EURACHEM PT WORKSHOP
PORTOROŽ - 12.10.2017

NOVEL ILC FOR ^{1}H, ^{31}P AND ^{19}F QNMR

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R&D Reference Materials
Sigma-Aldrich Production GmbH, Buchs SG, Switzerland

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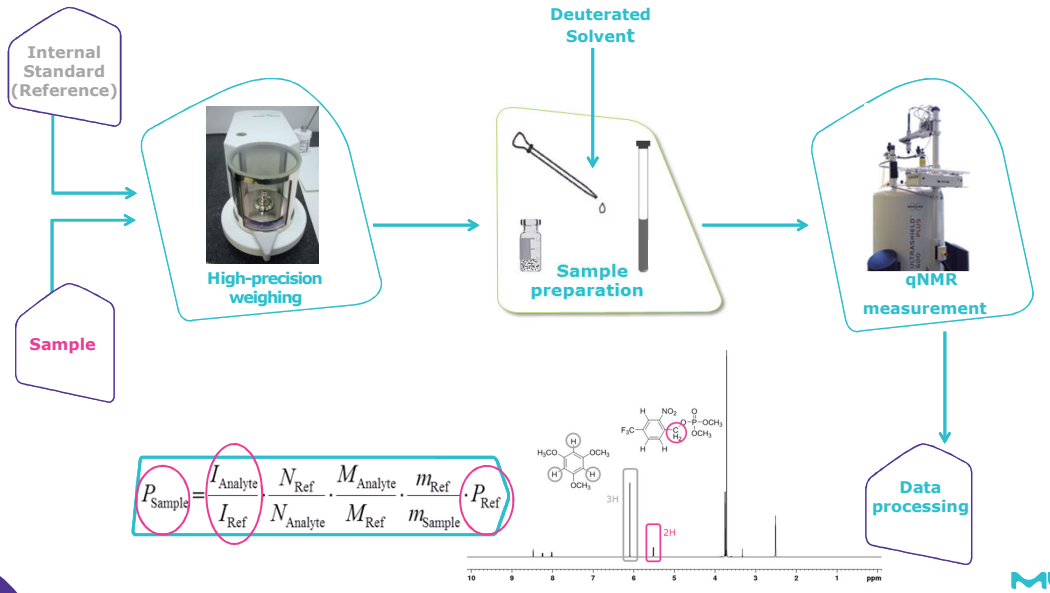
quantitative NMR
Accredited qNMR Certification @ Merck



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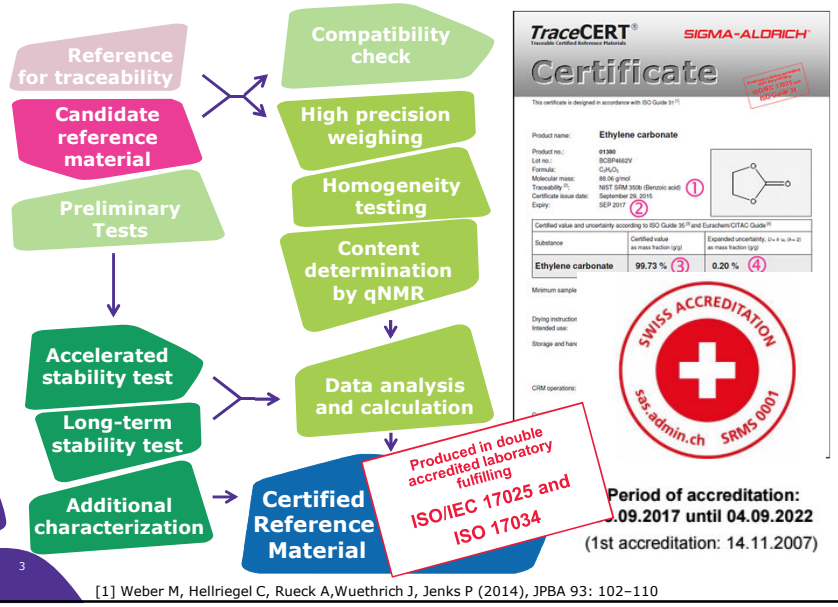
quantitative NMR
Accredited qNMR Certification @ Merck



Period of accreditation:
.09.2017 until 04.09.2022
(1st accreditation: 14.11.2007)

Certificate
(1) Traceability Statement
(2) Expiration Date
(3) Certified Value (g/g)
(4) Expanded Uncertainty (g/g)
(5) Signatures and Accreditation

Certified Reference Materials for…
- qNMR Analysis
- Method Validation
- Precision Control
- Bias Control
- Calibration

….

