USP-PMDA Workshop

Updates and Future Vision of qNMR at U.S. Pharmacopeia

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Scientific Liaison, General Chapters

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Brief History of Spectroscopy in USP-NF

USP 20-NF 15 (1980) – <761> Nuclear Magnetic Resonance Spectroscopy

with regard to ^{1}H is that the wide spectral range covered by ^{19}F scans makes it desirable to calibrate "zero" reference at some point paramagnetic (downfield) to the maximum field position. In this way, all the usable ^{19}F resonance signals are "on-chart" positive (upfield) to "lock-calibration zero."

Qualitative and Quantitative Analysis

NMR measurements are useful for a variety of analytical purposes. The various types of protons, fluorine atoms, carbon atoms, etc., each with a different environment, appear as different resonance signals with respect to their different chemical environments. The spectrum thus affords information about the molecular structure. The general multiplicity of each individual resonance (e.g., singlet, doublet, triplet) adds more structural information, and the combination of chemical shift and spin-coupling pattern enables the determination of (a) the number of the atoms being measured, (b) the chemical environment of each atom, (c) the structural and/or isomeric relationships, and (d) the presence of impurities. The integration of peak areas is an important step in interpretation since the ratio of areas yields the relative ratios of the various kinds of resonant nuclei. In addition, the integration may be extended to quantitative analysis.

The quantitative analysis of a compound by NMR exemplifies the use of a specific intimate property for measurement purposes. Once the position of a definite structural unit is known, the area of its resonance peak(s) can be related to that of other peaks, to obtain ratios of the various atoms represented in the spectrum. Quantitative estimation is limited largely by the accuracy and reproducibility of the built-in integrator that is usually part of the instrument recorder. By employing multiple integration tracings across the entire spectrum, as well as several independent analyses, a relative accuracy of ±2% can be achieved. If small parts of the scan yield the quantitative information of interest, these partial scans can be integrated at higher gain to improve the accuracy. The principal advantages of quantitative NMR are: (a) the intensity of a signal for a given nuclear isotope is proportional to the number of nuclei

Fig. 5.

the aromatic proton region (6.5 to 8.0 ppm) gives valuable data about the nature of aryl ring substituents, since *ortho*-, *meta*-, and *para*-protons show different coupling (8, 2, and about 0.5 Hz, respectively). A similar inspection of coupling in unsaturated systems gives data on isomer content (*cis*-HC═CH, 6 to 12 Hz; *trans*-HC═CH, 9 to 18 Hz), and conformational isomers have been successfully identified (ax–ax, 8 to 10 Hz; ax–eq, 2 to 5 Hz; and eq–eq, 1 to 4 Hz).

If alcoholic —OH is possibly present and has been noted by D_2O exchange, —OH coupling may be observed by scanning a test substance in DMSO-d_6. In this procedure, —CH_2OH shows a triplet, —CHR*OH* shows a doublet, and —CR_2OH shows a singlet.

If a complex spectrum is not interpretable by usual first-order rules, double resonance, as previously discussed, may be employed. Instruments used at 60 MHz or above should be equipped with homonuclear spin decouplers.

The spectra of compounds containing ethers, esters, ketones, etc., often may have resonance lines grouped in a narrow region of the spectrum (1.0 to 3.0 ppm) and are difficult to analyze because of peak overlap. In these cases it is often advantageous to add a shift reagent such as the dipyridine adduct of tris(2,2,6,6-tetramethyl-3,5-heptanedionato)europium(III) [Eu(dpm)$_3$], or tris-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionato)europium(III) [Eu(fod)$_3$], or their corresponding praseodymium compounds. [Eu(fod)$_3$] is the most useful, and in its presence, coordination occurs between the functional group and the transition metal complex causing a downfield shift of the resonance lines. The relative shift indicates the type of groups proximal to the protons.

Historical Story

qNMR Summit 2018 in Tokyo for ISO Application

More than 200 people from various scientific backgrounds gathered in Tokyo, Japan, on January 29-30th, 2018 to participate in “qNMR Summit in Tokyo”, the third International qNMR Summit. A major aspect of the summit revolved around the inclusion of quantitative NMR (qNMR) into ISO standard. The Summit included three different programs, including the international qNMR forum, **the USP qNMR Symposium in Tokyo**, and the International qNMR symposium.

Status of qNMR Applications

Moving qNMR from the Proof-of-principle Stage to USP Operation

qNMR fits very well in USP's 2020-2025 top-level Priorities.

