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Article

Determination of Ethanol content in alcoholic products by LF-NMR.

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**Supporting information**

**Matherials and methods**

Solvents and reagents (reagent grade) were all commercially available and used without further purification. 1H-NMR spectra were recorded on a Spinsolve HF 80 Multi-X Ultra benchtop NMR spectrometer, characterized by a magnetic field strenght of 80 MHz. The sample was introduced into a NMR glass tube and the operators used the pre-installed sequence PROTON 1D (1 minute) for the analyses. The sequence parameters were setted directly by the instrument.
A typical spectrum of the mixture consisting of the alcohol sample and salicylaldehyde is shown in **Figure S1.**



**Figure S1.** 1H-NMR spectrum of a mixture consisting of the alcohol sample and salicylaldehyde in 1:3 volume ratio

The ethanol content was expressed in sample is expressed in %w/w, calculated as:

$$C\_{x}= \frac{I\_{x}}{I\_{STD}}\*\frac{N\_{STD}}{N\_{x}}\*\frac{M\_{x}}{M\_{STD}}\*\frac{m\_{STD}}{m\_{x}}\*P\_{STD}$$

Where Cx is the ethanol content in w/w%, and Ix, Istd, NSTD, Nx, Mx, Mstd, mstd, mx and pstd are, respectively, the integral area, proton’s number generating peaks, molecular mass, sample and standard weights (in grams) and purity related to ethanol (x) and IS (std). Integrated signals ar referred to of methylene (or methyl) group of ethanol peak and aldehydic group of salicylaldehyde (IS) peaks.

 The conversion from w/w% to v/v % values is performed by multiplying the result by the ratio between sample and ethanol densities.

**Precision data**

The method’s precision was estimated by eleven indipendent repeatability determination on four different kind of alcoholic matrices, as explained in **Table S1.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **CH3Ethanol** | **CH3Ethanol** | **CH3Ethanol** | **CH3Ethanol** | **CH2 Ethanol** | **CH2 Ethanol** | **CH2 Ethanol** | **CH2 Ethanol** |
| **Anhydrous ethyl alcohol** | **Denatured ethyl alcohol (euro DG)** | **Crude alcohol** | **Hydroalcoholic mixture ethyl alcohol/water 80/20 %v/v** | **Anhydrous ethyl alcohol** | **Denatured ethyl alcohol (euro DG)** | **Crude alcohol** | **Hydroalcoholic mixture ethyl alcohol/water 80/20 %v/v** |
| 99.50 %v/v | 88.83 %v/v | 93.01 %v/v | 81.26 %v/v | 100.10 %v/v | 88.28 %v/v | 92.88 %v/v | 83.57 %v/v |
| 99.56 %v/v | **87**.**57 %v/v** | 91.95 %v/v | 81.08 %v/v | 100.14 %v/v | **86**.**98 %v/v** | 92.40 %v/v | 83.37 %v/v |
| 99.50 %v/v | 88.93 %v/v | 92.64 %v/v | **80**.**40 %v/v** | 100.05 %v/v | 88.48 %v/v | 92.42 %v/v | 82.67 %v/v |
| **99**.**25 %v/v** | 88.93 %v/v | 93.33 %v/v | 80.95 %v/v | 99.74 %v/v | 88.34 %v/v | 92.95 %v/v | 82.68 %v/v |
| **99**.**65 %v/v** | 89.37 %v/v | 92.13 %v/v | 81.26 %v/v | 100.24 %v/v | 88.69 %v/v | 92.29 %v/v | 82.99 %v/v |
| 99.47 %v/v | 88.94 %v/v | 93.17 %v/v | 81.28 %v/v | 99.99 %v/v | 88.41 %v/v | 93.09 %v/v | 83.30 %v/v |
| 99.47 %v/v | 88.97 %v/v | 92.78 %v/v | 81.59 %v/v | 100.03 %v/v | 88.33 %v/v | 93.82 %v/v | 83.72 %v/v |
| 99.53 %v/v | 89.19 %v/v | 92.66 %v/v | 81.38 %v/v | 100.06 %v/v | 88.65 %v/v | 92.48 %v/v | 83.38 %v/v |
| 99.50 %v/v | **89**.**00 %v/v** | 92.50 %v/v | 81.21 %v/v | 100.14 %v/v | **89**.**57 %v/v** | 92.19 %v/v | 83.48 %v/v |
| **99**.**82 %v/v** | **89**.**63 %v/v** | **94**.**75 %v/v** | 81.30 %v/v | **100**.**44 %v/v** | **88**.**97 %v/v** | 94.41 %v/v | 83.28 %v/v |
| **99**.**18 %v/v** | 88.92 %v/v | 93.49 %v/v | 81.04 %v/v | 99.79 %v/v | 88.31 %v/v | 93.20 %v/v | 82.64 %v/v |

