

New approach for wine authenticity screening by a cumulative ^1H and ^2H qNMR

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Abstract. New methodological approach for rapid control of wine authenticity without sample preparation, based on the quantitative NMR spectroscopy (qNMR) of the protium ^1H and deuterium ^2H nucleus is suggested. The content of dominant (e.g. water, ethanol) and some minor (e.g. glycerol, organic acids) components of wine are determined from qNMR ^1H spectra for authentication of molecular composition. The sum of all exchanging hydrogen atoms of wine's components provide the ^1H signal with a chemical shift of 4.8 ppm. Accounting for their content from ^1H spectrum allows us calculate the ^2H isotopic content in wine water from integral intensity of corresponding signal in the ^2H qNMR spectrum using an internal or external standard with a known content of the ^2H isotope. The possible addition of water can be found from comparison of this value with values of surface and/or ground waters from corresponding viticulture areas. This approach was used for white and red wines from the Black Sea region (Krasnodar area & Crimea peninsula). The ^2H contents in investigated wines range from 157 to 165 ppm. The maximum ^2H isotope content in surface waters does not exceed 148 ppm. A qNMR measurement of wine according to the proposed approach takes some minutes, that significantly exceeds the laboriousness of methods based on IRMS/SIRA (e.g. $\delta^{13}\text{C}$, $\delta^{18}\text{O}$). The error of qNMR measurements is less than 2.0%. The qNMR screening of deuterium (^2H) in ethanol can be used for detection of possible wine chaptalization. This approach is similar to the known SNIF-NMR method. The positive difference with this method is the use of minimal quantity of enriched ^2H standard and measurement of integral intensities of all signals instead of heights. It allows to reduce measurements' time as well as to measure the ^2H content of all fragments of ethanol molecules – CH_3^- , CH_2^- , OH -groups. The publication has been prepared with the support of the "RUDN University Program 5–100".

1. Introduction

Any aqueous-organic or aqueous-alcoholic solutions, in particular samples of alcoholic products, can be characterized by composition at different hierarchical levels – isotope, atomic, fragment and component. Each of these provides complementary information to authenticate them. Universal method for determining the composition of stable isotopes of elements is Isotope Ratio Mass Spectrometry/Stable Isotope Ratio Analysis (IRMS/SIRA). Classical methods of elemental analysis of organic systems are also universal, well developed and automated. The fragment composition of organic multicomponent systems is carried out by the methods of nuclear magnetic resonance spectroscopy (NMR) and infrared spectrometry or Raman scattering. The quantitative component composition of such objects is determined by various methods – chromatographic, electrophoretic, mass spectrometry, optical and NMR. The last one is the only direct method for determining the absolute molar content of aqueous-organic/aqueous-alcoholic solution components without the use of authentic standard samples.

2. Rapid screening of the wine component composition by ^1H NMR spectroscopy

^1H NMR spectroscopy for authentication of wine is currently employed to characterize wine in term of targeted components [1,2] as well as non targeted fingerprint metabolomic analysis [3,4]. No targeted method requires a large array of preliminary information that is not always available.

Screening analysis implies a simplicity or lack of sample preparation of analyte, quantitative assessment of the content of components, speed for making an immediate decision [5]. ^1H NMR is the ideal method for such screening of wines. The specificity of differences in the component composition of wines is determined by the geographical origin and some features of the region of grape growing, the composition of surface and ground water, soil, grape varieties, the technology of wine production. The latter factors are manifested in the content of minor components of wine, such as glycerol, organic and amino acids, etc. Registration of ^1H NMR spectrum provides for a short time (few minutes)

obtaining of a spectrum that allows calculating the water content (sum of all exchanging protons of groups OH and NH) and ethanol, glycerol, basic acids and residual carbohydrates. The calculation is based on measuring the signal areas of individual structural fragments of molecules of wine components. Here and below structural fragments are mean hydrogen-containing groups, which include only one atom other than hydrogen. For example, H₂O, H₂S, NH₃, CH₄, C₂H₄, C₆H₆ (benzene), C₆H₁₂ (cyclohexane) molecules contain only one chemically equivalent structural fragment, while the ethanol molecule contains three, and the glycerol molecule contains four. ¹H and ²H NMR spectra of any compound contain individual signals of each structural fragment, the integral intensity of which is directly proportional to their content. As a quantitative marker for measuring of minor components, it is convenient to use a strong-field satellite of signal CH₃ group of ethanol which has intensity 0.55% of main CH₃ signal. Although measuring the content of minor components is more effective in the absence of satellite signals with additional irradiation of the sample at a frequency of ¹³C nuclei.

