

# Development of standard solutions for qNMR

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## Introduction & Objective

AQARI (Accurate QuAntitative NMR with Internal reference substance) has been recently applied to purity determination of the official analytical method such as the Japanese Pharmacopoeia and Japan's Specifications and Standards for Food Additives because of absolute purity determination method with traceability to the International System of Units (SI).

A large number of reference materials for AQARI are being distributed in the commercial market by reagent makers. In addition, it has been reported that AQARI with standard solution was able to conduct absolute quantitation with accuracy of approximately less than or equal to 1% without using high-resolution balance like as AQARI with reference material.<sup>1)</sup>

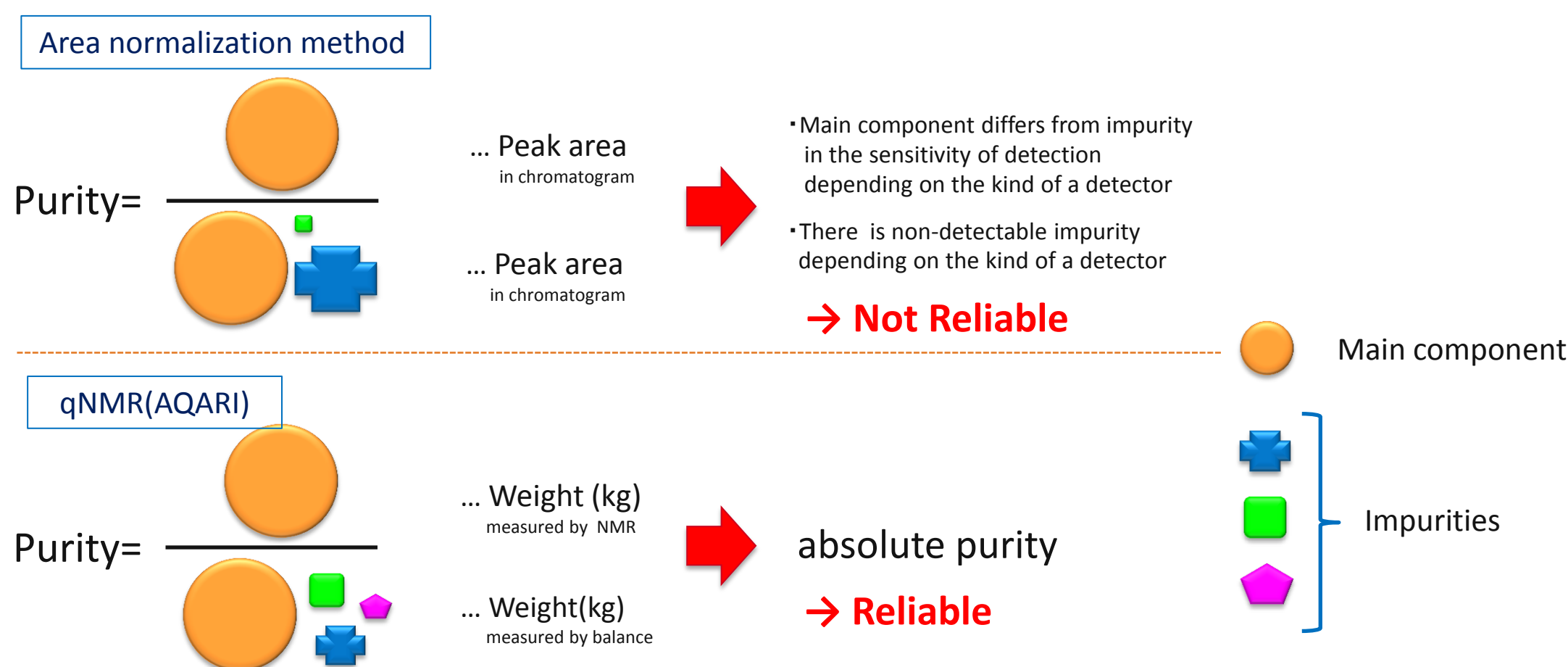
However, standard solution suitable for AQARI with concentration guaranteed is not currently being distributed.

For this reason, we have developed standard solutions for AQARI.

(1) Taichi Yamazaki, Takeshi Ohtsuki, Toru Miura, Takako Suematsu et al., Bunseikagaku, Vol. 63 (2014) No. 4 p. 323-329.

