qNMR in the Japanese Pharmacopoeia (JP), now and future

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What is quantitative NMR (qNMR) ?

Quantitative NMR (qNMR) is an absolute quantification method with SI*-traceability by utilizing internal standard (Primary ratio method).

***SI : International System of Units**

QNMR is able to absolutely determine the purity or the concentration of low molecular organic compounds.

Application（drugs, pesticides, natural products, food additives）

Joint Research group of qNMR studies since 2008

National Institute of Health Sciences (NIHS), Japan

The National Institute of Advanced Industrial Science and Technology (AIST)

Fujifilum-Wako Pure Chemical Industries, Ltd. (Reagent company)

JEOL Ltd. (Instrument company)

TSUMURA & CO. (Pharmaceutical company)

Researches aimed for adoption of qNMR in JP, especially for absolute quantification of JP marker compounds, have been continuing after 2014 by the JP experimental group, too.

Why JP started utilizing qNMR

JP starts utilizing qNMR for preparation of reference standards (marker compounds) for quantitative analyses of herbal medicines*1 or impurity of chemical drugs *2 from 2014.

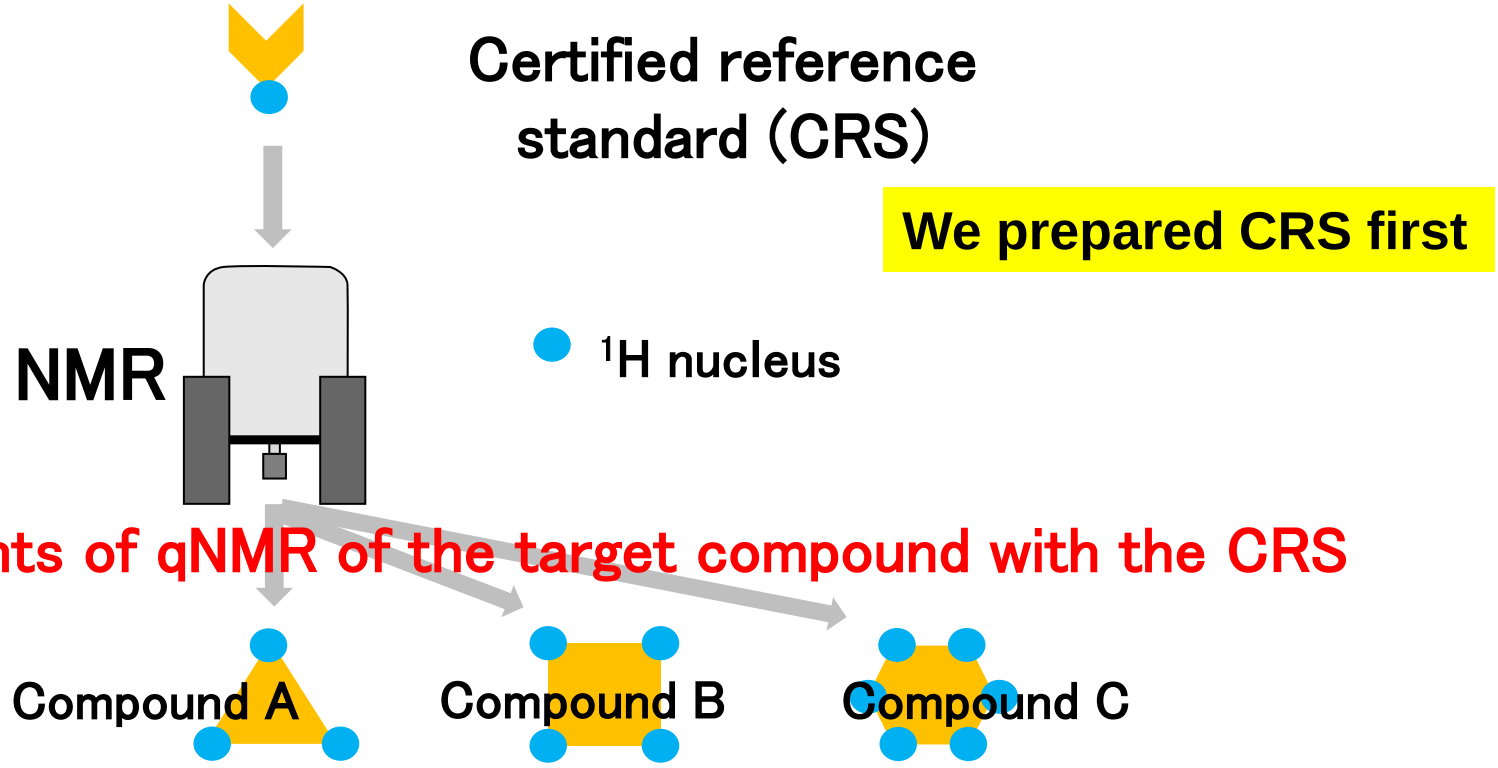
Because their preparation is difficult for following reasons.

