Development of analytical standards guaranteed by qNMR

Toru Miura1, Naoki Sugimoto2, Takako Suematsu3 and Yoko Yamada1
1Wako Pure Chemical Industries, Ltd., 2National Institute of Health Sciences (NIHS), 3JEOL Resonance Inc.

Abstract

qNMR using the internal reference substance with traceability to the International System of Units (SI), named as AQARI (Accurate Quantitative NMR with Internal reference substance), recently attracts attention as one of absolute purity determination methods, because of its efficiency and reliability. In fact, qNMR has already been utilized as the official analytical method, such as the Japanese Pharmacopoeia and Japan’s Specifications and Standards for Food Additives.

Although many analytical standards, of which the purities are guaranteed by area normalization method of GC or HPLC, are distributed in commercial reagent markets, few ones with certified absolute purities are available. For this reason, we had started to develop analytical standards with their absolute purity values determined using AQARI.

The most essential element in establishing the determination method using AQARI, is the certification of the purity of the internal reference substance. Therefore, we developed internal reference standards for AQARI for the first time in global market. As a result, we built up the system to prepare analytical standard with SI-traceability by using AQARI.

Next, we started to establish supply system of any compounds with absolute purity values determined by AQARI. We explored appropriate framework for sample preparation method, NMR parameter settings, sample distribution ways and selection of target signals of each compound. The explorations led us to good finding that qNMR was able to determine the purity of the natural organic compounds with the accuracy of approximately less than or equal to 1%. And then we established the supply system of the analytical standards such as pesticide, catechin, mycotoxin and so on, having absolute purity values.

Features of qNMR(AQARI)

- **Reliability : absolute quantitation method with SI traceability**
- **Efficiency**
  - Comparison of qNMR(AQARI) and area normalization method by GC or HPLC
  - Area-normalization method
    - Main component
    - Impurities
  - qNMR(AQARI)
    - Absolute purity → Reliable

Development of Certified Reference Materials(CRMs) for qNMR(AQARI)

Equation for purity calculation in qNMR(AQARI)

\[ P_{\text{relate}} = \frac{I_{\text{sample}}}{I_{\text{std}}} \times \frac{H_{\text{sample}}}{H_{\text{std}}} \times \frac{M_{\text{sample}}}{M_{\text{std}}} \times \frac{P_{\text{std}}}{P_{\text{std}}} \]

- Reliable CRM suitable for AQARI is needed for AQARI analysis with high accuracy.
- Therefore, we developed CRMs suitable for AQARI.

Experimental scheme

Example of Purity Determination of (-)-Epigallocatechin

- Sample preparation
- Analytical solvent
- Ultra-Micro Balance (10.000 mg)
- Data Process
- JNM-ECS400
- Observer nuleus 1H
- Decouple nuleus 13C
- field strength 400 MHz
- Spectral width 20 ppm
- DSP ON
- Offset 5 ... resolution 0.25 Hz
- Relaxation delay 60 s
- Probe temperature 25 ℃
- S/N ≧500
- Number of transition 8
- Dummy Scan 2

Example of purity determination

Acquired signal

Analysis by HPLC: 100.0 %

Traceability based on the number of nuclei

New Calibration system by qNMR(AQARI)

National Standards

- GC or HPLC
- Do not exist
- Traceability on the number of nuclei

Secondary Standards

- GC or HPLC
- Do not exist

Working Standards

- GC or HPLC
- Do not exist

Result

Quantitation

We can determine purity of over 400 Analytical Standards, such as catechin, pesticide, mycotoxin and Amino acid using by AQARI.

And most of these compounds have been already released as analytical standards with SI-traceability to reagent markets.