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Quantitative nuclear magnetic resonance spectroscopy — Purity determination of organic compounds used for foods and food products — General requirements for ^{1}H NMR internal standard method

Spectroscopie par résonance magnétique nucléaire quantitative — Détermination de la pureté des composés organiques utilisés dans les aliments et les produits alimentaires — Exigences générales pour la méthode de l'étalon interne par RMN ^{1}H

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Foreword

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This document was prepared by Technical Committee ISO/TC 34, *Food products*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Reliable quantification of food components is important for food safety and can be used as a measurement tool for food authenticity. Presently, chromatography such as gas chromatography (GC) and liquid chromatography (LC) is used in the majority of regulatory work associated with foods and food products. To obtain reliable quantification results with these methods, the use of certified reference materials (CRMs) is required, for which metrological traceability of the certified value, as measurement standards, is essential. However, obtaining such CRMs to fulfil these requirements is almost impossible in many cases as conventional methods that can establish metrological traceability, such as the mass balance, have limited applications. Therefore, the establishment of a simple, rapid, widely applicable and reliable purity quantification method, with a focus on the establishment of metrological traceability, for the characterization of measurement standards for food analyses is an essential. Quantitative nuclear magnetic resonance (qNMR) spectroscopy has been recognized as a quick and simple characterization method. The method is also recognized as metrologically traceable, and uses the purity from a CRM to determine the purity of other analytes. When a certified value of a CRM, whose value is stated as metrological traceable to the International System of Units (SI), is used as a measurement standard for qNMR, the determined purity value of the sample by qNMR can also be traceable to the SI through the CRM. qNMR, therefore, has the potential to provide the SI traceability to measurement standards relevant to food components.[10][17][36]

Quantitative nuclear magnetic resonance spectroscopy — Purity determination of organic compounds used for foods and food products — General requirements for ^{1}H NMR internal standard method

1 Scope

This document specifies general requirements and performance criteria for the determination of purity of organic compounds through the application of solution state proton (^{1}H) quantitative nuclear magnetic resonance (qNMR) spectroscopy using an internal standard method.

This document is applicable to bioactive compounds in functional foods, natural toxins, food additives and pesticides.

This document is applicable to the users pursuing metrological traceability of the measurement results.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
quantitative NMR
qNMR
quantitative analysis using NMR spectroscopy

3.2
proton quantitative NMR
^{1}H quantitative NMR
^{1}H qNMR
quantitative NMR (3.1) spectroscopy using proton (^{1}H) as the observed nucleus

3.3
qNMR procedure
predetermined workflow of quantitative analysis using *qNMR* (3.1) including sample solution preparation, data acquisition and data processing parameters that have been optimized and validated for a specific analyte

3.4
equilibrium magnetization
magnitude of the nuclear magnetization vector that is polarized in the sample after it has be placed into a static magnetic field

3.5
spin-lattice relaxation time
T_1
time needed for a set of spins of magnetically equivalent nuclei to attain macroscopic z-magnetisation, M_z, equilibrium in a magnetic field or to return to this equilibrium after excitation (by a radio frequency (RF) pulse)

Note 1 to entry: The recovery of M_z magnetisation is an exponential saturation process described by the Bloch-equation for M_z:

$$M_z(t) = M_z(t_{eq}) - [M_z(t_{eq}) - M_z(0)] \times \exp(-t/T_1)$$

where

$M_z(t)$ is the time function of M_z;
0 is the time zero;
t_{eq} is the time equilibrium has been achieved;
t can be any time between 0 and t_{eq}.

Note 2 to entry: Following a 90° pulse, 63 % of an ensemble of magnetically equivalent spins have relaxed after $1 \times T_1$, over 99 % of spins have relaxed after $5 \times T_1$.

3.6
free induction decay
FID
time-domain NMR signal that results from the precession of the nuclear magnetization vector inside the probe coil after application of an excitation RF pulse to a sample in a static magnetic field

3.7
shimming
process that is carried out to correct any inhomogeneities in the applied magnetic field during an NMR experiment

3.8
spectral width
SW
width of the spectrum after Fourier transformation

Note 1 to entry: It is given in Hz or ppm[1)].

Note 2 to entry: The axis for SW (x-axis) of an NMR spectrum is usually expressed as chemical shift in ppm scale. Resonance frequency of an NMR signal depends on the external magnetic field of the NMR instrument. Relationship between the resonance frequency in Hz and chemical shift, δ, in ppm is as follows:

$$\delta = \frac{\nu_s - \nu_r}{\nu_r}$$

where

δ is the NMR "chemical shift" of an individual signal, typically expressed in ppm scale;
ν_s is the absolute resonance frequency of the ^{1}H NMR signal for a sample measured in an NMR instrument, given in Hz;
ν_r is the absolute resonance frequency of the ^{1}H NMR signal for a chemical shift's reference compound signal measured in the instrument, given in Hz.