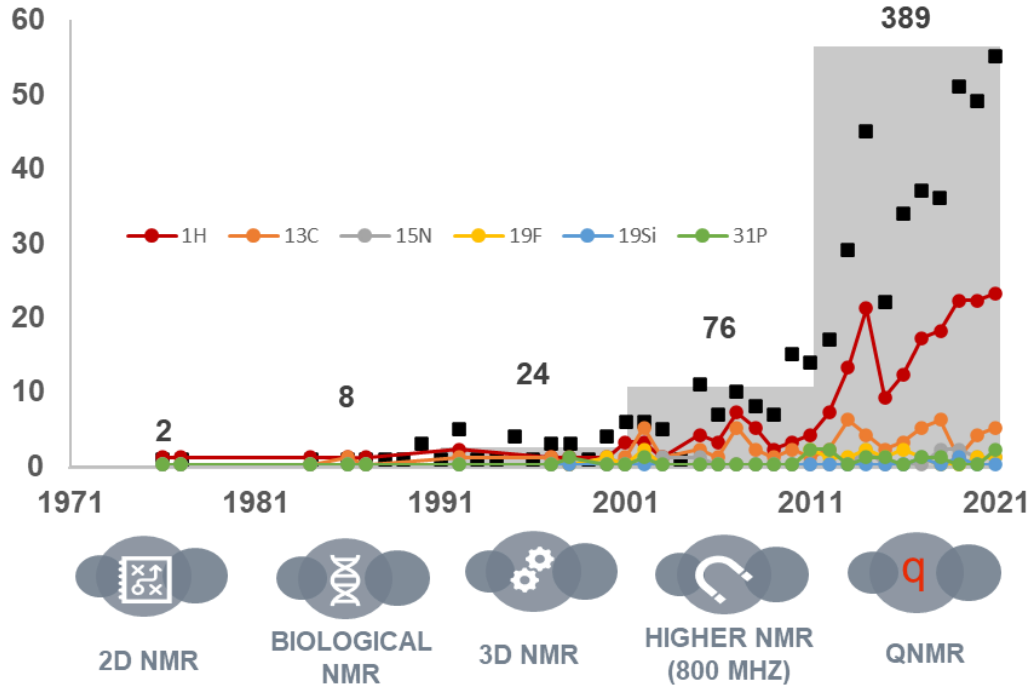
a) **Revision of General Chapters to update qNMR content and include benchtop NMR; Development of Solid-state NMR chapter.**

b) Leading the discussion on the need and appropriate mechanism for qNMR validation, e.g., **life cycle approach;**

c) **Continuing the investigation of the digital solution.**

d) Expanded use of qNMR for RS evaluation;

Number of Publications on qNMR



Number of publications on quantitative NMR since 1970 (■ in black, 499 items). Among these, ^{1}H qNMR (● in red, 296 items), ^{13}C qNMR (● in orange, 62 items), ^{15}N qNMR (● in gray, 12 items), ^{19}F qNMR (● in yellow, 15 items), ^{29}Si qNMR (● in blue, 2 items), and ^{31}P qNMR (● in green, 12 items). The data to be analyzed were retrieved through PubMed (National Center for Biotechnology Information, NCBI) on December 14, 2021. At the bottom, the chronological milestone line of NMR events is illustrated.

Book Chapter: Quantitative NMR in Qulality Control in Quality Control of Chinese Medicines-Strategies and Methods

Example: USP Reference Spectrum

Krill Oil

- **Analysis:**

Acquire the data outlined in *Data collection*. Minimally acquire the ^{1}H spectrum (fingerprint) of the *Sample solution* and the *Standard solution* as well as the quantitative ^{31}P spectrum of the *Sample solution* and the *Standard solution*. Record the resulting spectra and perform integration by hand or automated means on the quantitative ^{31}P NMR spectrum of the *Sample solution*. Integration of the peaks contained in the spectrum of the *Sample solution* must be performed such that the complete set of phospholipid peaks (as identified by comparison to the spectrum of the *Standard solution* and its **reference spectrum**) is included in the integration. The integration region for each signal must extend ±0.05 ppm on either side of the ^{31}P signal. Quantify the total phospholipids present, the phosphatidylcholine ether content, and the phosphatidylcholine content in the *Sample solution* using comparison to the concentration of the *Internal standard*. Compare the ^{1}H spectrum of the *Sample solution* to that of the *Standard solution* to determine the similarity of fingerprints according to which phospholipids identified in the **reference spectrum** of the *Standard solution* are present in the spectrum of the *Sample solution*.

Example: Reference Spectrum

European Directorate for the Quality of Medicines & HealthCare

Ph. Eur. Refer

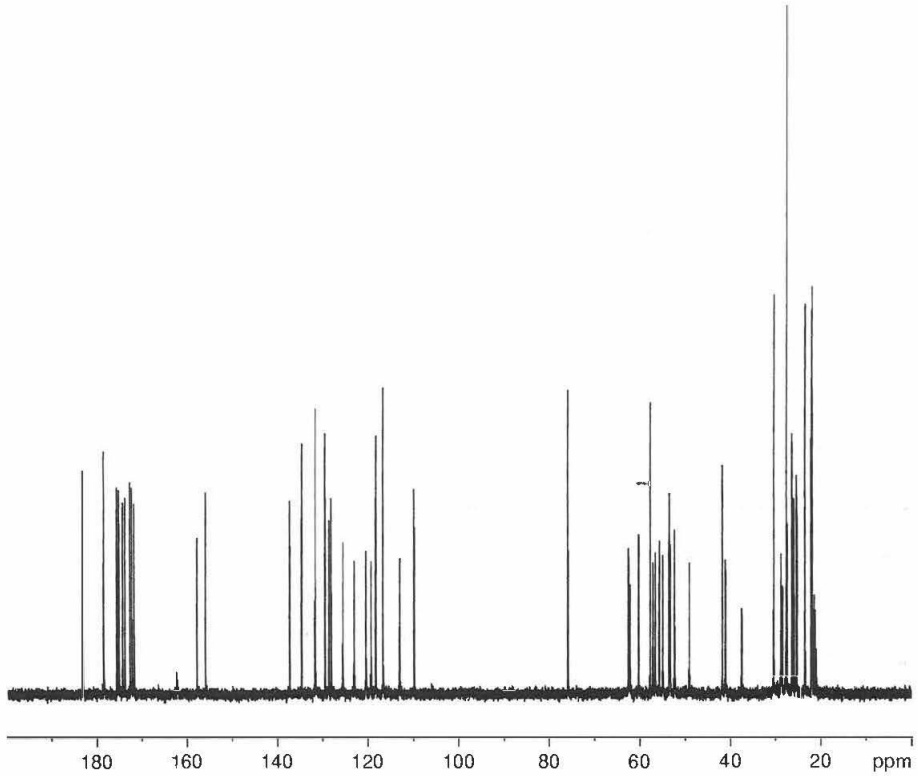
GOSEREL

Intended use

This official Reference Spectrum is to be used with the currently v
describing its suitable use.

