

Benchtop NMR Spectroscopy for Surfactant Quantification in Biologics

IFPAC 2025 - Case Examples of PAT - Real Time Release in Production & Rapid Testing

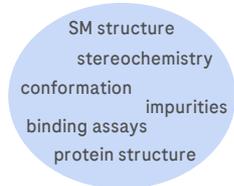
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Introduction to NMR in pharmaceutical industry

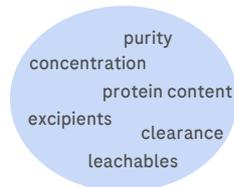
- NMR spectroscopy is a versatile tool commonly used in the pharma environment
 - Widely used in **R&D**: Structure elucidation, quantitative analysis, drug development for both **small and large molecules**
 - QC (mainly synthetic molecules): batch-to-batch consistency, purity and identity verification, integrity of biologics
 - So far very **limited use in GMP** environments due to complexity of operation and costs

- Paramount advantages of NMR spectroscopy
 - Absolute **quantification** through its inherent primary ratio characteristic
 - Detailed structural elucidation capabilities
 - Direct correlation of peaks to chemical structure
 - **Mixture analysis** possible (without chromatography)

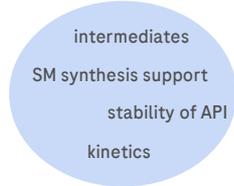
Structure elucidation



Quantitative NMR



Drug development and process optimization



Quality control



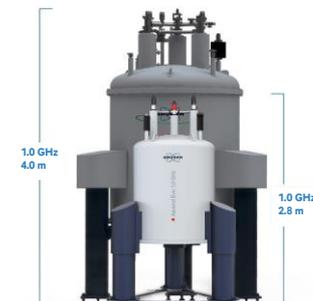
Benchtop NMR as an emerging tool for QC applications

- Benchtop NMR technology
 - Fits on a lab bench, small stray field. Possible on-site tool.
 - Much lower capital and maintenance costs (permanent magnet, no cryogenics needed)
 - Lower spectral resolution and lower sensitivity
 - Suitable for excipients and higher concentrations

- Recent advances in benchtop NMR instruments allow NMR technology to be **accessible for pharma QC**
 - Significant improvements in hardware (magnet design, RF coils) to enhance sensitivity and resolution
 - Easy sample preparation: e.g. no deuterated solvent needed
 - Simple-to-use software for end-users (no need to be an expert)
 - GMP compliance with end-to-end automation



Benchtop NMR spectrometer



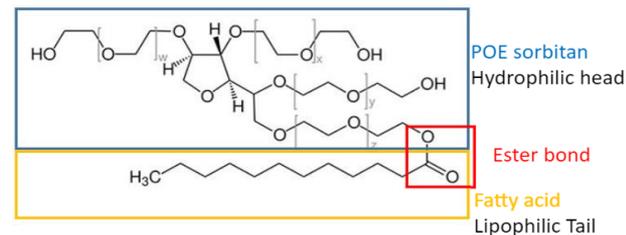
High-field NMR spectrometer

Benchtop NMR Use Case - Surfactant quantification

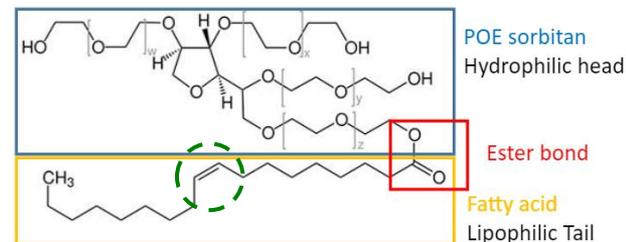
- Surfactants protect and stabilize proteins against interfacial stresses in biologic formulations
- **Surfactant content is a CQA** of the products
 - IPC: for conditioning buffer
 - QC: DS and DP release and stability testing
- Current method: HPLC-ELSD/CAD
 - Well-established gold standard for surfactant testing
 - Precision range of 1-7% RSD (intermediate: 3-7%)
 - Systematic multi-point calibration
 - Time and labor intensive preparation steps
 - Complex detector needed (ELSD/CAD)
- Challenges of surfactant quantification
 - Heterogeneous compounds with lot-to-lot variability
 - Low amounts in complex matrices with high concentrations of large molecules and other excipients
 - Stability of compounds

Commonly used surfactants for biologics

Polysorbate 20 (PS20)

