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Direct determination of deuterium of wide concentration range in water by Nuclear Magnetic Resonance

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ABSTRACT

Based on quantitative ^{1}H NMR and ^{2}H NMR techniques, a quantitative method of direct determination of deuterium concentration in water was proposed and validated. This is a simple, rapid, and sensitive method, with minimum sample preparation. The detection range of the proposed method was from natural abundance to nearly 100%, thus satisfying multiple analysis requirements.

1. Introduction

As a stable isotope, deuterated water is used in a wide range of domains, from agriculture, biological to chemistry or iatrology. Detection of low concentration deuterium in aqueous samples becomes increasingly important in biological tracer studies [1,2]. In metabolic studies, deuterated water is a tracer to measure the water space and turnover rate [3], gluconeogenesis [4–6] and lipogenesis [7] *in vivo*. Certain deuterated solvents, such as acetone-d6, are manufactured by monitoring remaining D_2O concentration in water to validate the exchange reaction degree [8]. High concentration deuterated water, used as a coolant and moderator in nuclear reactor, should be checked from time to time for safety reason, because it could easily absorb normal water moisture.

Many methods have been proposed to determine deuterated water concentration. For example, based on slightly different density of water, the pycnometer method, the falling drop method [9] and Cartesian diver technique were used to detect deuterium oxide fraction. However, the precision of the analytical results by these methods was easily interfered by the impurities and isotopes of ^{18}O. Some other methods have also been used, for instance, Fourier-transform Infrared spectroscopy (FT-IR), Mass Spectroscopy (MS) and Gas Chromatography (GC). The first related work using an IR spectrometer was done by Thornton et al. in 1950 [10]. Content of deuterium oxide could be determined with a precision of 0.03% in terms of relative standard deviation, but the detectable range was low ($<3\%$). Traditional Isotope Ratio Mass Spectrometry (IRMS) approach is to convert hydrogen of water to hydrogen gas either by heating water to 400 °C in the presence of zinc or a catalyst before determination [11,12]. Although the analytical results are usually reliable, the method is time-consuming and needed tedious labor [1]. Similar to MS, GC can determine the isotope of hydrogen as well [13], but is also inconvenient.

Nuclear Magnetic Resonance (NMR) has also been developed as a quantitative analytical method [14]. In principle, quantitative NMR (qNMR) should be amenable to all NMR sensitive nuclei without dimensional restriction. The principle of qNMR is that under certain condition, the signal area is directly proportional to the number of nuclei causing the peak. Site-specific natural isotope fractionation NMR (SNIF-NMR) is to determinate the isotope ratios at different sites in one molecule [15], which might deviate compared with statistical distribution [16]. The application of ^{2}H-qNMR is somehow limited because the deuterium signal is adopted as a lock in most NMR instruments nowadays. There are two solutions: operating the instrument without a lock or a special ^{19}F lock channel is installed. However, it is complicated to install and tune an expensive ^{19}F channel [17].

A serial of simple methods were established to detect different deuterium ratios in this work. For low enrichment (0.015%–6%), quantitative ^{2}H –NMR spectra were used without a lock process. Although it could face the problem of magnetic field drift, restriction of experiment time could reduce the risk efficiently.

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High concentration (5%–100%) deuterium could be calculated by ^{1}H –NMR spectra. It is a simple, rapid, accurate, and green analytical method for the determination of deuterium in a wide concentration range in water.

2. Experimental

2.1. Apparatus

All NMR experiments were carried out by using an Avance II-600 MHz NMR spectrometer (Bruker Company, Switzerland) equipped with a 5 mm TXI probe. ^{1}H –NMR spectra was operated at frequencies of 600.13 MHz and ^{2}H –NMR spectra at 92.12 MHz. All NMR data were processed by Topspin 2.0 software (Bruker Company, Switzerland).

The infrared experiments were recorded at Thermo Scientific Nicolet iS10 FT-IR spectrometer (Thermo Fisher Scientific Inc, USA) furnished with a DTGS probe (detection range: 7800–350 cm^{-1}).

2.2. Reagents and solutions

Deuterium oxide (D, 99.96%) and sodium 3-(trimethylsilyl) propionate-2,2,3,3-d_4 (TMSP) with 98% deuterated ratio were obtained from Cambridge Isotope Laboratories, Inc. Acetic acid (analytic grade, 99.5%) was purchased from Chengdu Changliao Chemical Company (Chengdu, China).