*1: They are natural product origin and separation cost is high.

*2: Even chemical drugs, synthetic cost of impurities is also high.

The qNMR is the “absolute” quantification method. If a NMR peak of a targeted compound is separated, no more purification is needed to obtain its purity value. Therefore, the cost is lower, comparing mass-balance method which requires several purification steps and determination of impurity values such as water content by Karl-Fischer’s method and inorganic contents by ignition method.

The attractive point of qNMR: Proliferation of SI traceable reference compounds by using qNMR



When qNMR of the targeted compound is measured with the CRS, the purity of the targeted one is available with SI traceability

Advertisement of certified reference standards with SI traceability for qNMR

NMR

NMR　qNMR

Wako

NMR　　NMIJ*）を通して SI にトレーサブルです。計量トレーサビリティを確保しているため、信頼性の高い、純度が保証された標準物質としてご使用いただけます。

*）NMIJ：（独）産業技術総合研究所計量標準総合センター

証明書
（製品 1 本毎に添付）

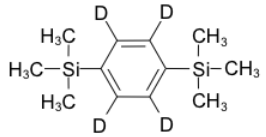
特　長

- 第三者（NMIJ）による純度保証（不純物が少なく、不確かさの小さな純度値を付加）
- 揮発性（昇華性）が低く、質量測定（秤量）がしやすい
- 測定対象物質と化学シフトが重ならない（0 ppm 付近）

qNMR 用内標準物質

●非水系溶媒用

1,4-BTMSB-d$_4$［1,4-Bis(trimethylsilyl)benzene-*d*$_4$］



分子式：分子量　＝ $C_{12}H_{18}D_4Si_2$：226.50

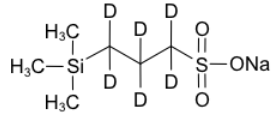
【Solubility】

Chloroform-*d*［$CDCl_3$］	：可溶
Acetone-*d*$_6$［$(CD_3)_2CO$］	：可溶
Methanol-*d*$_4$［CD_3OD］	：溶けにくい
Dimethyl Sulfoxide-*d*$_6$［DMSO-*d*$_6$］	：溶けにくい

*each 1 mg/mL, at 20℃

●水系溶媒用

DSS-d$_6$［Sodium 3-(Trimethylsilyl)-1-propane-1,1,2,2,3,3-*d*$_6$-sulfonate］



分子式：分子量　＝ $C_6H_9D_6NaO_3SSi$：224.36

【Solubility】

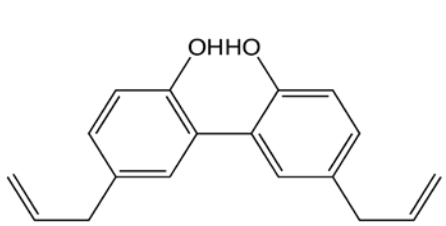
Deuterium Oxide［D_2O］	：可溶
Dimethyl Sulfoxide-*d*$_6$［DMSO- *d*$_6$］	：可溶

*each 1 mg/mL, at 20℃

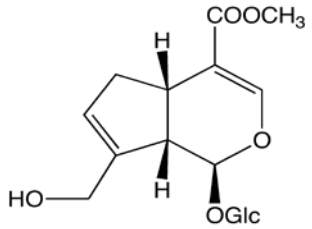
コード No.	品　　名	規　格	容　量	希望納入価格(円)
021-16441	1,4-BTMSB-d$_4$ Reference Material	Traceable Reference Material	50mg	30,000
048-31071	DSS-d$_6$ Reference Material	Traceable Reference Material	50mg	30,000

（K.S.）

Validation Studies of qNMR for chemical reagents used as reference standards for quantitative analyses of crude drugs in the Japanese Pharmacopoeia



Magnolol



Geniposide

Studies were performed in 5 independent laboratories

5 mg±10%

＋

1,4-BTMSB-d_4 1.0-1.1 mg

＋

d-solvent 1.0 mL

Each participant made 3 sample solutions

↓

The absolute purity of each sample was measured with qNMR by 3 times.