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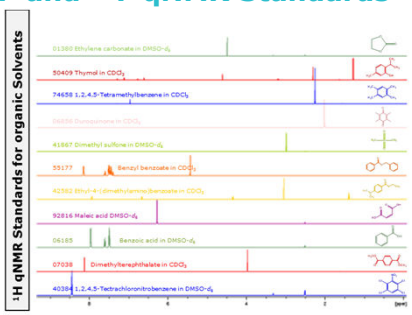
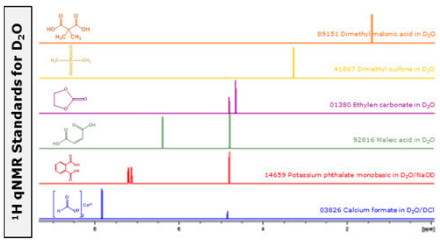
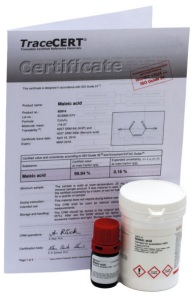
[1] Weber M, Hellriegel C, Rueck A,Wuethrich J, Jenks P (2014), JPBA 93: 102–110

certified reference materials
^{1}H, ^{31}P and ^{19}F qNMR Standards

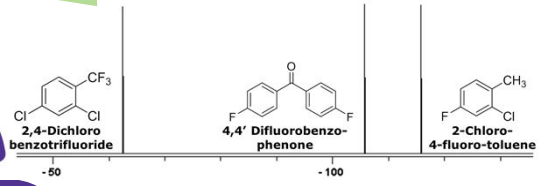
^{1}H



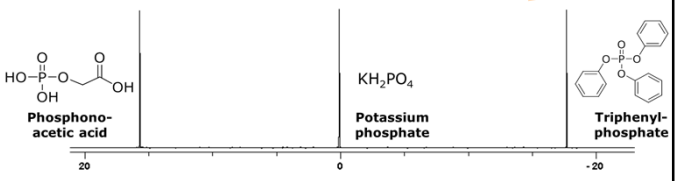




^{19}F



^{31}P



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certified reference materials
^{1}H, ^{31}P and ^{19}F qNMR Interlaboratory Comparison

- Few NMR/qNMR proficiency testing/Interlaboratory comparison schemes available
- Key comparisons from CCQM for metrological institutes (^{1}H)
- ^{31}P, ^{19}F schemes and multi nuclei molecule scheme not available so far
- Need for interlaboratory comparisons for laboratories accredited to ISO/IEC 17025

- ✓ 18 Participants (NMIs and commercial participants)
- ✓ Inclusion of ^{1}H, ^{19}F and ^{31}P series
- ✓ Possibility to take part in up to 5 different series – not mandatory to do all
- ✓ Multi nuclei analyte «FHP» can be used for ^{1}H, ^{19}F as well as ^{31}P determination → should yield similar results
- ✓ Comparable results obtained by quantitative NMR spectroscopy of ^{1}H, ^{19}F and ^{31}P atoms?
- ✓ Analytes and internal standard supplied by Merck, participants` own NMR solvents and equipment used
- ✓ First results available, further submissions ongoing

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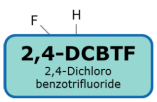
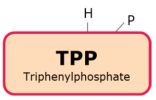
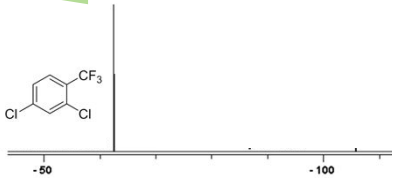
qNMR interlaboratory comparison
^{1}H, ^{31}P and ^{19}F qNMR Scheme

