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OIV International Organisation of Vine and Wine
Intergovernmental Organisation

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# Interlaboratory Study of Ethanol Usage as an Internal Standard in Direct Determination of Volatile Compounds in Alcoholic Products

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# Established analysis practice of spirit drinks – Traditional Internal Standard Method

**This method is recommended for official regulatory purposes by**

## European Commission

- Commission Regulation (EC) No. 2870/2000 of 19 December 2000 laying down Community reference methods for the analysis of spirits drinks (2000).

European Commission

## OIV

- Determination of the principal volatile substances of spirit drinks of viti-vinicultural origin, OIV method OIV-MA-BS-14 : R2009 (2009).
- Gas chromatographic determination of propan-2-ol, OIV method OIV-MA-BS-20 : R2009 (2009).

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## AOAC

- Alcohol (higher) and ethyl acetate in distilled liquors. Alternative gas chromatographic method. AOAC, Official Method 972.10 (2005).
- Methanol in Distilled Liquors. Gas chromatographic method AOAC, Official Method 972.11 (2005).

Official Methods of Analysis of AOAC INTERNATIONAL

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

## Method of Internal Standard. Traditional way

250 mL

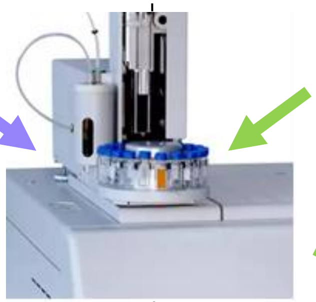
determine volume ethanol content

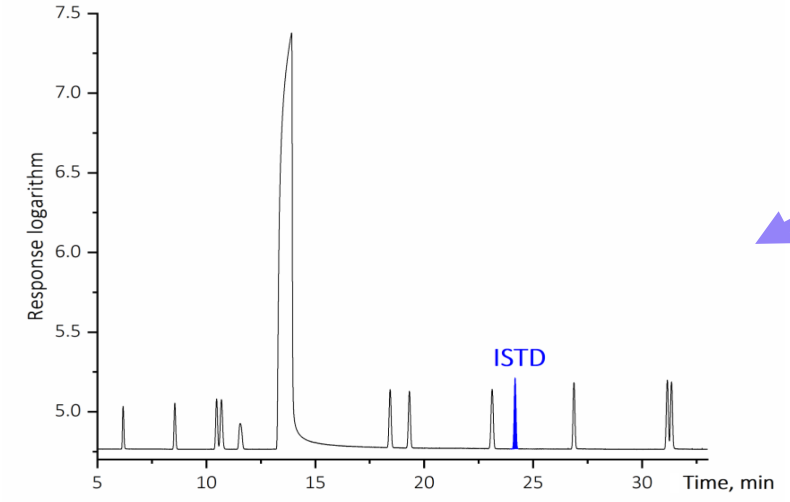

100 mL


1.0 mL

add Internal Standard

1.5 mL



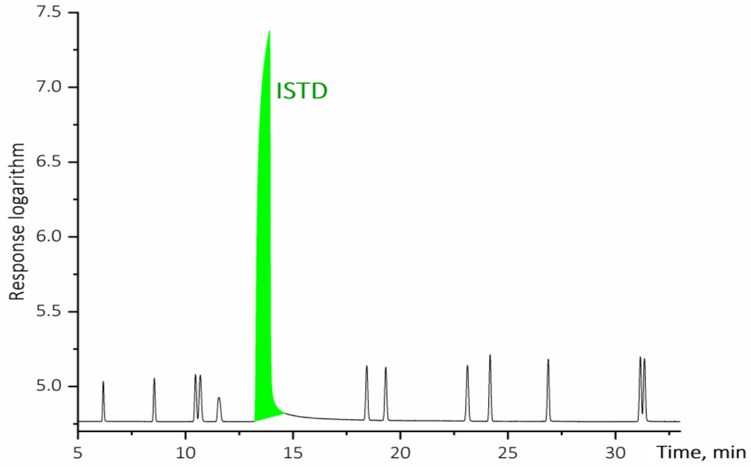



## Ethanol as Internal Standard. Novel way.


1.5 mL

# Direct determination

*JAFC*, ***61***, *2950–2956 (2013)*
https://doi.org/10.1021/jf3044956
*J. Chem. Metr.* ***12***, *59-69 (2018)*
https://doi.org/10.25135/jcm.14.18.02.063
*J. AOAC Int.* ***102***, *669–672 (2019)*
https://doi.org/10.5740/jaoacint.18-0258