## Reliability

- Comparison of qNMR(AQARI) and Area Normalization Method by GC or HPLC

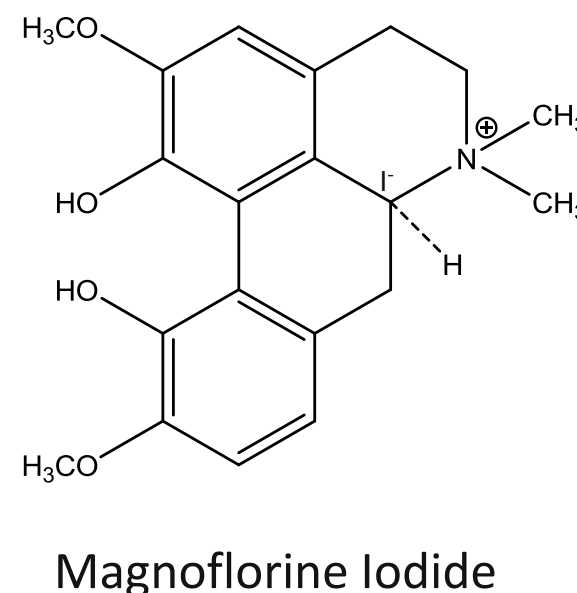
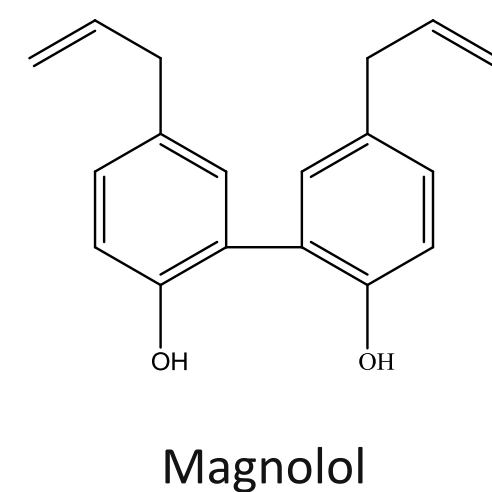
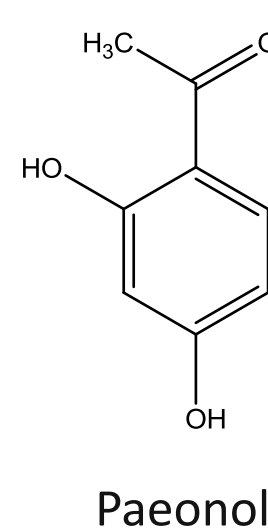
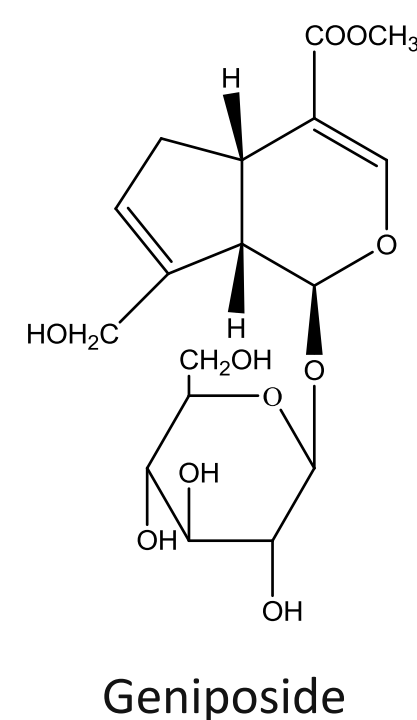


## Application to the Japanese Pharmacopoeia

AQARI has been adapted as a method for purity determination of reagents used for 4 standard reference materials for HPLC assay in the crude drug section of the Japanese Pharmacopoeia 16<sup>th</sup> supplement 2.



JP 16<sup>th</sup> supplement 2 was announced and enforced on Feb. 2014



Our facility(QC) was certified ISO17025 for qNMR analysis of these materials.

## Features of qNMR(AQARI)

### Reliability: absolute quantitation method with SI traceability

Moreover...

#### Simple method

#### Quickness

#### Efficiency

### Simple method

Determination of NMR measurement condition is almost not required.

### Quickness

Calibration curve for quantification is not required because of internal standard method.

### Efficiency

The reference substance that is the same as the analyte is not necessary.