**Table S1.** Raw data, analysed by 1H-NMR spectroscopy

The Shapiro-Wilk test **[1]** verifies whether the datasets distribution are normal, and this was verified for all datasets. Dixon's, Grubbs' one-value and two-value tests (applicable to normal distributions only), and Huber's median-based test **[2]** (applicable to both normal and non-normal distributions), verify the presence of outlier datas. All outlier datas are discarded and are reported in yellow in **Table** **S1**. Then, the homogeneity of the variances was checked using Fischer's **[3]** and Bartlett's tests **[4]:** the former is used when the same number of data is available for all levels; the latter in the opposite case. For the first, the variances obtained are statistically homogeneous if the following condition is met, defined in the **Equation S1:**

$$\frac{s\_{r max}^{2}}{s\_{r min}^{2}}\leq F\_{1-α, vmax=vmin}$$

**Equation S1.:** Fischer’s test formula

Where s2rmax and s2rmin are respectively the highest and lowest variance of repeatability among all investigated q-levels. The obtained variance ratio is compared with the tabulated values of Fischer's variable as a function of confidence level (a=0.05) and degrees of freedom nmax = nmin = n-1.

For the second, on the other hand, different numbers of data are available for all concentrations between the different q-levels of the measurand, homogeneity is checked using Bartlett's the parameter M is calculated, i.e. a variable distributed approximately as c2(n,1- a) with the following formula written in **Equation S2**:

$$M= \frac{1}{C}\* \left(v\_{T} ln\overbar{s\_{r}^{2}}- \sum\_{j=1}^{q}v\_{j}ln s\_{rj}^{2}\right)$$

$$C=1+\frac{1}{3(q-1)}\left[\left(\sum\_{j=1}^{q}\frac{1}{v\_{j}}\right)-\frac{1}{v\_{T}}\right]$$

**Equation S2.:** Bartlett’s test formula

**CH3 peaks:** The variances are not homogeneous, so different repeatabilities and uncertainties will be estimated for each level. For simplicity, the maximum typical deviation srmax was considered representative of the entire range of concentrations studied, hence a single repeatability and uncertainty will be derived.

**CH2 peaks:** We similarly repeat the same procedure for datas obtained by analysing the signals of the CH2 group. According to Bartlett's test, the following data sets in the table show homogeneous variances, so it is possible to assume a single type deviation representative of the four studied levels (q-levels), known as the pool-type deviation, calculated as follows:

$$\overbar{S\_{r}}= \sqrt{\frac{v\_{1}s\_{r1}^{2}+v\_{2}s\_{r2}^{2}+v\_{3}s\_{r3}^{2}+…v\_{q}s\_{rq}^{2}}{v\_{T}}}$$

where,

$v\_{1},v\_{2},v\_{j},v\_{q} $are the degrees of freedom of the variances for each level of the measurand (n=n-1), nT=$\sum\_{j=1}^{q}v\_{j }$is the sum of the degrees of freedom of the variances q.

The pool-type deviation is applicable for the entire range studied, defined by the q levels of the measurand. The repeatibility for each level was calculated as reported in **Equation S3** (**A**) and (**B**):

$$ \left(A\right) r\_{LAB CH3}= t\_{1-α,vT}s\_{r max}\sqrt{2}$$

$$\left(B\right) r\_{LAB CH2}= t\_{1-α,vT}\overbar{s\_{r}}\sqrt{2}$$

**Equation S3**. Repeatibilities equations for CH2 and CH3 peaks datas

Results are reported in **Table S2.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **sr max CH3 (%v/v)** | **srPOOL CH2 (%v/v)**  | **rLAB CH3 (%v/v)** | **rLAB CH2 (%v/v)** | **Student t** |
| 0.51 | 0.26 | 1.64 | 0.74 | 2 |

**Table S2**. Repeatability of the method for both CH2 and CH3 peaks

The uncertainty can be calculated from the data obtained in the laboratory, using Horwitz’s equation **[5]**, defined as:

$\left(A\right) σ\_{R}= 0,02\*C^{0,8495}$;

$$\left(B\right) U\_{H}=2\*σ\_{R}$$

|  |
| --- |
| **UH CH3 – CH2 (%v/v)** |
|  **2,00**  |
|  **1,84**  |
|  **1,90**  |
|  **1,76**  |

**Trueness**

*Comparison between results obtained by spectroscopic and cromatographic methods (3.2.2)*

Another check on trueness was performed by comparing the results of 1H-NMR method with those obtained by gas chromatographic quantification.

