Ethanol dosage in hydro-alcoholic gels.

Ayman Akhdar, Yael Hersant and Arnaud Gautier•

CNRS-UMR 62394, Université Clermont Auvergne, SIGMA,

ICCF, F-63000 Clermont-Ferrand, France.

Abstract

A method of determination of the ethanol content in hydro-alcoholics is reported. Based on quantitative ^{1}HNMR (q^{1}HNMR), the results are obtained rapidly and accurately. The method is available for viscous solutions to which other simpler technics do not apply.

Introduction

As the spread of the COVID-19 pandemic accelerates around the world, many low- and middle-income countries are still struggling to access the diagnostic tests they desperately need to control the disease. Moreover, passive protection methods such as masks and hydro-alcoholic gels are the first means of defence to avoid chaotic spread of the virus among the population. The World Health Organization (WHO) provides a protocol that was taken into consideration in many national legislations.[1] In France, at the beginning of the pandemic, we applied this protocol to fabricate several dozens of litters of hydro-alcoholic mixture at our institute and the content of ethanol in grams per grams of solution was determined by ^{1}H quantitative NMR (qNMR). If the content of ethanol in liquids gels can be approximatively measured using a simple alcohol-meter, this technic is less appropriate for viscous liquids containing gelling agents. We report here a method of ethanol content measurement using ^{1}H quantitative NMR (^{1}H qNMR). Our intention is to remind the scientific community of this well-known

• arnaud.gautier@uca.fr

technic, and of the fact that several samples obtained from abroad do not contain the adequate amount of ethanol.

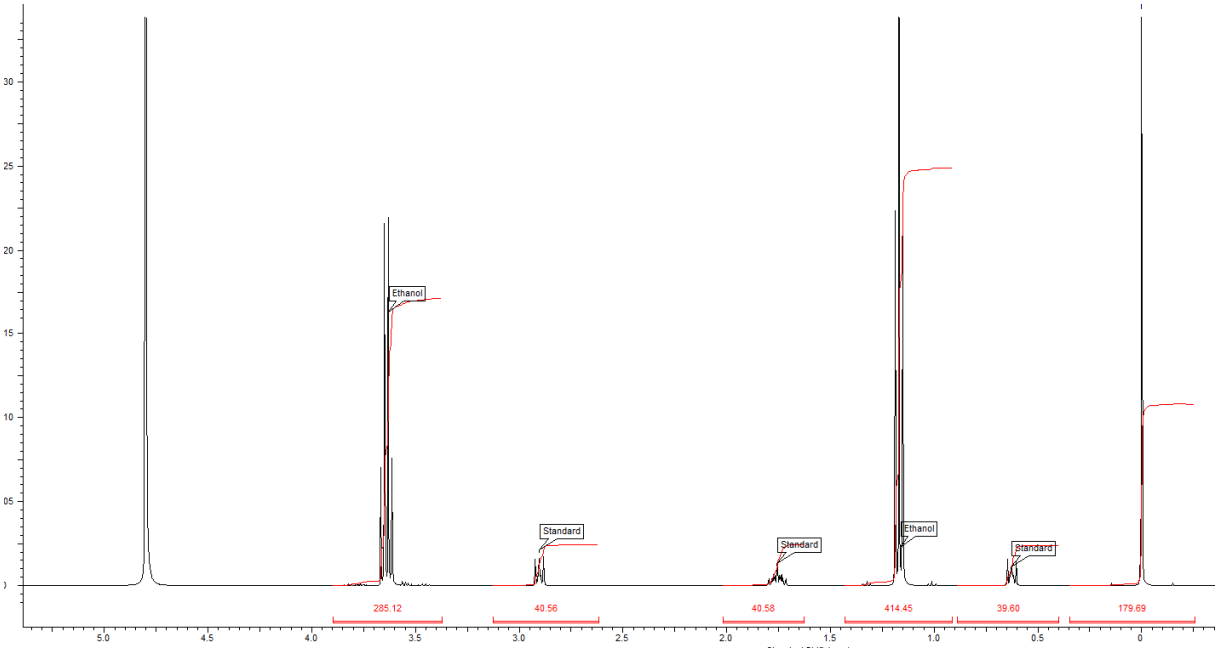
Quantitative NMR is a valuable method because the measured signals represent a direct measurement of the composition if correct inter-pulse delay are adequately chosen.[2] Therefore, in presence of an internal reference of known purity, the composition of a sample can be determined accurately using a single spectrum/ integration sequence (so-called absolute quantification). For this purpose, we used 3-(trimethylsilyl)-1-propanesulfonic acid di sodium salt (TSPA, CAS: 2039-96-5, 97% purity) as standard (also used as chemical shift reference) and D_2O for NMR solvent. After proper weighting of the standard and the gel, the mass of ethanol contained in the sample is calculated by equation 1:

$$masse(Ethanol) = \frac{46.1*15*0.97}{5*218.3} * \frac{\sum I^{Ethanol} * m^{std}}{\sum I^{Std}} \quad \text{Eq.1}$$

Where ΣInt stands for the sum of the integral of ethanol and standard, m is the weighted mass of the standard, 46.1 and 218.3 are the molar masses, 5 and 15 represent the total number of protons of ethanol and standard respectively; 0.97 is the purity of the standard.