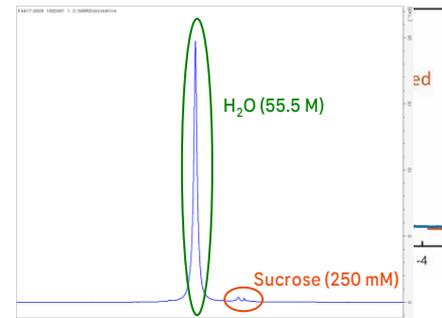


Polysorbate 80 (PS80)

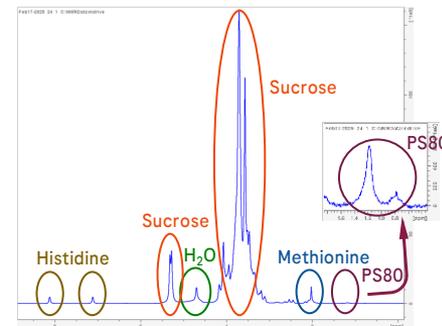


Benchtop ^1H qNMR method development for biologics

- Technical aspects
 - Protein suppression with CPMG filter in the pulse sequence
 - Solvent suppression (biologics are aqueous solutions)
 - External referencing with dimethyl sulfone (DMS) for single-point calibration
 - System - Suitability Test (SST) results simultaneously deduced from DMS measurement
 - Simple method transfer
- User-friendliness
 - Simple sample preparation - NMR tube filled with 500 μL sample.
 - Calibration is done automatically
 - Final output: concentration / result in a report
 - Suitable tool for at-line testing
- GMP qualification and Computer System Validation of NMR instruments



removes Mab signals to yield a clean baseline

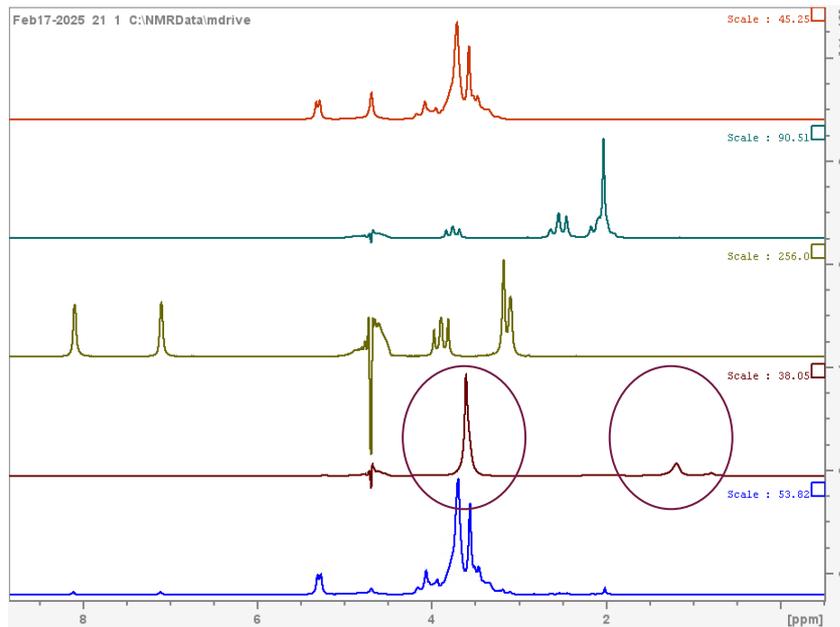


Results

Analytical performance according to requirements in ICH Q2

Specificity

- Selection of a representative signal of the analyte that does not show interference