¹H NMR spectrum gives direct quantitative information about the content of individual components in wine. However, it is not enough for the recognition of authentic wine, ¹H NMR spectrum identical to the spectrum of natural wine can correspond to an artificial mixture of water, ethanol and most minor components, in terms of their availability and known target spectrum. Those, ¹H NMR spectrum of adulterated product can be indistinguishable from genuine wine. In principle, a similar method of “synthesis of the image” by a combination of different compounds can provide adulteration of the elemental composition of wine, known from the elemental analysis for the corresponding region. Therefore, a fast screening of content of components of wine by ¹H NMR spectroscopy makes it possible to roughly evaluate the conformity of its composition to the declared origin, in particular on the content of ethanol, glycerol, acids and residual sugars. More importantly, registering spectrum in presence of a quantitatively introduced additive of an internal or an external standard (in a coaxial tube) allows to accurate measurement of integral intensities of the signal of ¹H nuclei participating in a proton exchange, which is 97% or more due to water protons. Knowledge of this exact value is necessary for accurate determination of ²H isotope content in wine water, as an indicator of the absence of exogenous water addition.

3. About hydrogen isotopes and ²H NMR

Hydrogen is represented in nature by two stable isotopes, corresponding to nuclides ¹H (protium) and ²H (deuterium) with an average prevalence of 99.985% and 0.015% (150 ppm), respectively. The natural content of ²H varies in the range from 40 to 200 ppm depending on the geographical location of substance, the source of its production and other reasons. Even in individual natural compound extracted from one plant, the relative content of deuterium in different hydrogen-containing structural fragments can differ by 4–5 times. In case of water, the range of variations in ²H content is 90–180 ppm.

Using ¹H NMR spectroscopy only to estimate absolute content of ²H isotope in the structural fragment of

molecules by reducing of integral intensities of signals ¹H of these structural fragments is impossible, since errors of their measurement are two orders of magnitude higher than the variations in the content of nucleus ²H (± 50 ppm, i.e., 0.005%). The use of ²H NMR spectroscopy directly for these purposes has some limitations since the combination of low natural content of ²H isotope (four orders of magnitude lower than ¹H) and the low relative sensitivity of the ²H NMR method (100 times relative to ¹H) reduces the actual sensitivity of the method 10⁶ times. However, the use of NMR equipment with high sensitivity and long-term stability removes this problem. Fourier spectrometers with operating frequencies for ²H nuclei 60 MHz and more allows receiving a ²H NMR signal with a signal-to-noise ratio of 100 for 1 g molecules up to 100 Da for one hour.

Precise measurement of ²H isotope content is promising for various areas of science: physics, chemistry, biology, medicine etc. For example, in the diagnosis of various pathologies according to the isotopic composition of hydrogen in biological fluids [6], differentiation of nature of juices and the products of their fermentation [7,8]. An important aspect of commodity examinations is the detection of adulteration of wines by adding water to them, the presence of exogenous ethanol and other components to imitate “fingerprint” of wine. To solve such problems, classical methods of IRMS/SIRA of hydrogen, carbon, and oxygen are widely used and discussed in a recent review [9]. Significantly less attention was paid to the methods of ²H NMR spectroscopy using external [6] or internal [10,11] standards for quantitative measurements.