## Development of Standard Solutions for qNMR(AQARI)

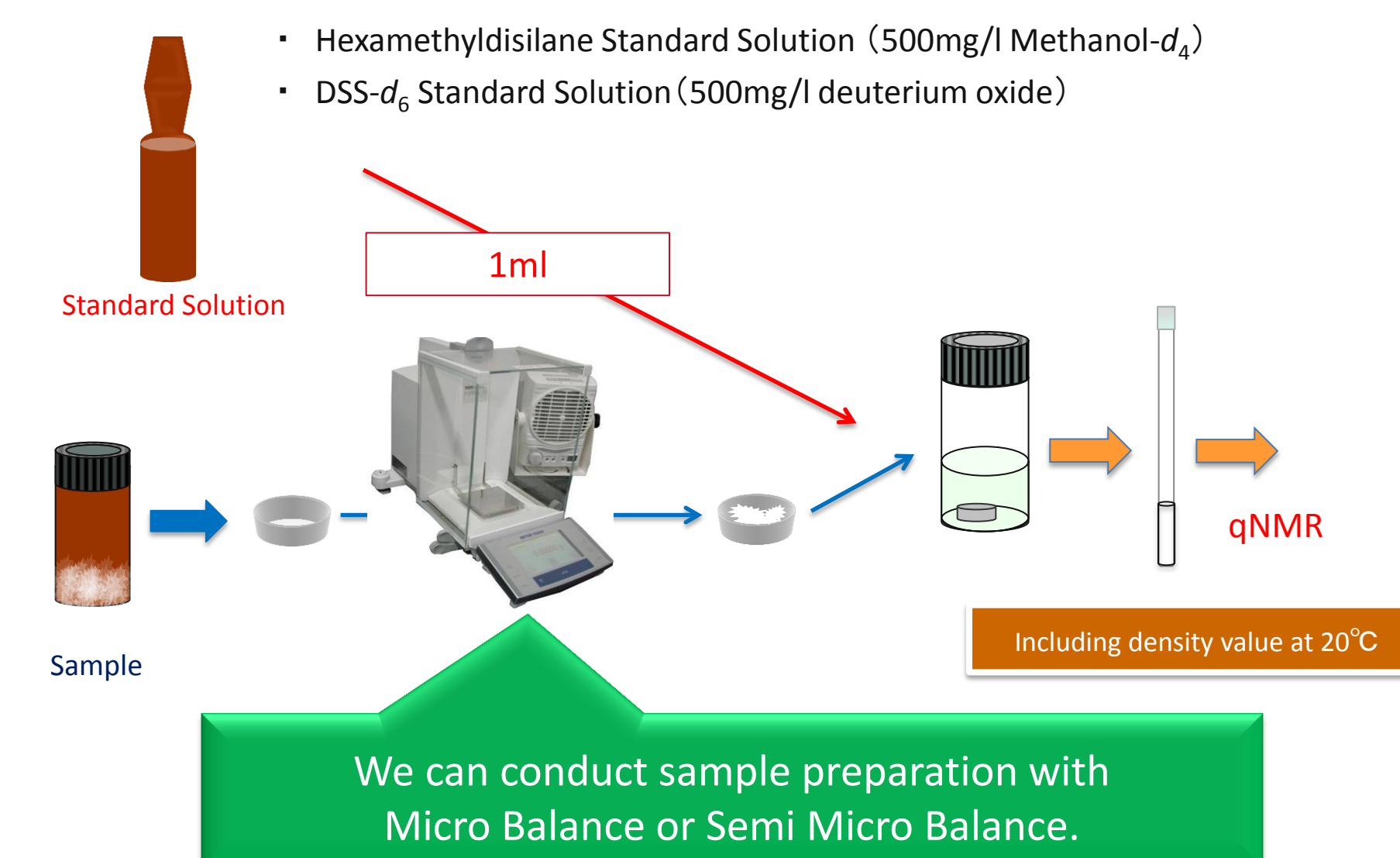
### Equation for purity calculation in qNMR(AQARI)