A serial of standard solutions were prepared by adding a certain volume of deuterated water (D, 99.96%) to pure water and then corresponding deuterium concentrations were calculated. A certain concentration TMSP was prepared by weighing TMSP powder accurately, then dissolving it in a polyethylene tube with the aid of an ultrasonicator. In ^{2}H –NMR experiments, 25 μL of 0.05 M TMSP (dissolved in deionized water) solution was added as internal reference. In ^{1}H –NMR experiments, 50 μL acetic acid or 100 μL 0.1 M TMSP (deuterated water as dissolvent) was used as internal reference.

2.3. ^{2}H –NMR spectroscopy

Delay time was set to 2.0 s after the longitudinal relaxation time (T1) determination of each ingredient. Lock power was switched off since deuterium was the very observation target. Free Induction Delays (FIDs) were acquired at 300 K without sample rotation with the following parameters: 64 pulses scanning for signal accumulation, P1 set to 180 μs with 6 dB (6.88 W) as Pl1 and receiver gain of 32, time domain being 256 k. The spectra were obtained by 90° pulse on a 100 ppm of sweep width with transmitter frequency offset (O1P) at 50 ppm. Each acquisition was repeated three times for average to reduce experimental error. Exponential function with a line broadening factor of 3 Hz was chosen to enhance the signal to noise (S/N) ratio. Followed by phase correction manually and automatic baseline correction, integration of ^{2}H signals was performed carefully by manually defining integration range (Fig. 1).

2.4. ^{1}H –NMR spectroscopy

In all ^{1}H experiments, a spectral width of 20 ppm, 64 k time domain, 16 scans and a receiver gain of 1 were used. 30 degree flip angle excitation pulse was performed at 300 K. Delay time was set to 20 s after T1 determination. Each sample was acquired 3 times, using exponential function (line broadening factor: 0.3 Hz) prior to Fourier Transform. After phase correction manually, an automation polynomial baseline correction was applied, as shown in Figs. 2 and 3.

