

Heteronuclear NMR Method as a Replacement for Classical POV Titration in the Determination of Hydroperoxides in Lipids and Lecithin

Prof. Dr. Bernd Diehl and Dr. Hendrik Henning

Who are we?



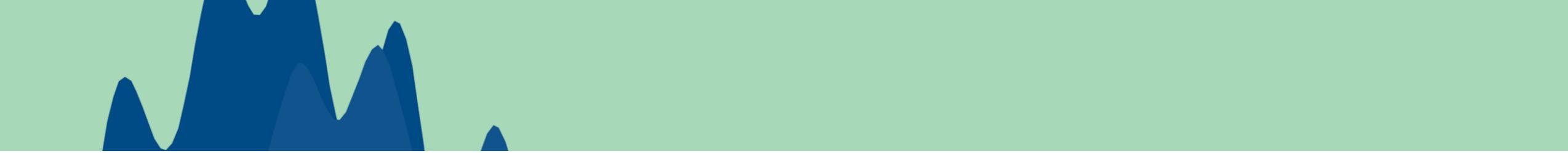
George
Hanna
qNMR
Award
Winner
2021



Bernstein | Diehl | Holzgrabe | Maiwald | Meyer | Monakhova | Schönberger

qNMR
the handbook

- SELECTIVITY
- CHEM. INERTNESS
- SOLUBILITY
- STABILITY
- RELAXATION
- SUPP. RESOLUTION



Understanding Peroxide Value (POV)

- Peroxide Value (POV): Measures the concentration of peroxides (in milliequivalents) per kilogram of material – an indicator of oxidative rancidity
- A higher POV indicates reduced shelf life and declining product quality
- Oxidation occurs when UV light or heat activates oxygen, leading to the breakdown of lipid double bonds
- Reactive oxygen species (ROS) can damage lipid-rich tissues like the brain, contributing to health risks

Historical and Modern Methods

- Some draw backs to these methods
- Official methods that titrate I_2 from reacted KI
 - AOAC 965.33 (1965 with a final update in 1969)
 - AOCS Cd 8b-90 (2009) Iso-octane and glacial acetic acid
 - ISO 3960:2017 (visual end point detection) and ISO 27107:2008 (automatic)
 - Hazardous chemicals, visual, and automatic titration
 - Iodine can add to double bonds
- Photometric determination of formed I_2
 - Measure I_3^- at 353 nm



- Iron based methods

- Ferrous Thiocyanate Method which goes red
- Ferrous Xylenol Orange (FOX) which goes from orange to purple

- Other methods

- Cadmium acetate
- Direct detection using FTIR
- Derivatization with Triphenylphosphine which is then detected using FTIR

Why choose NMR?

- Molar Ratios Directly Measurable (SI units)
- No Response Factor Dependencies
- Wide Concentration Range – Linearity retained across increasing concentrations
- Single Internal Standard for Multiple Analyses
- No Separation of Analytes Required
- Structural and Quantitative Data Measured Simultaneously
- Isotopic Analysis – e.g., ^1H , ^{31}P , ^{13}C , ^{19}F



POV Analysis @SYA

- We can perform ^{31}P -NMR analysis on phospholipids and perform POV using the same sample preparation
- The additional analysis is a lot cheaper saving our customers money!

Our method – ^{19}F direct quantification of POV

- Our method uses a patented “Swiss Army Knife” Internal Standard (TFPP) that selectively reacts with active oxygen species to form the oxide (TFPPO)
- The TFPP and TFPPO species are directly measured in the ^{19}F -NMR measurement
- Only 300mg of sample required
- We can detect values as low as other methods in the sub 0.1 meq O_2/kg range
- No interference as we focus on the ^{19}F spectrum!
- Duplicated across both sites in Germany and USA with great reproducibility
- Signal to Noise Spectra of $\sim 30,000:1$ with 128 scans on our BBFO 600 MHz cryo system