4. Detection of wine extension with water

The proof of authenticity and geographical origin of wine can be based on the correspondence of content of minor stable ²H, ¹³C, ¹⁷O, ¹⁸O isotopes to values characteristic of genuine wines of the corresponding region, which must be obtained for the individual components of wine – water, ethanol, glycerol, etc. Without separation, IRMS/SIRA method can only give the uninformative integral value of ²H/¹H (δ^2 H), ¹³C/¹²C (δ^{13} C) and ¹⁸O/¹⁶O (δ^{18} O) ratios of an entire set of components.

Due to processes of evapotranspiration in plants and some chemical exchange during fermentation of wine water is always enriched in heavy isotopic form versus water absorbed during ripening from the soil. Detection of significant (30% and more) water addition to wine by official methods are based on analysis of isotopic ratio ¹⁸O/¹⁶O (δ^{18} O) of wine water and comparison of this value with reference data from published scientific and/or official wine databank [12,13].

A known approach to identification of much smaller additives in a wine of exogenous water is based on the measurement of values of isotopic ratios ¹⁸O/¹⁶O by IRMS/SIRA method in ethanol and wine water [14]. In genuine wines, these values differ by a characteristic value, variations of which are small for a studied set of objects of analysis. As in the case of the official method, this approach requires complete extraction or equilibration of water molecules by reference substance. In a conventional application, it is necessary to carry out complete extraction of ethanol. This leads to main drawbacks of separation techniques – the complexity of

Table 1. Physical and chemical characteristics of Chardonnay wines (2016 crop).

No.	Ethanol, %, vol.	Sugar (as invert sugar), g/dm ³	Titrateable acids (as tartaric acid), g/dm ³	Volatile acids (as acetic acid), g/dm ³	Extract, g/dm ³	² H isotope in wine water, ppm
1	11.1	2.2	6.3	0.32	19.2	161.5
2	12.1	2.2	4.5	0.32	18.1	161.7
3	12.9	2.3	6.2	0.38	18.6	162.8
4	14.1	1.8	5.2	0.44	18.0	157.0
5	12.8	0.9	6.7	0.65	20.6	164.4
6	11.7	2.1	4.9	0.76	20.8	157.8
7	12.1	0.9	6.4	0.52	22.9	158.4
8	14.5	0.5	7.6	0.74	23.1	157.6
9	12.8	2.4	6.6	0.56	23.9	161.8

sample preparation, possible random errors due to errors in its implementation, as well as systematic, associated with the manifestation of kinetic and thermodynamic isotope effects in any separation processes. These reasons motivate the search for other methods without or with a minimum sample preparation.

NMR spectra of ¹H, ²H, ¹³C and ¹⁷O of wine contain separate signals of all structural fragments of its macro and micro components. However, the range of variations in the content of these carbon and oxygen isotopes is much less than that of hydrogen and is comparable with errors of their quantitative measurements. This makes their accurate quantitative measurement even more laborious than isolating individual components of a solution for IRMS/SIRA analysis. Therefore, rapid screening of isotopic composition is possible only for ²H isotope.

The water signal in ²H NMR spectrum has a characteristic position (4.8 ppm) and does not overlap with signals of other structural fragments of wine. Its intensity, as in the case of ¹H, even for wine containing 16% vol. of ethanol and 40 g/l of carbohydrates are more than 95% due to ²H nuclei of water. In the case of dry wines, about 98% of the intensity of this signal is determined by ²H nuclei of water, the rest is a contribution from ²H exchanging hydrogen atoms of hydroxyl groups of other wine components, primarily from ethanol and glycerol. Accounting for the contribution of the latter is quite simple from analysis of the ¹H NMR spectrum. Integral intensity of the ²H signal of wine water is a characteristic of the region of origin of grapes, climatic features and year of its production, fermentation technology. Its use for strict authentication of wine requires a corresponding database of genuine wines of a particular region and year of production. An alternative approach is to measure the value of ²H content in wine water and compare it with an average content of this isotope in surface and ground waters of corresponding region. The wine water must always have ²H isotope content at 8–15 ppm higher than in external water due to transpiration of light isotopes during the growing season and isotopic redistribution during fermentation.