$C_{Ethanol}$ = 789300 mg/L of pure ethanol

# Comparative analysis of the theoretical basis of two methods

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## EC 2870/2000

## Ethanol is Internal Standard

$$RRF_i^{3\text{-pentanol}} = \frac{C_i^{st}(mg/kg)}{A_i^{st}} / \frac{C_{3\text{-pentanol}}^{st}(mg/kg)}{A_{3\text{-pentanol}}^{st}} \longleftrightarrow RRF_i^{Ethanol} = \frac{C_i^{st}(mg/L\ AA)}{A_i^{st}} / \frac{\rho_{Ethanol}(mg/L\ AA)}{A_{Ethanol}^{st}} \quad (1)$$

$$C_i\,(mg/kg) = RRF_i^{3\text{-pentanol}} \cdot \frac{A_i}{A_{3\text{-pentanol}}} \cdot C_{3\text{-pentanol}}(mg/kg)$$

$$C_i\,(mg/L\ AA) = C_i\,(mg/kg) \cdot \rho_{test}(mg/L) / strength(\%\ v/v)$$

$$\longleftrightarrow C_i\,(mg/L\ AA) = RRF_i^{Ethanol} \cdot \frac{A_i}{A_{Ethanol}} \cdot \rho_{Ethanol}(mg/L\ AA) \quad (2)$$

$$\frac{u(C_i)}{C_i} = \sqrt{\left(\frac{u(RRF_i)}{RRF_i}\right)^2 + \left(\frac{u(A_i)}{A_i}\right)^2 + \left(\frac{u(A_{IS})}{A_{IS}}\right)^2 + \left(\frac{u(C_{IS})}{C_{IS}}\right)^2 + \left(\frac{u(\rho_{sample})}{\rho_{sample}}\right)^2 + \left(\frac{u(ABV(^v/_v))}{ABV(^v/_v)}\right)^2}$$

1 2 3 4 5 6

$>$

$$\frac{u(C_i)}{C_i} = \sqrt{\left(\frac{u(RRF_i)}{RRF_i}\right)^2 + \left(\frac{u(A_i)}{A_i}\right)^2 + \left(\frac{u(A_{Ethanol})}{A_{Ethanol}}\right)^2}$$

1 2 3

The presence of these **three additional terms (4, 5, 6)** indicates a higher uncertainty during the determination of concentrations of volatile compounds by the traditional method than "**Ethanol is Internal Standard**" method.

These additional terms are responsible for the uncertainty in the determination of concentration of internal standard, density and ABV value of a test sample.