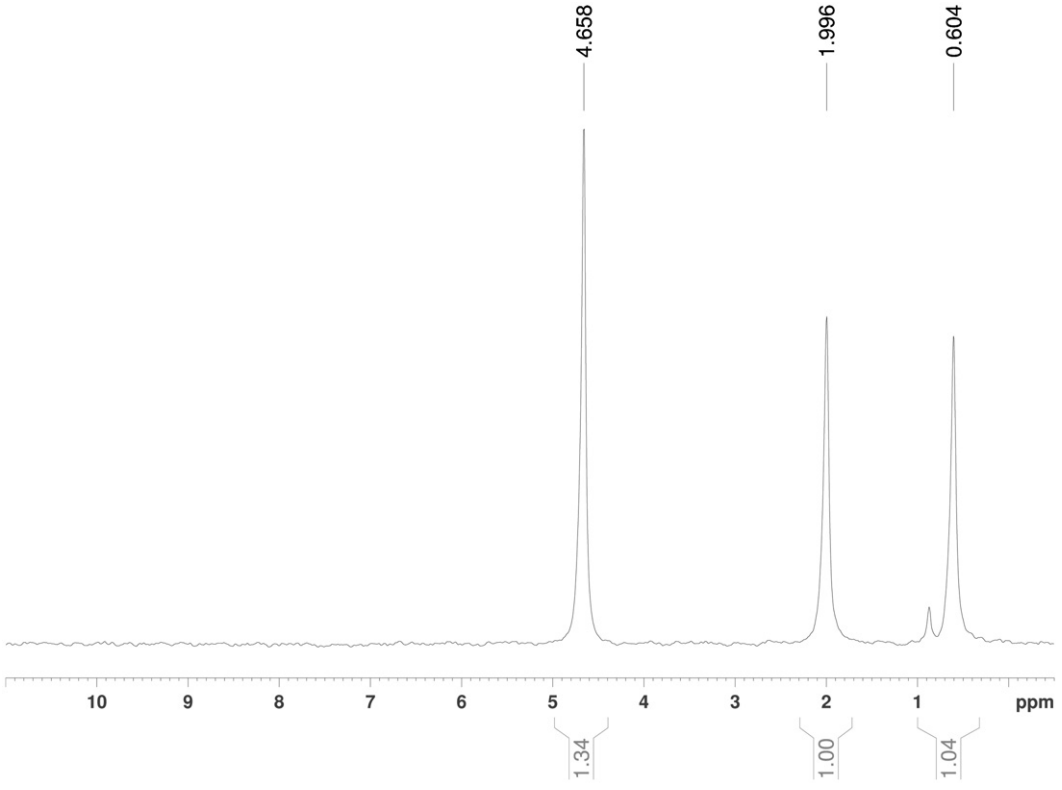

Fig. 1. ^{2}H NMR spectrum of deuterated water and internal reference TMSP. The single peak at 4.658 ppm is from deuterated oxide in water. The chemical shifts 0.604 and 1.996 ppm are the peaks of two kinds of deuterium in TMSP with nearly equivalent integration areas.

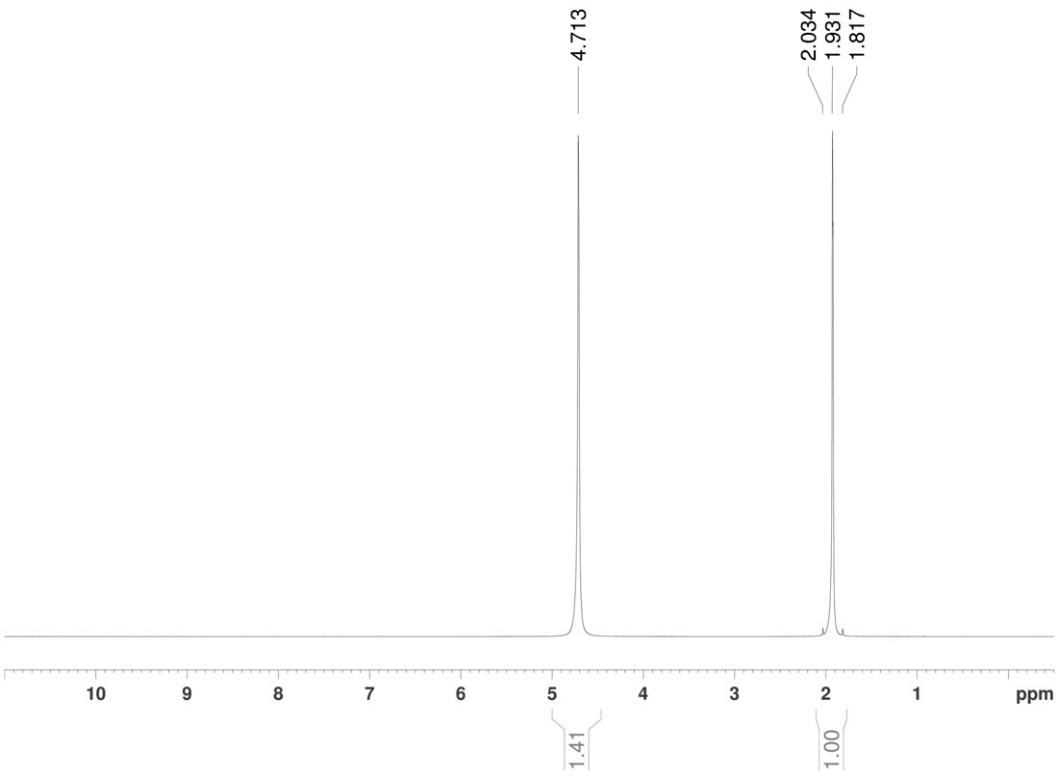

Fig. 2. ^{1}H NMR spectrum of H_2O and internal reference CH_3COOH. The signal peak at 4.713 ppm is from H_2O and the peak at 1.931 ppm is from the methyl in acetic acid, around which the two small peaks are satellite peaks caused by ^{13}C.

2.5. FT-IR spectroscopy

The sample was sandwiched between two CaF_2 windows (Φ30 mm × 2 mm, Tianguang Optical Instrument Company, Tianjin, China) with a 30 μm thick spacer.

Because D_2O could easily absorb moisture from air, the sample was quickly detected after preparation. After 16 times of background scans, FT-IR spectra were recorded 16 times for average as well.

3. Results and discussion

3.1. 90° Pulse calibration

Accurate 90 degree pulse could force macroscopic magnetization vector from z axis (direction of the magnetic field) exactly into x-y plane. 90° pulse should be calibrated after a period of time because of the magnetic field drift. Here, D_2O and ethylbenzene were employed as calibration targets and the 90° pulses of ^{2}H and ^{1}H were 180 μs and 7.6 μs, respectively.

