

Solutions for Innovation

qNMR

Quantitative Analysis by NMR

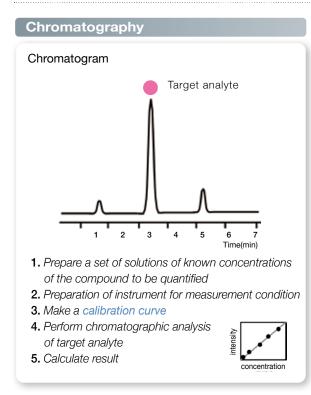


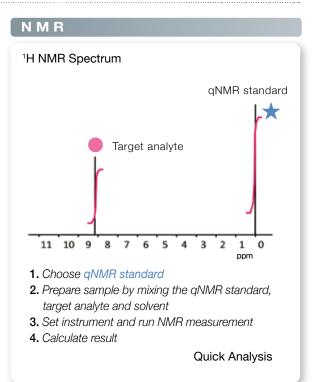
JEOL Ltd.

What is quantitative analysis using NMR(qNMR)?

Quick and accurate quantitative analysis is possible without using a reference material that is the same as the target analyte.

Comparison of Chromatography and NMR analysis Procedures





Features of qNMR

- 1. Versatility
 — Target analyte are typical organic compounds
 * Quantitative analysis of a compounds is possible, although its standard sample may not be available.
 2. Efficiency
 — The qNMR standard that is the same as the target analyte is not necessary.
 * One qNMR standard can be used for unlimited number of target analyte.
 3. Speed
 — Calibration curve is not required --> With qNMR it is possible to perform absolute quantification.
 * Usually, a few milligrams of sample is required.
 4. Reliability
 — SI (The International system of units) traceable analysis is possible.
 - 1 Why purity analysis by NMR is attracting attention

Improved reliability of quantitative analysis

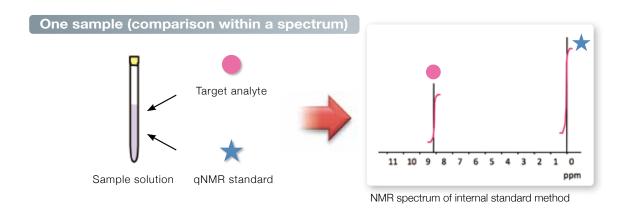
If the purity of the qNMR standard used in quantitative analysis is not known, qNMR can be used to determine the purity. By incorporating the absolute purity value into quantitative analysis calculation, it is possible to improve on the reliability of quantitative analysis obtained by chromatographic methods.

Variety of qNMR methods*1

There are two methods of quantitative analysis using NMR mainly.

Internal Standard Method

The presence of standards in the sample solution minimizes various factors that can lead to errors and allows for precision. This method is utilized for purity analysis.

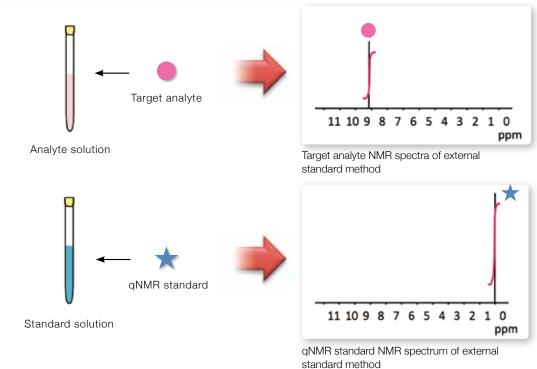


External Standard Method: PULCON*2

A standard solution is prepared and used separately from the sample solution, so that the contamination into the target analyte is minimized.

Various methods have been reported.

Two samples (comparison between 2 spectra)



^{*1} There are two basic quantitative method by NMR: the relative and absolute quantification. However, we only introduce the absolute quantification of analyte here.

^{*2} PUlse Length-based CONcentration measurement. A quantitative analysis method measurement of involving measurement of the standard and analyte sample separately and them comparing the signal areas.

qNMR workflow of internal standard method

Quantitative methods for the accurate determination of the purity or content of organic compounds.

qNMR is SI-traceable and accurate quantitative values.