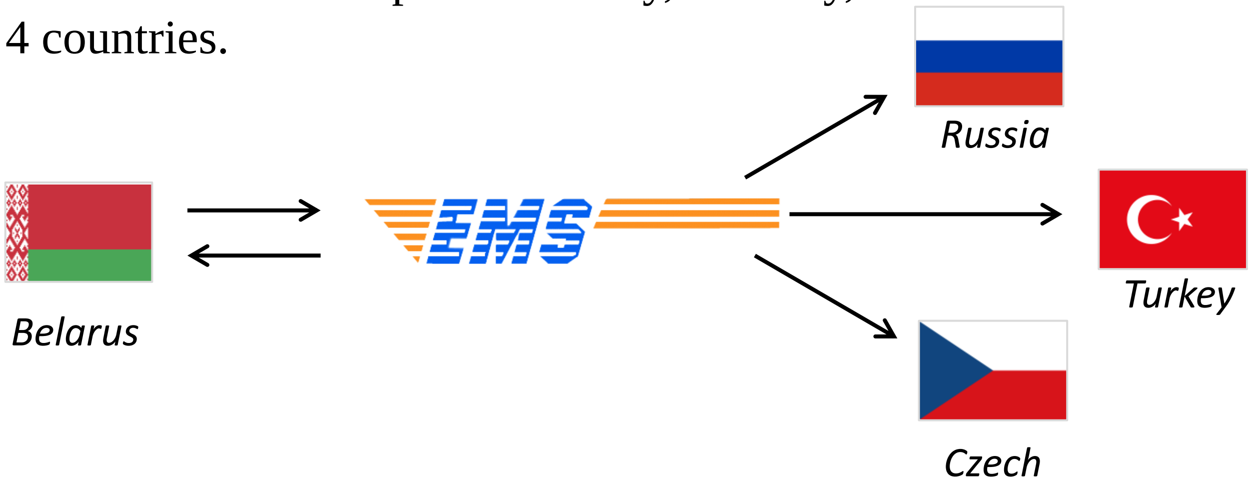
# Interlaboratory Study of the Method

A collaborative interlaboratory study (ILS) of the method of direct quantitation of volatile compounds in spirit drinks and alcoholic products – **“Ethanol as Internal Standard”** was performed in the beginning of 2019.

In one laboratory, a set of standard solutions was prepared using the gravimetric method in accordance with EC2870/2000 and purchased from a commercial source the samples of brandy, whiskey and vodka.

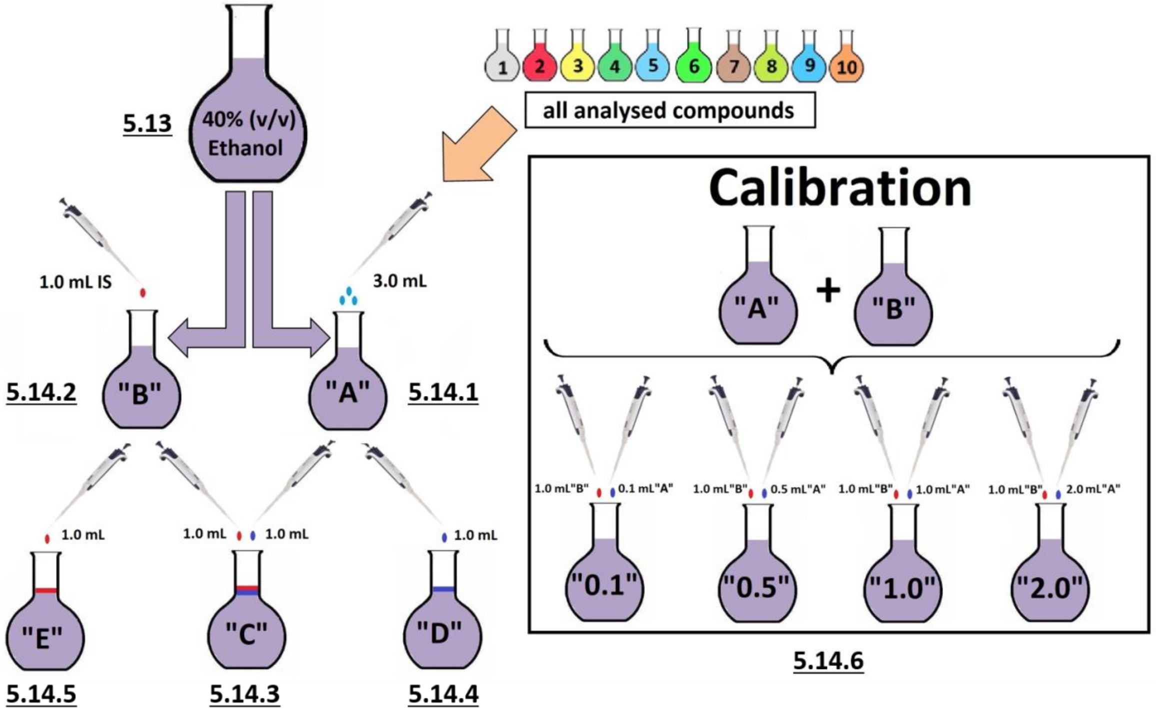
Five aqueous ethanol 40% (v/v) standard solutions contained acetaldehyde, methyl acetate, ethyl acetate, methanol, 2-propanol, 1-propanol, 2-methyl-1-propanol, 1-butanol and 3-methyl-1-butanol in concentrations from 10 mg/L AA to 500 mg/L AA.


