Implementation of qNMR in the Japanese Pharmacopoeia

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qNMR description in the JP

JP	Addition and Change	
JP16 Suppl-1 (2012.10.1)	① Listed in **General information/G5 Crude Drugs**/ "Quantitative Analytical Technique Utilizing NMR Spectroscopy and Its Application to Reagents in the JP" ● 参考情報に「核磁気共鳴（NMR）法を利用した定量技術と日本薬局方試薬への応用」を収載	➢ Added the paragraph on "Qualification of NMR equipment used for quantitative NMR" in the JP17 (2016.4.1) ● JP17で「定量NMRに使用する機器の性能の管理」を追加
JP16 Suppl-2 (2014.2.28)	① Listed in **General tests/<5.01> Crude drugs tests/ "10. Assay of Marker Compounds for the Assay of Crude Drugs and Extracts of Kampo Formulations Utilizing NMR Spectroscopy"** ● 生薬試験法に「核磁気共鳴（NMR）法を利用した生薬及び漢方処方エキスの定量指標成分の定量」を収載 ③ Listed the **reagents for assay of crude drugs determined by qNMR in General tests/<9.41> Reagents, test solutions** as "xx for assay or xx for assay 2 (Purity value by quantitative NMR)" ● qNMRで値付けされた生薬定量用試薬を収載（定量用もしくは定量用2（qNMR純度規定）） Principle of qNMR, supply of reference materials and software, and definition of SI traceable marker compound for HPLC assay	➢ Added the paragraph on qNMR to **General tests/<2.21> NMR Spectroscopy** in JP17 ● JP17で一般試験法NMRで定量NMRに関する項を追加 Brief introduction of qNMR General tests/<2.21 5. Experimental techniques of ^{1}H and ^{13}C NMR spectroscopy unt
JP18 (2021.6.7)	④ Changed ② [<5.01> Crude drugs tests/10. Assay of Marker Compounds for the Assay of Crude Drugs and Extracts of Kampo Formulations Utilizing NMR Spectroscopy] ● 生薬試験法「核磁気共鳴（NMR）法を利用した生薬及び漢方処方エキスの定量指標成分の定量」改正 **⑤ Description of qNMR in the guideline for drafting the JP** ● 日局原案作成要領におけるqNMRの記載	➢ Changed "internal reference compound" to "reference standard for qNMR" in ② [<5.01>/10] and ③ <9.41> ● 「内部基準物質」を「qNMR用基準物質」に変更 ➢ Added SN ratio in NMR to ② [<5.01>/10] ● NMRにおけるSN比の追記

[Ref] Goda Y., PMDRS (Pharmaceutical and Medical Device Regulatory Science), 48, 670-682 (2017)

Reagents determined by qNMR used as reference standards in the assay of crude drug section in the JP

No.	JP	Reagents		生薬，漢方処方エキス Crude drugs and Kampo formula extracts
1	JP16-2 (2014.2.28)	ゲニポシド	Geniposide	サンシシ (Gardenia Fruit)，サンシシ末 (Powdered Gardenia Fruit)，黄連解毒湯エキス (Orengedokuto Extract)，加味逍遥散エキス (Kamishoyosan Extract)，加味帰脾湯エキス (Kamikihito Extract)
2	JP16-2 (2014.2.28)	ペオノール	Paeonol	ボタンピ (Moutan Bark)，ボタンピ末 (Powdered Moutan Bark)
3	JP16-2 (2014.2.28)	マグノロール	Magnolol	コウボク (Magnolia Bark)，コウボク末 (Powdered Magnolia Bark)，半夏厚朴湯エキス (Hangekobokuto Extract)
4	JP16-2 (2014.2.28)	マグノフロリン	Magnoflorine	葛根湯加川芎辛夷エキス (Kakkontokasenkyushin'i Extract)
5	JP17 (2016.4.1)	レイン	Rhein	桃核承気湯エキス (Tokakujokito Extract)，乙字湯エキス (Otsujito Extract)
6	JP17 (2016.4.1)	ロスマリン酸	Rosmarinic acid	半夏厚朴湯エキス (Hangekobokuto Extract)
7	JP17 (2016.4.1)	サイコサポニンb2	Saikosaponin b2	加味帰脾湯エキス (Kamikihito Extract)，抑肝散エキス(Yokukansan Extract)，柴胡桂枝湯エキス (Saikokeishito Extract)，柴苓湯エキス (Saireito Extract)，柴朴湯エキス (Saibokuto Extract)，小柴胡湯エキス (Shosaikoto Extract)，大柴胡湯エキス (Daisaikoto Extract)，補中益気湯エキス (Hochuekkito Extract)，乙字湯エキス (Otsujito Extract)，
8	JP17 (2016.4.1)	*(E)*-ケイ皮酸	(*E*)-Cinnamic acid	桃核承気湯エキス (Tokakujokito Extract)，桂枝茯苓丸エキス (Keishibukuryogan Extract)，苓桂朮甘湯エキス (Ryokeijutsukanto Extract)，五苓散エキス (Goreisan Extract)
9	JP17-1 (2017.12.1)	[6]-ギンゲロール	[6]-Gingerol	ショウキョウ (Ginger)，ショウキョウ末 (Powdered Ginger)，真武湯エキス (Shimbuto Extract)，半夏厚朴湯エキス (Hangekobokuto Extract)
10	JP17-1 (2017.12.1)	ロガニン	Loganin	サンシュユ (Cornus Fruit)，八味地黄丸エキス (Hachimijiogan Extract)，牛車腎気丸エキス (Goshajinkigan Extract)
11	JP17-1 (2017.12.1)	[6]-ショウガオール	[6]-Shogaol	カンキョウ (Processed Ginger)
12	JP17-2 (2019.6.28)	*(E)*-フェルラ酸	(*E*)-Ferulic acid	当帰芍薬散エキス (Tokishakuyakusan Extract)
13	JP17-2 (2019.6.28)	10-ヒドロキシデセン酸	10-Hydroxy-2-(*E*)-decenoic acid	ローヤルゼリー (Royal Jelly)
14	JP17-2 (2019.6.28)	シノメニン	Sinomenine	防已黄耆湯エキス (Boiogito Extract)
15	JP17-2 (2019.6.28)	エボジアミン	Evodiamine	呉茱萸湯エキス (Goshuyuto Extract)
16	JP18 (2021.6.7)	マンギフェリン	Mangiferin	白虎加人参湯エキス (Byakkokaninjinto Extract)
17	JP18 (2021.6.7)	サイコサポニンa	Saikosaponin a	サイコ (Bupleurum Root)
18	JP18 (2021.6.7)	サイコサポニンd	Saikosaponin d	サイコ (Bupleurum Root)
19	JP18 (2021.6.7)	ジフェニルスルホン	Diphenyl sulfone	ソヨウ (Perilla Herb)（ペリルアルデヒド：RMS基準物質（内部基準物質）Internal reference compound of perillaldehyde

19 reagents evaluated by utilizing qNMR are listed as reference standards in the assay of **37 monographs** (crude drugs and Kampo formula extracts)

Geniposide for assay (General Tests /<9.41> Reagents, Test Solutions, JP18)

Geniposide for assay $C_{17}H_{24}O_{10}$ Use geniposide for thin-layer chromatography meeting the following additional specifications. Correct the content based on the amount (%) obtained in the assay.

Unity of peak—Dissolve 5 mg of geniposide for assay 2 in 50 mL of diluted methanol (1 in 2). To 1 mL of this solution add diluted methanol (1 in 2) to make 100 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and compare the absorption spectra of at least 3 points including the top of geniposide peak and around the two middle peak heights of before and after the top: no difference in form is observed between their spectra.

Operating conditions

Column, column temperature, mobile phase, and flow rate: P operating conditions in the Assay under Gardenia Fruit.

Detector: A photodiode array detector (wavelength: 240 spectrum: 220 – 400 nm).

System suitability

System performance: Proceed as directed in the system suitability in the Assay under Gardenia Fruit.

Solvent
Reference
Target signals

Assay—Weigh accurately 10 mg of geniposide for assay 2 and 1 mg of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy using an ultramicrobalance, dissolve in 1 mL of deuterated methanol for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Transfer the sample solution into an NMR tube 5 mm in outer diameter, measure ^{1}H-NMR as directed under Nuclear Magnetic Resonance Spectroscopy <*2.21*> and Crude Drugs Test <*5.01*> according to the following conditions, using 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as the reference standard for qNMR. Calculate the resonance intensities, A_1 (equivalent to 1 hydrogen) and A_2 (equivalent to 1 hydrogen), of the signals around δ 3.93 ppm and δ 4.06 ppm assuming the signal of the reference standard for qNMR as δ 0 ppm.

Amount (%) of geniposide ($C_{17}H_{24}O_{10}$)
$= M_S \times I \times P/(M \times N) \times 1.7147$

M: Amount (mg) of geniposide for assay taken
M_S: Amount (mg) of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy taken
I: Sum of the signal resonance intensities, A_1 and A_2, based on the signal resonance intensity of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy as 18.000
N: Sum of number of the hydrogen derived from A_1 and A_2
P: Purity (%) of 1,4-BTMSB-d_4 for nuclear magnetic resonance spectroscopy

Operating conditions

Apparatus: An apparatus of nuclear magnetic resonance spectrum measurement with ^{1}H resonance frequency of not less than 400 MHz.
Target nucleus: ^{1}H.
Digital resolution: 0.25 Hz or lower.
Measuring spectrum range: 20 ppm or upper, including between −5 ppm and 15 ppm.
Spinning: off.
Pulse angle: 90°.
^{13}C decoupling: on.
Delay time: Repeating pulse waiting time not less than 60 seconds.
Integrating times: 8 or more times.
Dummy scanning: 2 or more times.
Measuring temperature: A constant temperature betwe

Required detectivity

System suitability

Test for required detectability: When the procedure is conducted with the sample solution under the above-mentioned operating conditions, the S/N of the two signals around δ 3.93 ppm and δ 4.06 ppm is not less than 100.