Quantitative NMR analysis is possible by means of either internal or external standard method. The choice of these methods depends on the purpose of analysis.

Among the internal standard methods, a gNMR analysis that can quantitatively express the reliability of the analytical results and qualifies in principle as a primary standard measurement method is possible.

If reliability is the most important factor, e.g. purity, the

internal standard method is strongly recommended. In official methods such as Japanese Pharmacopoeia, the qNMR with SI-traceability applied qNMR method is used as a quantitative method to accurately determine purity and content,*3 and are used for evaluation of certified reference materials.*4

The internal standard method is employed by JEOL in the analysis operation from sample preparation to calculation.

Procedure



Accurate weighing of the qNMR standard and target analyte

Ensure reliability

The absolute value of the amount of the material can be obtained by measurements verified by the International System of Units(SI). This measurement method is called the primary standard measurement method, and NMR is one of them,*4

At qNMR analysis, certified standard material with SI traceability of reference materials (Certified reference material, CRM) can be used, enabling SI-traceable purity evaluations.



$oldsymbol{oldsymbol{1}}$ ISO standardization of qNMR method *5

Contributes to improved reliability in the quantitative analysis of organic compounds such as pharmaceuticals, reagents and food ingredients.

International standard for the quantitative purity determination of organic compounds using nuclear magnetic resonance (NMR) has now been adopted by ISO 24583, Quantitative nuclear magnetic resonance spectroscopy - Purity determination of organic compounds used for foods and food products -General requirements for ¹H NMR internal reference methods.-

The qNMR (quantitative NMR) method is an analysis method that enhances reliability. This international standard defines the measurement procedures for the qNMR method, which is a "quantitative analysis" method using NMR, and improves the reliability of the purity determination of organic compounds around the world. This will further promote the implementation of the gNMR method in society and contribute to the realization of a comfortable, safe and secure society.

The international standard was published by ISO on 19 December 2022.

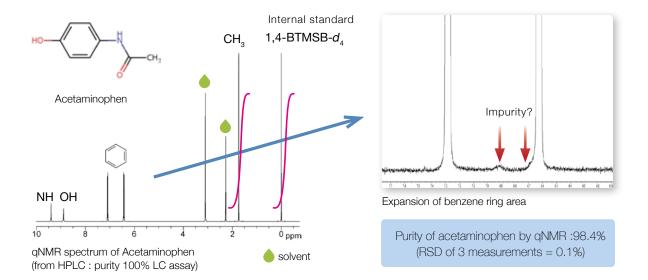
^{*3} THE JAPANESE PHARMACOPOEIA EIGHTEEN EDITION (THE MINISTRY OF HEALTH , LABOUR AND WELFARE)

^{*4} T.Ihara, T.Saito, JEOL News Vol47, No.1, 2012 - Realization of an Innovative Metrological Traceability using the Quantitative NMR Method

^{*5} ISO 24583: https://webdesk.jsa.or.jp/books/W11M0070/index English page is available.

Example of qNMR internal standard method

Purity Analysis of Acetaminophen



The result of the purity of acetaminophen reagent is shown here. 1,4-bis(trimethylsilyI)benzen- d_4 (1,4-BTMSB- d_4) was used as the internal standard material. *6

We decided to use the $^{1}\mathrm{H}$ signal of the $\mathrm{CH_{3}}$ group and the calculation was made according to the

formula(1) to determine a purity of 98.4%.

As such, qNMR enables purity analysis and confirmation of impurities simultaneously. In addition, it is possible to obtain information on changes of the sample over a period of time and it can be used in product quality control.