Results

The following spectrum depicts a typical qNMR of a home-made hydro-alcoholic solution using the WHO protocol. The amount of gel and TSPA is 34.69 and 18.14 mg respectively.



As it can be seen, ethanol and standard signals are well separated, allowing a clean integration. As the amount of gelling agent is usually lesser than a few percents, its signals are not visible. Only signals coming from the traces of glycerol are visible below the CH_2OH signal at 3.6 ppm, which makes its integration slightly overestimated. The accuracy of the measurement can be determined by the calculation of the deviation of each integration: the measured number of protons versus the theoretical number at each peak calculated by:

$$Dev = 100 * \left(\frac{Int * \sum Proton}{\sum Int} - NbProton\right) / NbProton \qquad \text{Eq.2}$$

Table 1.

Standard	TSPA:	18.14mg			Sum	Int/^{1}H	Mmol
δ(ppm)	2.8	1.65	0.52	0			
Number of ^{1}H	2	2	2	9	15		
Integration	40.56	40.58	39.6	179.7	300.4	**20.0**	**0.0831**
Deviation(%)[(a)]	-1.2	-1.3	-1.1	0.3			
Ethanol		34.69mg					

δ(ppm)	3.60	1.18					
Number of ^{1}H	2	3			5		
Integration	285.1	414.5			699.6	**139.9**	**0.563**
Deviation(%)[(a)]	1.9	-1.3					

A: Deviation more than 5% should be discarded.

Indeed, the number of mmol of ethanol contained in the mass of the sample is simply obtained from the cross product between the blue numbers. In our typical case, 34.69 mg of gel contains 0.58 mmol of ethanol which makes 26.8 mg. Therefore, the masse percentage is 75%.

Discussion

We followed the WHO protocol and recorded the masses of the constituents added: 833 mL ethanol (96%): 666 g; H2O2 (3%): 42mL of H_2O_2(3%): 42 g; 14.7mL of glycerol: 18 g; water to complete the 1000mL: 133 g. The total mass is therefore 859g. The mass percentage of ethanol in the gel is therefore 77.5%, in agreement with our findings (see WHO's quality control, Ref. 1, p3). During the last months, we have noticed that some gels do not fulfil WHO's requirements, some of them being so viscous that the use of an alcoholmeter becomes useless. Therefore, NMR determination of the mass percentage becomes an interesting alternative.[3]

Method

Sample Preparation. *Use of screw necked vials with PTFE closure is strongly recommended in order to minimize the ethanol evaporation.* Masses are weighed using a Mettler Toledo balance (0.01 mg accuracy) into a 4 mL flat bottom screw neck vial equipped with a centred hole, screw closure closed by a silicone PTFE (Macherey-Nagel, Germany). The standard is weighted first (Table 1) then 2 mL of D_2O is introduced. The vial is closed and gently shaken until all the solid dissolves. The gel is added (Table 1) into the vial with a syringe (Inject®-F 1 ml) equipped with a needle (Henke Sass Wolf, Germany, 0.6X25mm) through the silicone join in order to minimize the evaporation of ethanol. The

solution was shaken, then 600 µL were transferred into a 3 mm standard NMR tubes (Norell) for analysis.

Pulse Program. Sample Temperature: 19 °C (regulated ± 0.1 K). Single pulse, without carbon decoupling ('zg' with 90° pulse). Data Points (acquired): 64 K. NS=64. Relaxation delay: D1=60s. Acquisition Time: 4s. Spectral window for proton: SW=30ppm and O1: 7.5 ppm

Post-Acquisition Processing. Performed with ACDLABS software (1.2, academic version). Zero Filling: to 256K. Line Broadening: LB = 0.1 Hz. Phasing: manually. Baseline Correction: 6th order polynomial. For each signal measured a ratio signal/noise>100 is verified.

References

1. Guide to Local Production: WHO-recommended Handrub Formulations. https://www.who.int/gpsc/5may/Guide_to_Local_Production.pdf?ua=1

2. Pauli, G.F.; Chen, S.N.; Simmler, C.; Lankin, D.C.; Gödecke, T.; Jaki, B.U.; Friesen, J.B.; McAlpine, J.B.; Napolitano, J.G. Importance of purity evaluation and the potential of quantitative ^{1}H NMR as a purity assay. *J Med Chem*., **2014** *57*, 9220-31.

3. For some of these gels gathered from colleagues travelling from abroad, the mass percentage were found far away the WHO requirement, one of them reaching only 5-7%. We therefore recommend this procedure for the analysts having the possibility to access to the equipment described inhere.