^{1}H

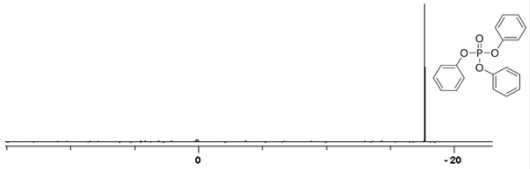




^{19}F



^{31}P



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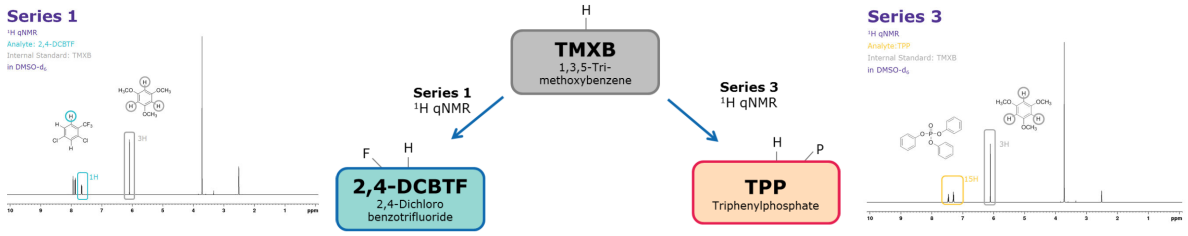
qNMR Interlaboratory Comparison
^{1}H, ^{31}P and ^{19}F qNMR Scheme



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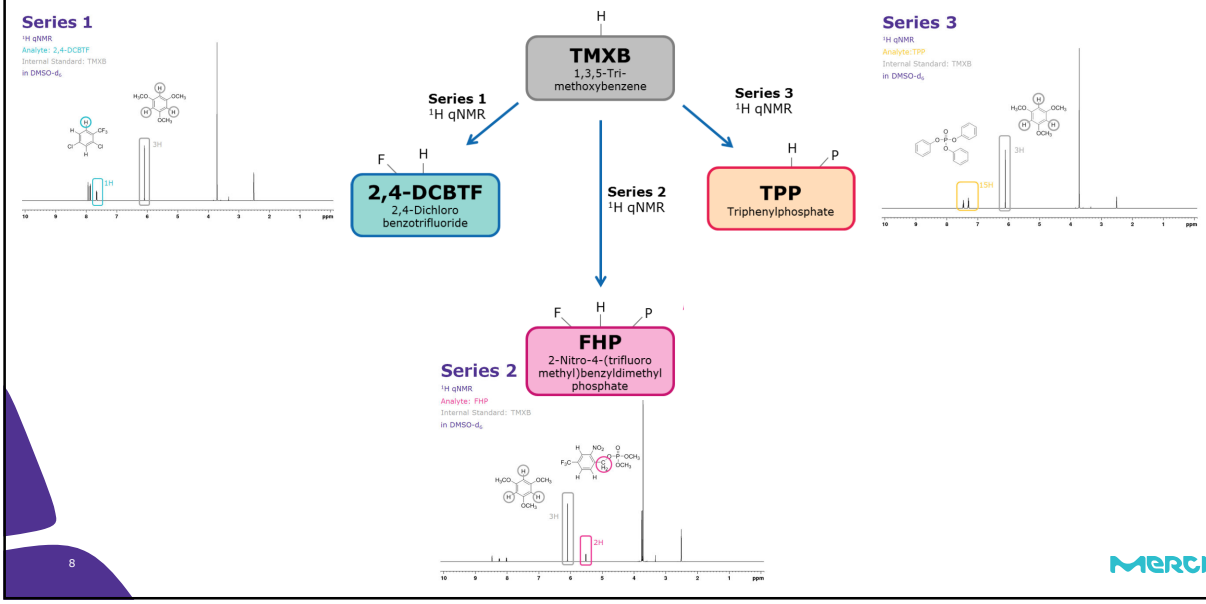
qNMR Interlaboratory Comparison
^{1}H, ^{31}P and ^{19}F qNMR Scheme