For the purpose, the same four matrices (anhydrous ethyl alcohol, denatured ethyl alcohol: euro DG, crude and Hydroalcoholic mixture ethyl alcohol-water 80 %v/v) were analysed also by the reference GC-FID method, carrying out six determination for each kind of sample. Although in the previous paragraph from the comparison with the ring test residual it was found that the signals of the -CH3 group do not give accurate results, they were included in this study as further evidence of the greater trueness of the results given by the -CH2 peak.

As reported in UNICHIM Manual **[6]**, the above approach is used when materials with an accepted reference value are not available.

Once the normality of the data distribution has been verified, the type deviations relative to the data set obtained by NMR and that of the gas chromatograph are calculated (alternately for the -CH3 and -CH2 signals) and homogeneity is checked by F-test at the probability level p=0.95. Raw chromatographics data are reported in **Table S3**.

|  |  |  |  |
| --- | --- | --- | --- |
| **Anhydrous ethyl alcohol (%v/v)** | **Denatured ethyl alcohol (euro DG) (%v/v)** | **Crude alcohol (%v/v)** | **Hydroalcoholic mixture ethyl alcohol/water 80/20 %v/v %v/v (%v/v)** |
| 100.84 | 92.89 | 82.88 | 87.92 |
| 100.85 | 92.22 | 82.45 | 88.1 |
| 100.73 | 92.63 | 82.74 | 88.21 |
| 100.73 | 93.2 | 82.75 | 87.94 |
| 100.9 | 93.01 | 82.76 | 88.08 |
| 100.7 | 92.44 | // | 88.2 |
| 101.11 | // | // | // |
| 100.78 | // | // | // |
| 101.15 | // | // | // |
| 100.84 | // | // | // |
| 101.05 | // | // | // |

**Table S3.** Raw data collected with CLEN/ILIADe 143:2023 version 25 July 2023 method

The deviation between the results obtained by the two methods is acceptable if the condition given in the equation reported below is met, reported in **Equation S4** **(A)**, **(B)** and **(C)**:

$\left(A\right) \left|∆\right|$*=* $\left|\overbar{x}\_{A}-\overbar{x}\_{N}\right|\leq t\_{p=1-α, v∆}\* s\_{∆}$

Where, if the F-test is satisfied:

$\left(B\right) s\_{∆}= \sqrt{\frac{s\_{A}^{2}\left(n\_{A}-1\right)+ s\_{N}^{2}(n\_{N}-1)}{n\_{A}+n\_{N}-2}\* \left(\frac{1}{n\_{A}}+\frac{1}{n\_{N}}\right)}$; $v\_{∆}= n\_{A}+n\_{N}-2$

If the F-test is not satisfied:

$\left(C\right) s\_{∆}= \sqrt{\frac{s\_{A}^{2}}{n\_{A}}+\frac{s\_{N}^{2}}{n\_{N}}}$ $v\_{∆}= \frac{s\_{∆}^{4}}{\frac{\left(^{s\_{A}^{2}}/\_{n\_{A}}\right)^{2}}{v\_{A}}+\frac{\left(^{s\_{N}^{2}}/\_{n\_{N}}\right)^{2}}{v\_{N}}}$

**Equation S4.** Formulas used to statistically compare the two datasets obtained by the two methods analysed (LF-NMR and GC-FID)