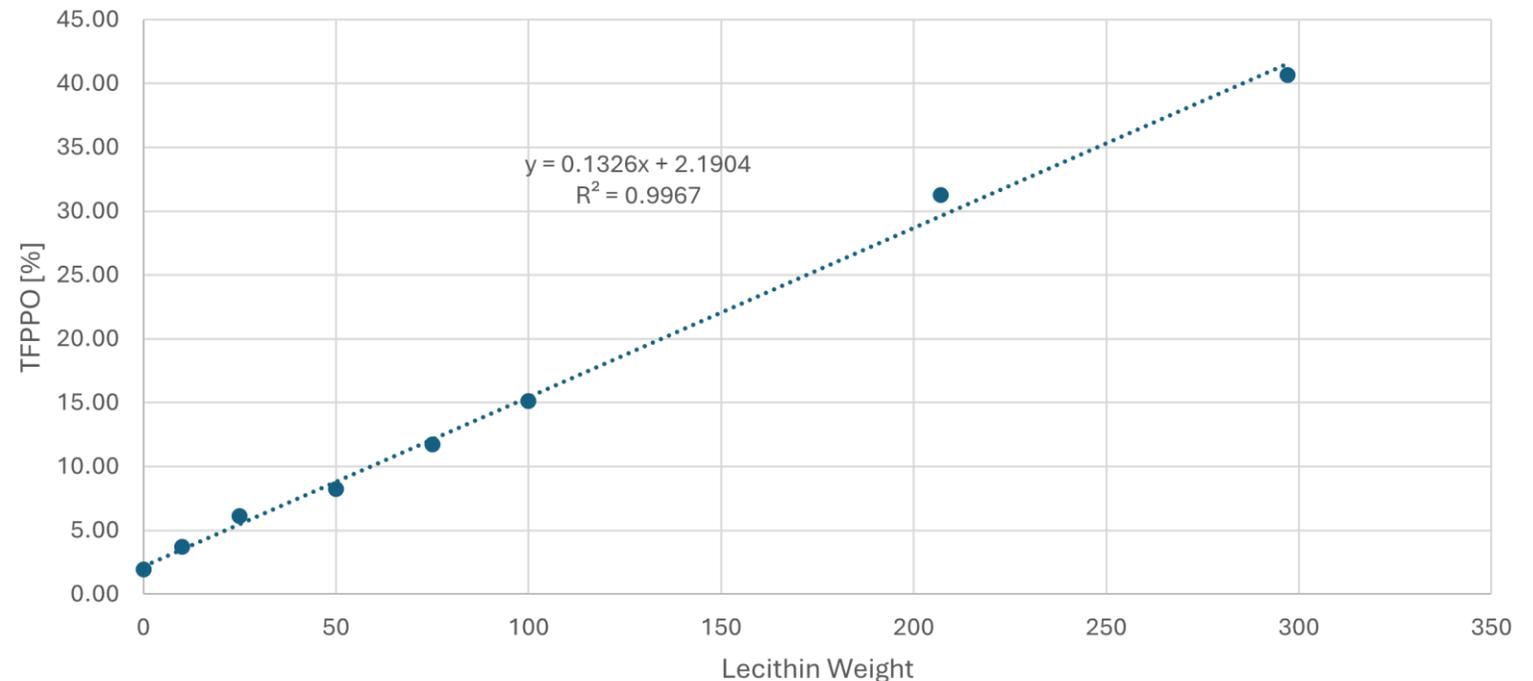
Linearity

TFPP		1 Kg = x mg	1000000		
mg/ml	MG	Oxidation [%]	Weight [mg]	TFPPO [%]	
7.815		65	352	67	Measurement
μMol		POV	43.0	2.2	Blank