Specificity

System performance: When the procedu the sample solution under the above-mentioned operating conditions, the two signals around δ 3.93 ppm and δ 4.06 ppm are not overlapped with any signal of obvious foreign substance, and the ratios of the resonance intensities, A_1/A_2, of each signal around δ 3.93 ppm and δ 4.06 ppm are between 0.99 and 1.01, respectively.

Repeatability

System repeatability: When the test is re ample solution under the above-mentioned operating conditions, the relative standard deviation of the ratio of the resonance intensity, A_1 or A_2, to that of the reference standard for qNMR is not more than 1.0%.

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Hygroscopicity of the standard products used for the crude drug test in the JP

Substantial weight change due to humidity change →High hygroscopicity

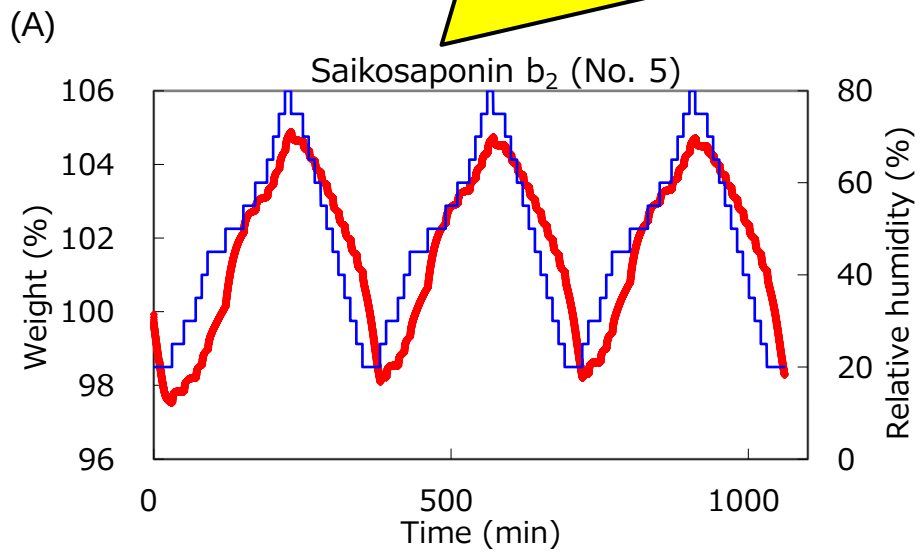
Table 1.

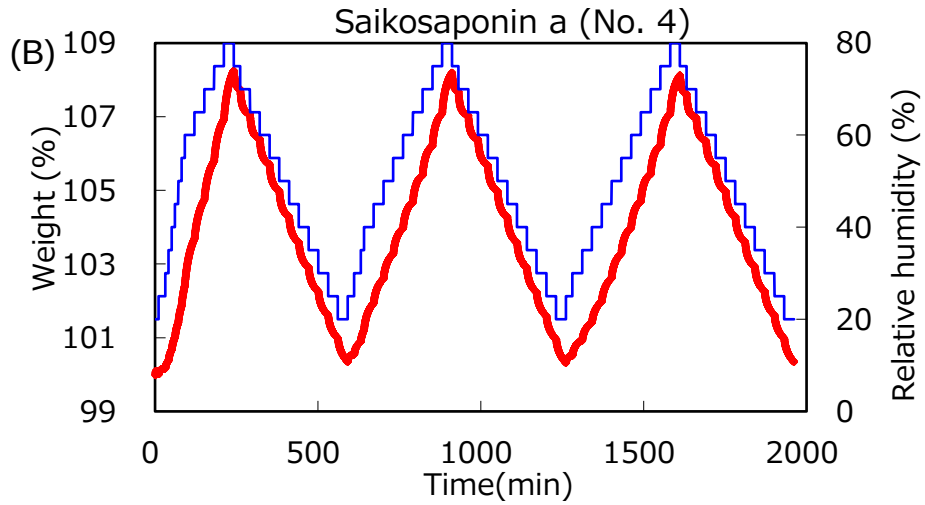
No.	Reagent	Code. No.	Lot. No.	TGA condition		Weight change Max(%)-Min(%)
				Temperature (℃)	Relative humidity (%)	
1	Ginsenoside Rb_1	072-06581	APG0894	25 ℃	20%→80%→20%×3	15.163
2	Ginsenoside Rg_1	076-05021	WEP7884	25 ℃	20%→80%→20%×3	9.350
3	Saikosaponin d	199-16311	TLL2003	25 ℃	20%→80%→20%×3	8.838
4	Saikosaponin a	192-16281	TLP6646	25 ℃	20%→80%→20%×3	8.272
5	Saikosaponin b_2	199-16171	TLJ5221	25 ℃	20%→80%→20%×3	7.358
6	Resibufogenin	185-02681	TLP5517	25 ℃	20%→80%→20%×3	6.600
7	Barbaloin	022-16971	TLL2557	25 ℃	20%→80%→20%×3	6.563
8	Bufalin	029-16981	TLP5516	25 ℃	20%→80%→20%×3	5.606
9	Rosmarinic acid	181-02661	TLM1354	25 ℃	20%→80%→20%×3	3.903
10	Hirsutine	085-09291	DCF1259	25 ℃	20%→80%→20%×3	1.729
11	Amygdalin	017-23571	DCL1634	25 ℃	20%→80%→20%×3	0.910
12	Hesperidin	085-09311	DCF1593	25 ℃	20%→80%→20%×3	0.686
13	(*E*)-Capsaicin	034-21121	WEJ5854	25 ℃	20%→80%→20%×3	0.168
14	Arbutin	019-23771	DCF1260	25 ℃	20%→80%→20%×3	0.114
15	(*E*)-Cinnamic acid	037-22211	DCF1261	25 ℃	20%→80%→20%×3	0.096
16	Dehydrocorydaline nitrate	045-31841	TLN6757	25 ℃	20%→80%→20%×3	0.095
17	Cinobufagin	037-21971	TLP5515	25 ℃	20%→80%→20%×3	0.078
18	Magnolol	130-16781	TLJ4901	25 ℃	20%→80%→20%×3	0.078
19	Rhynchophylline	188-02671	DCG6361	25 ℃	20%→80%→20%×3	0.073
20	10-Hydroxy-2-(*E*)-decenoic acid	081-09271	TLL2557	25 ℃	20%→80%→20%×3	0.069
21	Loganin	128-05931	TLP5514	25 ℃	20%→80%→20%×3	0.040

[Ref] Uchiyama N. *et al.,* YAKUGAKU ZASSHI, 140, 1063-1069 (2020)

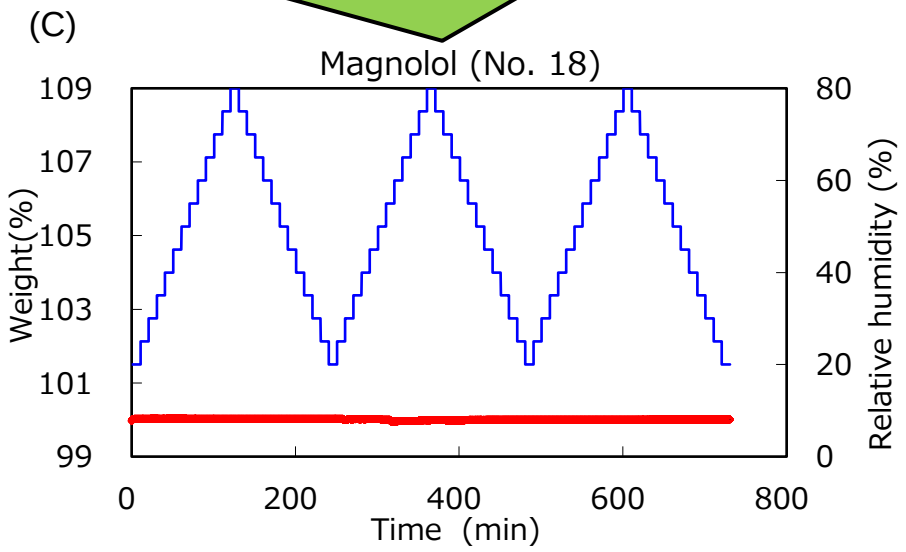
Water sorption-desorption analysis of the standard products used for the crude drug test in the JP