²H content of wine water is calculated by comparing the measured integral intensities of water signal of wine

with the signal of the water sample with a known content of ²H isotope, corrected for the decrease in ¹H signal integral intensity of wine water in relation to that of the pure water sample. This ensures that other components are accounted for. To obtain this correction from ¹H NMR spectrum, various standards can be used – internal (additive of the substance causing reference signal) or external (separate coaxial tube of smaller diameter inside the tube with analyte). Integral intensities of compared signals in spectra should be of the same order, and internal additives should be inert and low volatile. The main advantages of such cumulative screening for water addition detection are the lack of sample preparation, speed due to optimization of measuring procedure and sufficient precision for making a conclusion based on the magnitude of an excess of ²H content of wine water relative to water of the grape growing region. The proposed limitation of this approach is the same as the official methods of EU Regulation. It means that detection of a small percentage of water is difficult.

The approbation of cumulative screening of ²H isotope content in wine water is implemented for a series of natural wines from grapes from some geographic areas of the Krasnodar region. Information for this and other regions of Russia was previously unknown. As follows from a recent review [9] the systematization of such information for wines from other regions of the world is also absent.

5. Experimental approbation

Two series of measurements of ²H isotope content in wine water of the Krasnodar region of Russia were carried out. The first included 10 samples: 9 samples of red dry Cabernet Sauvignon wine produced from grapes of 2015 in its various parts, 1 sample of the same crop from Spain. Experimental parameters and the detailed results of this study were presented earlier [11].

In 2016, we selected 9 samples of grapes Cabernet Sauvignon and Chardonnay in various areas of the Krasnodar region in 30 kg each. The production of wines carried out according to a classical technology using the same yeast cultures for 10 days. Physical and chemical characteristics of wines are presented in Tables 1 and 2. All dry wines had rather high alcohol content – from 11.1 to 14.9% vol., which is due to high sugar accumulation in

Table 2. Physical and chemical characteristics of Cabernet Sauvignon wines (2016 crop).

No.	Ethanol, %, vol.	Sugar (as invert sugar), g/dm ³	Titrateable acids (as tartaric acid), g/dm ³	Volatile acids (as acetic acid), g/dm ³	Extract, g/dm ³	² H isotope in wine water, ppm
1	12.4	3.5	6.8	0.31	22.5	159.2
2	12.2	3.3	6.9	0.35	22.5	161.3
3	13.6	3.6	6.3	0.38	22.0	158.1
4	14.9	3.4	5.8	0.79	21.8	162.3
5	13.4	3.0	6.2	0.29	23.9	158.1
6	14.3	3.0	6.3	0.25	25.6	159.4
7	13.1	2.1	6.4	0.26	22.4	159.4
8	13.1	3.3	7.7	0.31	26.0	159.1
9	11.5	3.2	7.7	0.32	27.1	157.3

berries. High alcohol content in test samples was promoted as a high-quality wine and its further conservation of microbiological stability.

NMR spectra were recorded on JEOL JNM-ECA 600 (Japan) spectrometer, with operating frequencies on ¹H 600 MHz, and on ²H 92.1 MHz. Calibrated tubes with a diameter of 10 mm and a length of 178 mm were used. Wine samples were filtered to eliminate possible mechanical impurities. As a standard, a coaxial internal tube (external standard) with an external diameter of 3 mm filled with water containing an additive of 0.2 M dissolved europium (III) trifluoromethylsulfonate was used, that shifts a NMR signal of ²H of water in internal tube by 2 ppm in a weak field relative to water signal of analyte. Registration parameters of NMR spectra are presented in Table 3.

6. Results and discussion

The method of measuring ²H isotope content in wine water without its isolation based on cumulative screening of wine by qNMR ¹H and ²H was used first time by us in 2003 for searching of differences between Georgian wines and wines of other states [15]. Authentic wines of Georgia (Kakheti Region) were represented by Georgian side interested in protecting their products [15]. Samples of wines from other countries – Russia (Krasnodar region, as the geographic terrain closest to Georgia) and few of Western Europe countries (Germany, Italy, France and Spain) were purchased in Russian retail. The results obtained showed that ²H isotope content in water of Georgian wines has values in the range of 160–166 ppm, which is close to values for wines from France and Italy, somewhat higher values for wines from Germany and much less than for wine samples from Spain (about 170 ppm). In Russian wines values of ²H content in wine water is significantly lower and vary widely in a range of 146–158 ppm. This was explained as a combination of latitudinal and climatic factors and wine extension with water [15].

