

Development of analytical standards guaranteed by qNMR

Toru Miura¹, Naoki Sugimoto², Takako Suematsu³ and Yuko 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 substances 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 sets, sample distribution ways and selection of target signal(s) 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

Moreover...

Simple method

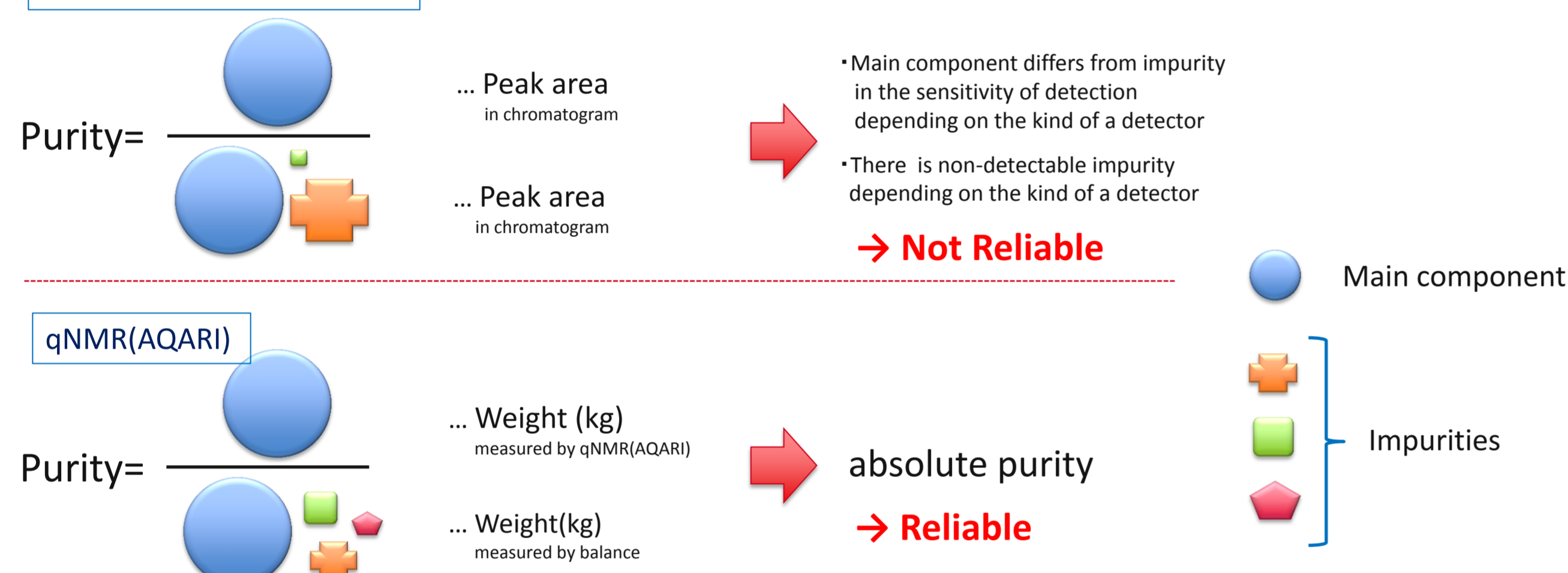
Quickness

Efficiency

Reliability

- Comparison of qNMR(AQARI) and area normalization method by GC or HPLC

Area normalization method



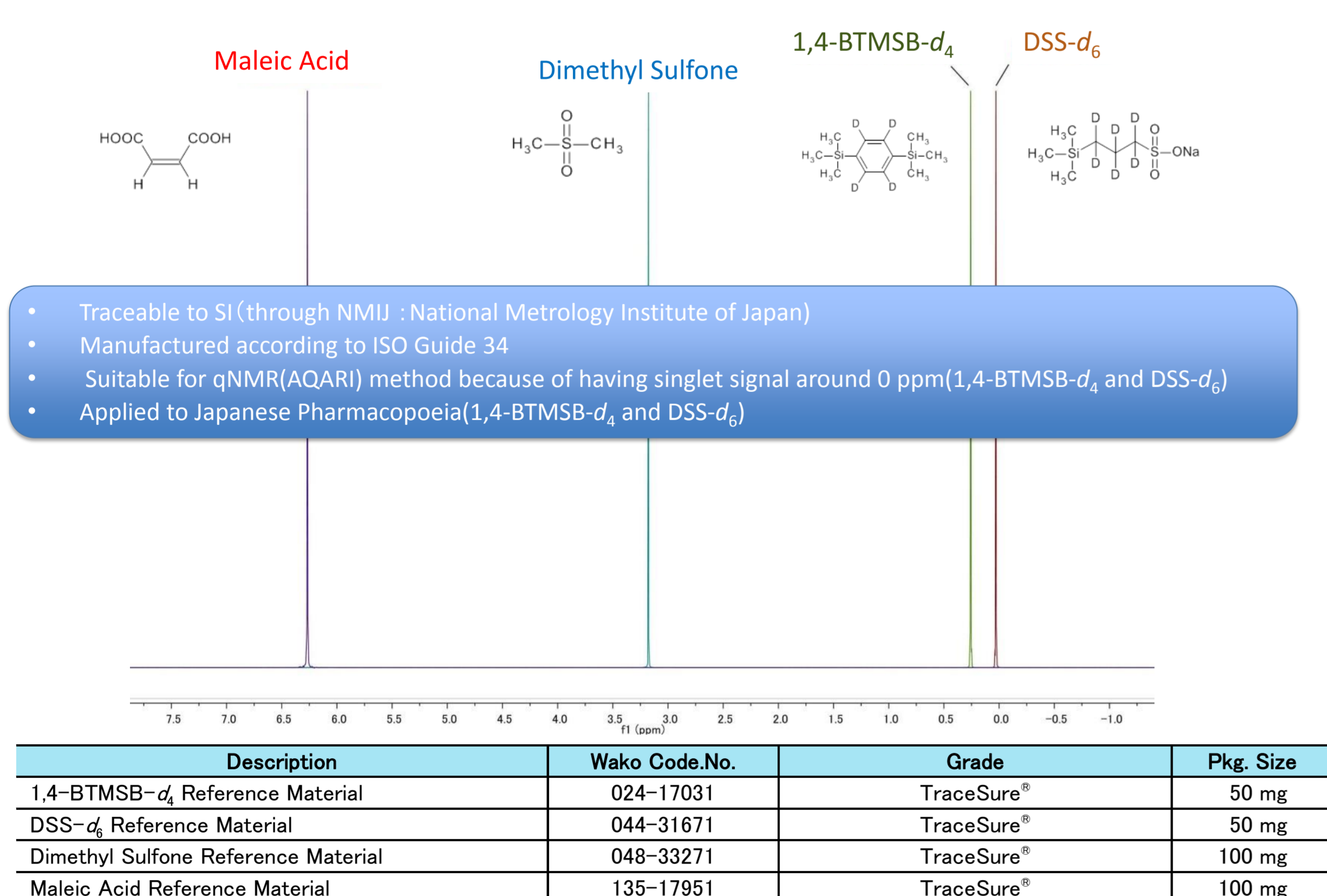
Development of Certified Reference Materials(CRMs) for qNMR(AQARI)

Equation for purity calculation in qNMR(AQARI)