↓

The results indicate that the purity of these compounds can be determined by qNMR with an accuracy of more than two significant digits when the molecular weight of the target reagent is around 300 with a weighed amount of about 10 mg

qNMR conditions

Instruments: 400-800 MHz
Spectral width: 20ppm
Center of spectrum: 5ppm
Pulse angle: 90°
Aq. time: 4 s
Digital resolution : 0.25 Hz
Delay time: 60 s
Temp. : ambient
Decoupling condition: MPF8
Number of scans: 8 times
Dummy scans: 2 times

J. Hosoe et al, Pharmaceutical and Medical Device Regulatory Science, 41, 960-970 (2011).

Reagents determined by qNMR used as reference standards in the assay of the crude drug section in the JP (1)

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)，抑肝散加陳皮半夏エキス(Yokukansankachimpihange Extract), 柴胡桂枝乾姜湯エキス (Saikokeishikankyoto 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)

Reagents determined by qNMR used as reference standards in the assay of the crude drug section in the JP (2)

No.	JP	Reagents		生薬，漢方処方エキス　Crude drugs and Kampo formula extracts
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), ペリルアルデヒド用RMS基準物質 (reference compound for HPLC quantification of perillaldehyde by RMS)
20	JP18　Suppl 1 (2022.12.12)	アミグダリン	Amygdalin	キョウニン (Apricot Kernel), トウニン (Peach Kernel), トウニン末 (Powdered Peach Kernel), 桂枝茯苓丸エキス (Keishibukuryogan Extract), 桃核承気湯エキス (Tokakujokito Extract), 麻黄湯エキス (Maoto Extract)
21	JP18　Suppl 1 (2022.12.12)	アルブチン	Arbutin	ウワウルシ (Bearberry Leaf), ウワウルシ流エキス (Uva Ursi Fluidextract)
22	JP18　Suppl 1 (2022.12.12)	デヒドロコリダリン硝化物	Dehydrocorydaline nitrate	エンゴサク (Corydalis Tuber)
23	JP18 Suppl 1 (2022.12.12)	ヒルスチン	Hirsutine	チョウトウコウ(Uncaria Hook)　釣藤散エキス(Chotosan Extract)　抑肝散エキス(Yokukansan Extract)
24	JP18 Suppl 1 (2022.12.12)	リンコフィリン	Rhynchophylline	チョウトウコウ(Uncaria Hook)　釣藤散エキス(Chotosan Extract)　抑肝散エキス(Yokukansan Extract)
25	JP18 Suppl 2 (2024.6.28)	アトラクチレノリドⅢ	Atractylenolide Ⅲ	当帰芍薬散エキス (Tokishakuyakusan Extract)
26	JP18 Suppl 2 (2024.6.28)	アトラクチロジン	Atractylodin	当帰芍薬散エキス (Tokishakuyakusan Extract)
27	JP18 Suppl 2 (2024.6.28)	安息香酸	Benzoic acid	牛車腎気丸エキス (Goshajinkigan Extract), 八味地黄丸エキス(Hachimijiogan Extract), 真武湯エキス (Shinbuto Extract), ブシアルカロイド用RMS基準物質 (reference compound for HPLC quantification of Aconite alkaloids by RMS)
28	JP19 (2026)	ヘスペリジン	Hesperidin	チンピ (Citrus Unshiu Peel), 補中益気湯エキス (Hochuekkito Extract), 六君子湯エキス (Rikkunshito Extract), 釣藤散エキス (Chotosan Extract), 抑肝散加陳皮半夏エキス (Yokukansankachinpihange Extract)

RMS (Relative Molar Sensitivity) method

27 reagents evaluated using qNMR have been listed as reference standards in the assay of 50 monographs (Crude drugs and Kampo formula extracts) until JP18-2

Introduction of qNMR into JP (1)

First Appearance	Original	Addition or Change
JP16-1 (2012.10)	Listed "Quantitative Analytical Technique Utilizing NMR Spectroscopy and Its Application to Reagents in the JP" at G5 Crude Drug sction in General information	Added the paragraph on "Qualification of NMR equipment used for quantitative NMR" in the JP17 (2016.4.1)
	参考情報生薬等の項に「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	JP17で「定量NMRに使用する機器の性能の管理」を追加
JP16-2 (2014.2)	Listed "10. Assay of Marker Compounds for the Assay of Crude Drugs and Extracts of Kampo Formulations Utilizing NMR Spectroscopy" at <5.01> Crude drugs tests section in General tests	
	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	
	Listed the reagents determined by qNMR for assay of crude drugs as "xx for assay o xx for assay 2 (Purity value by quantitative NMR)"at <9.41> Reagents, test solutions section in General tests	Until JP 18-2, 27 reagents determined by qNMR has been listed.
	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))	JP18-2までにqNMRで値付けされた27試薬を収載

[Ref] Goda Y., Pharmaceutical and Medical Device Regulatory Science, 48, 670-682 (2017);
Goda Y., Shoyakugaku Zasshi, 78, 51-55 (2024).