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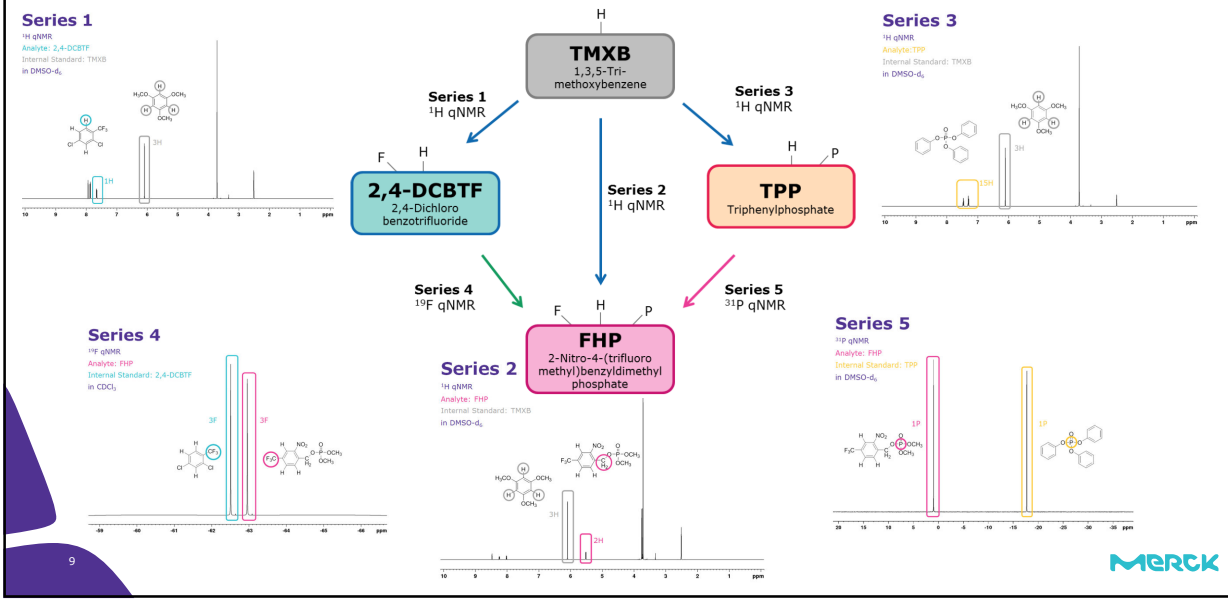
qNMR Interlaboratory Comparison

^{1}H, ^{31}P and ^{19}F qNMR Scheme



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qNMR Interlaboratory Comparison

Guidance for the Participants

Series #	Analyte	Internal Standard	Solvent	qNMR method
1	2,4-DCBTF	TMXB	DMSO-d_6	^{1}H qNMR
2	FHP	TMXB	DMSO-d_6	^{1}H qNMR
3	TPP	TMXB	DMSO-d_6	^{1}H qNMR
4	FHP	2,4-DCBTF	$CDCl_3$	^{19}F qNMR
5	FHP	TPP	DMSO-d_6	^{31}P qNMR

Table 1: Analytes, internal standards, solvents and qNMR methods for series 1-5.

Series #	ppm Signal Analyte	Number of ^{1}H/^{19}F/^{31}P atoms Analyte	ppm Signal Internal Standard	Number of ^{1}H/^{19}F/^{31}P Internal Standard
1	~ 7.7 ppm	1	~ 6.1 ppm	3
2	~ 5.5 ppm	2	~ 6.1 ppm	3
3	~ 7.1 – 7.6 ppm	15	~ 6.1 ppm	3
4	~ -63.0 ppm	3	~ -62.5 ppm	3
5	~ 0.9 ppm	1	~ -17.7 ppm	1

Table 3: Chemical shift (in ppm) and Number of ^{1}H, ^{19}F or ^{31}P atoms of signals intended for integration.

Series #	Number of samples	Repetitions per sample	mg TMXB	mg 2,4-DCBTF	mg TPP	mg FHP	Solvent µl
1	choose between 3 - 10	choose freely	Choose between 9 – 11 mg	Choose between 18 – 20 mg	-	-	DMSO-d_6 600 -1000 µl
2	choose between 3 - 10	choose freely	Choose between 8 – 10 mg	-	-	Choose between 10 – 12 mg	DMSO-d_6 600 -1000 µl
3	choose between 3 - 10	choose freely	Choose between 24 – 26 mg	-	Choose between 9 – 11 mg		DMSO-d_6 600 -1000 µl
4	choose between 3 - 10	choose freely	-	Choose between 6 – 8 mg	-	Choose between 10 – 12 mg	$CDCl_3$ 600 -1000 µl
5	choose between 3 - 10	choose freely	-	-	Choose between 9 – 11 mg	Choose between 9 – 11 mg	DMSO-d_6 600 -1000 µl

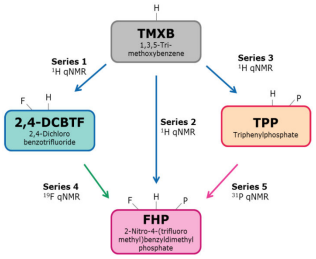
Table 2: Guideline for number of samples, repetitions per sample, amount of analyte and internal standard, solvent and solvent amount.