3.2. Selection of internal reference

An ideal internal reference should be highly pure, not or little toxic, stable, soluble and inexpensive in NMR experiments, easy to be eliminated for recycle. Most importantly, any signal of selected internal reference should not overlap with the target analyte's peak profiles. Various compounds preferably with singlet have been suggested as the internal standard. In ^{1}H-qNMR, maleic acid [18], 1,3,5-benzenetricarboxylic acid [19,20], the singlet in three different ^{1}H signals of anthracene [21], etacrynic acid (EA) [22] *etc.* have been employed. In this work, acetic acid was selected for use, since its ^{1}H signal can separate from water's completely and also it is of cheap and nontoxic characters. In ^{2}H-qNMR, N,N-tetramethylurea (TMU) with known D/H isotopic content [17] is an official standard (Community Bureau of References) and the tert-butyl sulfide [23] can also be used. In this paper, TMSP was chosen for use, which had two peaks but with nearly equal integration area.

3.3. Spin-lattice relaxation time

A suitable relaxation delay (D1) was one of the most important parameters in NMR experiment. Generally, D1 was set to 3 to 5 times of the longest T1 of every peak of this compound to avoid signal saturation. Especially in qNMR experiment, when D1 was 5 times longer than spin-lattice relaxation time T1, 99.3% initial magnetization was realigned along the magnetic direction [24]. An equilibrium magnetization state could be reconstructed between two excitation pulses, if a sufficiently long D1 was employed. Therefore, to develop a correct qNMR method, a proper D1 should be carefully optimized first.

Here, T1 of each compound was determined by the standard "Inversion-Recovery" sequence (180°-τ-90°) [25]. In ^{1}H experiment, T1 values for acetic acid and water were 0.76 s and 3.8 s, respectively; and D1 was set to 20 s. In ^{2}H NMR experiment, since T1 of D_2O and TMSP was 0.42 s and 0.15 s, respectively, D1 was selected to 2 s.

3.4. Calibration curve and limit of detection by NMR

NMR quantitative analysis was based on the changed proportionality of integration of different content in real samples and constant amount of internal reference. Here, the linearity of the NMR quantitative methods was evaluated by plotting the calibration curve: the relative integration area as a function of the concentration of deuterium.