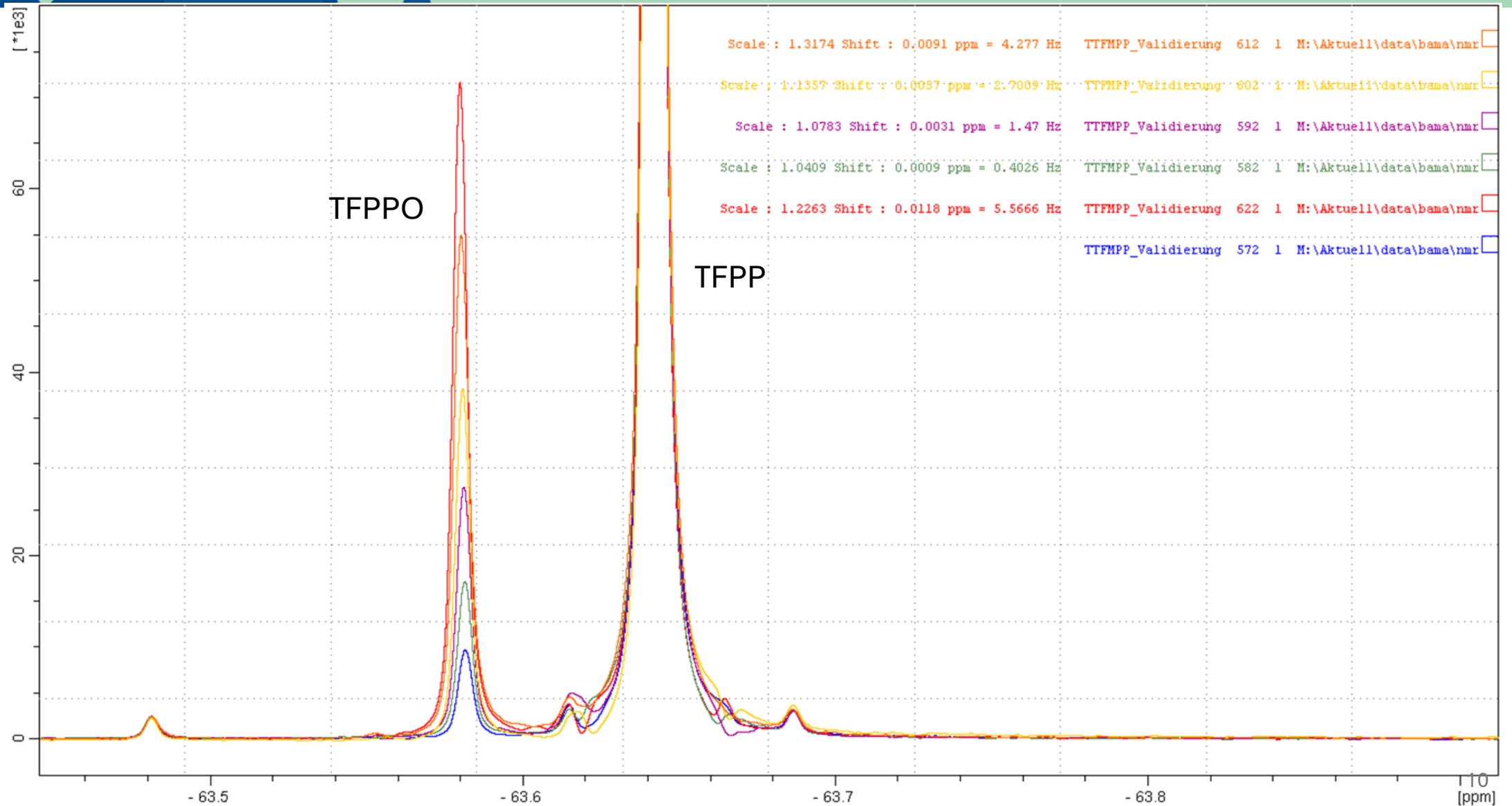
Example of an Excel calculation, the corresponding values are entered in the yellow fields, MW and μMol are only blank to hide the details of the Reference Item.