Formula(1)
$$P_{\text{sample}} = \frac{I_{\text{sample}}}{I_{\text{std}}} \times \frac{H_{\text{std}}}{H_{\text{sample}}} \times \frac{m_{\text{std}}}{m_{\text{sample}}} \times \frac{M_{\text{sample}}}{M_{\text{std}}} \times P_{\text{std}}$$

I= signal intensity(integral value), H= number of protons(number of hydrogen atoms in the functional group), m= mass(weight), M= Molar Mass , P= purity(%)

Introduction of qNMR related reagents

Code No.	Product	Grade	Package size
024-17031 020-17033	1,4-BTMSB-d ₄ Reference Material [CRM]	TraceSure®	50 mg 50 mg×4
044-31671 040-31673	DSS-d ₆ Reference Material [CRM]	TraceSure®	50 mg 50 mg×4
048-33271	Dimethyl Sulfone Reference Material [CRM]	TraceSure®	100 mg
135-17951	Maleic Acid Reference Material [CRM]	TraceSure®	100 mg
093-06731	4 Internal Standard Set for Quantitative NMR [CRM]	for qNMR	1 set
634-29181	3,5-Bis(trifluoromethyl)benzoic Acid for Quantitative NMR (1H, 19F)	NMIJ CRM 4601-c	200 mg
639-44151	1,4-Bis(trimethylsilyl)-2,3,5,6-tetrafluorobenzene for Quantitative NMR ($^{\rm 1H},^{\rm 19}{\rm F})$	NMIJ CRM 4602-a	100 mg
161-24661	Potassium Hydrogen Phthalate [CRM]	TraceSure®	50 g
041-33641	DSS- $d_{\rm 6}$ Standard Solution (500 mg/L Deuterium Oxide Solution)	for qNMR	1 mL×5 A

^{*6 1,4-}BTMSB- d_4 : Code No. 024-17031, 020-17033 Fujifilm Wako Pure Chemical Corporation

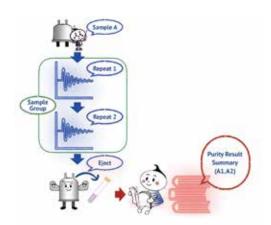
Information of qNMR Analysis System

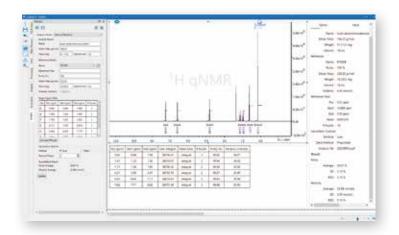
JEOL NMR Systems offer both internal and external standard method

Data Analysis Tool

JASON SMILEQ

SMILEQ(Spectral Management Interface Launching Engine for qNMR) is an automated qNMR analysis system provided by JASON(JEOL Analytical Software Network). (p.8)

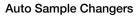




Optional

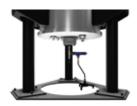
Long Time Low Temperature Unit Compressor Type

This ling tern low temperature unit a vortex tube. The achievable temperature of sample dependents on the specification of the compressor used, but the ability to easily reach a temperature of approx,25 degrees C below room temperature. It is suitable for long measurement of samples requiring low temperature.



Sample tubes of various diameters and special sample tubes can be transported safely. The JackBean type does not require the use of stairs when changing NMR sample. Pre-Cool and Pre-Heat types are also available for users who need to keep samples cool or pre-heat them.

Туре	Option
ASC24	
ASC30 (JackBean)	Pre-Cool Pre-Heat
ASC64 (JackBean)	
ASC100 (JackBean)	









Installation Case about qNMR and our support

FUJIFILM Wako Pure Chemical Corporation Tokyo plant

FUJIFILM Wako Pure Chemical Corporation has started production of certified reference material (CRM) using ISO 24584 (qNMR), first in the world. *1

The qNMR (quantitative NMR) method has been stipulated in Japanese Industrial Standards (JIS), and an International Organization for Standardization (ISO) method for sample analysis. The ISO method for qNMR was established through collaborative research by multiple organizations including FUJIFILM Wako Pure Chemical Corporation and JEOL Ltd.

*1 As of October 30th, 2023

qNMR can be used for purity assay even without a reference material identical to the target analyte, and has the advantage that the analysis time is shorter than other methods. This means that qNMR can also be applied for analyte for which a reference material has not yet been, such as new drugs.