Sucrose

Methionine

Histidine

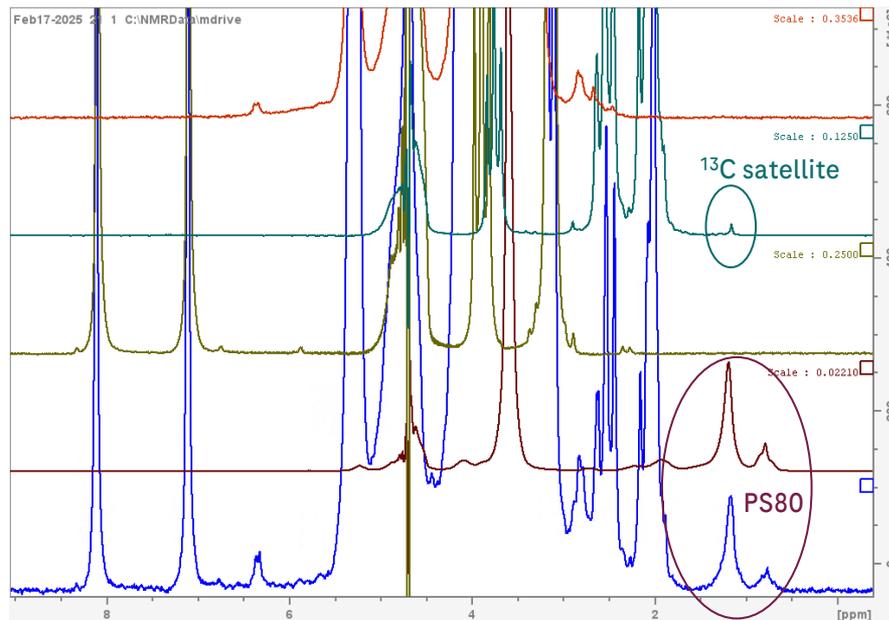
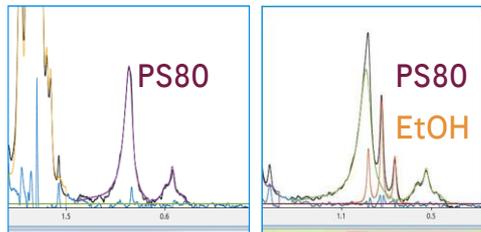
PS80

Complete
formulation

Analytical performance according to requirements in ICH Q2

Specificity

- Selection of a representative signal of the analyte that does not show interference
- Acquisition control: ^{13}C decoupling to remove satellite signals
- The Bruker Advanced Chemical Profiling (ACP) tool allows deconvolution of overlapping signals during automated processing:



Sucrose

Methionine

Histidine

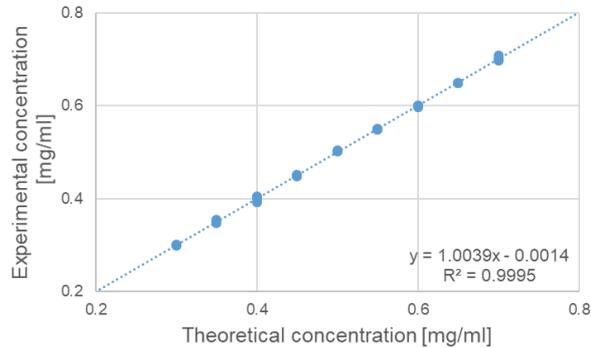
PS80

Complete formulation

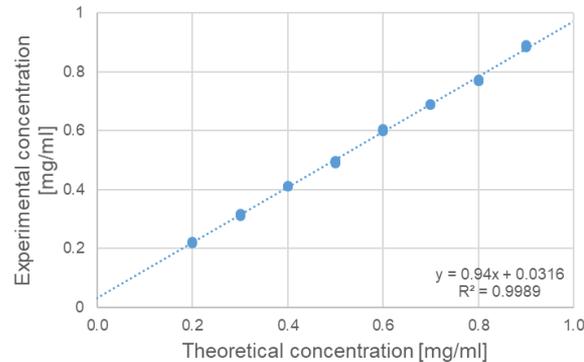
Analytical performance according to requirements in ICH Q2

Linearity and Accuracy

Linearity of PS20 content in a model formulation containing excipients and Mab



Linearity of PS80 content in a model formulation containing excipients and Mab



Example for PS80 quantification at 8 levels: accuracy

Concentration from preparation [mg/ml]	Experimental concentration from triplicates [mg/ml]	Recovery (%)
0.2	0.220	110.2
0.3	0.314	104.7
0.4	0.411	102.7
0.5	0.495	98.9
0.6	0.601	100.2
0.7	0.689	98.4
0.8	0.771	96.4
0.9	0.887	98.6

- With the current setup of 20 mins we are approaching LOQ with 0.2 mg/ml
- Possibility to increase sensitivity: number of scans / longer experiments

Analytical performance according to requirements in ICH Q2

Precision by repeatability

- Fewer variable factors contributing to method precision compared to HPLC

- Repeatability at different levels
 - NMR: RSD is **1.0% or less** for levels above 0.3 mg/ml
 - max RSD for all levels
 - NMR: 1.5 - 3.5%
 - Improves with longer measurement
 - HPLC: 1.0-7.0%