Additional values:

Name	MW [g/mol]	Formula	Density [kg*m^{-3}]	T1 times [s]	Purity [g/g]	U [g/g]
TMXB	168.19	$C_9H_{12}O_3$	1041	^{1}H: 2.6	99.96 %	0.12 %
2,4-DCBTF	215.00	$C_7H_3F_3Cl_2$	1453	^{1}H: 4.5 ^{19}F: 2.3	-	-
TPP	326.28	$C_{18}H_{15}O_4P$	1206	^{1}H: 3.7 ^{31}P: 1.2	-	-
FHP	329.17	$C_{10}H_{11}F_3NO_5P$	1200	^{1}H: 1.0 ^{19}F: 1.4 ^{31}P: 2.3	-	-

Table 4: Additional values for TMXB, 2,4-DCBTF, TPP and FHP. MW = Molecular weight, T1 = T1 relaxation time, Purity = certified purity value as mass fraction, U = expanded uncertainty as mass fraction (g/g) ($u_{combined}$*k (k=2), confidence level 95%, recommended minimal sample amount 10 mg).

qNMR interlaboratory comparison
Results from Pre-tests



Series #	internal standard	tested molecule	qNMR method	Mass fraction (g/g)	Expanded uncertainty as mass fraction (g/g)
1	TMXB	**DCBTF**	^{1}H qNMR	**99.56 %**	**0.16 %**
2	TMXB	**FHP**	^{1}H qNMR	**99.50 %**	**0.21 %**
3	TMXB	**TPP**	^{1}H qNMR	**99.99 %**	**0.15 %**
4	DCBTF	**FHP**	^{19}F qNMR	**99.50 %**	**0.33 %**
5	TPP	**FHP**	^{31}P qNMR	**99.51 %**	**0.27 %**

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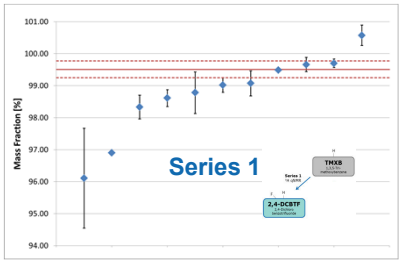
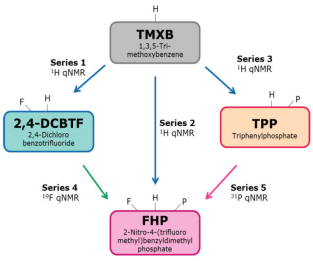
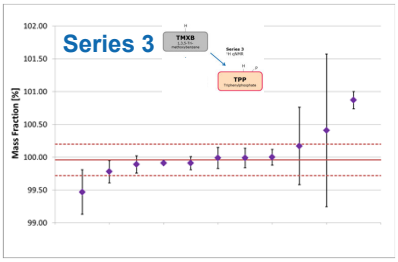
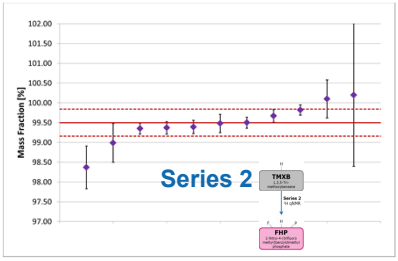
qNMR interlaboratory comparison
^{1}H qNMR Results

Red = Reference value ± uncertainty (k=2)









- Series 1 (2,4-DCBTF) = liquid CRM
- Series 2, 3 = solid CRMs
- Handling/Weighing 2,4-DCBTF difficult for some participants
- Measurement uncertainties need a closer look
- Wide distribution of values in series 1

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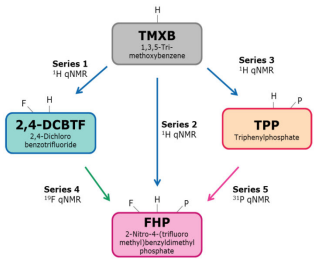
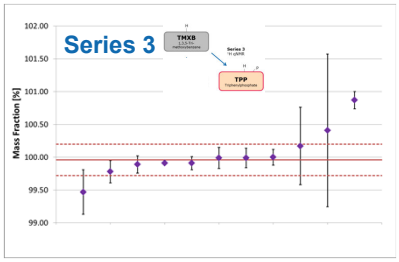
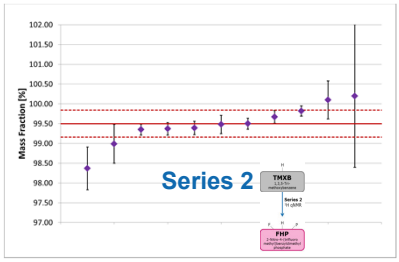
qNMR Interlaboratory Comparison
^{31}P qNMR Results