Introduction of qNMR into JP (2)

First Appearance	Original	Addition or Change
JP17 (2016.3)	Added the paragraph on qNMR to General tests <2.21> NMR Spectroscopy	Changed the name of quatitative reference compounds from "internal reference standard" to "qNMR reference standard" in JP18 according to JIS and added the description about S/N ratio in JP18
	JP17で一般試験法NMRに定量NMRに関する項を追加,	JP18で JISに従い内部基準物質からqNMR基準物質に名称変変更, さらにS/N比に関する記述を追加
JP18 (2021.6)	Added detailed description on qNMR in the guideline for drafting the JP	
	局方記載要領にqNMRに関する詳細な内容を追加	
JP19 (2026)	Listed "Reference NMR Spectrum Based on 1H Spin Information and Application to Reagents in the Japanese Pharmacopoeia" at G5 Crude Drug sction in General information	
	(qNMR測定で得られた)1Hスピン情報に基づいた参照NMRスペクトルと日本薬局方試薬への応用を収載 (*HiFSAの応用)	

HiFSA: ^{1}H iterative Full Spin Analysis; qNMRで得られたスペクトルから推定されたスピン情報(化学シフトと相関核、スピン-スピン結合定数)に基づき、反復計算ソフトを利用し線形フィッティングを行い、極めて正確なスピン情報を得る手法. スピン情報は、磁場サイズに依存しないので、このデータを元に、任意の磁場サイズに対応するスペクトルが得ることができる. また、そのデータだけで、ジアステレオマーなどを区別する精緻な確認試験に応用できる.

＊ P.S. Achanta, et al., J. Pharm. Biomed. Anal., 192, 113601 (2021)

Expansion of qNMR utilization to chemical drug fields

From JP17, JP allowed to use impurity standards for quantification of organic impurities in chemical drugs and the JP expert committee prepared the document concerning the draft “Quality Standards of the Japanese Pharmacopoeia Reference Standard” in the guideline for drafting the JP18 and the documents refer to qNMR.

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
記載上の
① 標準
を記
② 試験
と．
③ 試験
指定
④ 標準
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 perf
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**

qNMR description in the guideline for drafting JP18

Points to consider（留意点）when describing the quantitative ^{1}H NMR measurement in the section "9.41 Reagents, Test Solutions" in General Tests or "Form 2" in Standard Quality Standards

6.2　定量 1H 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	Points to consider when describing the quantitative 1H NMR measurement in the section "9.41 Reagents, Test Solutions" in General Tests or "Form 2" in Standard Quality Standards
6.2.1　qNMR 試料溶液の調製方法	**6.2.1 Process for preparing qNMR sample solutions**
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 measured (analyte)
6.2.1.1.2　qNMR 用基準物質の情報	6.2.1.1.2 Information on reference materials 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 chemical shift standard substances (if necessary)
6.2.1.1.4　qNMR 測定溶媒の情報	6.2.1.1.4 Information on qNMR measuring solvents
6.2.1.2　試料溶液の調製方法	6.2.1.2 Preparation method for sample solution
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	Warm and humid information when weighing the actual sample, and the method and warm and humid, if controlled
6.2.2　qNMR 測定	**6.2.2 qNMR determination**
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 Eligibility of the device to be used (qualified for qNMR determination)
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 reproducibility, system performance, and confirmation of detection)
6.2.2.2. qNMR 測定条件	6.2.2.2. qNMR assay conditions
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 during 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 Observation range (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 (actual measurement information)
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 during 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 (actual measurement information)
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 (actual measurement information)
6.2.2.2.11　測定温度(実際の測定時の情報)	6.2.2.2.11 Measured temperature (information at actual measurement)
6.2.2.3　qNMR 解析条件	6.2.2.3 qNMR analysis conditions
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 Signal information for quantitative measurement**
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	**The reason why that signal was chosen, the integration extent of each signal used for quantitation (in ppm representation) is indicated.**
(溶媒を選択した理由を示す)	**(The reason why that solvet was chosen is indicated.)**
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 Calculation formula
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

