_02030/bioconf-oiv2019_02030.html
6. *Journal of Mass Spectrometry,* **2019**, https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/jms.4493
7. *Food Control*, **2021**, doi.org/10.1016/j.foodcont.2020.107528
8. *Food Chemistry*, **2020**, https://doi.org/10.1016/j.foodchem.2020.128107
9. *Food Analytical Methods*, **2021,** https://doi.org/10.1007/s12161-021-02047-8
10. *Journal of Chemical Metrology,* **2021**, https://www.acgpubs.org/doc/20211226124535A2-66-JCM-2111-2259.pdf
11. *Journal of Food Composition and Analysis*, **2022,** https://doi.org/10.1016/j.jfca.2022.104772
12. Improved document COMMISSION REGULATION EC2870/2000 https://elab.bsu.by/download.php?id=308
13. Improved document OIV-MA-BS-14, https://elab.bsu.by/download.php?id=312
14. Improved document OIV-MA-AS312-03A https://elab.bsu.by/download.php?id=317
15. Improved document OIV-MA-AS315-27 https://elab.bsu.by/download.php?id=316
16. Improved document Indian Standard 3572-2005, https://elab.bsu.by/download.php?id=315
17. Improved document Norma Mexicana NMX-V-005-NORMEX-2013 https://elab.bsu.by/download.php?id=311
18. Improved document National standards of People's Republic of China GB/T 15038 https://elab.bsu.by/download.php?id=309
19 and GB/T 11858 https://elab.bsu.by/download.php?id=307
20. Improved document AOAC Official Method 972.10 (USA), https://elab.bsu.by/download.php?id=306
21. Improved document AOAC Official Method 972.11 (USA), https://elab.bsu.by/download.php?id=305

Technical support: https://elab.bsu.by/elib/?i=11
E-mail: elab@inp.bsu.by