Calculations was done considering the NMR raw datas reported in **Table S1**. The results were reported in **Table S4** for the -CH3 and -CH2 signals.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | $$\overbar{x}\_{N}$$ | $\overbar{x}\_{A}$**-CH3 signal** | $$\left|\overbar{x}\_{A}-\overbar{x}\_{N}\right|$$**-CH3 signal** | $$t\_{}\* s\_{∆}$$**-CH3 signal** | **Acceptability** | $\overbar{x}\_{A}$**-CH2 signal** | $$\left|\overbar{x}\_{A}-\overbar{x}\_{N}\right|$$**-CH2 signal** | $$t\_{}\* s\_{∆}$$**-CH2 signal** | **Acceptability** |
| **Anhydrous ethyl alcohol**  | 100.88 | 99.49 | 1.39  | 0.16 | **NO** | 100.03 | 0.85 | 0.14 | **NO** |
| **Denatured ethyl alcohol (euro DG)**  | 88.08 | 89.07 | 1.00  | 0.26 | **NO** | 88.28 | 0.20 | 0.40 | **YES** |
| **Crude alcohol**  | 92.73 | 92.76 | 0.03  | 0.56 | **YES** | 92.92 | 0.19 | 0.65 | **YES** |
| **Hydroalcoholic mixture ethyl alcohol/water 80/20 %v/v** | 82.72 | 81.32 | 1.40  | 0.25 | **NO** | 82.99 | 0.26 | 0.33 | **YES** |

**Table S4.** Comparison between two methods acceptability criteria

*Comparison with real samples*

The comparison between NMR and GC methods was also carried out on 25 real crude heads-tails alcohol samples, with a 85-96 %vol range alcohol content, analysed in duplicate by each technique.

The values of repeatability for CH3 and CH2 results are, respectively, rLAB= 1.45 %v/v and rLAB = 0.74 %v/v Firstly, for all samples was verified the repeatability condition for each duplicate determination according to the equation :

$$∆\_{m}\leq r\_{LAB}$$

Raw datas are reported in **Table S5**.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample (LIMS/YEAR)** | **Dm CH3****(%v/v)** | **rLAB CH3****(%v/v)** | **RESULT** | **Sample (LIMS/YEAR)** | **Dm CH3****(%v/v)** | **rLAB CH3****(%v/v)** | **RESULT** |
| **862/23** | 0.16 | 1.45 | **POS** | **1101/23** | 0.25 | 1.45 | **POS** |
| **868/23** | 0.13 | 1.45 | **POS** | **1105/23** | 0.00 | 1.45 | **POS** |
| **982/23** | 0.08 | 1.45 | **POS** | **1106/23** | 0.29 | 1.45 | **POS** |
| **1081/23** | 0.09 | 1.45 | **POS** | **1107/23** | 0.19 | 1.45 | **POS** |
| **1044/23** | 0.04 | 1.45 | **POS** | **1204/23** | 0.27 | 1.45 | **POS** |
| **984/23** | 0.06 | 1.45 | **POS** | **1173/23** | 0.13 | 1.45 | **POS** |
| **939/23** | 0.34 | 1.45 | **POS** | **1198/23** | 0.63 | 1.45 | **POS** |
| **800/23** | 0.05 | 1.45 | **POS** | **1199/23** | 0.17 | 1.45 | **POS** |
| **801/23** | 0.1 | 1.45 | **POS** | **266/24** | 0.64 | 1.45 | **POS** |
| **987/23** | 0.43 | 1.45 | **POS** | **267/24** | 0.14 | 1.45 | **POS** |
| **989/23** | 0.31 | 1.45 | **POS** | **273/24** | 0.11 | 1.45 | **POS** |
| **1097/23** | 0.01 | 1.45 | **POS** | **275/24** | 0.52 | 1.45 | **POS** |
| **1100/23** | 0.35 | 1.45 | **POS** | // | // | // |  |
| **Sample (LIMS/YEAR)** | **Dm CH2****(%v/v)** | **rLAB CH2****(%v/v)** | **RESULT** | **Sample (LIMS/YEAR)** | **Dm CH2****(%v/v)** | **rLAB CH2****(%v/v)** | **RESULT** |
| **862/23** | 0.05 | 0.74 | **POS** | **1101/23** | 0.22 | 0.74 | **POS** |
| **868/23** | 0.01 | 0.74 | **POS** | **1105/23** | 0.04 | 0.74 | **POS** |
| **982/23** | 0.12 | 0.74 | **POS** | **1106/23** | 0.29 | 0.74 | **POS** |
| **1081/23** | 0.16 | 0.74 | **POS** | **1107/23** | 0.16 | 0.74 | **POS** |
| **1044/23** | 0.09 | 0.74 | **POS** | **1204/23** | 0.37 | 0.74 | **POS** |
| **984/23** | 0.03 | 0.74 | **POS** | **1173/23** | 0.12 | 0.74 | **POS** |
| **939/23** | 0.21 | 0.74 | **POS** | **1198/23** | 0.64 | 0.74 | **POS** |
| **800/23** | 0.00 | 0.74 | **POS** | **1199/23** | 0.05 | 0.74 | **POS** |
| **801/23** | 0.06 | 0.74 | **POS** | **266/24** | 0.42 | 0.74 | **POS** |
| **987/23** | 0.39 | 0.74 | **POS** | **267/24** | 0.33 | 0.74 | **POS** |
| **989/23** | 0.24 | 0.74 | **POS** | **273/24** | 0.03 | 0.74 | **POS** |
| **1097/23** | 0.14 | 0.74 | **POS** | **275/24** | 0.46 | 0.74 | **POS** |
| **1100/23** | 0.28 | 0.74 | **POS** | // | // | // |  |