Substantial weight change due to humidity change
→High hygroscopicity





Less remarkable weight change due to humidity change
→Low hygroscopicity



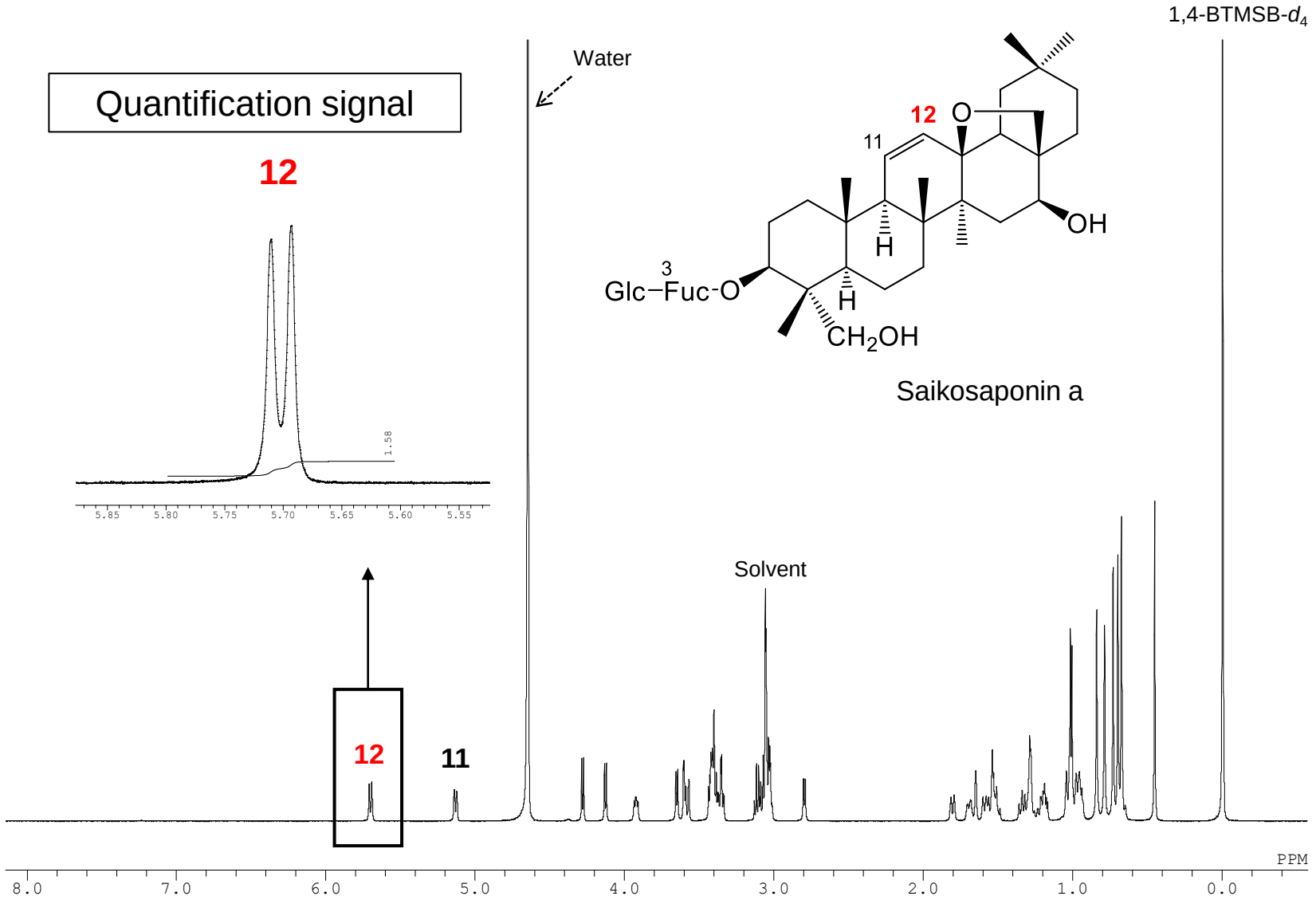
Red line: weight (%); blue line: relative humidity (%)

The hygroscopicity of reagents affects the purity of compounds, based on the extent of increase in their water content.
→The optimal and reproducible sample preparation methods for qNMR of hygroscopic reagents were studied.

[Ref] Uchiyama N. *et al.,* YAKUGAKU ZASSHI, 140, 1063-1069 (2020)

【Hygroscopic reagent】 Saikosaponin a (SSA)

^{1}H-qNMR spectrum of SSA in methanol-d_4）



[Ref] Uchiyama N. *et al.,* Chem. Pharm. Bull, 69(1), 26-31 (2021)

【Hygroscopic reagent】 Saikosaponin a (SSA)

Table 1. Purity (%) of saikosaponin a, prepared under **non-controlled humidity** conditions in three independent laboratories

Laboratory	A	B	C	Average of three labs
Average (%)	86.37	90.18	91.81	**89.45**
SD (%)	0.13	0.20	0.13	**2.79**

Non-controlled humidity conditions

High variation in purity

Table 2. Purity (%) of saikosaponin a, prepared **after controlling humidity** conditions across three independent laboratories

Laboratory	A	B	E	Average of three labs
Average (%)	89.29	89.36	89.20	**89.28**
SD (%)	0.20	0.10	0.16	**0.076**

After controlling humidity conditions

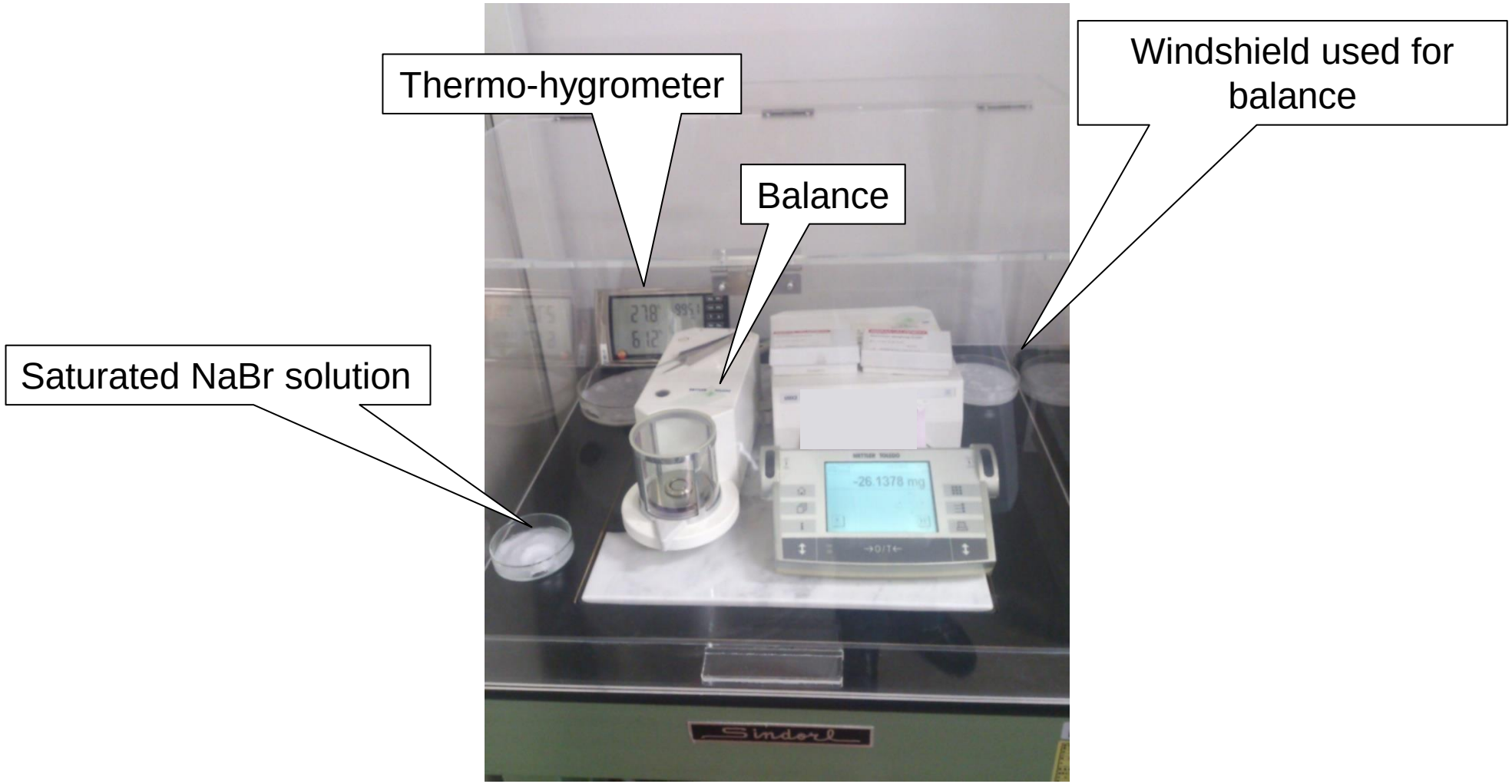
Low variation in purity

飽和NaBr水溶液にて調湿（温度20 ℃, 湿度60%, 4時間）後, 秤量も同条件で実施.
Weighing of the reagent after 4 h of humidity adjustment (at approximately 60% humidity) using a saturated NaBr solution at 22–23 ℃

[Ref] Uchiyama N. *et al.*, Chem. Pharm. Bull, 69(1), 26-31 (2021)

Humidity control method

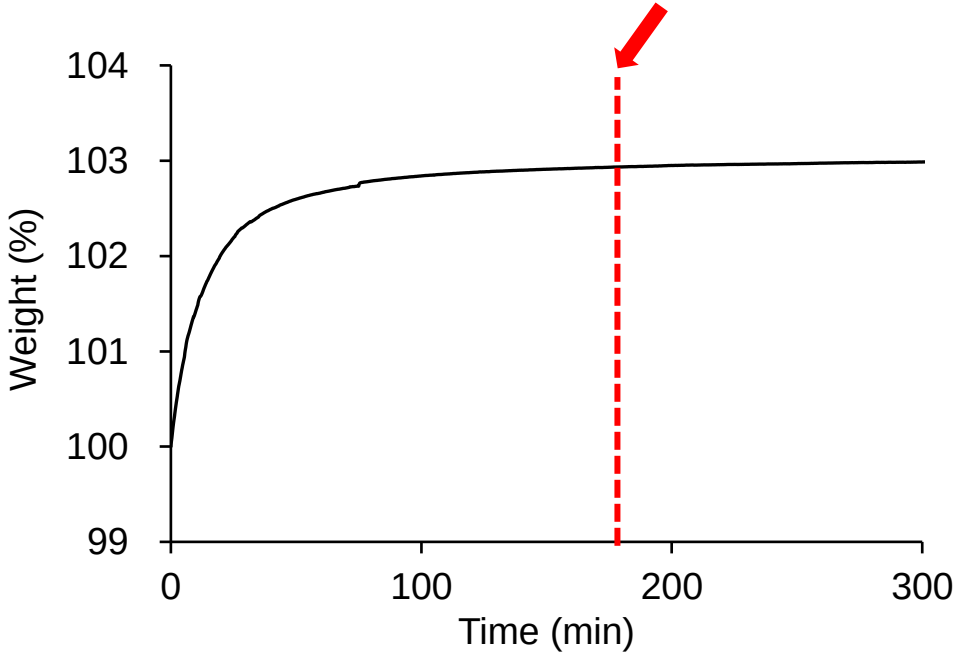
(1) Humidity control using a saturated NaBr solution



【Hygroscopic reagent】 Saikosaponin a (SSA)

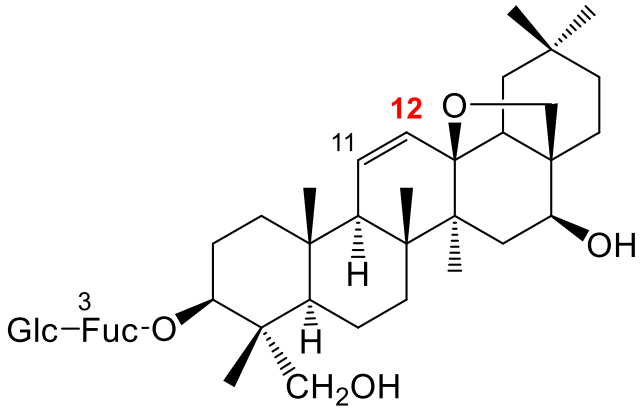
Examination of equilibration time of weight of SSA a at 20℃/60%RH

The rate of weight change was less than 0.1% per h after 3 h (180 min).