FUJIFILM Wako Pure Chemical Corporation is a supplier of reference material and certified reference material (RMs/CRMs) for qNMR. These reference materials will be standards for quantitative analysis when measuring together with the target analyte. As another feature of qNMR is that a single reference material can be used for a number of analytes.

FUJIFILM Wako Chemical Corporation has contributed to the standardization of qNMR and has introduced qNMR into their quality control processes. They have a plan to provide a sufficient number of RMs/CRMs for general analysis and are preparing to meet the diverse demands of users. Through these efforts, they are working to disseminate qNMR and support its users.



JNM-ECZL400

FUJIFILM Wako Pure Chemical Corporation currently uses three JEOL NMR systems, one of which is the latest JNM-ECZL400S, which can be used for automated qNMR, in order to analyse many analytes quickly and efficiently. We also carry out regular inspections to ensure stable operation of the NMR system. (Interview, November 2023)

You can find out more details on our website. https://www.jeol.co.jp/products/technology_cases/cases/nmr_12.html

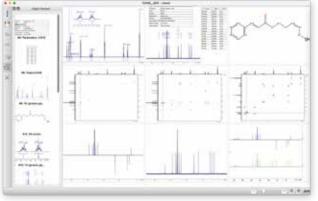


JASON(JEOL Analytical Software Network) is next-generation NMR analysis software focused on the automated processing, analysis and reporting of NMR data.

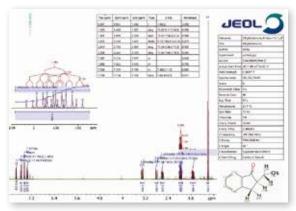
Highly flexible interface and extensive analysis functions

JASON's intuitive interface facilitates a variety of NMR analyses. In addition to basic 1D/2D-NMR data processing, JASON offers versatile functions such as multiplet analysis, peak deconvolution, ¹H, ¹³C chemical shift prediction, automatic attribution and spin simulation.

One of JASON's special features is its canvas. Each NMR data set can be linked to each other using the object link function, providing seamless support from spectral analysis to report generation.



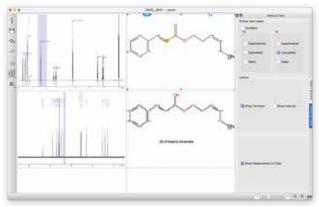
JASON Canvas



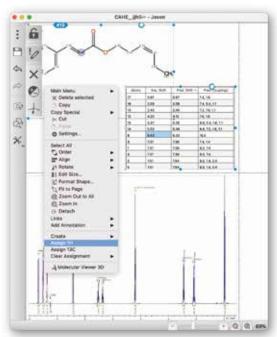
Example for report of J-coupling patterns

¹H, ¹³C Chemical shift prediction

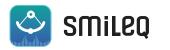
JASON can predict chemical shifts for input chemical structures. Using the predicted ¹H and ¹³C chemical shifts, the structural formula and the actual NMR spectra can be compared with each other. Furthermore, NMR spectra can be automatically assigned from the predicted chemical shift information. Easily chose Auto assignment [¹H] or [¹³C] from the menu and the signal assignment results are displayed on the NMR spectrum.



¹H and ¹³C Chemical shift prediction



The result of automatic assignment for ¹H NMR spectrum





SMILEQ (Spectral Management Interface Launching Engine for Q-NMR) is an automated qNMR measurement and analysis system, where measurements with the NMR software Delta and gNMR analysis with JASON are carried out seamlessly and automatically in an integrated manner.

Measurement & Analysis in one Step

All qNMR measurements, data processing and reporting for the internal standard method are performed automatically. qNMR measurement conditions and FID data measured with Delta are properly data processed via JASON's SMILEQ plug-in, and quantitative values are calculated and reported automatically.

The conditions for qNMR analysis can be managed centrally using the Delta qNMR seamless tool, while the SMILEQ system enables user-independent, automated and optimised qNMR analysis.