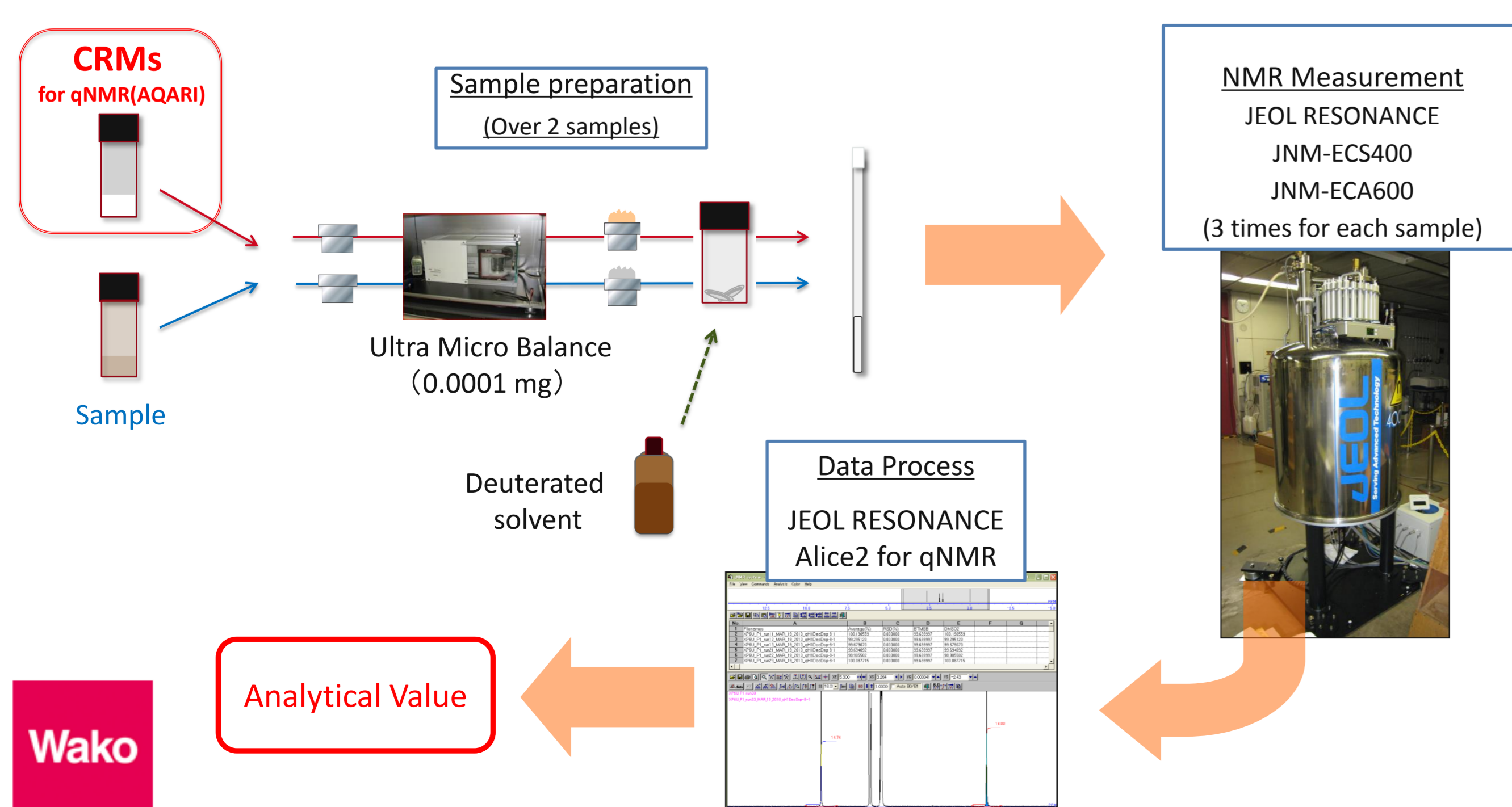
$$P_{\text{analyte}} = \frac{I_{\text{analyte}}}{I_{\text{std}}} \times \frac{H_{\text{std}}}{H_{\text{analyte}}} \times \frac{m_{\text{std}}}{m_{\text{analyte}}} \times \frac{M_{\text{analyte}}}{M_{\text{std}}} \times P_{\text{std}}$$

I: Integral area
H: Number of protons
M: Molecular mass
m: Weighing value
P: Purity