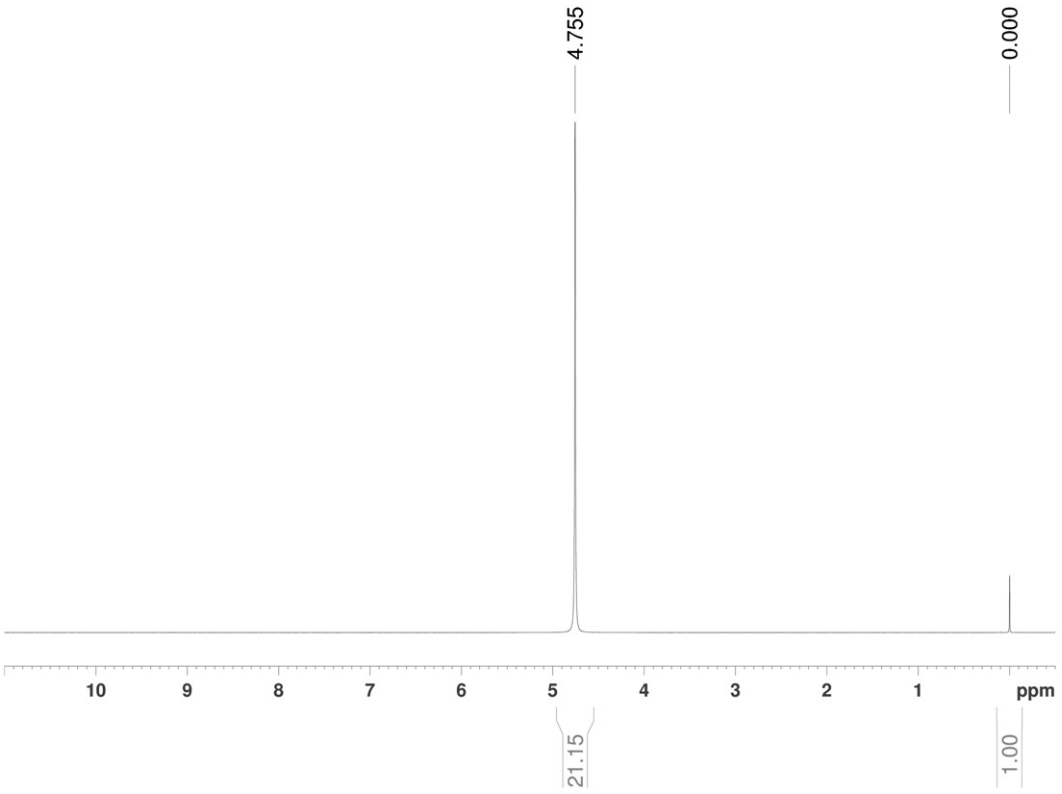

Fig. 3. ^{1}H NMR spectrum of H_2O and internal reference TMSP. The peak at 4.755 ppm is from H_2O while the peak at 0 ppm is caused by TMSP.

In this work, the calibration curve by NMR was divided into three parts: (a) because hydrogen is the most sensitive nuclear in NMR experiment and its spin number is 1/2, ^{1}H NMR calibration curve was investigated by adding TMSP powder when D_2O ratio was above 90% (H_2O ratio below 10%, as shown in Fig. 4); (b) if deuterium content was between 5% and 90%, acetic acid was used as the internal reference, which was much cheaper than TMSP (see Fig. 2, $R^2=0.9988$). For 50 μL acetic acid would occupy 10% volume of total sample, the maximum deuterium concentration of this method could only reach 90%, as shown in Fig. 5; and (c) the low D_2O concentration ($<5\%$) usually could not be detected accurately by ^{1}H NMR. Below certain deuterium concentration, the signal of H_2O was intensive, causing a large relative integration area ratio compared with the internal reference, thus leading to inaccurate result. Therefore, we tried to study deuterium spectra in low deuterium concentration samples directly. In NMR, the receptivity of deuterium is approximately 9.65×10^{-3} times smaller than that of hydrogen due to its exceptionally low natural abundance, 0.015%. The NMR receptivity is a presentation of the sensitivity of the NMR signal of that nuclide at natural abundance, relative to that of the proton assigned the value of 100% [26]. For the same concentration sample, since deuterium spin number is 1, its peak is wider than proton signal. Fig. 6 was the calibration curve for the detection of low concentration of deuterium in water by ^{2}H NMR. It was estimated that from natural abundance 0.015% to 6%, deuterium oxide in water could be determined with excellent linearity ($R^2=0.9991$).

In qNMR, the definition of limit of detection (LOD) is usually the concentration that could be detected with a signal-to-noise (S/N) ratio of 10 [27]. According to the S/N of natural abundance D_2O signal with 64 scans, the LOD was calculated to be 0.0048% deuterium in water (in volume). The experimental results illustrated that qNMR could detect D_2O concentration over a very wide range, from 0.0048% to almost 100%.

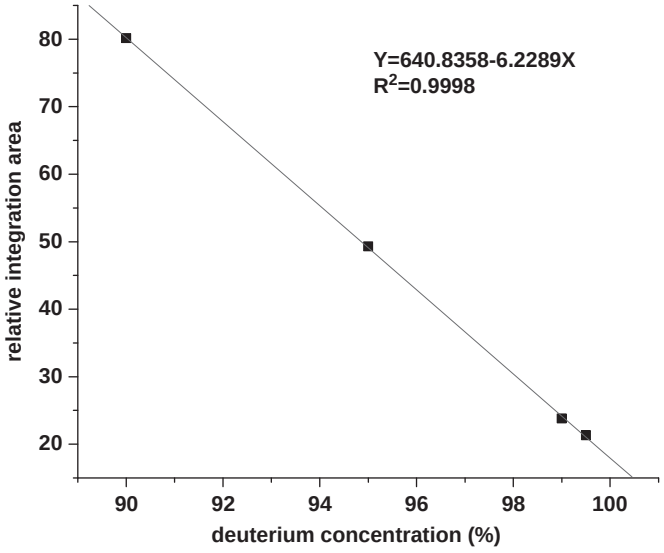

Fig. 4. Calibration curve of ^{1}H NMR spectra with TMSP as internal reference. The deuterium concentration is from 90% to nearly 100%.