In 2017 an analysis of 9 samples of Cabernet Sauvignon wine (2015 crop) of the Krasnodar region from among the nominees of the Russian wine award was conducted. ²H isotope content of water varied in the range of 159–166 ppm in them with one exception

Table 3. Registration parameters of NMR ¹H and ²H spectra.

Parameter	¹ H	² H
The angle of the excitation pulse	90°	90°
Acquisition time	3.5 sec	1.9 sec
Relaxation delay	17 sec	0.5 sec*
Spectrum width	15 ppm	25 ppm
Points number	32 K	4 K
Scans number	8	256
Exponential multiplication	0.2 Hz	2.0 Hz

Notice: * Value for wine water (relaxation delay is 7 sec in case of measurement of the ²H content in CH₃- and CH₂- groups of ethanol molecules).

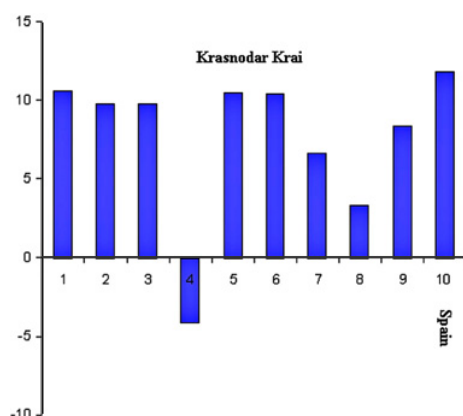


Figure 1. Results of determination of ²H isotope content of water in wines of the 2015 crop (“0” value corresponds to water standard SMOW 155.76 ppm).

(Fig. 1). Similar values have had some wines from Crimea peninsula. The highest value was again found to Spanish wine (168 ppm) [11]. Comparison of the results of measurements of ²H content of water in wines of 2002 and 2015 allowed establishing that the reason for the low content of ²H in the first case was wine extension with water.

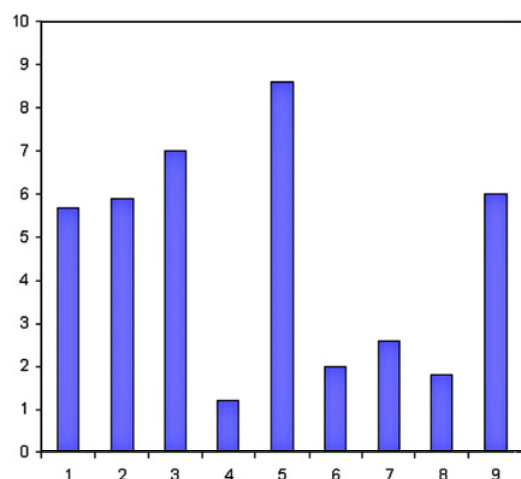


Figure 2. Results of determination of ²H isotope content of water in 9 samples of Cabernet Sauvignon wine 2016 (“0” value corresponds to reference water SMOW 155.76 ppm).

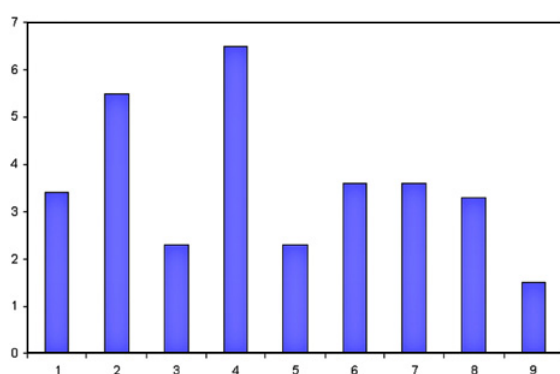


Figure 3. Results of determination of ²H isotope content of water in 9 samples of Chardonnay wine 2016 (“0” value corresponds to reference water SMOW 155.76 ppm).