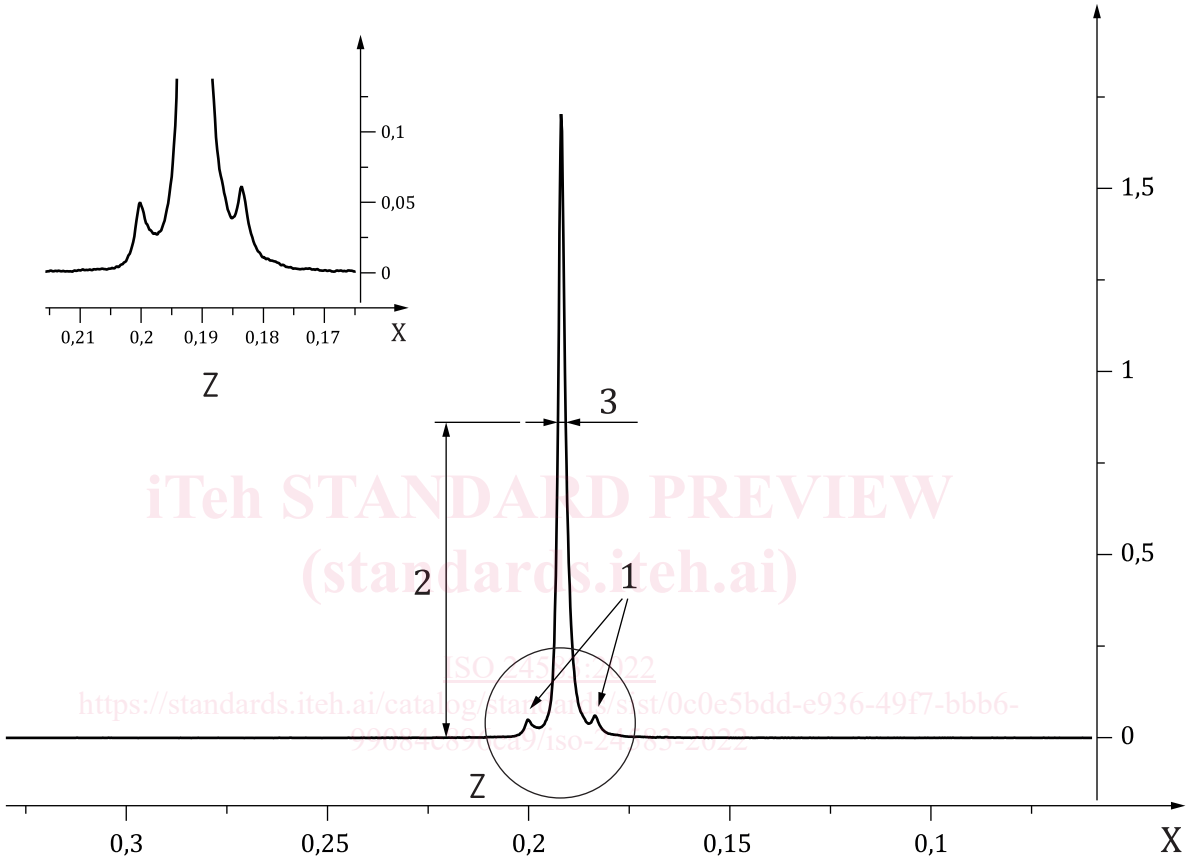
Since the difference in the numerator is usually in Hz and the denominator in MHz, δ is expressed in ppm.

1) ppm = parts per million.

3.9
full width at half maximum
FWHM
width of a line shape at half the maximum signal intensity

Note 1 to entry: It is expressed in Hz.

Note 2 to entry: Figure 1 illustrates FWHM. The example signal is from 1,4-bis(trimethylsilyl)(D_4)benzene (see Table B.1).



Key

X chemical shift (ppm)
Z chemical shift (ppm)
1 ^{29}Si satellite signal
2 half height of signal
3 full signal width at half maximum height

Figure 1 — Illustration of FWHM

3.10
flip angle
pulse angle
pulse flip angle
tilt angle of the bulk nuclear magnetization vector, relative to the static magnetic field after applying an RF pulse of specific duration and amplitude in a static magnetic field at thermal equilibrium

Note 1 to entry: This non-equilibrium magnetization can be induced by applying an RF pulse of sufficient excitation bandwidth and carrier frequency near the Larmor frequency of the nuclear spins.