3.5. Calibration curve and limit of detection by FT-IR

Anti-symmetric and symmetric stretching vibration band of D_2O appeared at 2495.5 cm^{-1}, as shown in Fig. 7. According to Beer-Lambert law, when a beam of monochromatic radiation passes through thin medium, the intensity absorption is directly proportional to the sample concentration at a certain thickness. Fig. 8 is the calibration curve obtained by the IR absorbance at 2495.5 cm^{-1} from corresponding deuterium concentration.

FT-IR calibration curve was also constructed of D_2O ratio in the range of 0.5%–7%. When below 0.5%, the D_2O absorbance at

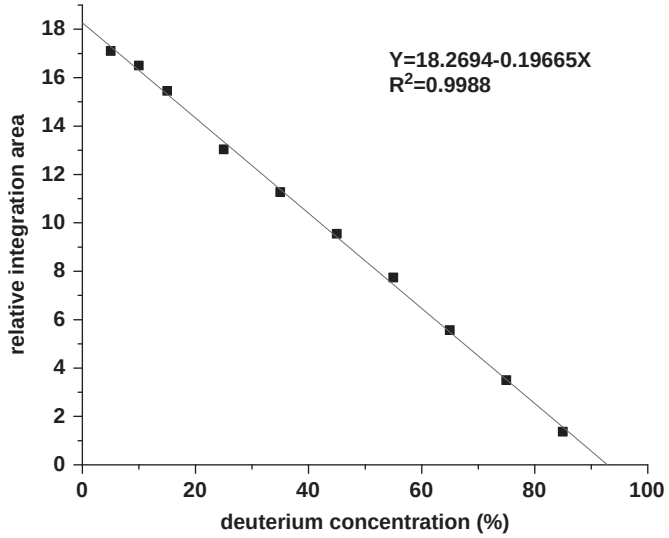

Fig. 5. Calibration curve of ^{1}H NMR spectra with CH_3COOH as internal reference. The deuterium concentration is from 5% to 90%.

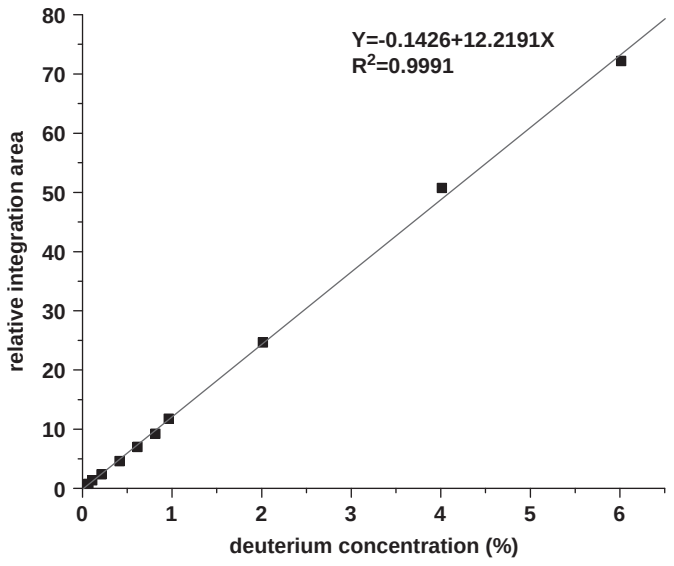

Fig. 6. Calibration curve of ^{2}H NMR spectra. The deuterium concentration is from natural abundant 0.015% to 6%.

2495.5 cm^{-1} could not be recorded accurately due to the interference from the huge signal of H_2O at 3400 cm^{-1}. When concentration was higher than 7%, the observe peak of D_2O would saturate.

3.6. Comparison of ^{2}H NMR and FT-IR determination

To compare FT-IR and ^{2}H qNMR methods, seven unknown samples were measured simultaneously and the analytical results were satisfactory (Table 1).

3.7. Analysis of water samples

Seven water samples were spiked with deuterium oxide at concentrations of 0.10%, 1.00%, 5.64%, 13.69%, 48.00%, 71.05% and 97.68%, respectively, and detected by quantitative ^{2}H NMR or ^{1}H NMR in triplicate, according to their concentration. The recoveries of deuterium from seven water samples were in the range from 95.0% to 110% (Table 2).