サイコサポニンaの絶対純度を測定するには，温度20℃，湿度60%の条件下で3時間以上調湿後，秤量も同湿度条件で行うことが適当と考えられた.

The humidity control conditions for SSA were set at 3 h or more at 20℃ and 60% humidity.



Saikosaponin a

[Ref] Uchiyama N. *et al.,* Chem. Pharm. Bull, 69(1), 26-31 (2021)

【Hygroscopic reagent】 Saikosaponin a (SSA)

Table 1. Purity (%) of saikosaponin a, prepared under **non-controlled humidity** conditions in three independent laboratories

Laboratory	A	B	C	Average of three labs
Average (%)	86.37	90.18	91.81	**89.45**
SD (%)	0.13	0.20	0.13	**2.79**

Non-controlled humidity conditions

High variation in purity

Table 2. Purity (%) of saikosaponin a, prepared **after controlling humidity** conditions across three independent laboratories

Laboratory	A	B	E	Average of three labs
Average (%)	89.29	89.36	89.20	**89.28**
SD (%)	0.20	0.10	0.16	**0.076**

After controlling humidity conditions

Low variation in purity

飽和NaBr水溶液にて調湿（温度20℃，湿度60%，4時間）後，秤量も同条件で実施.

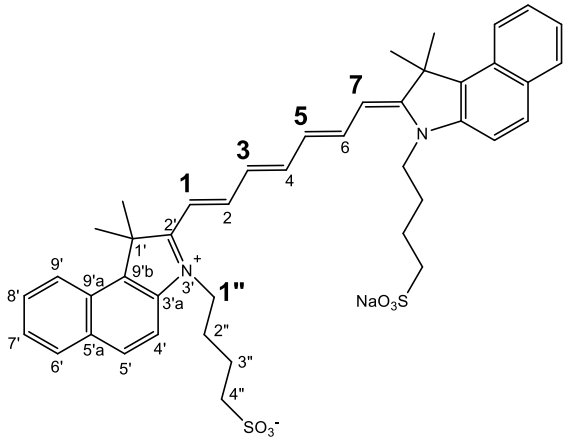
Weighing of the reagent after 4 h of humidity adjustment (at approximately 60% humidity) using a saturated NaBr solution at 20℃

[Ref] Uchiyama N. *et al.,* Chem. Pharm. Bull, 69(1), 26-31 (2021)

【Hygroscopic reagent】 Indocyanine green (ICG)

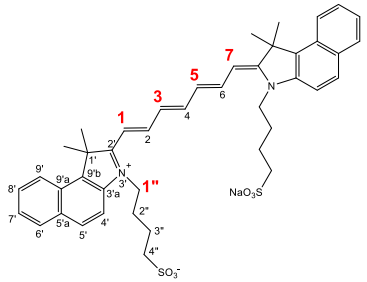
Indocyanine green (ICG), a fluorescence angiography agent listed in the Japanese Pharmaceutical Codex 2002 (JPC 2002)

- Recently, we studied the adaptation of indocyanine green (ICG), a fluorescence angiography agent listed in the Japanese Pharmaceutical Codex 2002 (JPC 2002), into the JP.
- **ICG is a hygroscopic substance**, and its official reference standard can be purchased from the Pharmaceutical and Medical Device Regulatory Science Society of Japan (PMRJ) for use as a non-pharmacopoeial reference standard (non-PRS).
- **The NMR spectrum of non-PRS ICG,** obtained **after storage in a drying chamber** at 105 ℃ for 2 h, revealed that a persistent presence of **residual ethanol** (data not shown). This suggested that it was difficult to use non-PRS ICG as a reference standard because its purity could not be calculated using the mass balance method. Therefore, we aimed to use qNMR to directly determine its absolute purity, which is necessary for its use as a HPLC reference standard in the JP.
- We performed **qNMR of ICG, with or without humidity control, using a saturated sodium bromide (NaBr) solution and a constant temperature and humidity box** and compared the results obtained.



Indocyanine Green (ICG)
$C_{43}H_{47}N_2NaO_6S_2$: 774.96

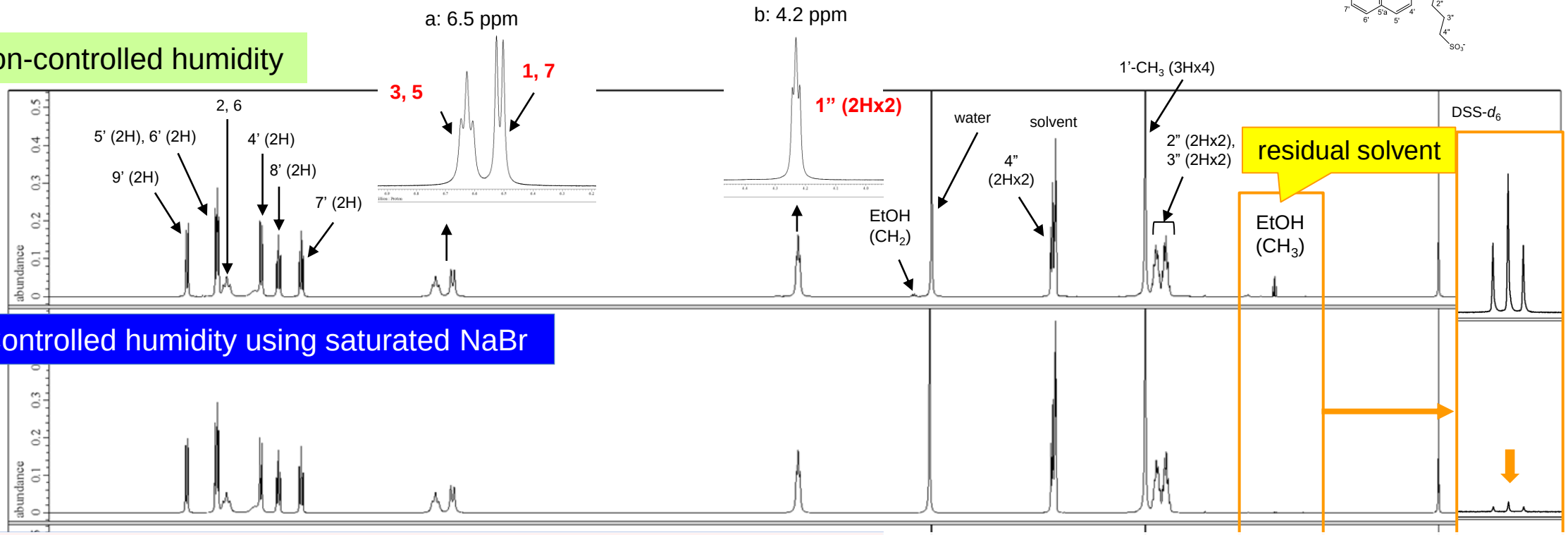
^{1}H-qNMR spectra of ICG in DMSO-d_6 under three different humidity conditions



(1) Non-controlled humidity

(2) Controlled humidity using saturated NaBr

(3) Controlled humidity using a constant temperature and humidity box



Condition	Purity of **ICG** average (%)	SD (%)	Labs		Purity of **ethanol** average (%)	SD (%)
(1) Under non-controlled humidity	**86.12**	**2.70**	4	×	**0.26**	**0.11**
(2) Controlled humidity (saturated NaBr)	**84.19**	**0.47**	5	△	**N.D.**	**N.D.**