3.11
repetition time
T_r
time period from the application of the first RF pulse of a pulse sequence until the same pulse is applied again in the subsequent transient

3.12
number of transients
NT
number of scans
number of times that a Fourier transformation-NMR experiment is repeated with the resulting *FIDs* (3.6) accumulated/summed to improve the signal-to-noise ratio of the NMR spectrum

3.13
decoupling
NMR experimental technique to eliminate spin-spin coupling

Note 1 to entry: In this document, only heteronuclear decoupling, e.g. decoupling of ^{1}H-^{13}C spin-spin coupling, is considered.

3.14
dummy scan
steady state scan
transient that is performed to establish a steady state of the magnetization, all parts of the NMR experiment are carried out (e.g. RF pulses, delays, pulsed field gradients), but no data is recorded

3.15
satellite signal
signals arising from fraction of sample containing another NMR active nucleus showing the coupling to this nucleus

3.16
zero filling
insertion of zero values at the end of an *FID* (3.6) signal prior to Fourier transformation, a means to increase the frequency domain resolution of an NMR spectrum

3.17
phase correction
mathematical procedure used to restore a pure absorption lineshape over the whole NMR spectrum

3.18
baseline correction
mathematical procedure used to correct distortions in the baseline of an NMR spectrum

3.19
spectral resolution
degree of distinction and separation of signals used in quantitative analysis

3.20
integrated area
signal area
peak area
integration value of the signal interval between the baseline of the signal and the resonance signal

3.21
minimum weight
smallest sample quantity required for a weighment to just achieve a specified relative accuracy of weighing

[SOURCE: EURAMET Calibration Guide No.18 Version 4.0[9]]

3.22
repeatability
measurement precision under a set of repeatability conditions of measurement

[SOURCE: ISO/IEC Guide 99:2007[5], 2.21]

3.23
calibration
operation that, under specified conditions, in a first step, establishes a relation between the quantity values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties and, in a second step, uses this information to establish a relation for obtaining a measurement result from an indication

[SOURCE: ISO/IEC Guide 99:2007[5], 2.39, modified — The notes to entry have been deleted.]

3.24
internal standard
material used as a measurement standard for the purity evaluation for a *qNMR procedure* (3.3) in solution together with a sample

Note 1 to entry: A schematic illustration is given in Figure 2.

3.25
qNMR standard
component of an *internal standard* (3.24) used as the measurement standard for a *qNMR procedure* (3.3)

Note 1 to entry: A schematic illustration is given in Figure 2.

3.26
receiver gain
RG
amplification ratio of the signal by the receiver

3.27
line-broadening
mathematical processing technique by which the *FID* (3.6) is manipulated by exponential function in order to improve the signal-to-noise ratio at the expense of resolution

4 Principles

4.1 General

NMR is one of the most useful techniques for the structure elucidation of organic compounds due to three important features:

a) chemical shifts of resonance signals;

b) spin-spin couplings by neighbouring non-equivalent NMR active nuclei;

c) the proportionality between the integrated area and the number of corresponding ^{1}H nuclei.

The first and third features play an important role in comparing different integrated areas quantitatively. The integrated area is directly proportional to the size of the population of ^{1}H nuclei causing this resonance signal, if the experimental conditions are optimized correctly. Since the integrated areas of two distinct resonances are usually well separated due to their respective chemical shifts it becomes possible to determine the molar ratios of chemical substances or structural moieties giving rise to the signals. In other words, all signals in the spectrum can be assigned to chemical (sub-)structures of the analyte and the qNMR standard. Therefore, if an internal standard, with a certified purity value of identified structure, is added to the sample solution that contains an analyte of known structure,

the purity of the analyte in sample can be determined from the relationship derived in Formulae (3) and (4).

For the i^{th} signal in the ^1H NMR spectrum of a single analyte compound in a sample, a integrated area I_i can be expressed as Formula (1):

$$I_i \propto N_i \frac{m}{VM} P \sin\beta \frac{1-e^{-T_r/T_{1i}}}{1-e^{-T_r/T_{1i}}(\cos\beta)} M_z(t_{eq}) \quad (1)$$

where

I_i is the integrated area of the i^{th} signal of the compound;

N_i is the number of resonating protons for the integrated area of the i^{th} signal (I_i);

V is the volume of the sample solution;

m is the mass of the sample;

M is the molar mass of the compound;

P is the purity (mass fraction) of the compound;

β is the excitation flip angle;

T_{1i} is the spin-lattice relaxation time of the i^{th} ^1H signal of the compound;

T_r is the repetition time;

$M_z(t_{eq})$ is the equilibrium magnetization.

The relaxation times T_{1i} can be different for the ^1H nuclei of different signals.