**Table S5**. Repeatability tests for real alcoholic samples received in the laboratory in the last two years

This first test carried out for all the real samples reproduced above confirms that for the duplicate test, repeatability is confirmed on both the CH3 and CH2 signals.

Hence, the comparison between NMR and GC analysis was evaluated by the **Equation S5** for each analysed sample.

$$\frac{∆\_{x}}{\sqrt{u\_{\overbar{x}}^{2}+u\_{rif}^{2}}}\leq t\_{p,v}$$

**Equation S5** t-test applied on NMR and densimeter reference value on anhydrous ethyl alcohol sample

Where Dx is the difference in absolute value between results obtained with NMR and GC; u$\overbar{x}$ and urif are respectively the uncertainties associated to NMR and to gascromatographic method.

Raw data are reported in **Table S6**.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample (LIMS/YEAR)** | **x CH3 (%v/v)** | **xrif (%v/v)** | **Dx** | **ux (CH3)** | **urif** | $$\frac{∆\_{x}}{\sqrt{u\_{\overbar{x}}^{2}+u\_{rif}^{2}}}$$ | **t** | **RESULT** |
| **862/23** | 91.80 | 92.93 | 1.13 | 0.5125 | 0.254 | 0.95 | 2.23 | POS |
| **868/23** | 91.21 | 94.24 | 3.04 | 0.5125 | 0.254 | 2.54 | 2.23 | **NEG** |
| **982/23** | 91.90 | 92.70 | 0.80 | 0.5125 | 0.254 | 0.67 | 2.23 | POS |
| **1081/23** | 94.37 | 94.10 | 0.13 | 0.5125 | 0.254 | 0.22 | 2.23 | POS |
| **1044/23** | 92.54 | 93.46 | 0.92 | 0.5125 | 0.254 | 0.77 | 2.23 | POS |
| **984/23** | 93.06 | 93.30 | 0.84 | 0.5125 | 0.254 | 0.20 | 2.23 | POS |
| **939/23** | 92.97 | 94.76 | 1.79 | 0.5125 | 0.254 | 1.50 | 2.23 | POS |
| **800/23** | 92.59 | 93.33 | 0.74 | 0.5125 | 0.254 | 0.62 | 2.23 | POS |
| **801/23** | 92.45 | 92.77 | 0.32 | 0.5125 | 0.254 | 0.27 | 2.23 | POS |
| **987/23** | 92.86 | 93.40 | 0.55 | 0.5125 | 0.254 | 0.46 | 2.23 | POS |
| **989/23** | 89.80 | 88.90 | 0.91 | 0.5125 | 0.254 | 0.75 | 2.23 | POS |
| **1097/23** | 90.04 | 91.10 | 1.07 | 0.5125 | 0.254 | 0.89 | 2.23 | POS |
| **1100/23** | 95.87 | 96.30 | 0.43 | 0.5125 | 0.254 | 0.36 | 2.23 | POS |
| **1101/23** | 90.30 | 91.11 | 0.81 | 0.5125 | 0.254 | 0.68 | 2.23 | POS |
| **1105/23** | 94.39 | 93.80 | 0.33 | 0.5125 | 0.254 | 0.49 | 2.23 | POS |
| **1106/23** | 90.34 | 91.26 | 0.92 | 0.5125 | 0.254 | 0.78 | 2.23 | POS |
| **1107/23** | 90.37 | 90.99 | 0.63 | 0.5125 | 0.254 | 0.52 | 2.23 | POS |
| **1204/23** | 90.36 | 92.20 | 1.08 | 0.5125 | 0.254 | 1.55 | 2.23 | POS |
| **1173/23** | 90.20 | 92.40 | 1.51 | 0.5125 | 0.254 | 1.85 | 2.23 | POS |
| **1198/23** | 94.71 | 95.40 | 0.70 | 0.5125 | 0.254 | 0.58 | 2.23 | POS |