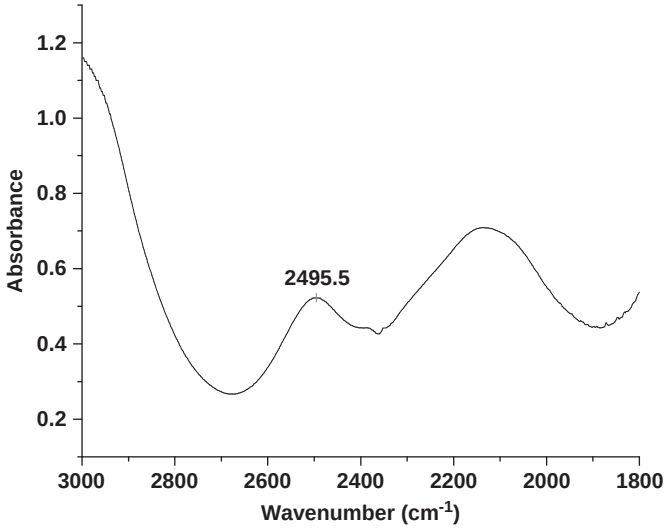

Fig. 7. Signal at 2495.5 cm^{-1} wavenumber is the anti-symmetric and symmetric stretching vibration band of D_2O.

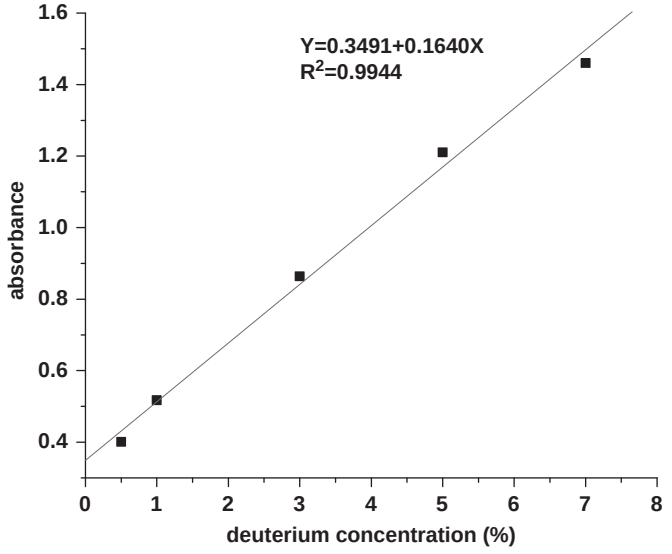

Fig. 8. The calibration curve range is from 0.5% to 7%, obtained by the IR absorbance at 2495.5 cm^{-1} from corresponding deuterium concentration.

Table 1
Analytical results of deuterium in water by qNMR and by FT-IR.

Number of sample	Real Concentration (%)	Concentration determined by NMR (%)	Concentration determined by FT-IR (%)
No. 1	0.71	0.79 ± 0.01[a]	0.85 ± 0.02
No. 2	1.20	1.16 ± 0.03	1.14 ± 0.05
No. 3	2.35	2.31 ± 0.02	2.35 ± 0.18
No. 4	3.51	3.50 ± 0.06	3.33 ± 0.11
No. 5	4.51	4.50 ± 0.18	4.36 ± 0.09
No. 6	5.21	5.32 ± 0.10	5.21 ± 0.46
No. 7	5.81	6.00 ± 0.14	6.06 ± 0.07

[a] Average + standard deviation ($n=3$).

4. Conclusion

In this work, a sensitive, selective and accurate quantitative ^{2}H NMR and ^{1}H NMR method was established for direct determination

Table 2
Recoveries of determining deuterium in water by qNMR.

Number of sample	Added deuterium concentration (%)	Detect method	Found deuterium concentration (%)	Recovery ratio (%)
No. 1	0.10	^{2}H –NMR	0.11 ± 0.01[a]	110
No. 2	1.00	^{2}H –NMR	0.95 ± 0.05	95.0
No. 3	5.64	^{2}H –NMR	5.57 ± 0.15	98.8
No. 4	13.69	^{1}H –NMR	13.70 ± 0.54	100
No. 5	48.00	^{1}H –NMR	48.49 ± 0.01	101
No. 6	71.05	^{1}H –NMR	70.47 ± 0.11	99.2
No. 7	97.68	^{1}H –NMR	97.62 ± 0.05	99.9

[b] Average + standard deviation ($n=3$).

of deuterium oxide in water by using a 600 MHz NMR spectrometer. After parameters optimization, a wide concentration range of deuterium was determined, from natural abundance to nearly 100% (in volume). The method is simple, fast and relatively green.

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