Further information about the Reference Standards is available in the on-line catalogue

Catalogue code: Y0000126 Revision 01 Date of issue:



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FIDRES 0.376760 Hz
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DE 6.00 usec
TE 298.0 K
D12 0.00002000 sec
PL13 19.00 dB
D1 0.69999999 sec
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PCPD2 90.00 usec
SFO2 400.1316005 MHz
NUC2 1H
PL2 –3.00 dB
PL12 18.00 dB
P1 7.70 usec
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LB 1.00 Hz
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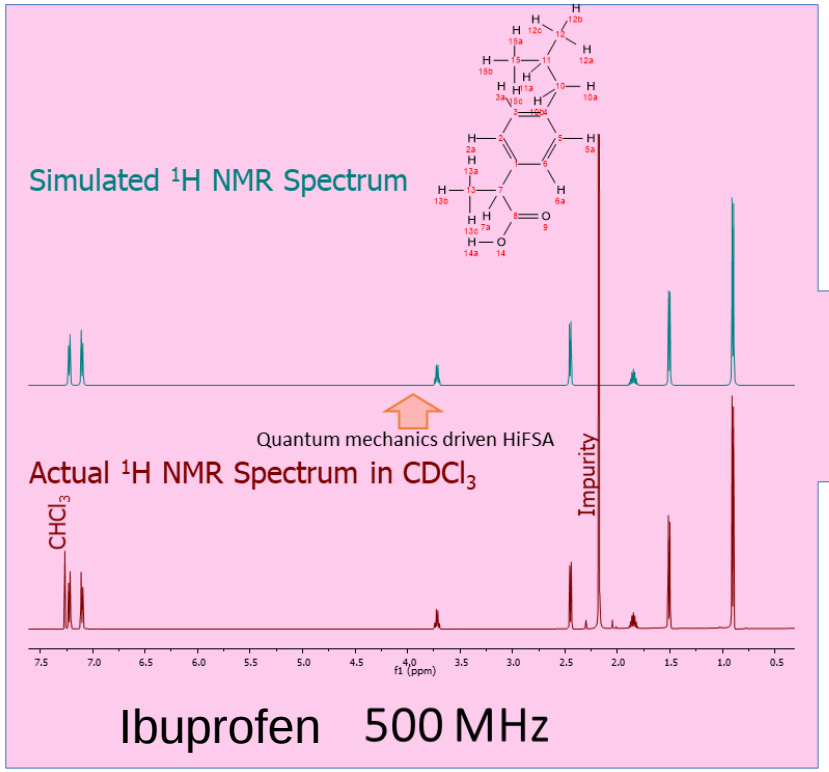
Catalogue code: Y0000126 Revision 01 Date of issue: 11/12/2007 Page 2/2

Example: Digitized Reference Spectrum

Quantum Mechanics Spectral Analysis (QMSA)