To obtain reliable results on ²H isotope content in wines of Krasnodar region, 9 red and 9 white wines from grapes (2016 crop) of different areas of the Krasnodar region were made under standard conditions and ²H isotope content in water was measured. Measurement of ground water isotopic composition in areas where grapes were collected was also conducted. In all cases, the content of isotope ²H in ground water does not exceed 148 ppm, whereas its content in wine water of studied samples ranges from 157 to 164 ppm, i.e. more by 9–16 ppm (Figs. 2 and 3). The sum of experimental measurement errors of signal intensities in ¹H and ²H spectra of analytes does not exceed ±1 ppm.

This suggests that for authentic wines from this region in 2016 the isotope ²H content value of wine water should not be less than 156 ppm, i.e. content of ²H in reference water SMOW (155.76 ppm). The analysis of an additional set of 20 commercial wines and wines from the Krasnodar region (2015–2018 crops) confirm such conclusion of the minimum value in the content ²H of authentic wine water from this region.

The estimated criterion of the authenticity for wines from the Krasnodar region wines is not unique. It is easy to calculate that adding about 30% of water containing

148 ppm ²H isotope to an authentic wine with an ethanol content of 12% vol. and ²H isotope content of 160 ppm in wine water produces an adulterated product with an ethanol content of about 10% vol. and content of ²H isotope about 156 ppm, which is within the authentication range. Consequently, represented procedure which is like official methods according to the EU legislation [16], effective enough to detect wine adulterations. However, its labour intensity is disproportionately lower, since does not require sample preparation. Wine extension with water from other regions of Russia, in which content of ²H isotope in water is usually lower by 4–8 ppm, can be detected with significantly fewer water additives.

There is a known approach for detecting the addition of exogenous water to wine is the method of differential measurement of ¹⁸O/¹⁶O ratio in separated water and wine ethanol using IRMS/SIRA technique, which does not require any reference samples but uses complicated sample preparation technique [14]. It can be assumed that use of a similar approach for ²H NMR of ethanol of wine with a comparison of values of ²H content in CH₃-, CH₂- and OH-groups of ethanol molecule ((D/H)_I, (D/H)_{II} and (D/H)_{III}) may be more informative [11].

Going beyond the question of wine adulteration with water, it should be noted that presented method of fast cumulative screening of ²H isotope content in water is universal for any aqueous-alcoholic or aqueous-organic solutions, for example, foodstuffs (e.g. mineral water, juices, drinks, wine, brandies), biological fluids, pharmaceuticals, etc. To identify the raw material nature of ethanol in wine, we have applied the ALKOSCAN method [17], which uses the same sample preparation and the same approach as SNIF-NMR developed earlier [18], but which has a few fundamental differences that expand its capabilities. The most important of them is the measurement of signal integral intensity instead of their heights, which makes it possible to implement it on any NMR spectrometers without stabilization by ¹⁹F signal and allows measuring the content of ²H isotope in the OH group of ethanol. The use of external or internal standards enriched with ²H isotope for calibration allows minimizing its quantity and halving the time of each measurement on any spectrometer.

7. Conclusion

A new approach for screening of wine authenticity by qNMR ¹H & ²H has been proposed, which allows a measurement of ²H isotope content in wine water without sample preparation. This allows to make a conclusion about wine authenticity or to identify significant wine extension with water. Having predictive capabilities comparable to the official EU/OIV methods, a new method is significantly simpler and faster. It is applicable for determinations of ²H isotope in water of any aqueous-alcoholic or aqueous-organic solutions. To identify the use of exogenous carbohydrates in the production of wine, a method like SNIF-NMR is convenient, but it has some positive differences that ensure the expansion of its informativeness, universality and sensitivity. Further detailed studies will be conducted on wines from the Crimea peninsula.

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