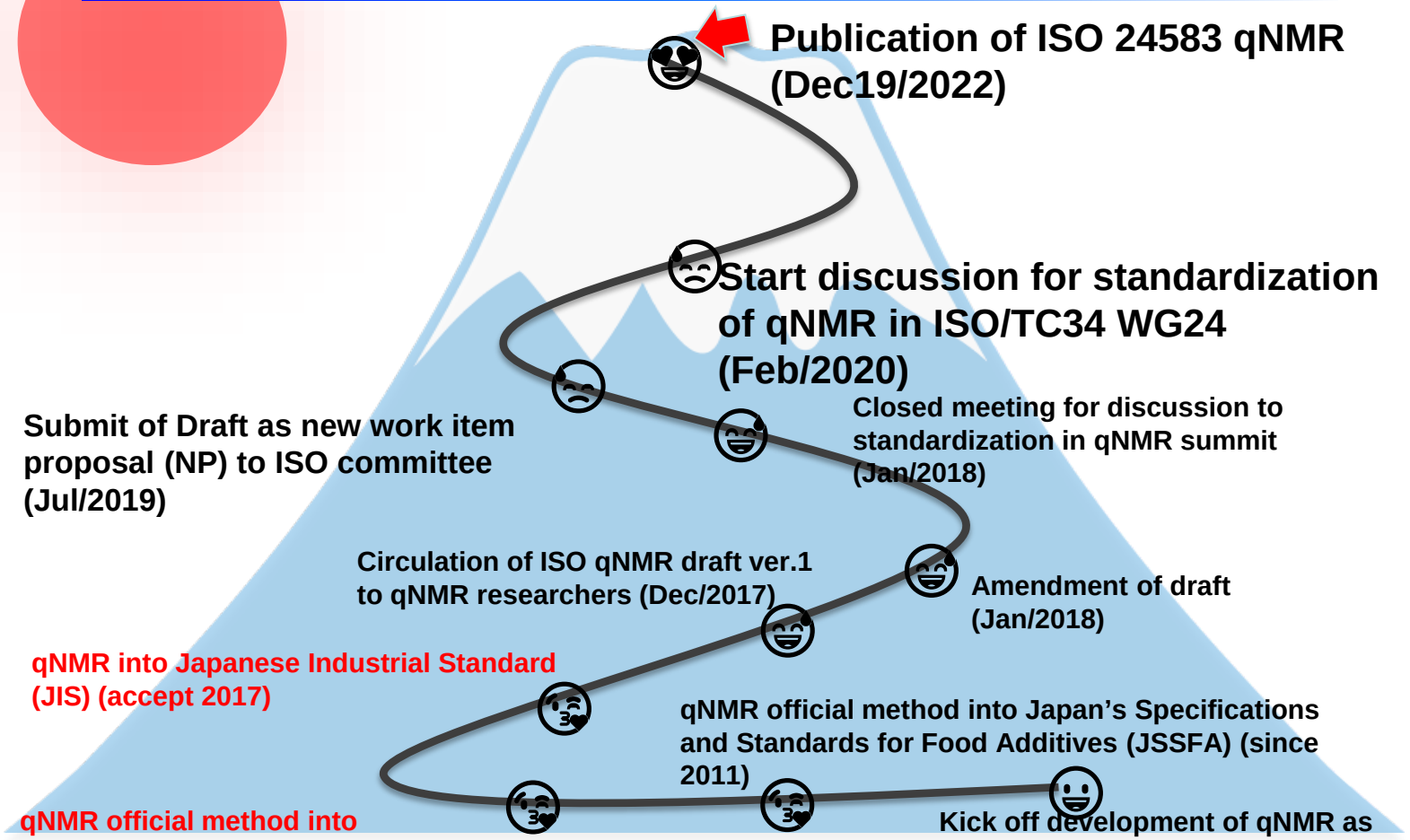
Key Issues for progress of qNMR in pharmaceutical laboratories

The acceptancy of qNMR data to regulatory authorities and recognition of this fact in pharmaceutical companies are most important.

The authorities who have never used qNMR seems to be somewhat difficult to understand NMR characteristics. Normally they image chromatographic charts other than spectroscopic ones when they think quantification. But there are big difference.