| **1199/23** | 90.49 | 88.40 | 2.08 | 0.5125 | 0.254 | 1.75 | 2.23 | POS |
| **266/24** | 88.71 | 92.50 | 4.27 | 0.5125 | 0.254 | 3.18 | 2.23 | **NEG** |
| **267/24** | 89.12 | 92.40 | 3.85 | 0.5125 | 0.254 | 2.75 | 2.23 | **NEG** |
| **273/24** | 86.00 | 90.70 | 4.70 | 0.5125 | 0.254 | 3.94 | 2.23 | **NEG** |
| **275/24** | 90.82 | 95.00 | 3.91 | 0.5125 | 0.254 | 3.51 | 2.23 | **NEG** |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample (LIMS/YEAR)** | **x CH2 (%v/v)** | **xrif (%v/v)** | **Dx** | **ux (CH2)** | **urif** | $$\frac{∆\_{x}}{\sqrt{u\_{\overbar{x}}^{2}+u\_{rif}^{2}}}$$ | **t** | **RESULT** |
| **862/23** | 92.33 | 92.93 | 0.61 | 0.2631 | 0.254 | 0.51 | 2.23 | POS |
| **868/23** | 93.55 | 94.24 | 0.69 | 0.2631 | 0.254 | 0.58 | 2.23 | POS |
| **982/23** | 92.53 | 92.70 | 0.17 | 0.2631 | 0.254 | 0.14 | 2.23 | POS |
| **1081/23** | 94.98 | 94.10 | 0.75 | 0.2631 | 0.254 | 0.74 | 2.23 | POS |
| **1044/23** | 94.15 | 93.46 | 0.69 | 0.2631 | 0.254 | 0.57 | 2.23 | POS |
| **984/23** | 94.33 | 93.30 | 0.42 | 0.2631 | 0.254 | 0.86 | 2.23 | POS |
| **939/23** | 94.61 | 94.76 | 0.16 | 0.2631 | 0.254 | 0.13 | 2.23 | POS |
| **800/23** | 93.26 | 93.33 | 0.07 | 0.2631 | 0.254 | 0.06 | 2.23 | POS |
| **801/23** | 93.15 | 92.77 | 0.38 | 0.2631 | 0.254 | 0.32 | 2.23 | POS |
| **987/23** | 93.67 | 93.40 | 0.26 | 0.2631 | 0.254 | 0.22 | 2.23 | POS |
| **989/23** | 90.95 | 88.90 | 0.25 | 0.2631 | 0.254 | 1.72 | 2.23 | POS |
| **1097/23** | 91.06 | 91.10 | 0.04 | 0.2631 | 0.254 | 0.03 | 2.23 | POS |
| **1100/23** | 96.53 | 96.30 | 0.23 | 0.2631 | 0.254 | 0.19 | 2.23 | POS |
| **1101/23** | 91.17 | 91.11 | 0.06 | 0.2631 | 0.254 | 0.05 | 2.23 | POS |
| **1105/23** | 94.77 | 93.80 | 0.71 | 0.2631 | 0.254 | 0.81 | 2.23 | POS |
| **1106/23** | 91.13 | 91.26 | 0.14 | 0.2631 | 0.254 | 0.11 | 2.23 | POS |
| **1107/23** | 91.13 | 90.99 | 0.14 | 0.2631 | 0.254 | 0.12 | 2.23 | POS |
| **1204/23** | 90.95 | 92.20 | 0.49 | 0.2631 | 0.254 | 1.05 | 2.23 | POS |
| **1173/23** | 90.92 | 92.40 | 0.78 | 0.2631 | 0.254 | 1.24 | 2.23 | POS |
| **1198/23** | 95.16 | 95.40 | 0.24 | 0.2631 | 0.254 | 0.20 | 2.23 | POS |
| **1199/23** | 88.23 | 88.40 | 0.18 | 0.2631 | 0.254 | 0.15 | 2.23 | POS |
| **266/24** | 93.44 | 92.50 | 0.46 | 0.2631 | 0.254 | 0.79 | 2.23 | POS |
| **267/24** | 93.66 | 92.40 | 0.69 | 0.2631 | 0.254 | 1.06 | 2.23 | POS |
| **273/24** | 90.56 | 90.70 | 0.14 | 0.2631 | 0.254 | 0.12 | 2.23 | POS |
| **275/24** | 93.95 | 95.00 | 0.78 | 0.2631 | 0.254 | 0.88 | 2.23 | POS |