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

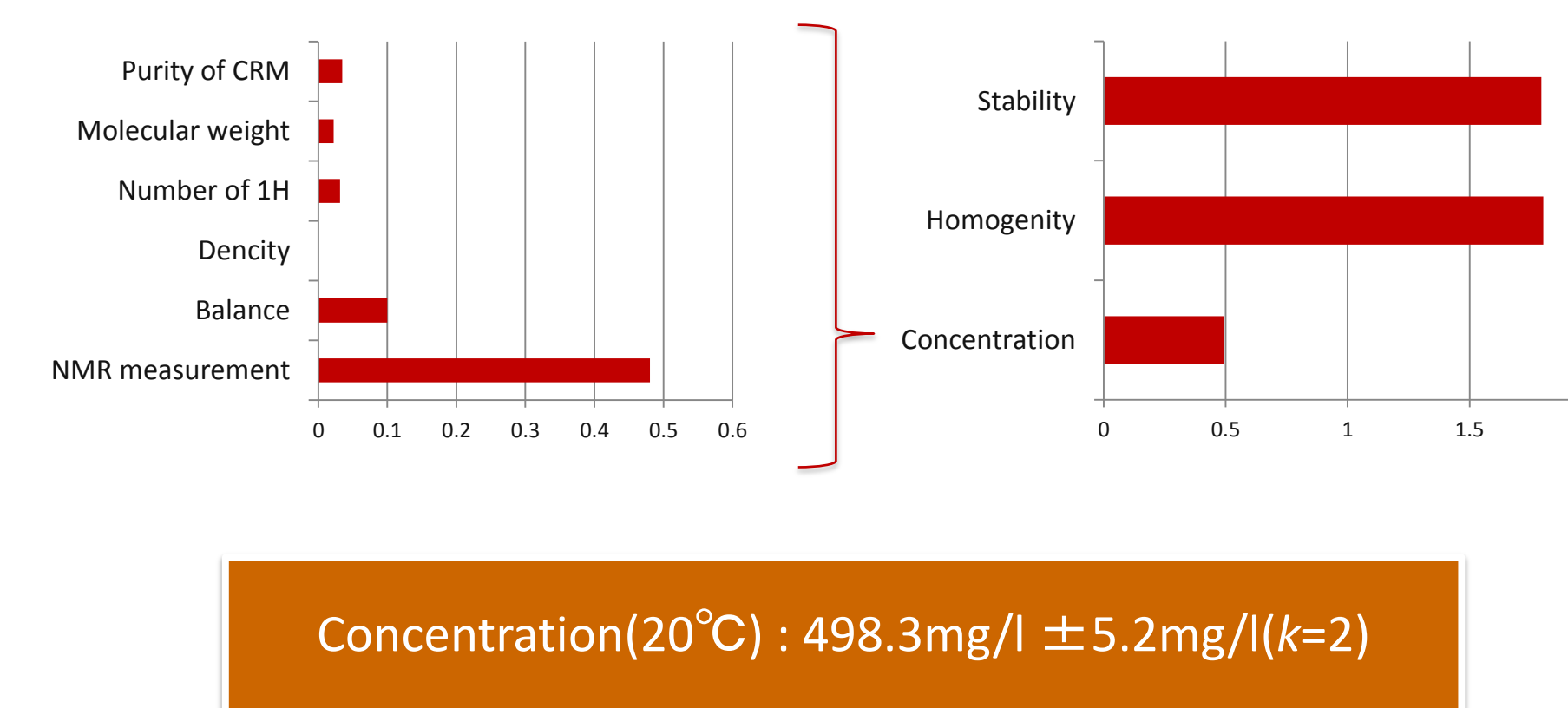
$I$  : Integral area  
 $H$  : Number of protons  
 $M$  : Molecular mass  
 $m$  : Weighing value  
 $P$  : Purity

Reliable Internal Standard (I.S.) suitable for AQARI is needed for AQARI analysis with high accuracy.