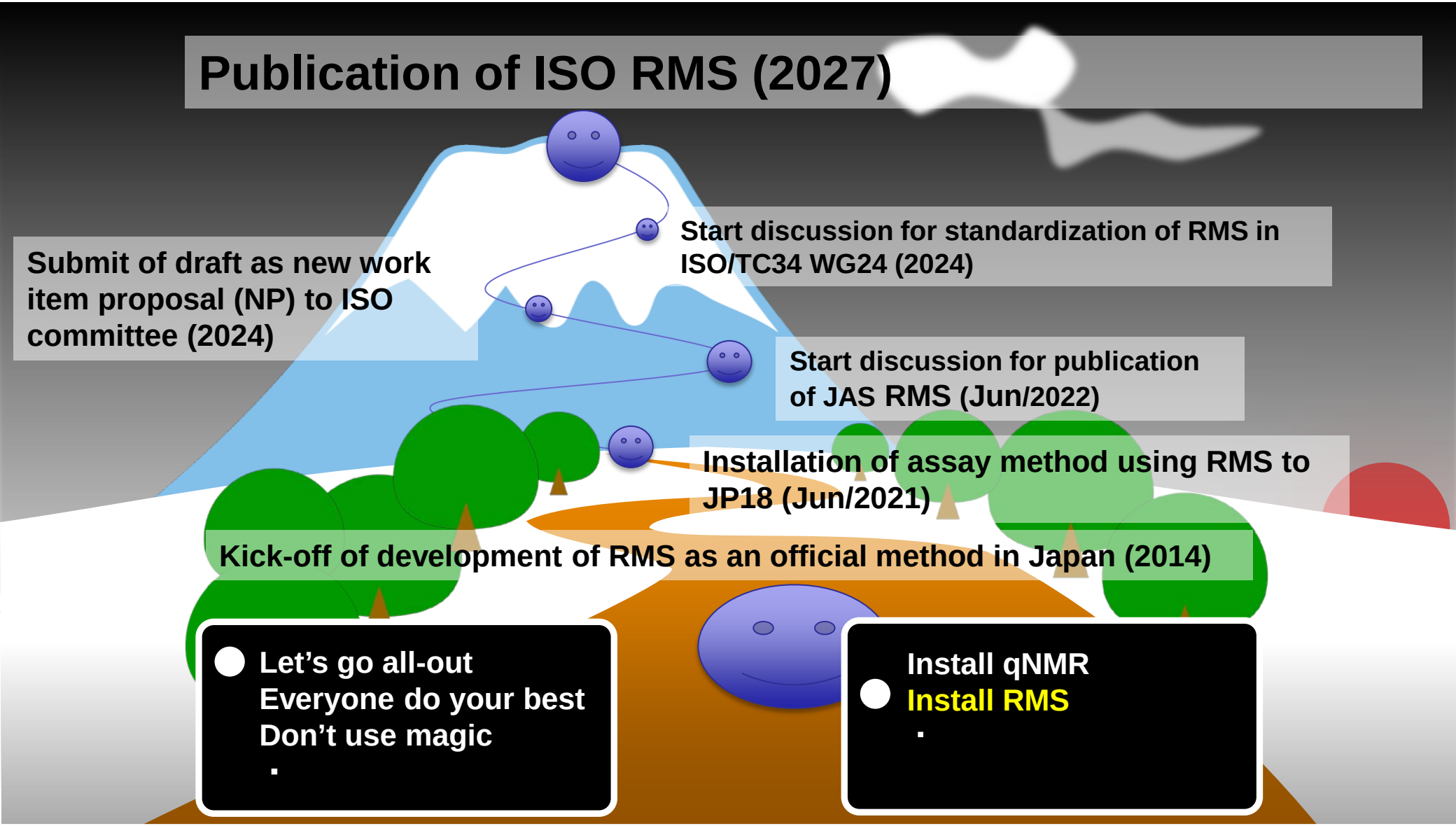
A slide used at USP meeting in 2016

Road map for ISO standardization of qNMR



杉本博士のスライドを借用

In parallel with aggressive adoption of qNMR in JP, our joint research group made much effort to list qNMR in ISO and in 2022 ISO 24583 qNMR was published.



Slide by Dr. Sugimoto

What is ^{1}H iterative Full Spin Analysis (HiFSA)

by Guido F. Pauli, UIC, Chicago (IL)

HiFSA is an **NMR methodology** which allows the complete interpretation of the complex resonances (also known as "multiplets") typically found in ^{1}H NMR spectra of molecules, such as organic compounds and natural products.
HiFSA can be performed using any 1D ^{1}H NMR data set, provided that the resolution is adequate. It typically requires an iterative process, which can be performed manually with a spin simulation tool, or (semi-)automed using a computational tool such as the PERCH software.

https://gfp.people.uic.edu/rt/hifsa.htm

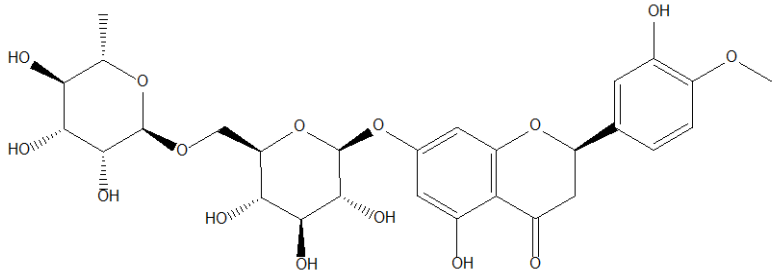
What is HiFSA in JP

by Yukihiro Goda

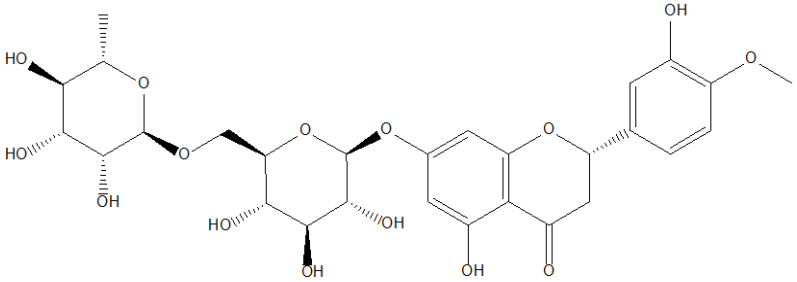
HiFSA is the method that provides extremely accurate ^{1}H spin information (chemical shifts, correlated nuclei and spin-spin coupling constants) on the basis of ^{1}H spin information elucidated from ^{1}H qNMR spectrum. It utilizes software products for iterative calculation for linear fitting. ^{1}H spin information is independent on magnetic field size. Therefore, it provides corresponding accurate spectrum of a targeted compound by ^{1}H NMR using any magnetic field size. ^{1}H-NMR spectrum sensitively varies, based on ^{1}H spin information, in other words, structure of targeted organic compound. Thus, accurate ^{1}H spin information is applicable to identification test which discriminates diastereomers that are not discriminated by other method, such as hesperidin and epi-hesperidin.