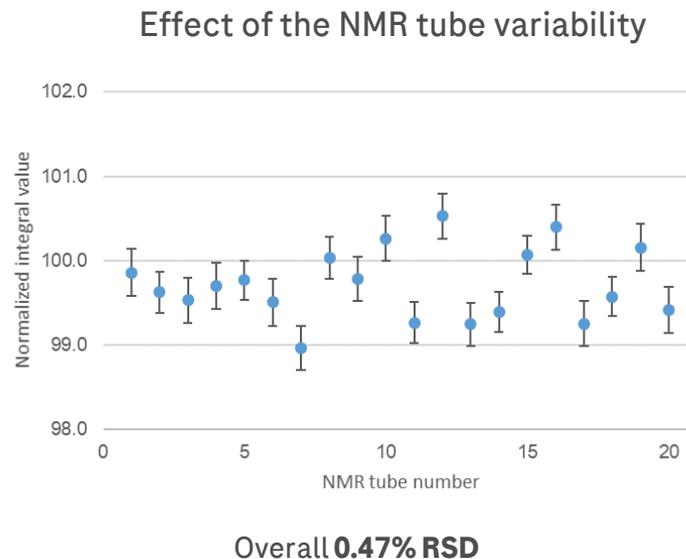
Example for PS80 quantification at 8 levels: precision

Concentration from preparation [mg/ml]	Experimental concentration from triplicates [mg/ml]	RSD [%] from triplicates
0.2	0.220	1.6
0.3	0.314	1.4
0.4	0.411	0.7
0.5	0.495	1.0
0.6	0.601	0.7
0.7	0.689	0.1
0.8	0.771	0.5
0.9	0.887	0.6

Analytical performance according to requirements in ICH Q2

Robustness

- No major factors affecting the method performance were found so far
- Influence of analyst is negligible
 - Simple sample preparation
 - Automated analysis
- External standard method: NMR tube quality is a key factor
 - Acceptable contribution to variability
- Regular automated shimming in place to maintain homogeneity of magnetic field
 - SST tests are performed to verify lineshape and magnetic field homogeneity

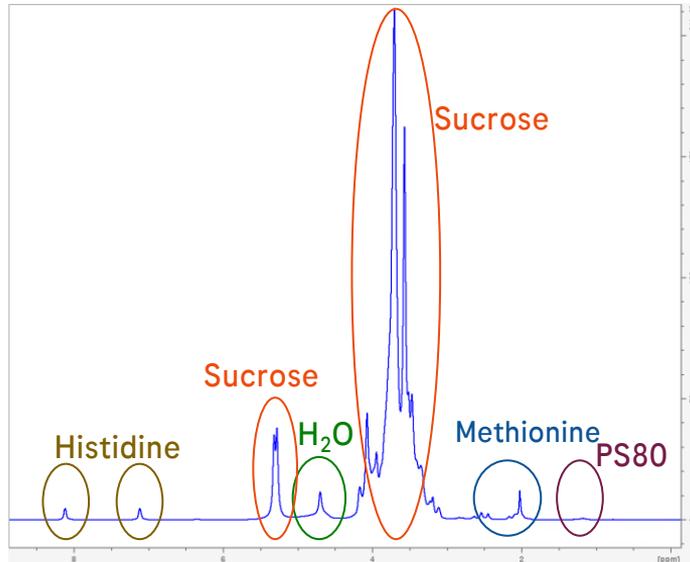


Outlook I

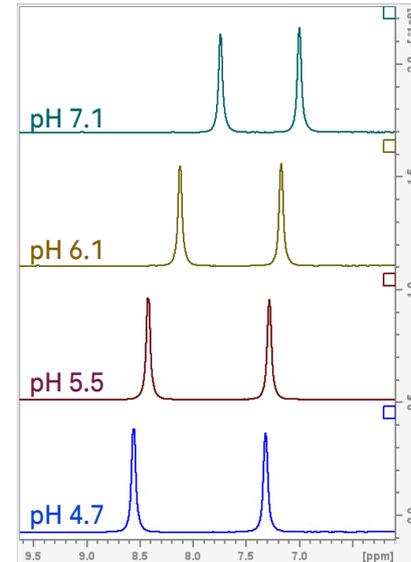
NMR as a potential Multi Attribute Method

Simultaneous quantification of **other excipients**

Osmolality: estimation from sucrose and other formulation components



pH sensitive signal of the Histidine buffering agent: pH indicator and part of the formulation