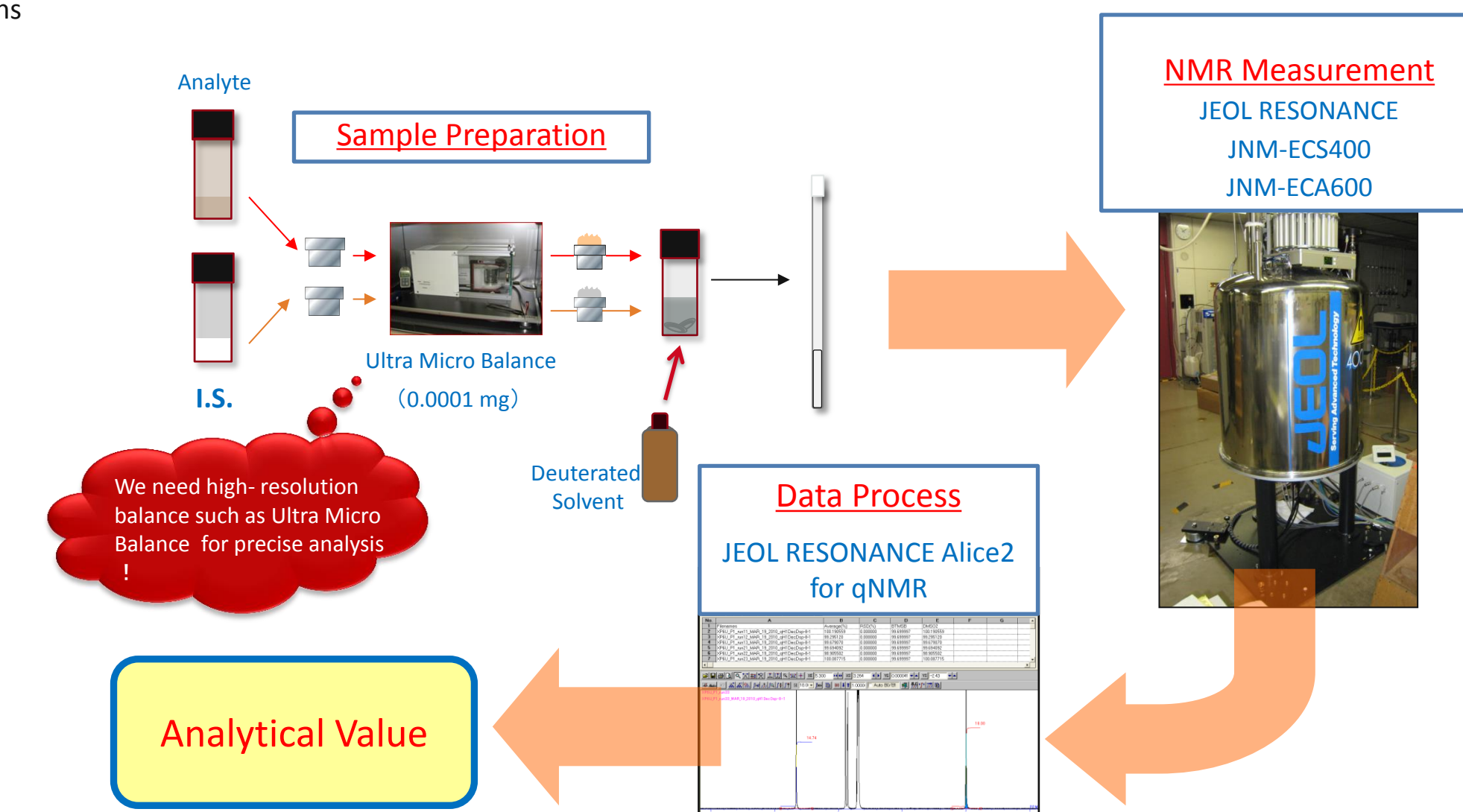
### New Experimental scheme of qNMR(AQARI) with Standard Solution