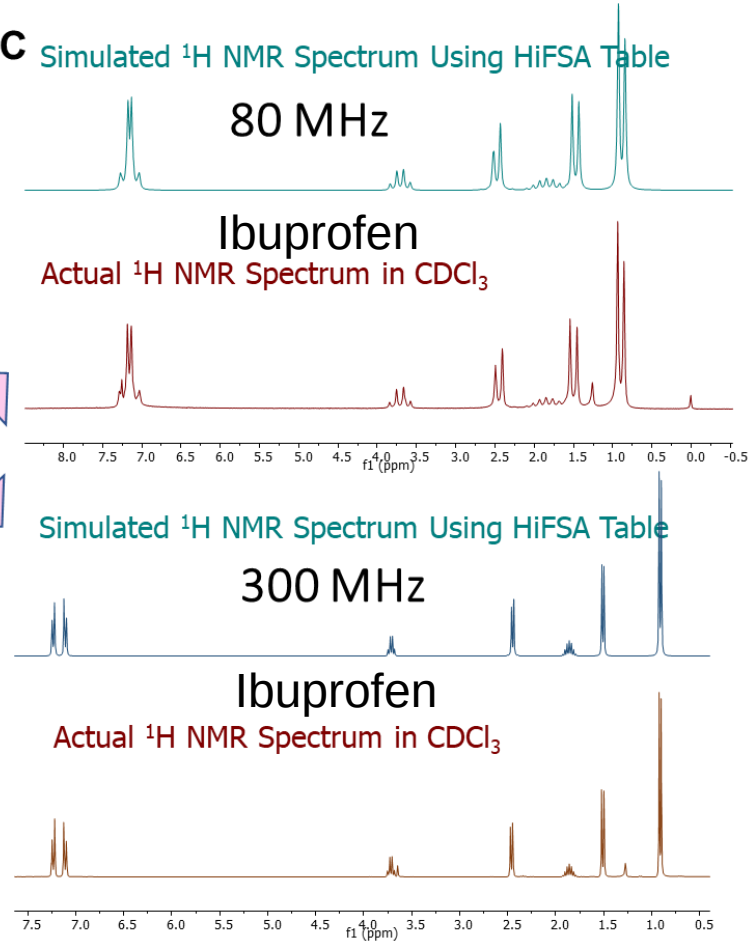
A



B

Atom	Shift (ppm)	J (Hz)	
2a CH	7.25	J(2a-3a)	7.50
		J(2a-5a)	0.50
		J(2a-6a)	1.50
		J(2a-7a)	0.62
3a CH	7.19	J(3a-5a)	1.50
		J(3a-2a)	7.50
		J(3a-6a)	0.50
5a CH	7.19	J(5a-3a)	1.50
		J(5a-2a)	0.50
		J(5a-6a)	7.50
6a CH	7.25	J(6a-3a)	0.50
		J(6a-5a)	7.50
		J(6a-2a)	1.50
		J(6a-7a)	0.62
7a CH	3.68	J(7a-13a,13b,13c)	6.80
		J(7a-2a)	0.62
		J(7a-6a)	0.62
10a CH2	2.49	J(10a-10b)	-12.40
		J(10a-11a)	7.00
10b CH2	2.44	J(10b-10a)	-12.40
		J(10b-11a)	7.00
11a CH	1.84	J(11a-12a,12b,12c)	6.80
		J(11a-15a,15b,15c)	6.80
		J(11a-10a)	7.00
		J(11a-10b)	7.00
12a,12b,12c CH3	0.87	J(12a,12b,12c)	6.98
		J(12a,12b,12c-11a)	6.80
13a,13b,13c CH3	1.42	J(13a,13b,13c)	7.70
		J(13a,13b,13c-7a)	6.80
15a,15b,15c CH3	0.87	J(15a,15b,15c)	6.98
		J(15a,15b,15c-11a)	6.80

C



Quantum Mechanics Spectral Analysis (A and B - QMSA)

Resonance frequency (Hz) = δ (ppm) × spectroscopy frequency [MHz] (B and C, Data Analysis)

A Digital Platform for ^{1}H NMR Data Analysis

Three pillars in the digital platform

- Standardized qNMR procedure

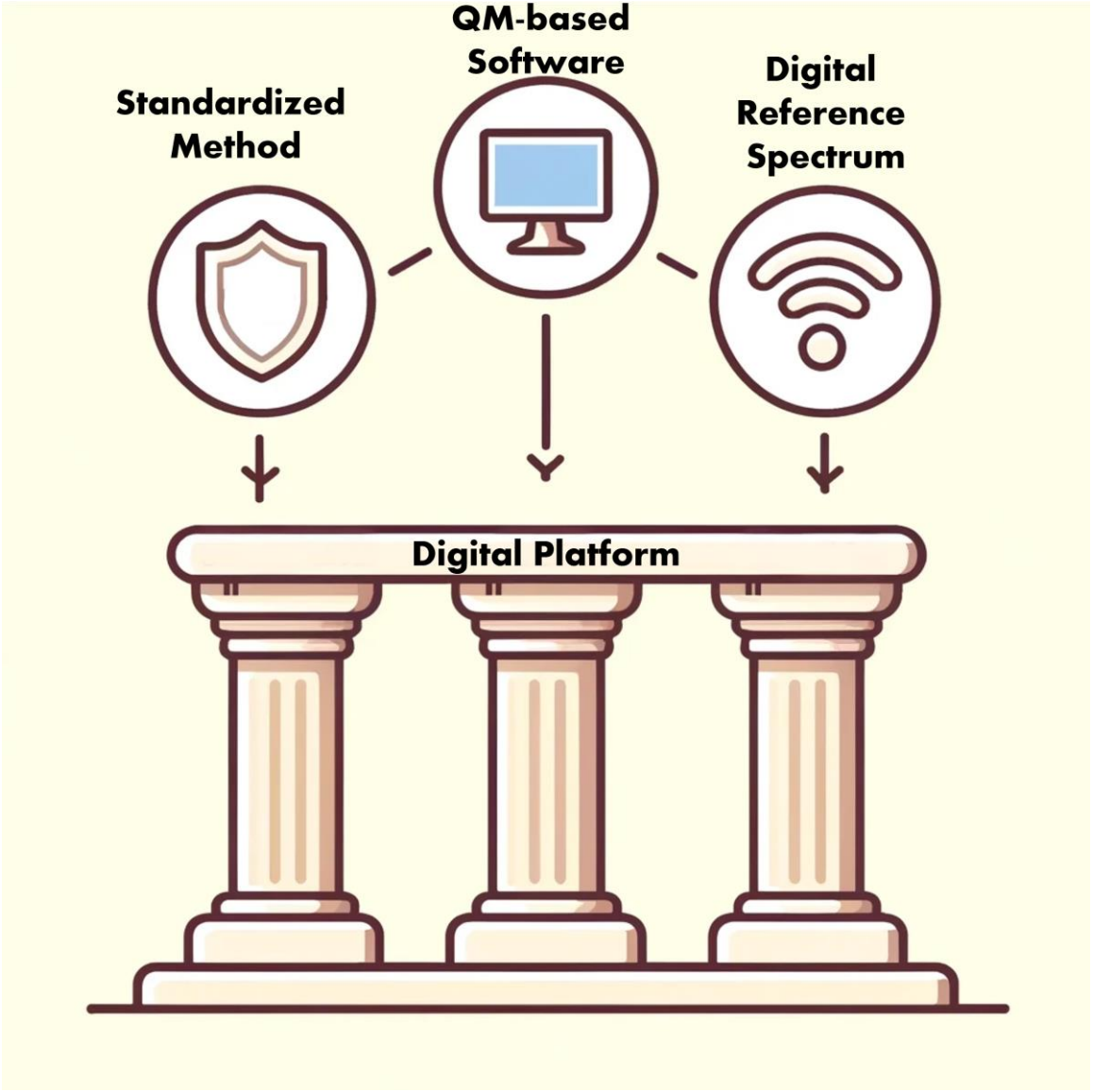
- sample preparation, data acquisition and analysis, experimental spectrum