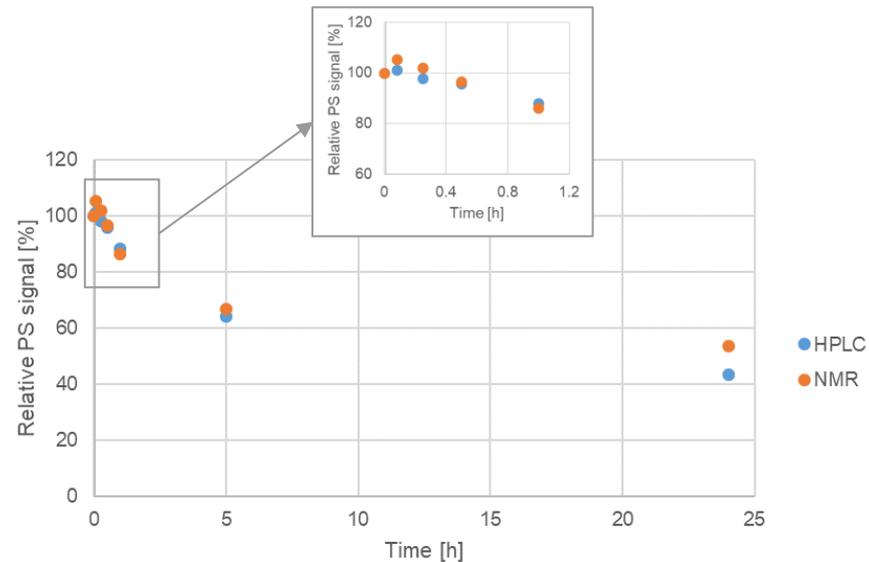


Outlook II

Excipient degradation monitoring in stability studies

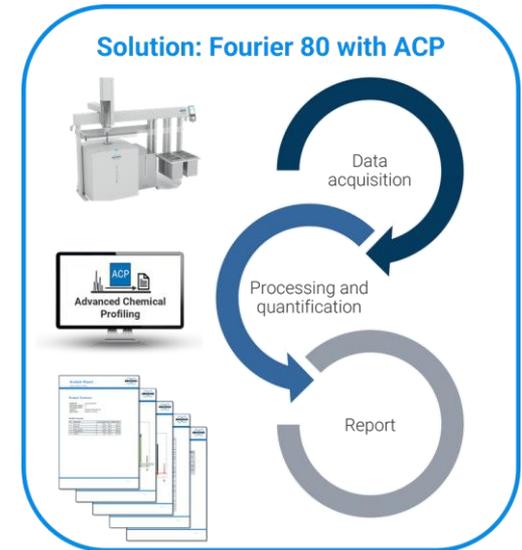
- Surfactant degradation is a potential risk in product stability and safety
- Monitoring of the degradation of excipients in stability studies is currently performed by HPLC
- Benchtop NMR was tested and compared to HPLC to follow **enzymatic degradation** of PS20
- NMR is able to monitor surfactant stability similarly to HPLC for ester hydrolysis

Enzymatic degradation of PS20 in a model formulation



Summary

- The benchtop NMR method offers an effective approach for the quantification of excipients in biologic drugs
- The method allows full automation and minimal end-user interaction
- Method performance for surfactant quantification in line with current industry standards and internal specifications
 - Initial results suggest even superior precision
- Potential multi attribute method with ability to detect surfactant stability issues



Authors and Acknowledgements

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Tomasz Baginski

Bruker BioSpin

Valentin Poirier



Doing now what patients need next

Doing now what patients need next

Analytical performance according to requirements in ICH Q2

Precision

Repeatability at one level

- Calculated from 6 single independent measurement
- RSD **0.9 - 1.4 %** for PS20 or PS80 in different commercial samples
- Not tested for HPLC

Intermediate precision

- **1.1%** for PS80 0.5 mg/ml in model formulation from **single measurements**
- HPLC: intermediate precision range for excipients of **3-7%** from duplicates (in line with observations of industry peers)

Kojima design for HPLC intermediate precision

Test	Instrument	Analyst	Column
1	A	a	α
2	A	b	α
3	A	a	β
4	B	b	α
5	B	a	β
6	B	b	β

Kojima design for NMR intermediate precision

Test	Instrument	NMR tube	Day	Value
1	A	a	α	0.05124
2	A	b	α	0.04987
3	A	a	β	0.05125
4	B	b	α	0.05022
5	B	a	β	0.05089
6	B	b	β	0.05085