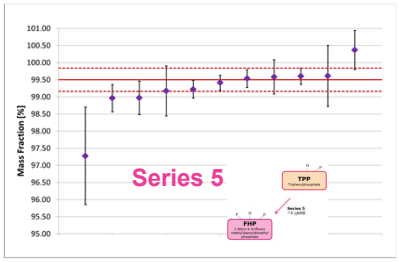
Red = Reference value ± uncertainty (k=2)





- Series 5 (FHP) = solid CRM
- Median similar to Series 2
- Measurement uncertainties need a closer look





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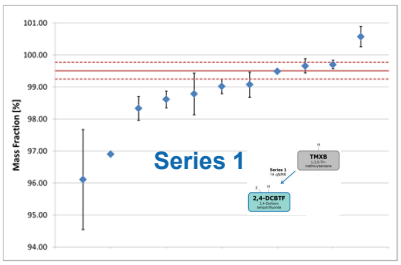
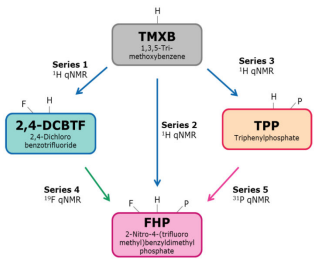
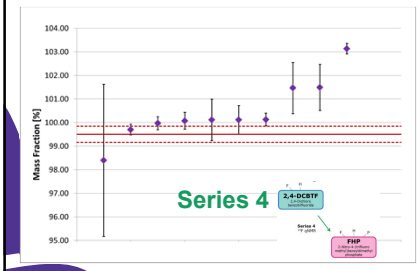
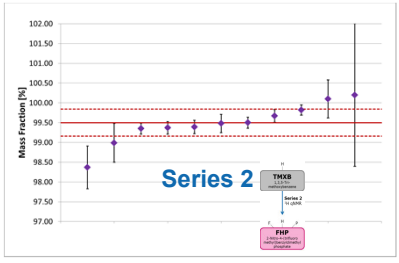
qNMR Interlaboratory Comparison
^{19}F qNMR Results

Red = Reference value ± uncertainty (k=2)









- Series 4 (FP) = solid CRM
- Results higher than from series 2
- ^{19}F measurement setup critical: spectral width, transmitter frequency offset…
- ^{19}F qNMR not well established yet in many laboratories
- Measurement uncertainties and possible outliers need a closer look

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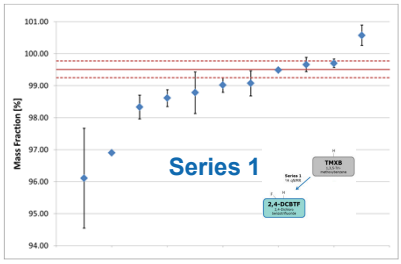
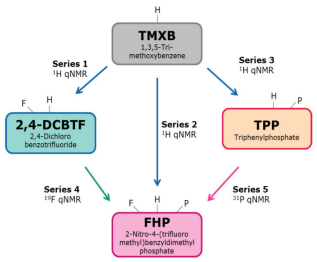
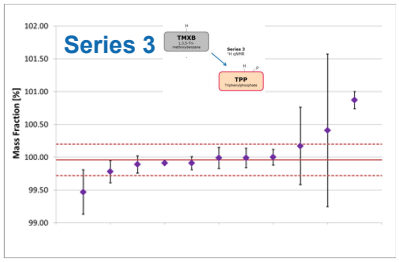
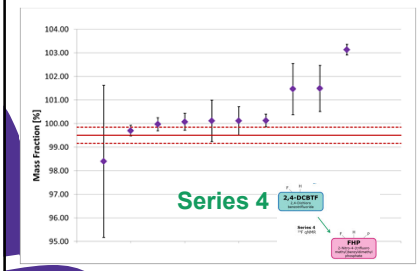
qNMR Interlaboratory Comparison
Result Overview