qNMRで得られたスペクトルから推定されたスピン情報（化学シフトと相関核、スピン-スピン結合定数）に基づき、反復計算ソフトを利用し線形フィッティングを行い、極めて正確なスピン情報を得る手法. スピン情報は、磁場サイズに依存しないので、このデータを元に、任意の磁場サイズに対応するスペクトルが得ることができる. ^{1}H-NMRスペクトルは、スピン情報、言い換えれば物質の構造に基づき鋭敏に変化する。従って、そのデータだけで、他の手法では対応出来ないジアステレオマー, 例えばhesperidinとそのエピマーなども区別する精緻な確認試験に応用できる。

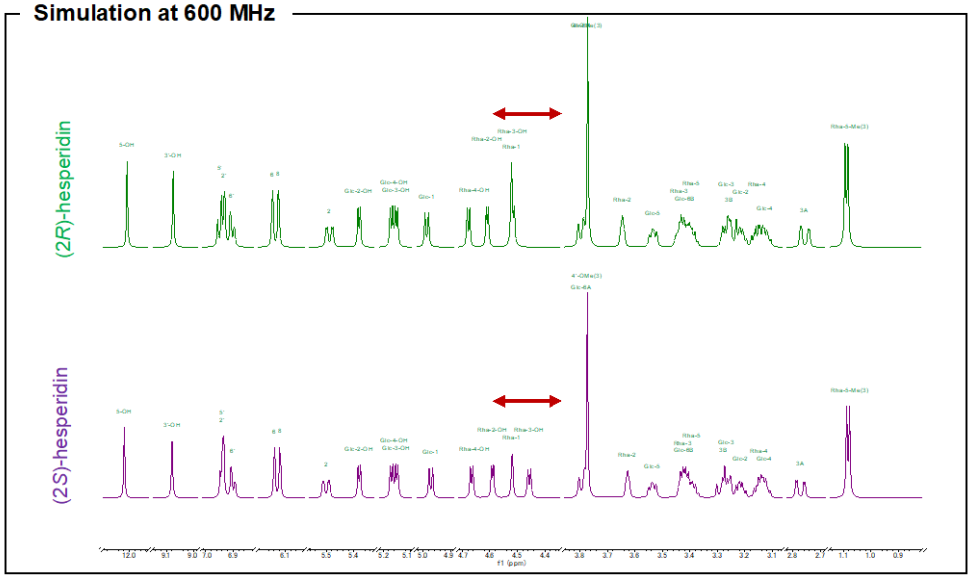
^{1}H-NMR spectra (at 600 MHz and 60 MHz) of hesperidin (S-form) and epi-hesperidin (R-form), and their structures

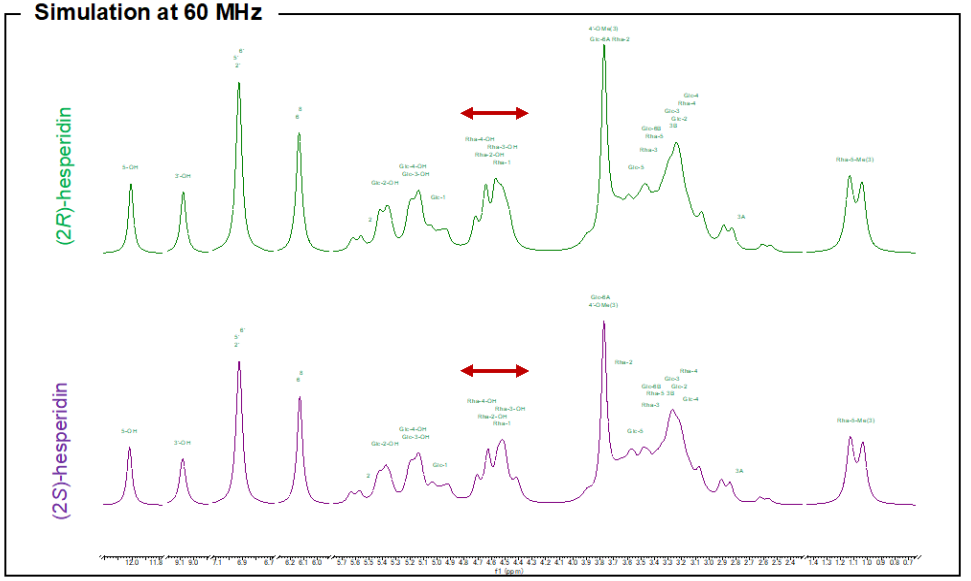


Epi-hesperidin (R-form)



Hesperidin (S-form)