- A database (or Spectral Library)

- retrieving relevant *digital Reference Spectrum* in the spectral library

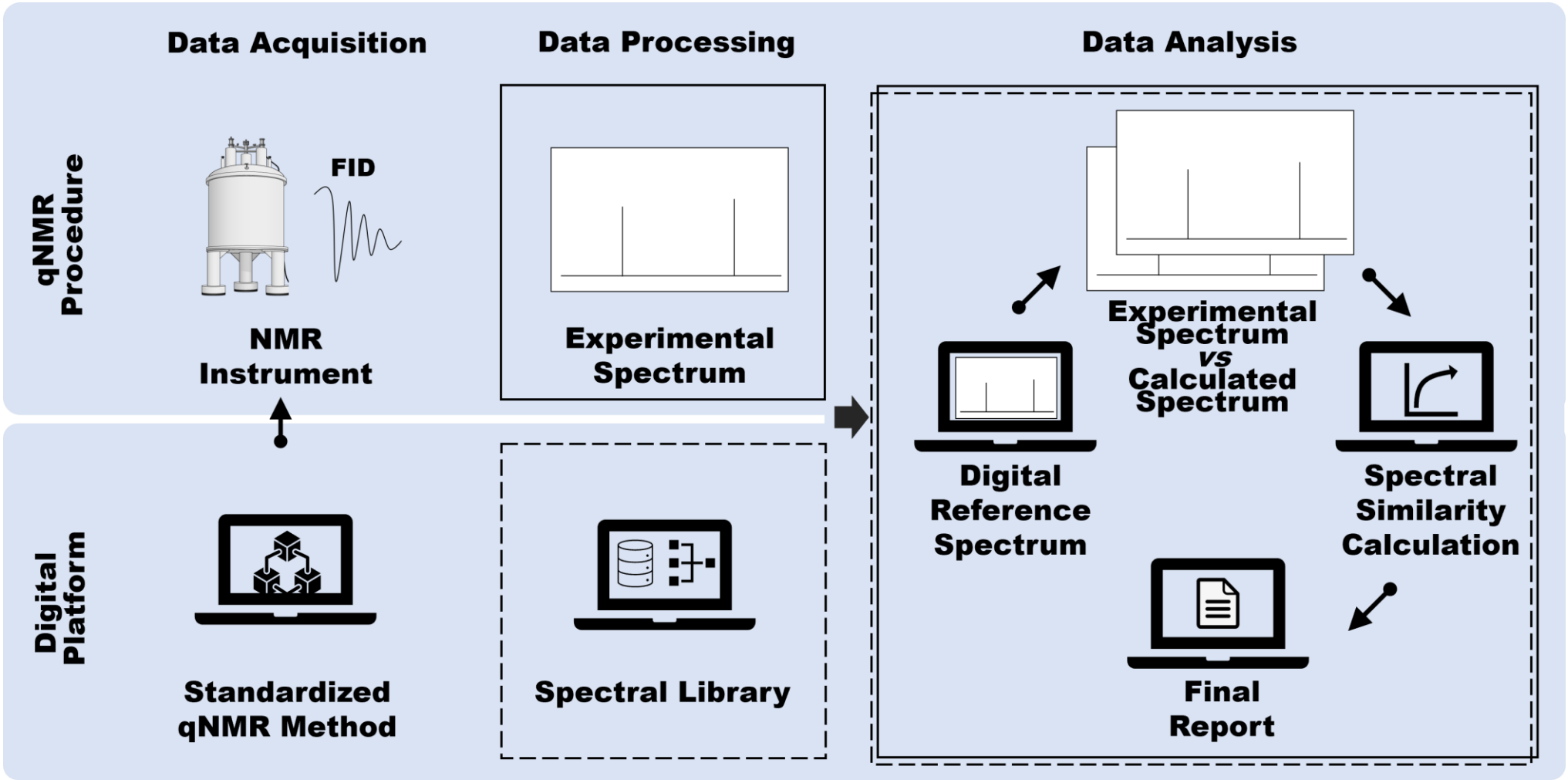
- QM-based software as an interactive interface