Prepared sets of 5 standard solutions and samples of brandy, whiskey, vodka where were sent by EMS mail to all 9 participants from 4 countries.

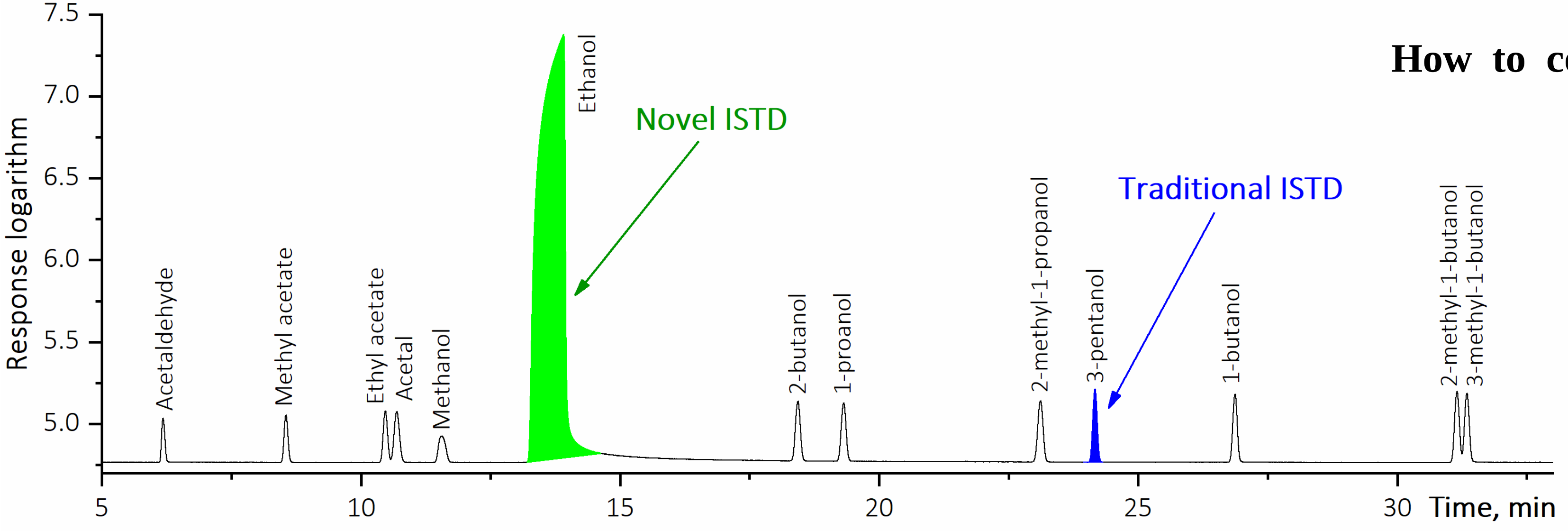


# Preparation of standard solutions of volatile compounds in accordance with EC 2870/2000

| 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 |
| 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** |