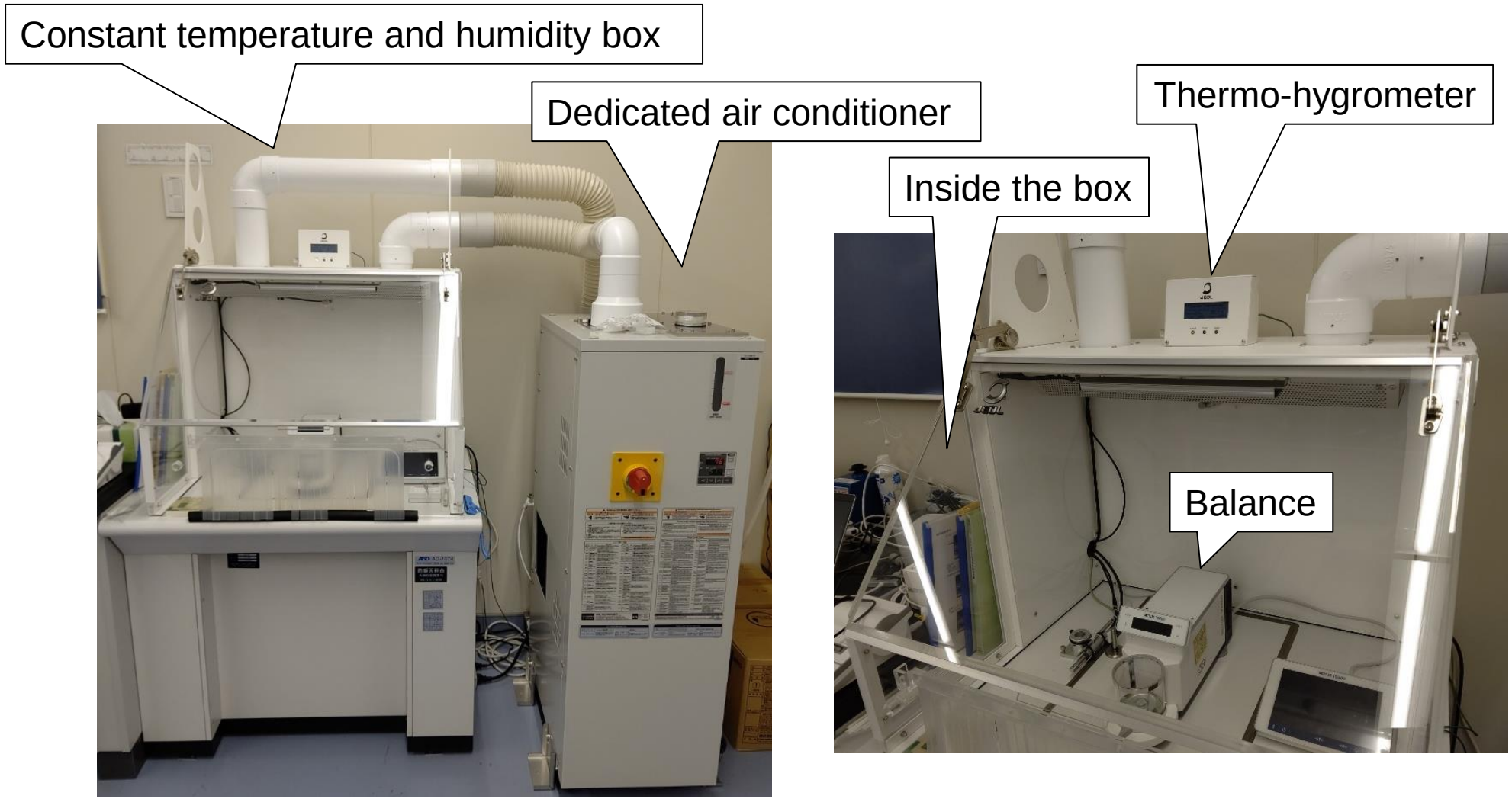
High variation in purity

Low variation in purity

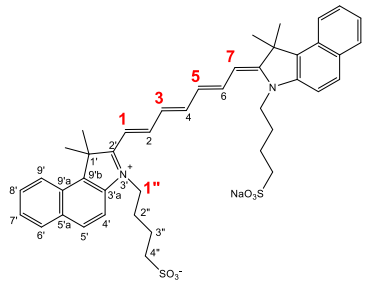
[Ref] Uchiyama N., et al., Chem. Pharm. Bull., 69(1), 118-123 (2021)

Humidity control method

(2) Humidity control in a constant temperature and humidity box



^{1}H-qNMR spectra of ICG in DMSO-d_6 under three different humidity conditions



(1) Non-controlled humidity

(2) Controlled humidity using saturated NaBr

(3) Controlled humidity using a constant temperature and humidity box

a: 6.5 ppm

b: 4.2 ppm

3, 5

1, 7

1" (2Hx2)

2, 6

5' (2H), 6' (2H)

4' (2H)

9' (2H)

8' (2H)

7' (2H)

1'-CH_3 (3Hx4)

water

solvent

4" (2Hx2)

2" (2Hx2), 3" (2Hx2)

EtOH (CH_2)

EtOH (CH_3)

DSS-d_6

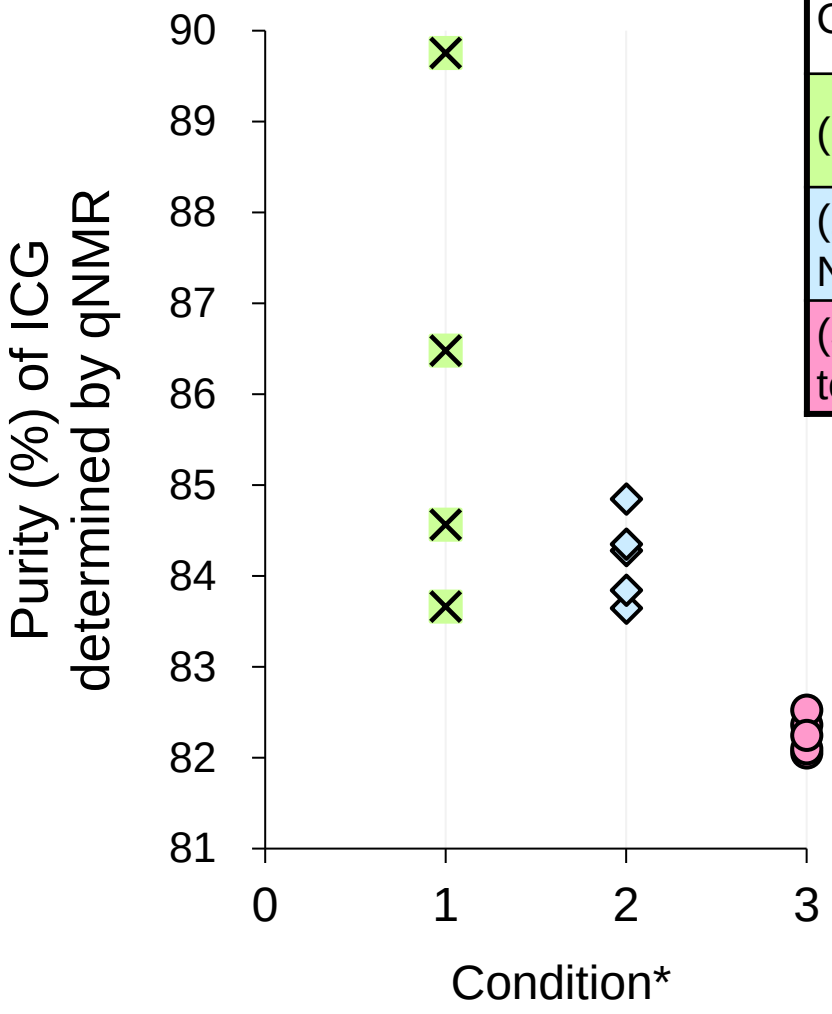
residual solvent

abundance

X : parts per Million : Proton

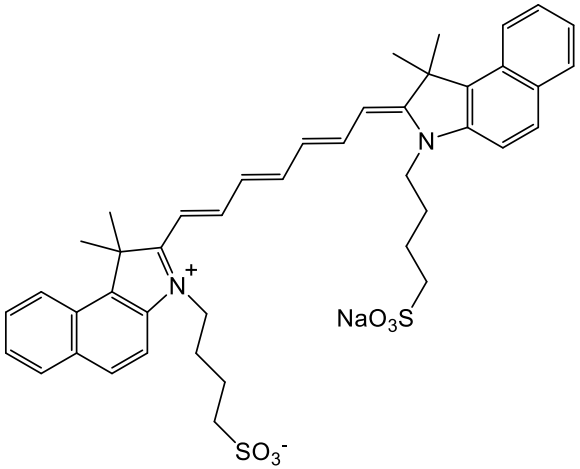
[Ref] Uchiyama N., et al., Chem. Pharm. Bull., 69(1), 118-123 (2021)

Comparison of absolute ICG purities under three different humidity conditions



Condition	Purity average (%)	SD (%)	Labs	
(1) Under non-controlled humidity	**86.12**	**2.70**	4	×
(2) Controlled humidity (saturated NaBr)	**84.19**	**0.47**	5	△
(3) Controlled humidity (a constant temperature and humidity box)	**82.26**	**0.19**	5	○

The lowest variability under controlled humidity in a constant temperature and humidity box.



Hygroscopic substance
Indocyanine green (ICG)

*1: Under non-controlled humidity conditions; 2: controlled humidity using a saturated NaBr solution; 3: controlled humidity in a constant temperature and humidity box

[Ref] Uchiyama N., et al., Chem. Pharm. Bull., 69(1), 118-123 (2021)

CONTENTS

INTRODUCTION

- Perillaldehyde (PRL) is an essential oil component derived from perilla (*Perilla frutescens* Britton), and is a characteristic compound of perilla herbs (蘇葉) listed in the JP17.

JP XVII
Crude Drugs and Related Drugs

Perilla Herb

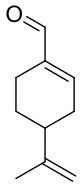
Perillae Herba

ソヨウ

Perilla Herb is the leaves and the tips of branches of *Perilla frutescens* Britton var. *crispa* W. Deane (*Labiatae*).

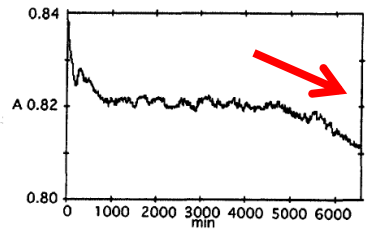
It contains not less than 0.08% of perillaldehyde, calculated on the basis of dried material.

Perilla Herb



Perillaldehyde (PRL)

- Quantitative analysis of PRL content in perilla herbs was performed by conducting HPLC, using an analytical standard of PRL. However, PRL undergoes rapid decomposition, especially in methanol.