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



Experimental scheme

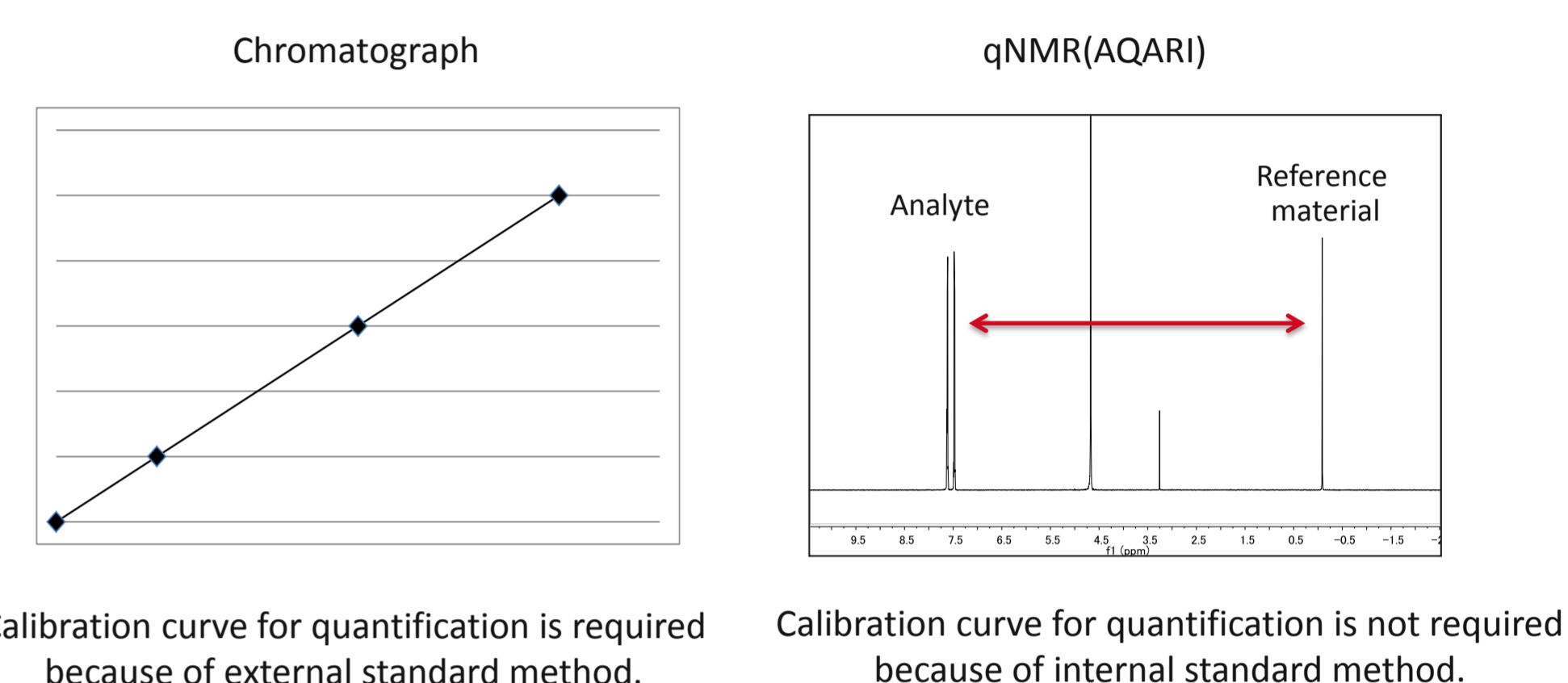