^{1}H spin information which will be described in JP as identification test of hesperidin

Chemical shifts [nucleus] (number of ^{1}H) 化学シフト[核] (水素数)
δ1.11182 ppm [A] (3H), δ2.80136 ppm [B] (1H), δ3.15711 ppm [C] (1H), δ3.17565 ppm [D] (1H), δ3.24400 ppm [E] (1H), δ3.29629 ppm [F] (1H), δ3.30644 ppm [G] (1H), δ3.42110 ppm [H] (1H), δ3.44899 ppm [I] (1H), δ3.45467 ppm [J] (1H), δ3.56471 ppm [K] (1H), δ3.65522 ppm [L] (1H), δ3.80242 ppm [M] (3H), δ3.82225 ppm [N] (1H), δ4.48433 ppm [O] (1H), δ4.54744 ppm [P] (1H), δ4.61724 ppm [Q] (1H), δ4.69205 ppm [R] (1H), δ5.00132 ppm [S] (1H), δ5.17791 ppm [T] (1H), δ5.19648 ppm [U] (1H), δ5.40646 ppm [V] (1H), δ5.53032 ppm [W] (1H), δ6.14721 ppm [X] (1H), δ6.16759 ppm [Y] (1H), δ6.92979 ppm [Z] (1H), δ6.96759 ppm [AA] (1H), δ6.96854 ppm [AB] (1H), δ9.10744 ppm [AC] (1H), δ12.04673 ppm [AD] (1H).

Spin-spin coupling constants [correlated nuclei] スピンースピン結合定数 [相関核]
6.200 Hz [A-H], –17.135 Hz [B-G], 3.177 Hz [B-W], 8.840 Hz [C-F], 9.887 Hz [C-K], 5.440 Hz [C-U], 9.341 Hz [D-H], 9.361 Hz [D-J], 5.577 Hz [D-R], 9.067 Hz [E-F], 7.812 Hz [E-S], 5.212 Hz [E-V], 5.169 Hz [F-T], 12.288 Hz [G-W], 6.257 Hz [I-K], –11.338 Hz [I-N], 3.400 Hz [J-L], 6.126 Hz [J-O], 1.852 Hz [K-N], 1.604 Hz [L-P], 4.373 Hz [L-Q], –0.538 Hz [W-Z], –0.268 Hz [W-AA], 2.223 Hz [X-Y], 2.177 Hz [Z-AA], 8.351 Hz [Z-AB], 0.268 Hz [AA-AB].

Present utilization of qNMR in and around JP

In JP

・Quantification of reference standards for assay and impurity tests 定量用標準物質の純度値付け

・Identification of reagents 試薬の確認試験

Around JP

・Quantification of reference standards for assay and impurity tests in the approval application 承認申請書中の定量試験や不純物試験の標準物質の純度値付け

・Impurity control of drug substance during synthetic process of JP products 合成過程での不純物コントロール

・Quantification of drug substances in chemical drug products 化学医薬品の定量試験

QNMR is a non-destructive quantification method. Therefore, it is convenient as a accurate or more strict substitute of a HPLC quantification analysis in JP when assay of many kind of drug products should be performed (such as removed drug sample test), because qNMR continuously quantify different substances without washing column and equipment.

Also, we have already confirmed that qNMR is useful for quantification of a drug substance in a injection preparation. 注射剤中の薬物の定量

Future utilization of qNMR in and around JP

Continuation

・Quantification of reference standards for assay and impurity tests 定量用標準物質

RMS (Relative Molar Sensitivity) method is especially useful for impurity standards the preparation of which is difficult, considering cost and time.

・Identification of reagents by HifSA 試薬の確認試験

New

・Identification test of drug substances by HifSA (Cost effective) 原薬の確認試験

・Direct quantification of impurities in drug substances and products.原薬と製剤純度試験

We have already confirmed that qNMR is useful for quantification of solvent impurity in drug substances and drug products.

Impurity quantification by non ^{1}H qNMR (^{31}P and ^{19}F).

^{31}P and ^{19}F nuclei exist in pharmaceuticals including new modality (siRNA and oligonucleotide) ones with high probability. We have already established quantification of drug substances by ^{31}P qNMR* and ^{19}F qNMR*. Their spectra are simper than corresponding ^{1}H qNMR ones and impurity test is normally limit test with one significant figure such as not more than 0.1%. These facts may overcome sensitivity problem and it is of interest to apply non ^{1}H qNMR to directly quantify organic impurity in drug substances.

*N. Uchiyama, Y. Goda et al., Chem. Pharm. Bull., 69 (2021) 26–31; idem ibid. 69 (2021) 118–123; idem ibid. 69 (2021) 630–638; idem ibid., 70 (2022) 892–900; idem ibid. 72 (2024) 36–40; idem Journal of Pharmaceutical and Biomedical Analysis Open, accepted.

Thank you for your attention