**Table S6**. Resulsts obtained from the comparison between two methods (GC-FID and LF-NMR)

**Robustness**

Robustness represents capacity of an analytical procedure to produce unbiased results when small changes in the experimental conditions are made voluntarily. Considering the proposed method, some procedural changes may occur only during sample preparation, therefore different ratios weighing between sample an internal standard were tested for robistness, using the OFAT (one-factor-at-time) approach.

Sample preparation consists of weighing a mixture of internal standard (salicylaldehyde) and alcohol sample in a 3:1 ratio. It was tested a 2:1 and a 4:1 ratios.

The differences Δi, in absolute value, between the average of the results obtained by applying the method with the chosen variations and the average of the results obtained with the nominal conditions were calculated. If Δi is significantly different from zero, the **Equation S6** must besatisfied:

$$\left|∆\_{i}\right|\leq t\_{1-α, veff}\*\sqrt{\frac{s^{2}\left(n-1\right)+ s^{'2}\left(n^{'}-1\right)}{n+n^{'}-2}\* \left(\frac{1}{n}+\frac{1}{n^{'}}\right)}$$

**Equation S6.** OFAT approach

Where:

$∆\_{i}=$ difference between the average of the results obtained by performing the original method and the varied method;

**s**= standard deviation of the replicas obtained by performing the method under the original conditions;

**s’**= standard deviation of the replicas obtained by performing the variant method;

**n**= number of replications performed with the original method;

**n'**= number of replications performed with the varied method;

veff= n+n’-2;

t1-a, veff= Student variable tabulated at the chosen probability level (p=0.95) and the respective degrees of freedom veff.

If the equation is satisfied, the method can be called robust for the parameter considered.

|  |
| --- |
| **Standard Conditions (Vsample/VSI 1:3) – 6 measures****%v/v (l/hl a.a.)** |
| **Ethanol CH3** | 92.12 | 92.75 | 92.83 | 92.07 | 92.37 | 92.28 |
| **Ethanol CH2** | 92.30 | 92.74 | 92.83 | 92.16 | 92.38 | 92.09 |
| **1 Modification (Vsample/VSI 1:2) – 6 measures****%v/v (l/hl a.a.)** |
| **Ethanol CH3** | 92.15 | 92.06 | 92.33 | 92.24 | 92.12 | 92.40 |
| **Ethanol CH2** | 93.32 | 93.01 | 93.57 | 92.93 | 93.07 | 93.30 |
| **2 Modification (Vsample/VSI 1:4) – 6 measures****%v/v (l/hl a.a.)** |
| **Ethanol CH3** | 92.68 | 92.91 | 93.26 | 92.87 | 92.85 | 92.72 |
| **Ethanol CH2** | 92.37 | 92.58 | 92.83 | 92.54 | 92.61 | 92.40 |

The parameters calculated to assess the robustness of the method for the two variations above can be found in the **Table S7.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Modification 1****(Vsample/VSI 1:4)** **CH3** | **Modification 2****(Vsample/VSI 1:2)****CH3** | **Modification 1****(Vsample/VSI 1:4)** **CH2** | **Modification 2****(Vsample/VSI 1:2)****CH2** |
| **n** | 5 | 5 | 6 | 6 |
| **n’** | 6 | 6 | 6 | 6 |
| **νeff** | 9 | 9 | 10 | 10 |
| **S** | 0.27 | 0.27 | 0.31 | 0.31 |
| **s’** | 0.13 | 0.20 | 0.24 | 0.17 |
| **t Student** | 2.26 | 2.26 | 2.23 | 2.26 |
| **Δi** | 0.11 | 0.4 | 0.8 | 0.05 |
| **Coeff. Student** | 0.10 | 0.1 | 0.4 | 0.32 |
| **Result** | **NEG** | **NEG** | **NEG** | POS |

**Table S7.** Results obtained with OFAT approach

In all cases studied, the method is not very robust. Consequently, the ratio of the sample volume to the volume of internal standard used is a critical parameter, so it must be kept equal.

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