- displaying comparisons between experimental and calculated spectra



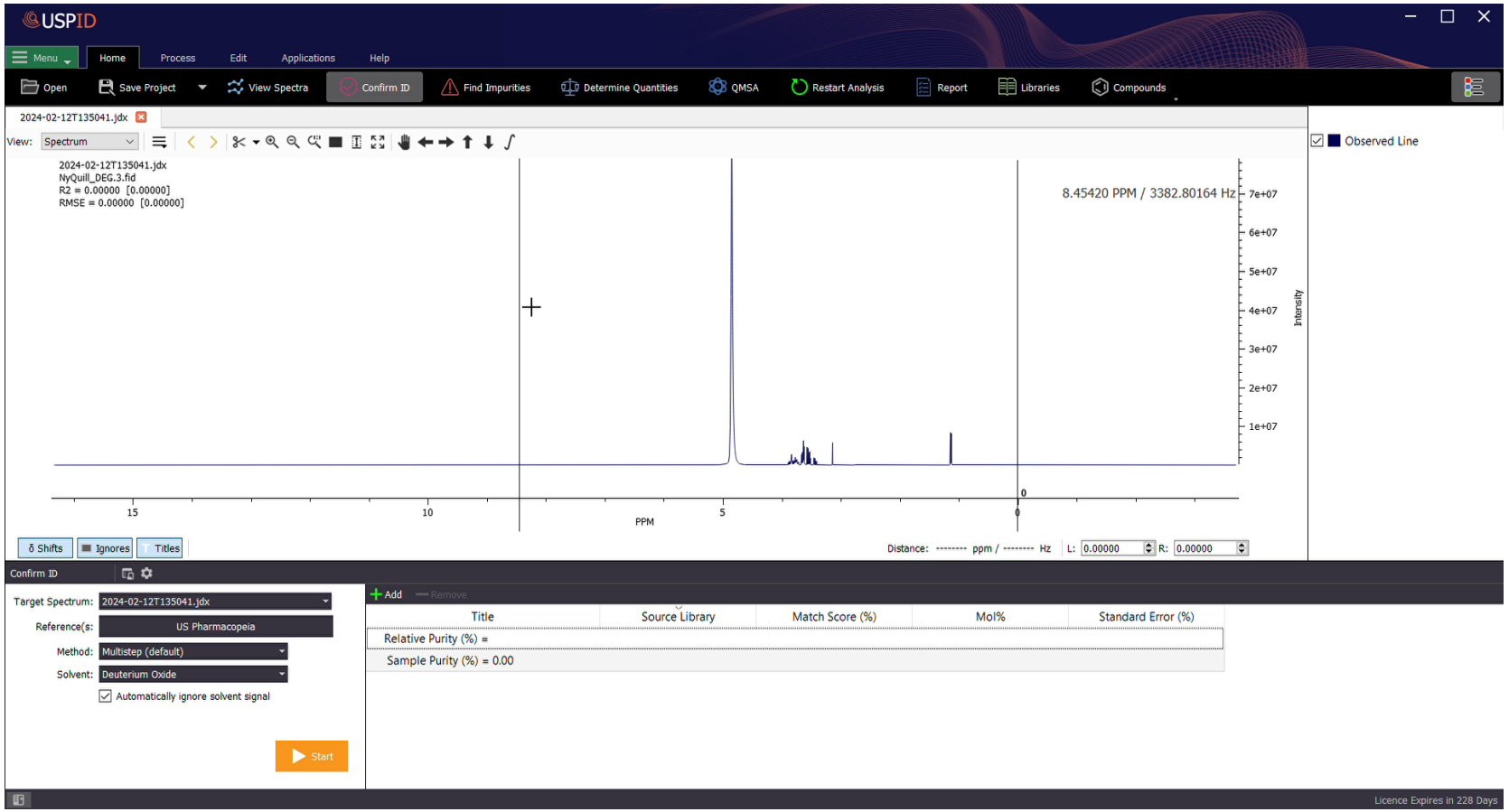
Design of Digital Platform

Workstream and three pillars in the digital platform



Case study of qualitative analysis

Diethylene glycol in Syrup Sample



Case study of Quantitative Analysis

Ascorbic acid – Repeatability Test and Linear Test

- In a repeatability test, six solutions of the test item will be prepared and measured in quick succession. Approx. 30 mg of ascorbic acid and 10 mg of the internal standard $DMSO_2$ were dissolved in 0.8 mL D_2O. The results, in terms of mass fraction (content, %), are provided.
- To assess the data analyzed by two different software, i.e., Topspin and digital platform software, both a t-test and an F-test were conducted, the outcomes of which are detailed. The Topspin represents traditional integral methodology; and the digital platform software demonstrates automatic QMSA approach.

Test item	content (%) Topspin	content (%) Digital Platform
A	99.15	99.11
B	99.62	99.73
C	99.60	100.07
D	99.56	100.12
E	99.50	99.59
F	99.26	99.39

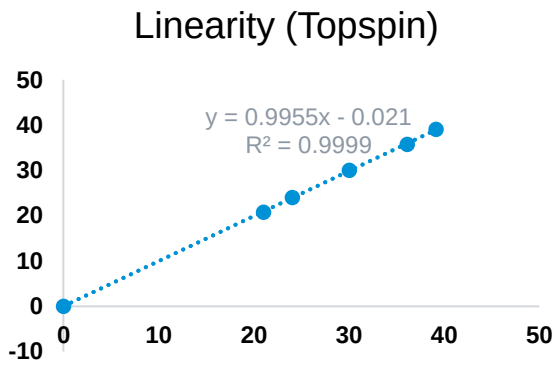
Case study of quantitative analysis

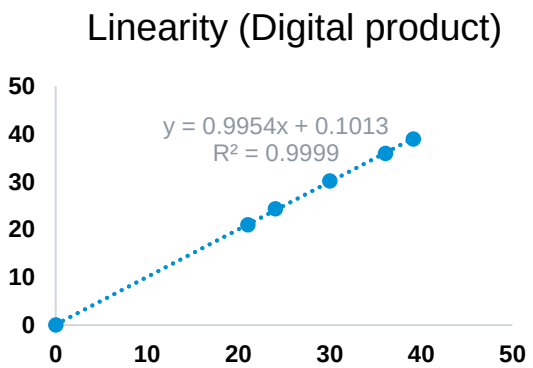
Ascorbic acid – Repeatability Test and Linear Test