Setting for experimental condition: Delta

The measurement conditions, analysis conditions and sample information can be specified on one screen

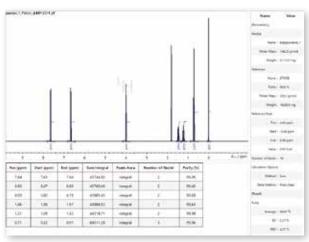


Analysis templates and a reference material library are available



Repeated, sequential or non-sequential measurement sequences can be defined

Data management



Easy-to-understand reports with qNMR analysis results and measurement conditions at a same time



Management of FID, pdf file and JASON format file created for each measurement



If re-analysis is required, individual analysis is possible with the SMILEQ plugin



Statistical results (average, SD,RSD) for all results can be created as a summary report in the case of multiple samples or repeated measurements (p.9)





Optimization for SOP development

SMILEQ is an automated analysis system that can manage all gNMR analyses via the Delta qNMR seamless tool. Standard Operating Procedures (SOP) support is also available, making SMILEQ ideal for quality control and other work environments with a large number of analysis samples.

Supported versions

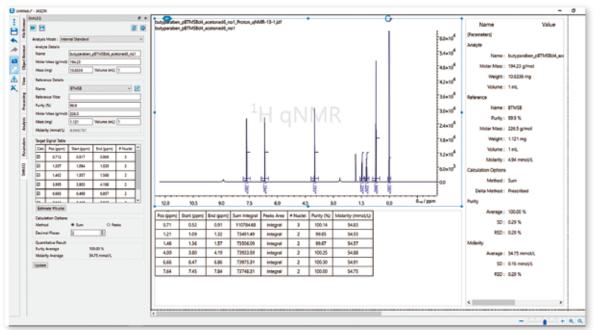
- -Delta 6.1 or later
- -JASON 1.3 or later
- SMILEQ_plugin 1.0 or later
- * The gNMR seamless tool is available in Smart Mode of Delta

Reporting functionality of the SMILEQ plug-in

The measurement data can also be processed and analysed for qNMR on your own PC. Statistical results from measurements of multiple samples and repeated measurements, as well as ISO 24583 reports, can also be generated automatically.

Internal standard and external standard analysis

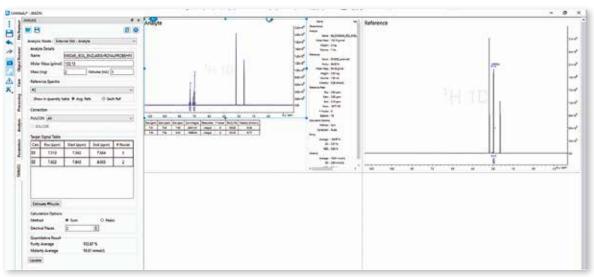
Perform internal standard qNMR analysis



Example of individual report

Internal reference: Enter reference and analyte details all one panel for simple qNMR analysis. Multiplet NMR standard signals can be used for analysis.

Perform external standard qNMR analysis



Example of individual report

SMILEQ can use external standards allowing routine qNMR analyses across different samples.

PULCON and SOLCOR corrections can be applied to obtain accurate concentrations.

The use of multiple reference spectra is also supported.



Summary reports of multiple samples and repeat measurements.

Summary Report (Purity)

Shiple name	AN		Amil	Anti	Aur	50	650	Air	90
butyl parahydronybenzoate1	7.86	99.48	99.57	99.95	99.67	0.25	0.25	15.76	0.19
	6.90	99.63	99.56	99.88	99.69	0.17	0.17		
	121	99.58	99.59	100.02	99.73	0.25	0.25		
	1.41	99.94	99.99	100.02	99.99	0.04	0.04		
	0.86	99.62	99.70	99.82	99.78	10.07	0.07		
	421	99.57	99.58	99.95	99.70	0.21	0.21		
butyl parahydroxybenzoateZ	7.86	99.93	99.59	99.28	99.60	0.33	0.33	99.72	0.25
	630	99.79	99.55	99.43	99.59	0.18	0.18		
	1,71	100.03	99.75	99.35	99.71	0.34	0.34		
	1.41	100.00	99.94	99.64	99.92	0.08	0.08		
	0.86	99.82	99.78	99.65	99.75	0.09	0.09		
	4.21	100.13	99.73	99.37	99.74	0.38	0.39		
butyl parahydrosybenzoate3	7.86	99.65	99.36	99.21	99.41	0.22	0.23	99.62	0.20
	5.30	99.61	99.55	99.32	99.49	0.15	0.15		
	1.71	99.83	99.53	99.48	99.61	0.19	0.19		
	1,41	99.85	99.88	99.94	99.89	0.05	0.05		
	0.86	99.73	99.74	99.74	99,74	0.00	0.00		
	421	99.70	99.61	99.45	99.58	0.13	0.13		