Simple method

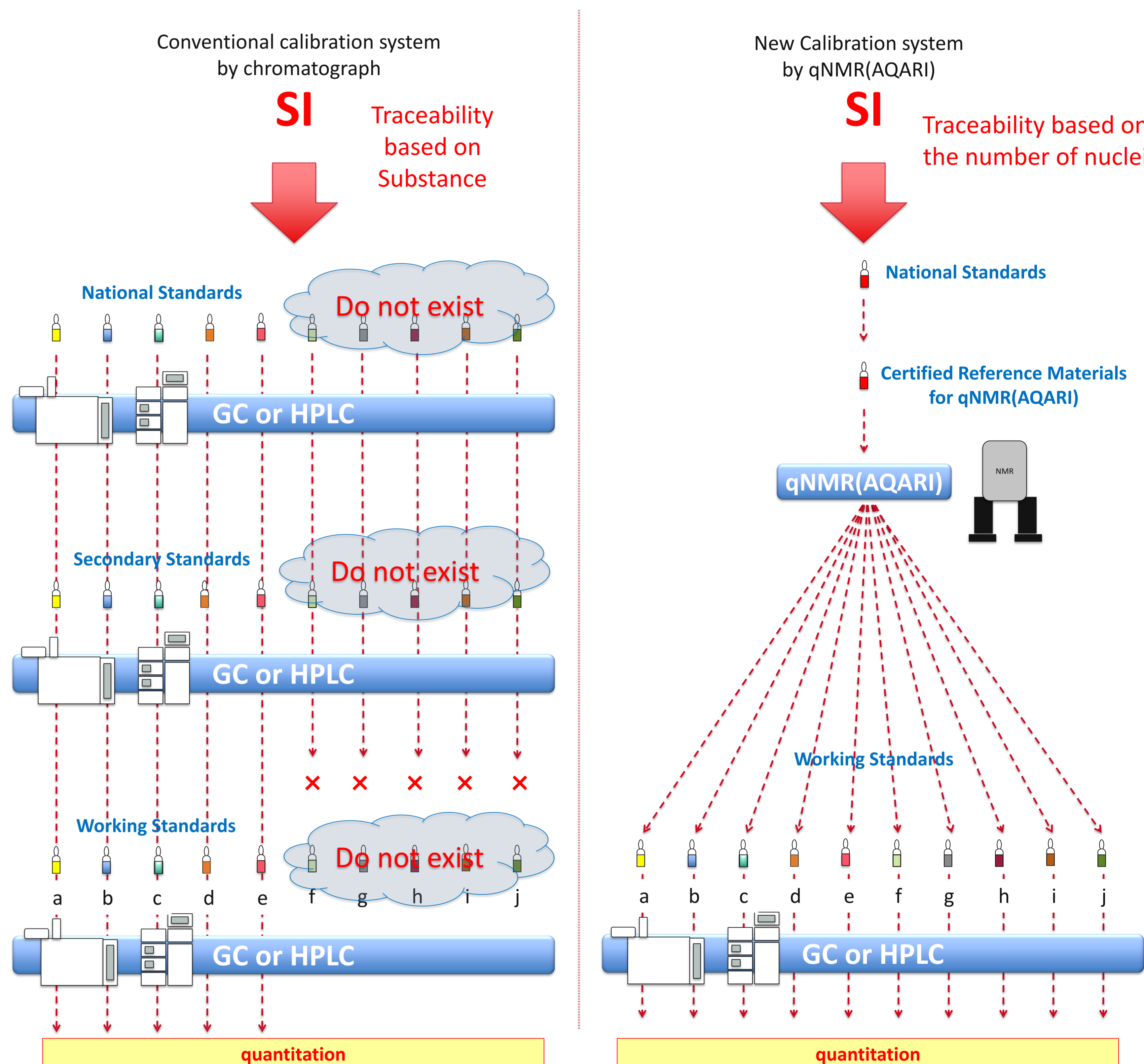
Determination of NMR measurement condition is almost not required.