Variability Analysis

	Topspin	Digital product
Mean	99.45	99.67
Variance	0.038	0.153
Observations	6	6
t-Test Results		
Statistic	-1.2320	
p-value	0.2461	
F-Test Results		
Statistic	0.2513	
p-value	0.0779	

Linearity results

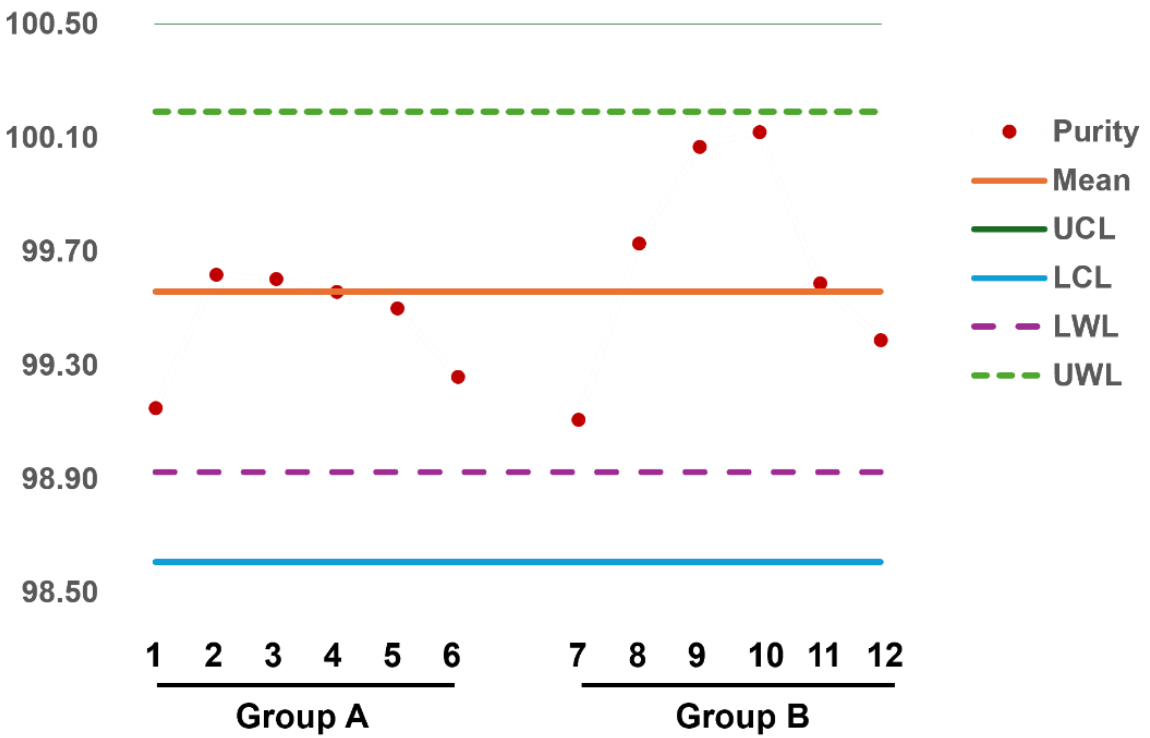




Case study of quantitative analysis

Ascorbic acid as an example

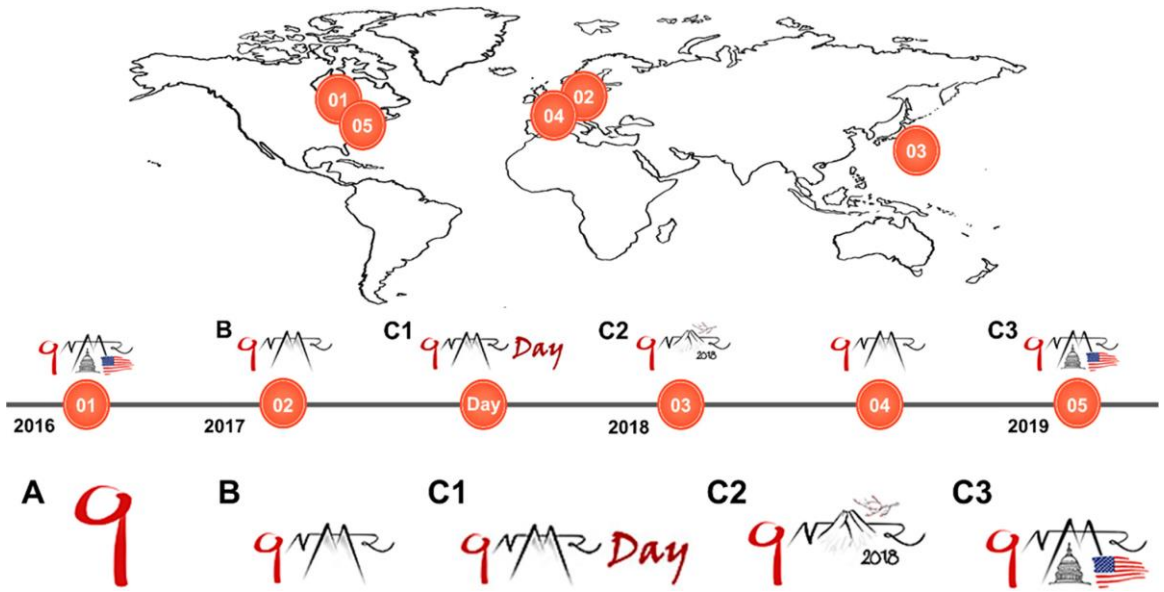
Control Chart



- All data were within the warning level and therefore accepted.
- The results obtained using the digital product got a mean value that were closer to the reference value of 99.7%.
- This includes continued algorithm updates and improvements in compatibility with computer systems.
- This case study represents initial step in using the control chart. The control chart will be used to monitor the digital product procedure performance, providing rules commonly used to make decisions.