Example of summary report

The JASON summary report shows the average purity and standard deviation per signal used in the quantification calculations for the each qNMR results. The average purity and standard deviation for each sample group is also displayed.

The table on the left shows the results of triplicate measurements for each of the three Butylparaben samples. The results of the quantification calculations for each repeat measurement and the average purity and standard deviation for each sample can be seen.

ISO 24583 qNMR analysis Report *7

Uncertainty of the Purity (according to ISO 24583)

Simple			847		Service .		Responsables		Aff Sample activises		
Serge toron	No.			487	in	## /#1	700	781	780	(80 (A)	
- Intyl	7.86	39.45	W57	99.79	99.62	678	99.72	0.14	99.60	(CA	
penahydronyberspeek	6.00	943	9536	99.79	29.66	612					
1.0	1.91	99.06	91.15	99.83	99.67	0.14					
	1.45	2925	79.39	10000	99.96	100					
	2.86	99.82	99.70	99.79	99:77	606					
	4.21	99.58	99.56	99,77	99.64	619					
lwy	7.86	99.91	90.60	99.30	19.62	631	9679 610	0.12			
pelelydroytercoste	6.00	99.78	88	99.44	19.60	817					
	1.01	100.01	9181	99.34	99.74	633					
	3.41	99.99	98.94	59,64	99.91	506					
	1.86	99.62	91.79	99.81	99.76	6.09					
	4.21	100.12	91.00	96.36	MEST	8.16					
paraly de confessiones in	7.86	99.65	99.25	99.17	19.36	625	96.59 0.79				
	6.00	99.61	9130	1931	99.47	675					
	1.01	9930	79.41	99.44	99.56	539					
	141	79.65	78.87	99.94	79.69	605					
	7.86	99.73	9175	9671	99.75	600					
	4.21	99.70	95.50	99.41	99.54	815					

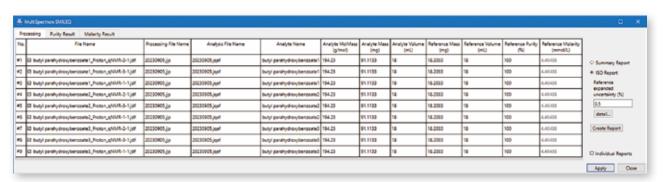
Example of the qNMR analysis report accordance with ISO 24583

Uncertainty Budget (according to ISO 24583)

	- Annie pfunctions	dist	-	Standard programs	Asserted - Parison	Reserve parentary processing
12AR размененій;	Measurement repeatability	. 2	99.77	0.36	1.00	0.10
	Variations from different signals selected	*	9958	0.18	1.00	
	Variations from ght/AR sample solution propositions	. %	2140	0.00	1.00	tos
Purity of standard	The purity of the internal standard used		HOUSE	. 81	1.00	8.35
				Continued	serviced uncomplete (16)	1.0
					Coverage factor	- 1
				10	perded occurrancy (N)	0.97

Report output according to ISO 24583 is available for quantitative calculations, which describes the uncertainty in repeated measurements, the uncertainty in the signal used for the calculation and the uncertainty in the conditioned sample. For qNMR analysis results, the range of quantitation values obtained can be more clearly evaluated.

Automatic batch analysis of multiple data set



Data processing, generating of summary reports and ISO reports for multiple data can be managed together in the 'MultiSpectrum SMILEQ' window. Simply select the Reports menu and reports for multiple data are automatically generated.

^{*7} Supported version is JASON 3.1 and later, SMILEQ-plugin 2.0 and later



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