Lecithin Test Item Weight [mg]	¹⁹ F TFPPO [%]
0	1.92
10	3.73
25	6.11
50	8.24
75	11.75
100	15.14
207	31.26
297	40.68

Linearity of the POV determination of lecithin



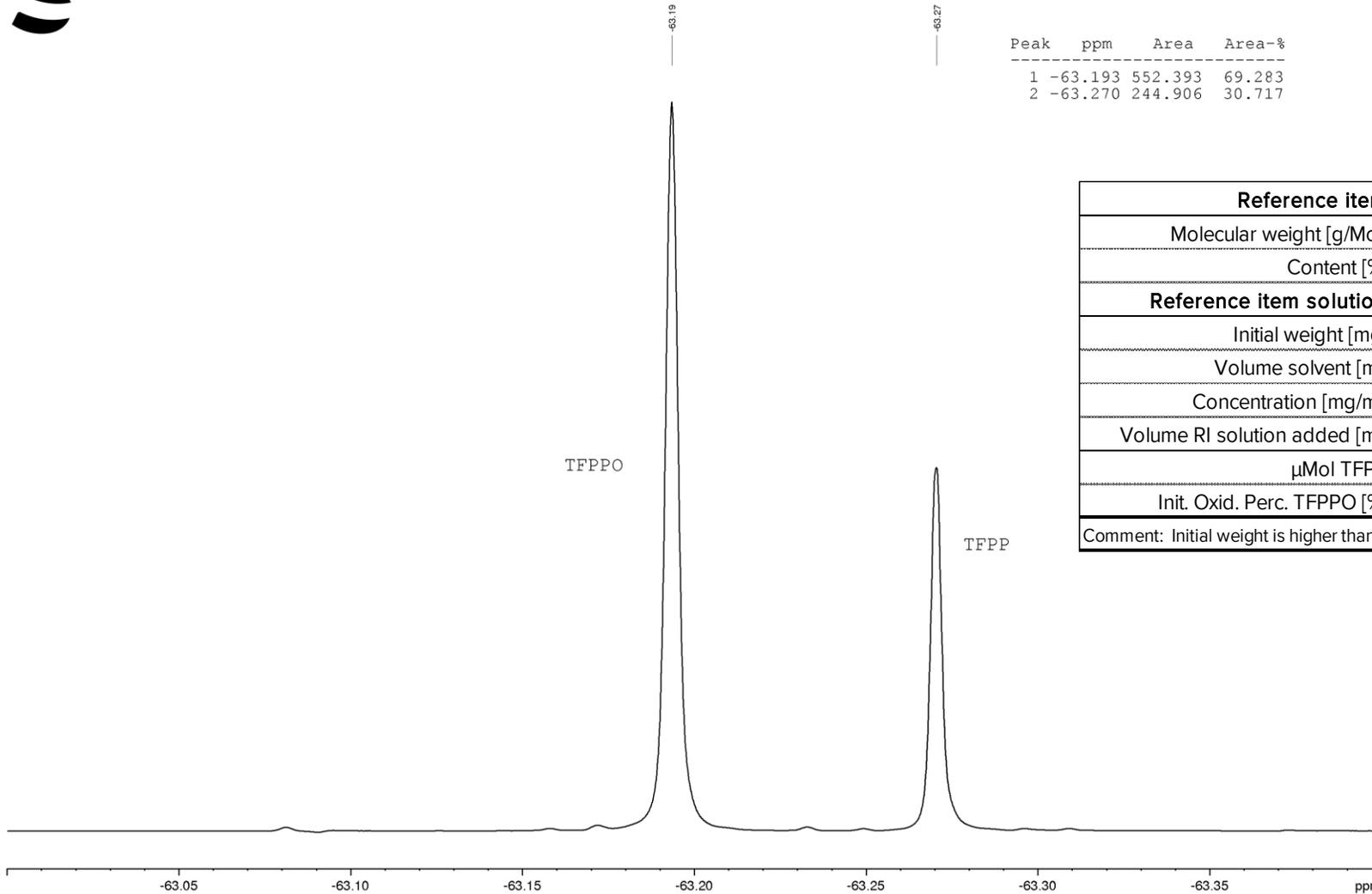
Increase in TFPPO signal