# Measurements of standard solution “C” to calculate factors $RRF_i^{3\text{-pentanol}}$ and $RRF_i^{Ethahol}$




**How to convert your concentration to mg/L AA ?**

Use for **mg/kg**

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

Use for **mg/L**

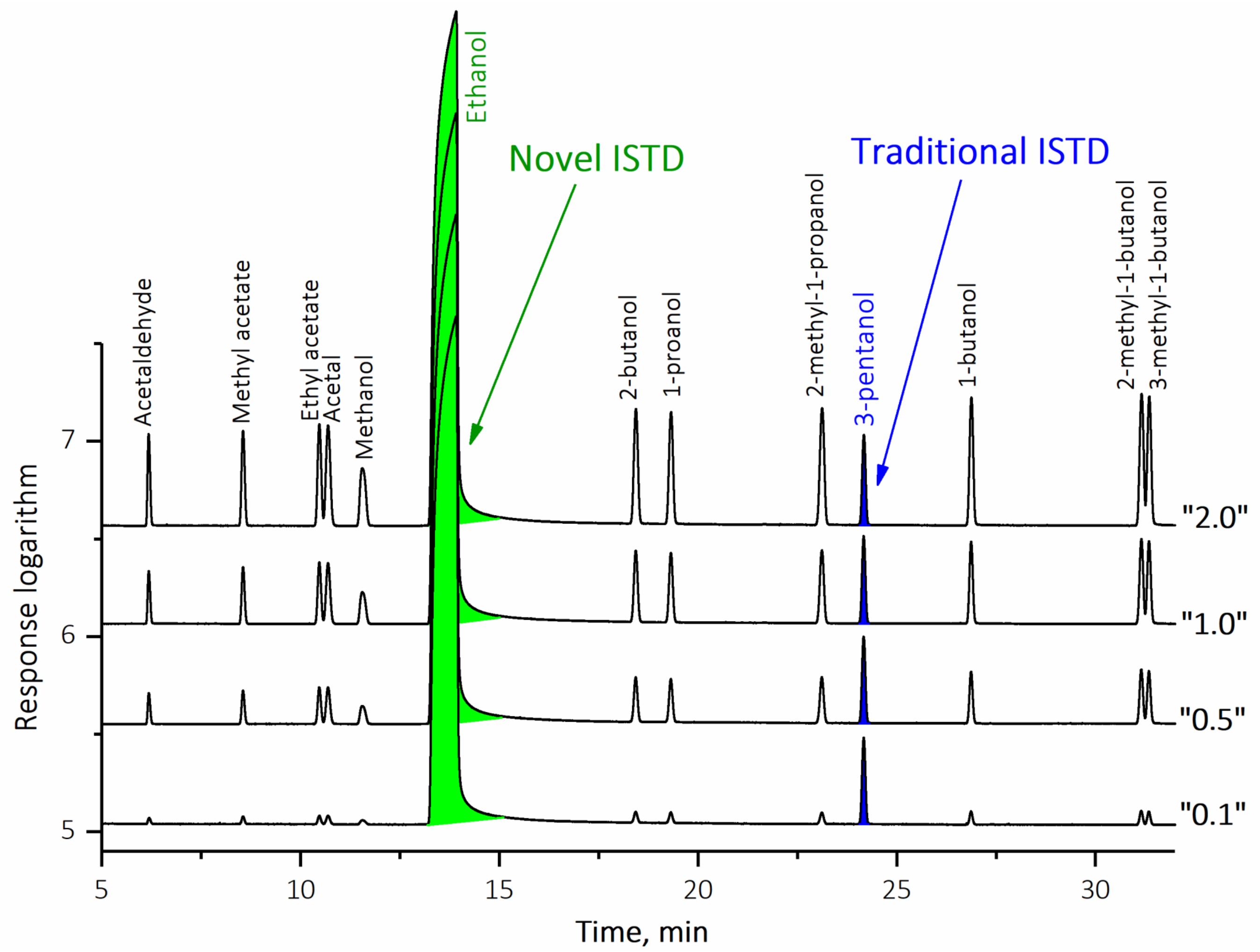
$$C_i\,(mg/L\ AA) = \frac{C_i\,(mg/L)}{strength(\%\ v/v)}$$

| No | Compound | Time, min | Response, a.u. | C, mg/L AA | C, mg/L | C, mg/kg | $RRF^{3\text{-pentanol}}$ | $RRF^{Ethanol}$ |
|---|---|---|---|---|---|---|---|---|
| 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 | 2.023 | 1.161 |
| 6 | **Ethanol** | **13.915** | **60396.000** | **789300** | | | 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 |

$$RRF_i^{Ethanol} = \frac{C_i^{st}(mg/L\ AA)}{A_i^{st}(a.u.)} \Big/ \frac{\rho_{Ethanol}(mg/L\ AA)}{A_{Ethanol}^{st}(a.u.)}$$

$$RRF_i^{3\text{-pentanol}} = \frac{C_i^{st}(mg/kg)}{A_i^{st}(a.u.)} \Big/ \frac{C_{3\text{-pentanol}}^{st}(mg/kg)}{A_{3\text{-pentanol}}^{st}(a.u.)}$$