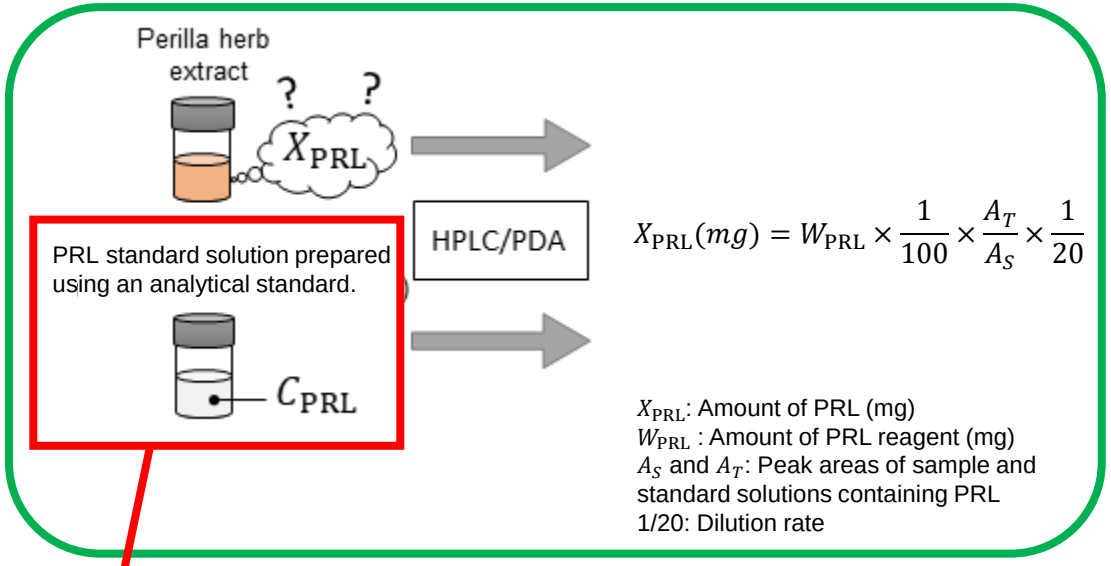


PRL decreased by 3.6% over a period of 4.5 days.

(UV 230 nm)
Fuchino et al. (2010) Japan. J. Pharmacog 64(1), 7-14

Time course of decrease in PRL content in methanol solution

Quantitative determination of PRL by the JP17 assay



The exact concentration of the analytical standard of PRL is unknown due to its easy decomposability.

- To overcome this problem, we adopted an alternative quantitative method using a single reference compound with **relative molar sensitivity (RMS)**.

[1] Nishizaki Y, et al., Food Hyg Saf Sci 2018; 59: 1-10. [2] Masumoto N, et al., J Nat Med 2019; 73, 566–576.

How to determine the relative molar sensitivity (RMS)

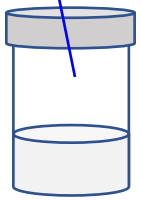
- Determine the ratio of the chromatography detector sensitivities to equal molar amounts of analyte and reference material

Analyte
(PRL)

Reference
(diphenyl sulfone: DPS)

[1]

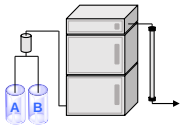
Weighing value



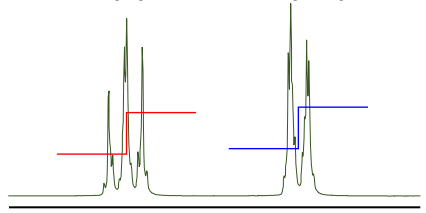
Mix analyte and reference in deuterium solvent

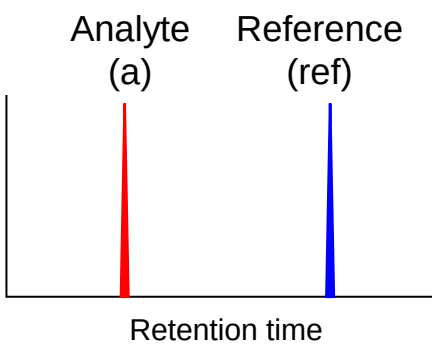
qNMR

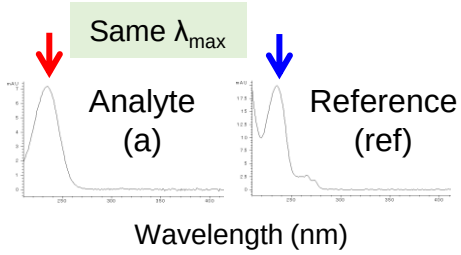
Dilution



HPLC/PDA







[2]

$$R_m = \frac{S_a}{S_{ref}} \times \frac{N_{ref}}{N_A}$$

S: signal area;
N: number of Hs;
A: peak area;
R: ratio

[3]

$$R_r = \frac{A_a}{A_{ref}}$$

Molar ratio

Response ratio

[4]

$$\boldsymbol{RMS} = \frac{R_r}{R_m}$$

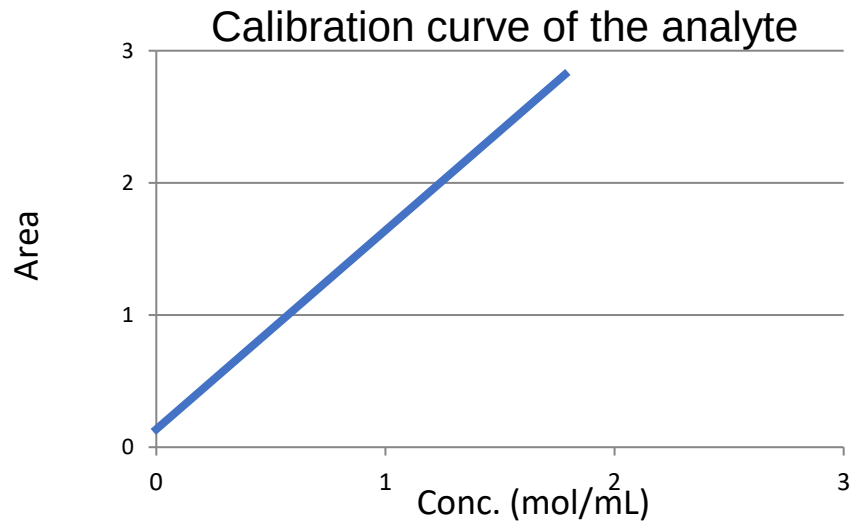
Expansion the use of RMS into regulated compounds and unstable compounds

Suitable candidate for the reference

1. Chemically stable
2. High purity
3. Low cost
4. Same absorption maximum (λ_{max}) as the analyte

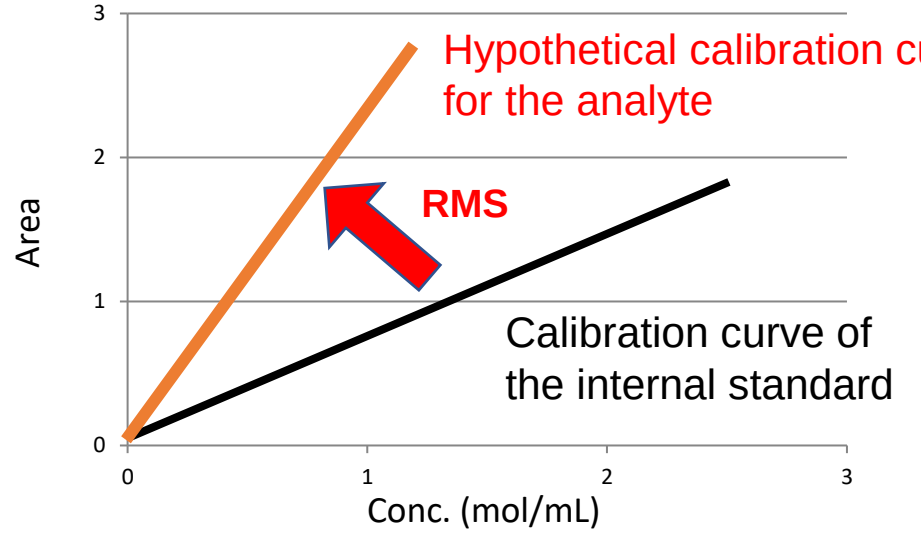
Differences between conventional chromatography and SR-RMS-chromatography

Conventional methods
Calibration curves using reference standards of the analytes



Plot an absolute calibration curve based on the data of the concentration and peak area, using the standard of the analyte, and measure the analyte concentration under the same conditions→**reference standard of the analytes for assay are required**

SR-RMS-chromatography
Calibration curve using RMS



Hypothetical calibration curves utilizing RMS to internal standards of known purity→ **reference standard of the analytes for assay are not required**

- When each compound demonstrates a proportional relationship with the amount of material and the peak area, the absolute calibration curve for the other can be deduced from the relationship between a calibration curve and the RMS.

Assay for PRL content in Perilla Herb in the JP18

[Ref] Masumoto N, et al., J Nat Med 73, 566–576 (2019)

1. Preparation of the sample and standard solutions

Analyte
Perilla Herb
(PRL)

ca. 0.2 g

Add 20 mL methanol
Shake for 10 min
Centrifuge

Supernatant | Residue

Add 20 mL methanol
Shake for 10 min
Centrifuge

Supernatant | Residue

Add methanol
50 mL
Sample solution*

Reference
(DPS)

10 mg

Weigh precisely,
Dissolve in
methanol 100 mL

100 mL
Standard solution

*according to the assay of Perilla Herb listed in the JP17

2. Quantitative determination of PRL using RMS

The amount of PRL (X_{PRL}, mg) is calculated using the following equation,

$$X_{PRL} = \frac{A_{PRL}}{A_{DPS}} \times \frac{m_{PRL}}{m_{DPS}} \times W_{DPS} \times \frac{1}{20} \times \frac{1}{RMS}$$

$$\frac{150.22\ (\text{MW of PRL})}{218.27\ (\text{MW of DFS})} \times \frac{1}{0.983\ (RMS)} = 0.700$$

$$X_{PRL} = \frac{A_{PRL}}{A_{DPS}} \times W_{DPS} \times \frac{1}{20} \times 0.700$$

Where *m* represents the molecular weight and W_{DPS} represents the amount of DPS used in the assay.