This formula suggests that N_i and T_{1i} are the only terms that depend on different signals. When $T_r >> T_{1i}$ is met, 1-exp($-T_r/T_{1i}$) becomes unity. Therefore, when T_r for an experimental parameter is set to sufficiently longer than the longest T_1 among signals of interest, the ratio of integrated areas can be proportional to N_i. When this relationship is applied to one compound, all parameters except I and N are common factors. Therefore, the I and N should only be considered. This is the basic principle for the proportionality of integrated areas for one compound that can be summarized in the Formula (2):

$$\frac{I_1}{I_2} = \frac{N_1}{N_2} \quad (2)$$

where

I_1 is the first integrated area of the compound;

I_2 is the second integrated area of the compound;

N_1 is the number of resonating protons for the integrated area of the first signal (I_1);

N_2 is the number of resonating protons for the integrated area of the second signal (I_2).

4.2 Conventions on the sample solutions for the qNMR procedure

In this document, the following conventions are used:

— A sample solution for the qNMR procedure is a mixture of a sample and an internal standard dissolved in a solvent or mixture of solvents.

— The sample (S) consists of a main component, which is the target component for the purity determination, and other components; in this context these are impurities. Hereafter, analyte (A) is referred to as the main component of the sample.

— The internal standard (IS) is a material used as a standard of the qNMR method. The internal standard also consists of a main component for the qNMR method, and other components (impurities). Hereafter, qNMR standard (Q) is referred to as the main component of the internal standard.

Figure 2 is a schematic illustration of the conventions.

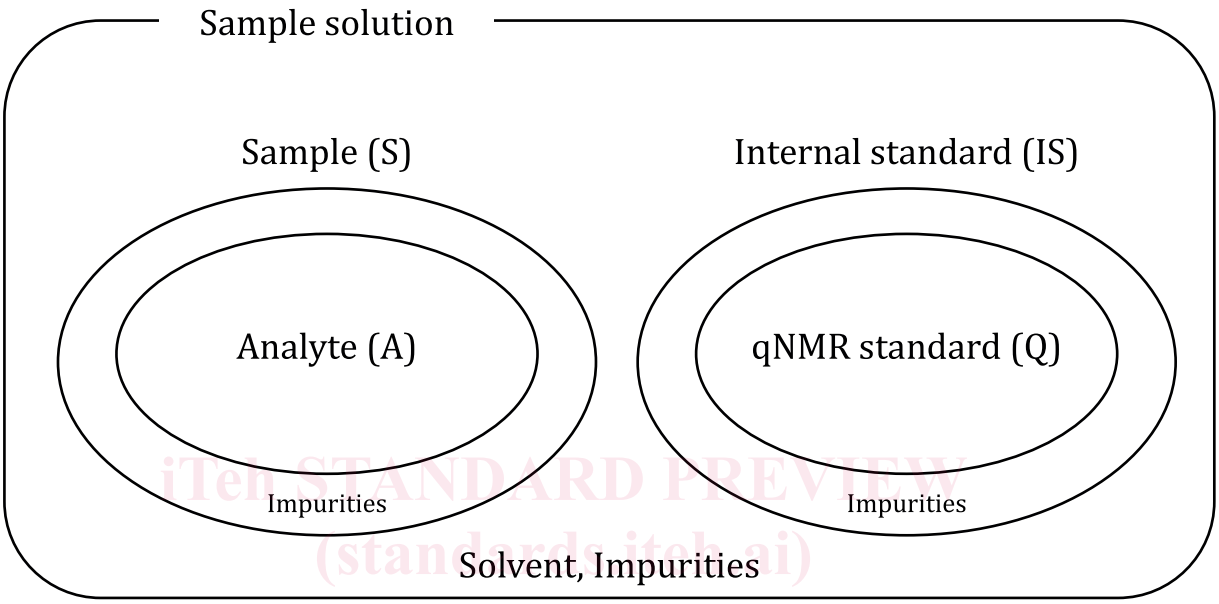


Figure 2 — Schematic illustration of the sample solution

4.3 ^{1}H quantitative NMR (^{1}H qNMR)

Formula (2) can also be applied to signals derived from two compounds in a sample solution. When the purity is determined by qNMR, the two compounds in the sample solution correspond to an analyte in the sample and the qNMR standard in an internal standard. Figure 2 is a schematic illustration of the sample solution for such a case. The sample (S) and the internal standard (IS) both consist of main components and impurities. Since the analyte and the qNMR standard (Q) are the main components, the purities can be expressed by percentages of mass fraction (kg/kg) of the sample and the internal standard.

When the longest T_{1i} satisfies with $T_r >> T_{1i}$ for the two compounds, Formula (3) can be derived from Formula (1):

$$\frac{I_{Q_j}}{I_{A_i}} = \frac{N_{Q_j}}{N_{A_i}} \times \frac{m_{IS}}{m_S} \times \frac{M_A}{M_Q} \times \frac{P_{IS}}{P_S} \tag{3}$$

where

A is the analyte in the sample;

Q is the qNMR standard in the internal standard;

S is the sample;

IS is the internal standard;