# Measurements of standard solutions “0.1”, “0.5”, “1.0”, “2.0” for evaluation of trueness (bias)




## Results

The analysis of obtained experimental data shows:

1. The bias of the method is insignificant at the significance level α = 5 % (ISO 5725-4, s. 4.7.2 ).
2. The linearity of the detector response for all analysed compounds was $R^2 \geq 0.995$.
3. The expanded uncertainty values were $U < 10\%$.
4. The method eliminates manual procedure of IS addition into both calibration standard solutions and spirit drinks.

Obtained high metrological characteristics of the method indicate the possibility for recommendation of this method for official regulatory purposes.

KELLY ET AL.: JOURNAL OF AOAC INTERNATIONAL VOL. 82, NO. 6, 1999 1375

FOOD COMPOSITION AND ADDITIVES

## Gas Chromatographic Determination of Volatile Congeners in Spirit Drinks: Interlaboratory Study

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**An interlaboratory study of a gas chromatographic (GC) method for the determination of volatile congeners in spirit drinks was conducted; 31 laboratories from 8 countries took part in the study. The method uses GC with flame ionization detection and incorporates several quality control measures which permit the choice of chromatographic system and conditions to be selected by the user. Spirit drink samples were prepared and sent to participants as 10 blind duplicate or split-level test materials 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). The precision of the method for 9 of the 10 analytes was well within the range predicted by the Horwitz equation. The precision of the most volatile analyte, ethanal, was just above statistically predicted levels. This method is recommended for official regulatory purposes.**

Council Regulation (EEC) No. 1576/89 (1) defines the description and composition of spirit drinks. The European Commission are currently drawing up legislation (2) that will prescribe methods of analysis to be used to monitor compliance with 1576/89.

Congeners are volatile substances formed along with ethanol during fermentation and maturation of spirit drinks and can be used to provide both qualitative and quantitative information for labelling purposes. In addition proposed European legislation specifically defines the volatile congener component of volatile substances as comprising the sum of: ethanal (acetaldehyde) and the ethanal fraction contained in 1,1-diethoxyethane (acetal) expressed as ethanal, and the sum of propan-1-ol (*n*-propanol), 2-methylpropan-1-ol (isobutyl alcohol), butan-1-ol (*n*-butanol), butan-2-ol (sec-butanol), 2-methylbutan-1-ol (active amyl alcohol) and 3-methylbutan-1-ol (isoamyl alcohol). Regulation 1576/89 also prescribes limits for methanol in wine spirit, brandy, grape marc, and fruit spirit drinks. The objective of this work, sponsored by the European Commission, was to formally validate methodology that would be suitable for use in monitoring compliance with 1576/89.

**Interlaboratory Study**

Collaborators from 31 laboratories in France, Germany, Greece, The Netherlands, Portugal, Spain, Ireland, and the United Kingdom took part in the study.

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# Need for an Improved Interlaboratory Study

For recognition of new method for regulatory purposes it is necessary to conduct official Interlaboratory Study.

Certainly, under patronage of OIV.

The method can be validated by analogy to the Interlaboratory Study as it was done in **1999**.

It is important to note that for the interlaboratory validation of the method the initial experimental data from the following tests can be used.

1) LGC (formerly the Laboratory of the Government Chemist) prepares certified reference material, such as LGC5100 Whisky-Congeners, for the world market regularly. Before the reference material can be sold interlaboratory experiments are performed. In these experiments 10 mL aliquots of commercially available whiskey are measured by 16 profile authoritative laboratories. Thus, this procedure can be also used for method validation by contacting LGC.

2) Regularly, twice a year, the Bureau National Interprofessionnel du Cognac carries out interlaboratory comparisons for the quality control of cognac in which more than 20 profile laboratories take part.

# Thank you for your attention !