Detect : UV 234 nm; column : ODS (4.6 × 150 mm, 5 μm)
Solvent : water/acetonitrile =13/7; flow rate : 1.0 mL/min

Sample and standard solutions are analyzed by conducting HPLC, using the same conditions as those used for RMS.

CONTENTS

qNMR description in the guideline for drafting the JP18

「日本薬局方標準品品質標準」原案に関する資料

（様式−標2）

［標準品の名称］
標準品の構造式

［分子式及び分子量］
［化学名，CAS 番号］
性状：外観
確認試験
示性値
純度試験
乾燥減量または水分

定量法
（*通例，液体クロマトグラフィーによる試験法等に基づいたマスバランス法で純度評価を行い、純度の補正係数を求め、標準品の秤取量はこの補正係数を用いて補正する．そのため、滴定法や定量 NMR 測定法などの絶対定量法は必要に応じて記載することで差支えない．*）
マスバランス法での純度評価は原則以下のとおりとする．
（原則として、類縁物質、残留溶媒、強熱残分の混在量を控除項目とし、次式で求める．）
純度（乾燥物又は脱水物）（%）＝{100% −（強熱残分%＋残留溶媒%）} ×（100%−類縁物質%）／100

（備考）作成にあっては，書式の外枠を設定する必要はない
記載上の留意点
① 標準品原料
を記載する
② 試験方法に
と．
③ 試験方法の
指定しても
④ 標準品原料
造機関より問い合わせがあった場合に適切に対応すること．
⑤ 定量法に qNMR を記載する場合は、原案作成要領第一部「6.1 定量 ^{1}H NMR 測定法」の例を参考として，定量の実施状況について正確に記載すること．
⑥ 定量 1H NMR 測定法の記載に際しては、原案作成要領第一部の「6.2 定量 ^{1}H NMR 測定法の一般試験法「9.41 試薬・試液」の項、又は標準品品質標準の「様式−標2」への記載に際しての留意点」に基づき，各情報を別紙に記載して提出すること．

qNMRによる定量の記載方法は，こちらに示されている
The requesting information on qNMR description in the assay is shown here as points of consider for entry

Document concerning the draft “Quality Standards of the Japanese Pharmacopoeia Reference Standard”

(FormStd-2)

[Name of Reference standard]
Structure formula of Reference Standard

[Molecular formula and molecular mass]
[Chemical name, CAS registry number]
Description: appearance
Identification
Specific physical and/or chemical values
Purity
Loss on drying or Water

Assay

Usually, purity assessments are performed according to the Mass Balance Method based on the Liquid Chromatography method to calculate the correction factor for purity and to perform rectifications for the amount of the reference standard using the provided correction factor. Therefore, **absolute assays such as titration and quantitative NMR assays can be described as needed.**

qNMRによる定量法の記載も認められている
Usage of qNMR is permitted as an absolute assay

(Remarks) Points to consider for entry
(5) If a qNMR is included in the assay, the status of the assay should be accurately described, referring to the example of **"6.1 quantitative ^{1}H NMR measurement"** of the guideline for drafting the JP.
(6) When describing the detailed information on quantitative ^{1}H NMR, submit the data in a separate form based on **“6.2 Points to consider when describing the quantitative ^{1}H NMR measurement” in the section of "9.41 Reagents, Test Solutions" in General Tests, or "Form 2" in quality standards of quantitative reference materials”**, of the guideline for drafting the JP18

Description which should be consulted if a ^{1}H qNMR is included in the assay

6.1　定量 ^{1}H NMR 測定法	6.1 Quantitative ^{1}H NMR measurement
^{1}H NMRによる定量では、測定対象の化合物とSIトレーサブルな純度既知のqNMR用基準物質をそれぞれ精密に量り、重水素化溶媒に溶解した溶液で^{1}H NMR測定を行う. 得られたスペクトル上に観測される測定 対象の化合物とqNMR用基準物質に由来するピーク面積、プロトン数、調製質量及び分子量の関係から、定量値を算出する.	For the quantification by ^{1}H NMR, the target analyte and the SI traceable reference standard for qNMR of known purity are accurately weighed, and ^{1}H NMR is measured using a solution dissolved in deuterated solvent. The quantitative value is calculated based on the relation between the signal area, number of proton, weights and molecular weight derived from the target analyte and the reference standard for qNMR observed in spectrum.
[例] 定量法 ウルトラミクロ化学はかりを用い、本品A mg及びqNMR用基準物質yy B mgをそれぞれ精密に量り、核磁気共鳴スペクトル測定用重水素化zz C mLに溶かし、試料溶液とする. この液を外径5 mmのNMR試料管に入れ、核磁気共鳴スペクトル測定用yyを化学シフト基準物質として、次の試験条件で核磁気共鳴スペクトル測定法(〈2.21〉及び〈5.01〉)により、^{1}H NMRを測定する.	[Examples] Weigh accurately A mg of xx for assay and B mg of the reference standard for qNMR Y for nuclear magnetic resonance spectroscopy, using an ultramicrobalance. Dissolve in C mL of deuterated D for nuclear magnetic resonance spectroscopy and use this solution as the sample solution. Transfer the sample solution into an NMR tube (outer diameter: 5 mm), measure ^{1}H-NMR as per methods described under Nuclear Magnetic Resonance Spectroscopy <2.21> and Crude Drugs Test <5.01>, according to the following conditions, using yy for nuclear magnetic resonance spectroscopy as the reference for chemical shifts.
化学シフト基準物質のシグナルをδ 0 ppmとし、δ ○.○○ ppm及びδ △.△△ ppm付近のそれぞれのシグナルの面積強度A1 (水素 数●に相当)及びA2 (水素数■に相当)を算出する。	Calculate the resonance intensities, A1 (equivalent to O hydrogen) and A2 (equivalent to O hydrogen), of the signals around δ O.OO ppm and δ O.OO ppm assuming the signal of the reference standard for qNMR as δ 0 ppm.
計算式	Calculation
試験条件	**Operating conditions**
装置:1H共鳴周波数400 MHz以上の核磁気共鳴スペクトル測定装置	Apparatus: An apparatus of nuclear magnetic resonance spectrum measurement having 1H resonance frequency of not less than 400 MHz.
測定対象とする核:1H	Target nucleus : 1H
デジタル分解能:0.25 Hz以下 観測スペクトル幅:−5 ～ 15 ppmを含む20 ppm以上	Digital resolution: 0.25 Hz or lower Measuring spectrum range: 20 ppm or upper, including between -5 and 15 ppm
スピニング:オフ	Spinning: off
パルス角:90°	Pulse angle: 90°
^{13}C核デカップリング:あり	^{13}C decoupling: on
遅延時間:繰り返しパルス待ち時間60秒以上	Delay time: Repeating pulse waiting time not less than 60 seconds.
積算回数:8回以上	Integrating times: 8 or more times.
ダミースキャン:2回以上	Dummy scanning: 2 or more times.
測定温度:20 ～ 30℃の一定温度	Measuring temperature: A constant temperature between 20°C and 30°C.
システム適合性	System suitability

Sample solution preparation

Calculation

Operating conditions

Description which should be consulted when describing the detailed information on ^{1}H qNMR

6.2　定量 ^{1}H NMR 測定法の一般試験法「9.41　試薬・試液」の項、又は標準品品質標準の「様式−標2」への記載に際しての留意点	**6.2 Points to consider when describing the quantitative ^{1}H NMR measurement" in the section of "9.41 Reagents, Test Solutions" in General Tests, or "Form 2" in quality standards of quantitative reference materials"**
6.2.1　qNMR 試料溶液の調製方法	**6.2.1 qNMR sample solution preparation**
6.2.1.1　試料	6.2.1.1 Sample
6.2.1.1.1　測定対象物質(分析種)に関する情報	6.2.1.1.1 Information on substances to be analyzed (analyte)
6.2.1.1.2　qNMR 用基準物質の情報	6.2.1.1.2 Information on reference standard for qNMR
6.2.1.1.3　化学シフト基準物質(必要な場合)の情報	6.2.1.1.3 Information on reference standard for chemical shift (if necessary)
6.2.1.1.4　qNMR 測定溶媒の情報	6.2.1.1.4 Information on qNMR solvents
6.2.1.2　試料溶液の調製方法	6.2.1.2 Sample solution preparation
6.2.1.3　使用天秤情報	6.2.1.3 Balance information
6.2.1.4　秤量情報	6.2.1.4 Weighing information
実際の試料秤量時の温湿度情報、調湿した場合はその方法と温湿度	Temperature and humidity information when weighing the sample, and the method and temperature and humidity, if controlled
6.2.2　qNMR 測定	**6.2.2 qNMR measurement**
6.2.2.1　使用機器の適格性(qNMR 測定に関する適格性が確認されていること)	6.2.2.1 Suitability of the apparatus (qualified for qNMR measurement)
6.2.2.1.1　システム適合性試験要件(システムの再現性、システムの性能、検出の確認)	6.2.2.1.1 System suitability testing requirements (system repeatability, system performance, and detection confirmation)
6.2.2.2. qNMR 測定条件	6.2.2.2. qNMR measurement condition settings
6.2.2.2.1　測定核	6.2.2.2.1 Measured nucleus
6.2.2.2.2　磁場サイズ(実際の測定時の機器名)	6.2.2.2.2 Magnetic field size (instrument name for actual measurement)
6.2.2.2.3　デジタル分解能(実際の測定時の情報)	6.2.2.2.3 Digital resolution (information at actual measurement)
6.2.2.2.4　観測範囲(実際の測定時のスペクトル中心とスペクトル幅)	6.2.2.2.4 Spectral region (spectral center and spectral width at actual measurement)
6.2.2.2.5　スピニング情報(実際の測定時の情報)	6.2.2.2.5 Spinning information (information at actual measurement)
6.2.2.2.6　パルス角(実際の測定時の情報)	6.2.2.2.6 Pulse angle (information at actual measurement)
6.2.2.2.7　デカップリング情報(実際の測定時の情報、デカップリングパルスシークエンスとオフセット値も記載する)	6.2.2.2.7 Decoupling information (also describes the actual measurement information, decoupling pulse sequence and offset value)
6.2.2.2.8　遅延時間(実際の測定時の情報)	6.2.2.2.8 Delay time (information at actual measurement)
6.2.2.2.9　積算回数と SN 比(実際の測定時の情報)	6.2.2.2.9 Integrating times and SN ratio (information at actual measurement)
6.2.2.2.10　ダミースキャン回数(実際の測定時の情報)	6.2.2.2.10 Number of dummy scans (information at actual measurement)
6.2.2.2.11　測定温度(実際の測定時の情報)	6.2.2.2.11 Measurement temperature (information at actual measurement)
6.2.2.3　qNMR 解析条件	6.2.2.3 qNMR analysis condition settings
6.2.2.3.1　qNMR スペクトル	6.2.2.3.1 qNMR spectra
6.2.2.3.2　定量測定対象シグナル情報	6.2.2.3.2 Target signal information for quantification
そのシグナルを選択した理由、定量に用いた各シグナルの積分範囲(ppm 表示)を示す	Shows the reasons for selecting the signal and the integration range of each signal used for the quantification (expressed in ppm)
(溶媒を選択した理由を示す)	Shows the reasons for selecting the solvent
6.2.2.3.3　データ処理条件	6.2.2.3.3 Data processing conditions
6.2.2.3.4　計算式	6.2.2.3.4 Calculations
6.2.2.3.5　定量結果および精度情報	6.2.2.3.5 Quantitative results and precision information