### Evaluation of uncertainty of DSS-d<sub>6</sub> Standard Solution



### Conventional Experimental scheme of qNMR(AQARI) with CRMs



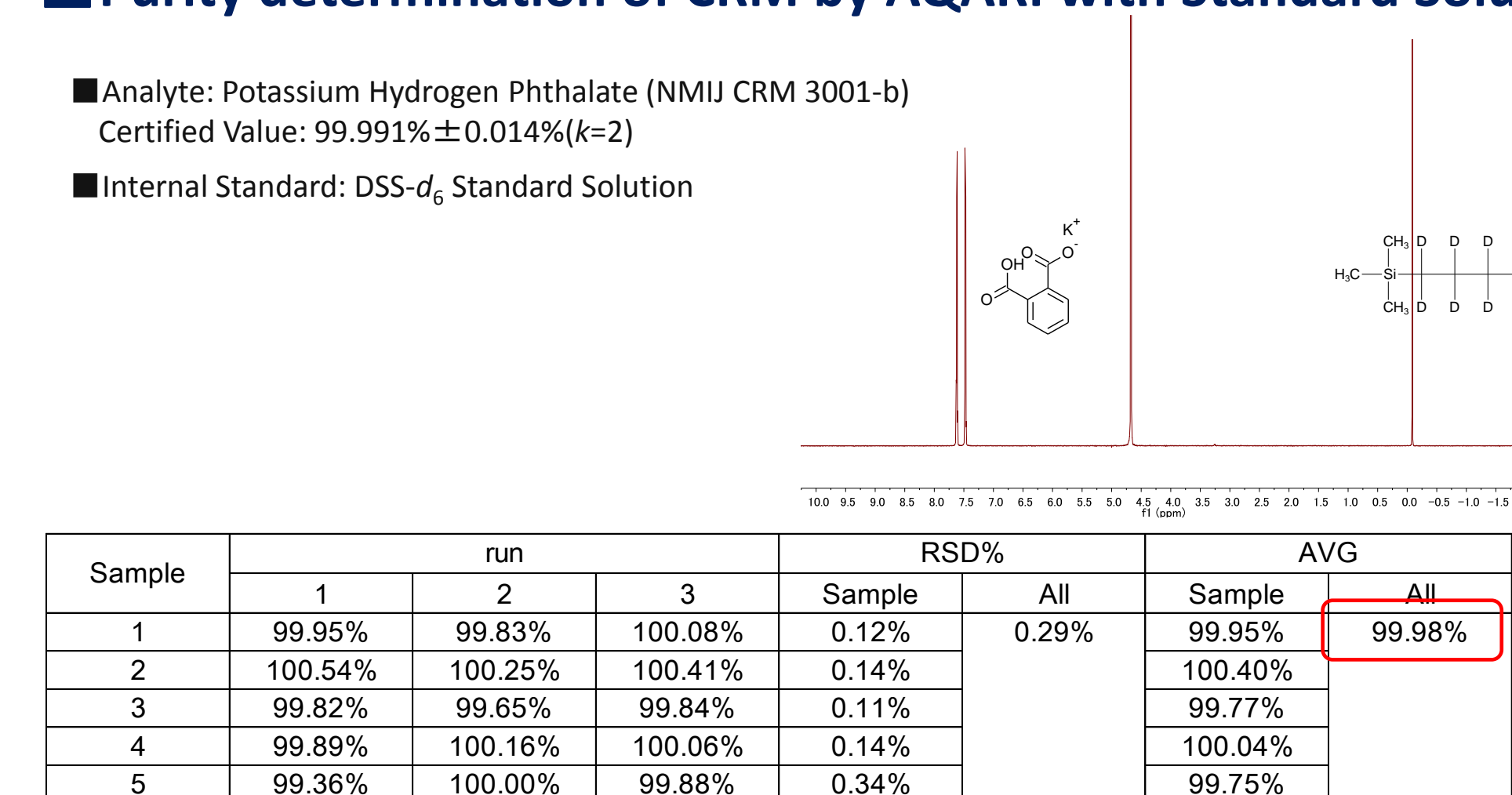
### Development of Standard Solution for qNMR(AQARI)

Wako Cat. No.	Product Name	Grade	Pkg. Size
041-33641	DSS-d <sub>6</sub> Standard Solution (500mg/L D <sub>2</sub> O)	for qNMR	1ml × 5A
085-10161	Hexamethyldisilane Standard Solution (500mg/l Methanol-d <sub>4</sub> Solution)	For qNMR	1ml × 5A

- Traceable to SI (through NMII : National Metrology Institute of Japan)
- Suitable for qNMR (AQARI) because of having singlet signal around 0 ppm

### Purity determination of CRM by AQARI with Standard Solution

- Analyte: Potassium Hydrogen Phthalate (NMIJ CRM 3001-b)  
Certified Value: 99.991%±0.014%(k=2)
- Internal Standard: DSS-d<sub>6</sub> Standard Solution



## Result

We have developed standard solutions suitable for AQARI named as DSS-d<sub>6</sub> Standard Solution (500mg/l Deuterium Oxide Solution) and Hexamethyldisilane Standard Solution (500 mg/l Methanol-d<sub>4</sub> Solution) with SI traceability through National Metrology Institute of Japan (NMIJ) and expanded uncertainty. Both the standard solutions are suitable for AQARI because of having singlet signal around 0 ppm.