NMR	JNM-ECS400
Observer nucleus	¹ H
Decouple nucleus	¹³ C
field strength	400 MHz
Spectral width	20 ppm
DSP	ON
Offset	5 ppm
Flip angle	90°
Acquisition time	4 s
Digital resolution	0.25 Hz
Relaxation delay	60 s
Probe temperature	25 °C
S/N	≥ 500
Number of transition	8
Dummy Scan	2

Quickness

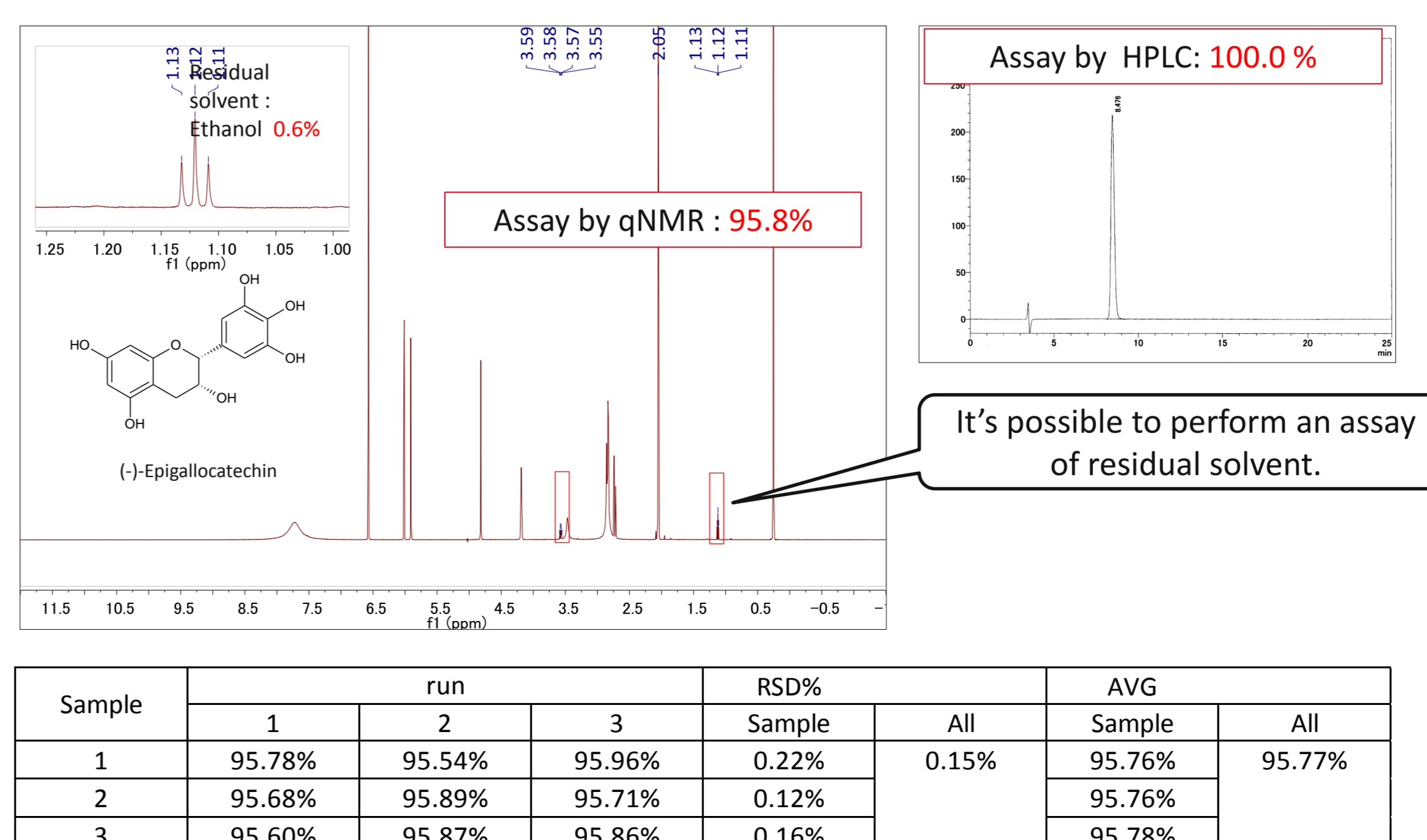


Efficiency



Result

Example of Purity Determination of (-)-Epigallocatechin



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.