CONTENTS

Applications of qNMR

- In certain cases, qNMR is performed for conducting assays on the following:
  (1) referential standard materials of API
  (2) reagent for impurity assay
  (3) reagent for system suitability testing

- Discussion points in the review are as follows:
  - The performance of testing method should be adequate for the usage of target material. Generally, higher precision and accuracy are expected for (1) compared with that for (2) or (3).
  - Parameter sets and testing conditions should be specified to assure performance of the testing method. These should be included in the Application Form.
  - The traceability of reference standards, evaluated by the National Institute, should be maintained.

Example of AF description-1

[Reagent for impurity assay]

Weigh accurately 10 mg of Impurity-X for assay and 1 mg of the reference standard for qNMR-Y. Dissolve in 0.5 mL of deuterated DMSO for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Measure ^{1}H-NMR spectrum as per methods described under Nuclear Magnetic Resonance Spectroscopy <2.21>, using TMS for nuclear magnetic resonance spectroscopy as the internal chemical shift reference compound, assuming the signal as δ 0 ppm. Calculate the resonance intensity Y (equivalent to 9 hydrogen) of the signal around δ 0.1 ppm and X (equivalent to 1 hydrogen) of the signal around δ 6.3 ppm.

Equation for calculation

Reference standard for qNMR Y: Comply with the JP General Tests (reagents and test solutions <9.41>).

-> Detailed evaluation process should be described in CTD Module 2.

Operating conditions:

Spectrometer: FT-NMR, not less than 400 MHz.

Conditions should be described in a similar manner to those described for the JP General Tests (reagents and test solutions <9.41>).

Example of AF description-2

[Referential standard materials (RSM) of API]

Weigh accurately 10 mg of RSM-X for assay and 1 mg of the reference standard for qNMR Y, dissolve in 0.5 mL of deuterated methanol for nuclear magnetic resonance spectroscopy, and use this solution as the sample solution. Measure ^{1}H-NMR spectrum as per methods described under Nuclear Magnetic Resonance Spectroscopy <2.21>, using TMS for nuclear magnetic resonance spectroscopy as the internal chemical shift reference compound, assuming the signal as δ 0 ppm. Calculate the resonance intensity Y (equivalent to 9 hydrogens) of the singlet signal around δ 0.1 ppm and X (equivalent to 1 hydrogen) of the singlet signal around δ 6.3 ppm.

-> Rationale for selecting the testing conditions, such as solvent, target signal, and so on, should be described in CTD Module 2.

Equation for calculation

Reference standard for qNMR Y:

Comply with the JP General Tests (reagents and test solutions <9.41>). Purity of Y is evaluated by NIST reference standard Z.

-> Detailed evaluation process should be described in CTD Module 2.

Purity of deuterated methanol for nuclear magnetic resonance spectroscopy: not less than XX%

Operating conditions:

Spectrometer: FT-NMR, not less than 400 MHz.

Conditions should be described in a similar manner to those described for the JP General Tests (reagents and test solutions <9.41>)

CONTENTS

Comparison of the descriptions related to qNMR between the JP, USP, and EP

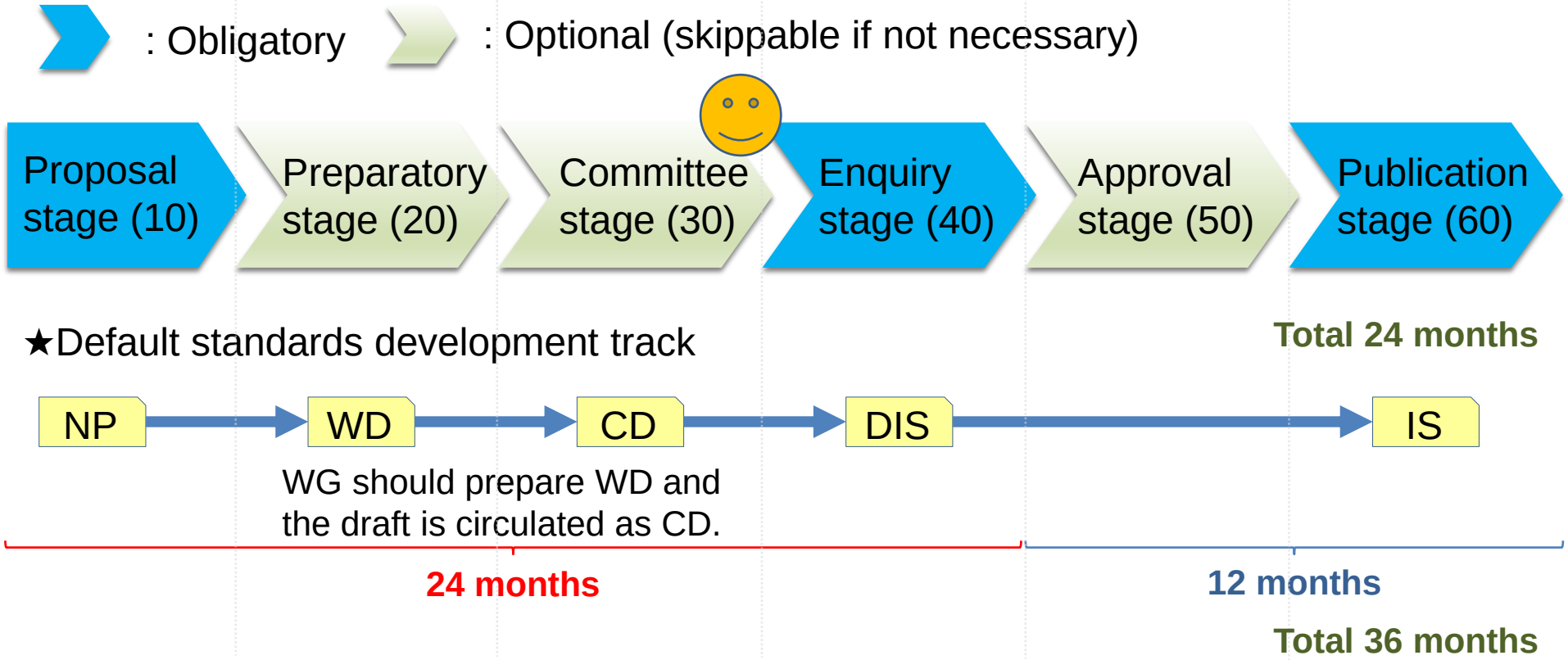
	JP18	USP43	EP10
NMR and qNMR	**General Tests**/ Spectroscopic Methods/ **<2.21> Nuclear Magnetic Resonance Spectroscopy** 5. Experimental techniques of ^{1}H and ^{13}C NMR spectroscopy **<5.01> Crude Drugs Test** 10. Assay of Marker Compounds for the Assay of Crude Drugs and Extracts of Kampo Formulations Utilizing NMR Spectroscopy 10.1. Principle of Quantitative Analytical Technique Utilizing NMR Spectroscopy 10.2. Supply of Reference Materials and Software for Quantitative NMR 10.3. Marker Compounds for Assay and Reference Substances for Quantitative Analysis of Crude Drugs and Extracts of Kampo Formulations in the JP 10.4. Precautions for Performing qNMR	**General Chapter**/ **<761> Nuclear Magnetic Resonance Spectroscopy/** • Qualification of NMR instruments • Qualitative and quantitative NMR analysis • Validation and verification of NMR analytical procedures **General Information**/ **<1761> Application of Nuclear Magnetic Resonance Spectroscopy/** • Quantitative Applications	**General Tests**/ **<2.2.33> Nuclear Magnetic Resonance Spectroscopy/** • Quantitative analysis
Liquid NMR	Description	Description	Description
Solid NMR	**No** description	Description <1761>	Description

The use of qNMR is noted in the Crude Drugs section

The JP and EP are somewhat structurally similar, whereas structure of the USP is different.

Current stage for developing qNMR standard by ISO/TC34

ISO/CD24583:
Quantitative nuclear magnetic resonance spectroscopy — Purity determination of organic compounds used for foods and food products — General requirements



NP: New Work Item Proposal, WD: Working Draft, CD: Committee Draft, DIS: Draft International Standard, IS: International Standard, WG: Working Group

Thank you for your attention