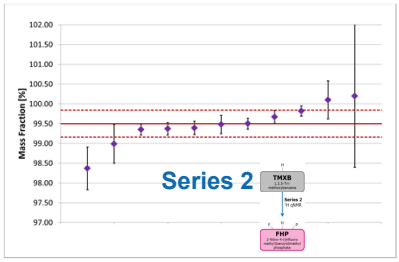
Red = Reference value ± uncertainty (k=2)

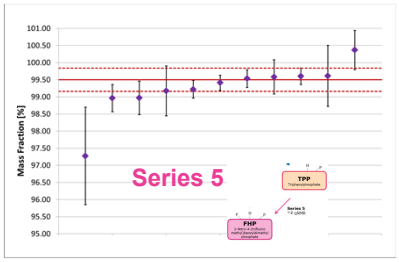












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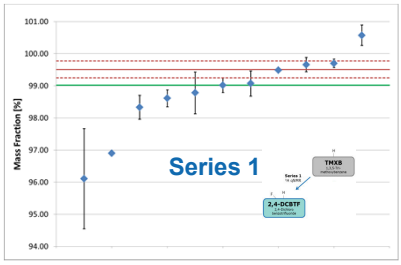
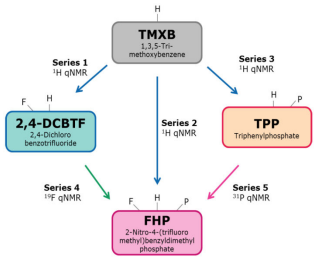
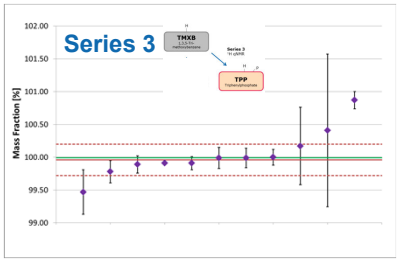
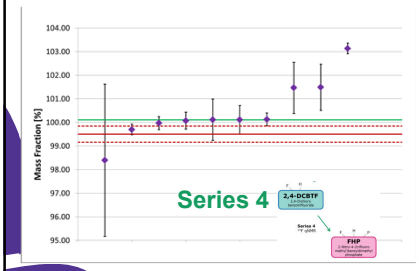
qNMR Interlaboratory Comparison
Result Overview

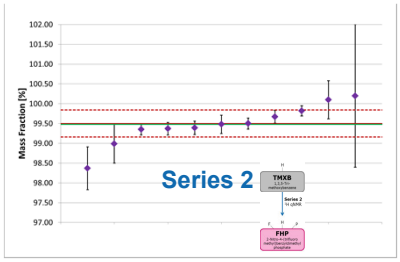
Red = Reference value ± uncertainty (k=2)
Green = Median

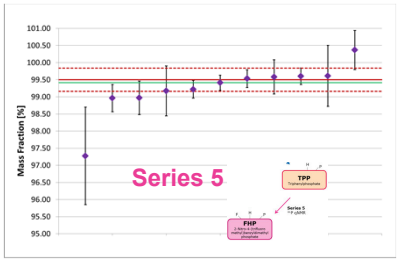












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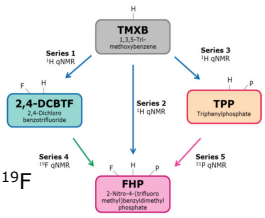
qNMR interlaboratory comparison
Outcome and Outlook



Outcome:

- Measurement series 2/3/5 generated more consistent results than series 1/4 (liquid, ^{19}F incorporated)
- ^{19}F qNMR results depend very much on measurement parameters chosen
- Sample preparation as important part of the measurement
- Influence of weighing process – a very precise balance with sufficient readability is a must (9,7428 mg vs 9,7 mg…) → higher measurement uncertainty
- Not all laboratories routinely calculate measurement uncertainty

Outlook:

- Detailed evaluation of the ILC series in progress
- Apply different Reference value possibilities, especially for 2,4-DCBTF
- Identify and summarize critical points, e.g. in ^{19}F measurement
- Mass Balance examination of the analytes
- Offer further qNMR PT schemes integrated in the Merck PT platform under our ISO 17043 accreditation
- Expand portfolio of PT schemes to meet demands from customers, e.g. in the field of microbiology

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NOVEL ILC FOR 1H, 31P AND 19F QNMR

Special Thanks to QuoData for the support

quo data

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