Cohost qNMR activities

An example: qNMR summits 1.0-6.0



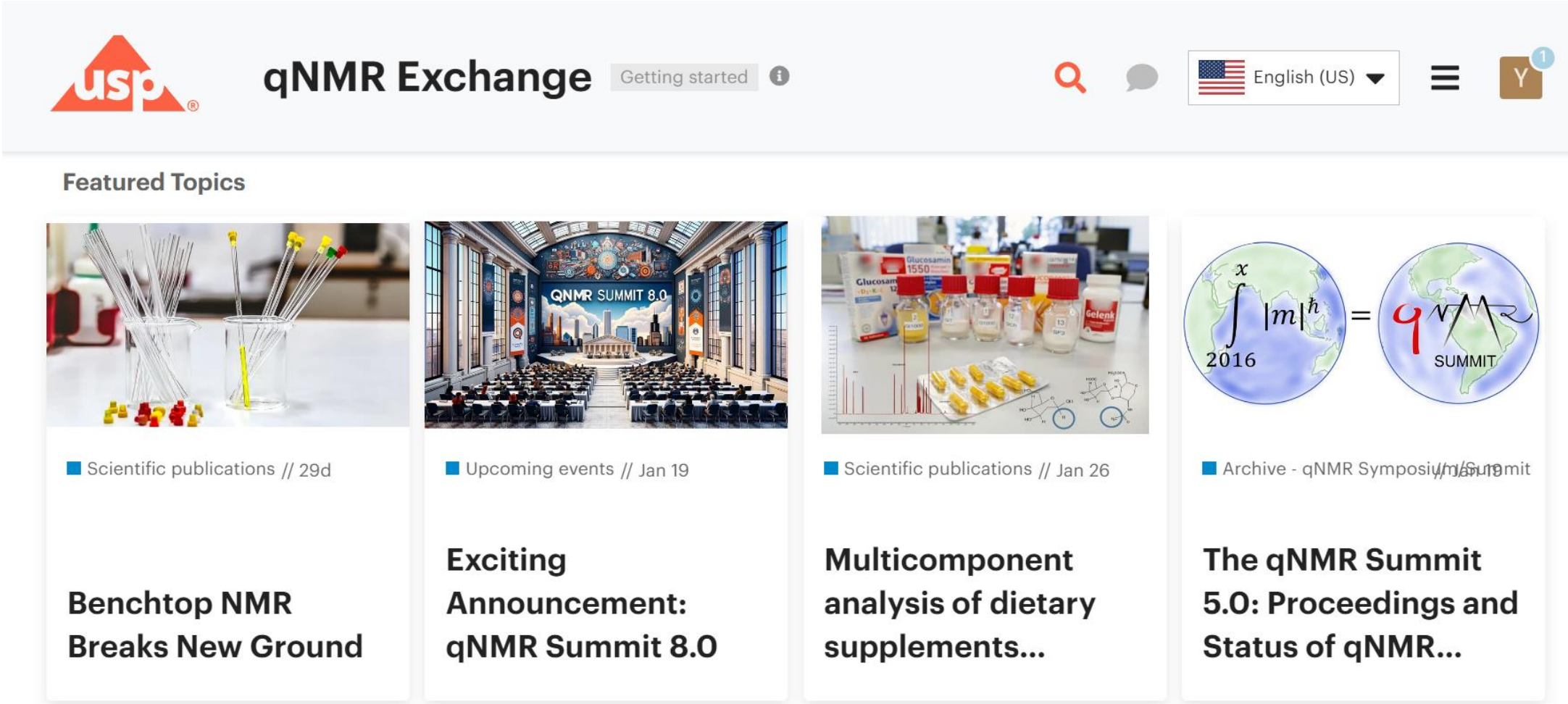
(A) The q artwork and (B) the qNMR Summit logo, as well as (C) the individual qNMR Summit logos of the 2016 qNMR Day in Bari, Italy (panel (C1)), the 2017 qNMR Summit 3.0 in Tokyo, Japan (panel (C2)), and the qNMR Summits 1.0 and 5.0 in Rockville, MD, USA (panel (C3)).

qNMR Summit	date	hosts[a]	location
1.0	October 2016	USP and CENAPT	Rockville, MD, USA
2.0	March 2017	BAM	Berlin, Germany
qNMR Day	November 2017	GIDRM	Bari, Italy
3.0	January 2018	METI	Tokyo, Japan
4.0	October 2018	University of Würzburg	Würzburg, Germany
5.0	October 2019	USP and CENAPT	Rockville, MD, USA
6.0	October 5–7, 2021	USP and CENAPT	virtual event
7.0	August 15,16, 2024	USP, CENAPT and Dominican U.	Chicago, IL, USA

[a]CENAPT, Center for Natural Product Technologies at UIC; BAM, Federal Institute for Materials Research and Testing; GIDRM, Gruppo Italiano Discussione Risonanze Mognetiche; METI, Ministry of Economy, Trade and Industry.

Engagement and Education for Scientific Community

https://qnmr.usp.org/



Expert Volunteers help power USP's impact on global public health

Serving on Expert Committees, Panels and Sub-Committees, they collaborate to develop quality standards and other solutions that help build a more resilient supply of quality medicines.

Apply and amplify your impact

Questions

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The standard of trust