Showcase a few examples
Let's get a POV scale for good
and bad

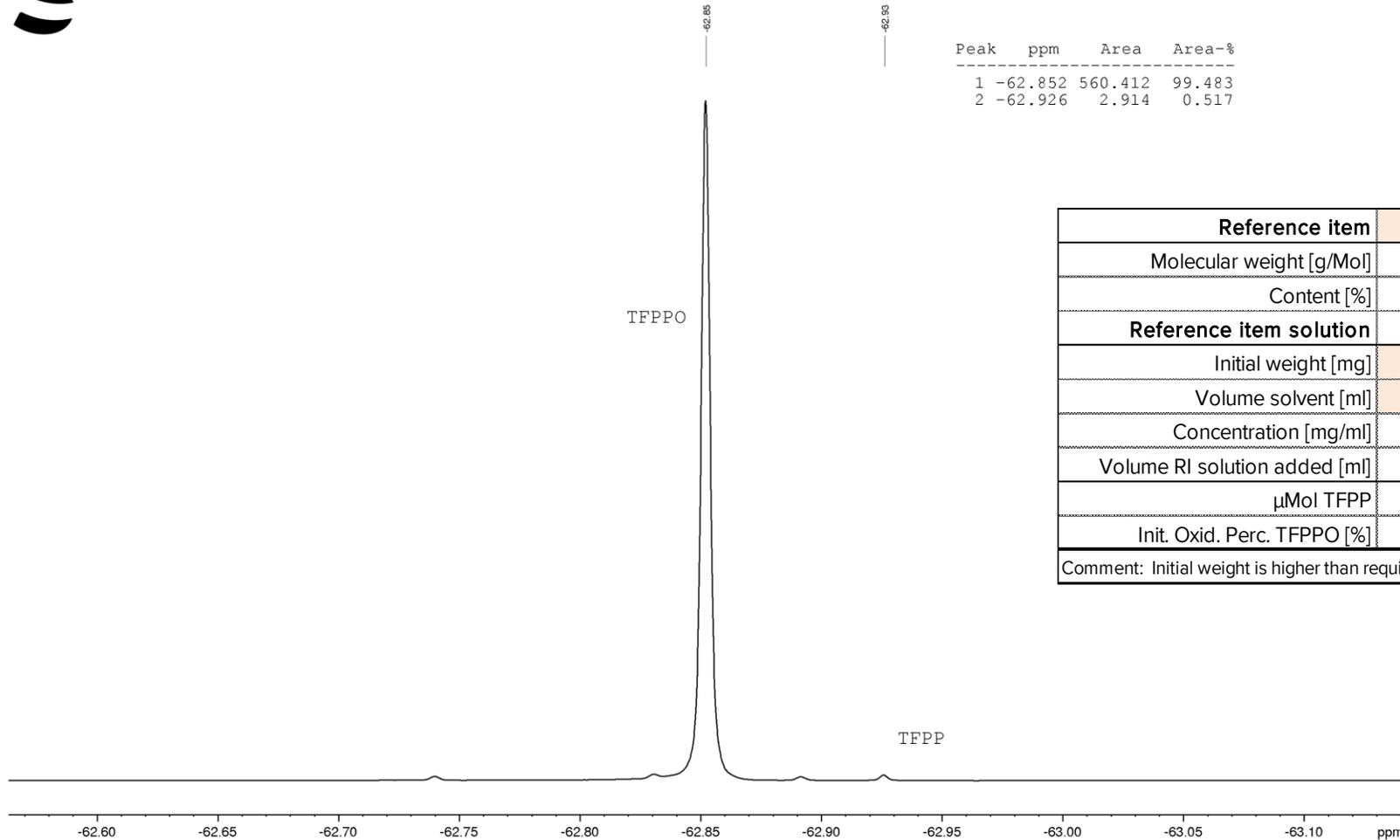
Old reheated clarified butter



Peak	ppm	Area	Area-%
1	-63.193	552.393	69.283
2	-63.270	244.906	30.717

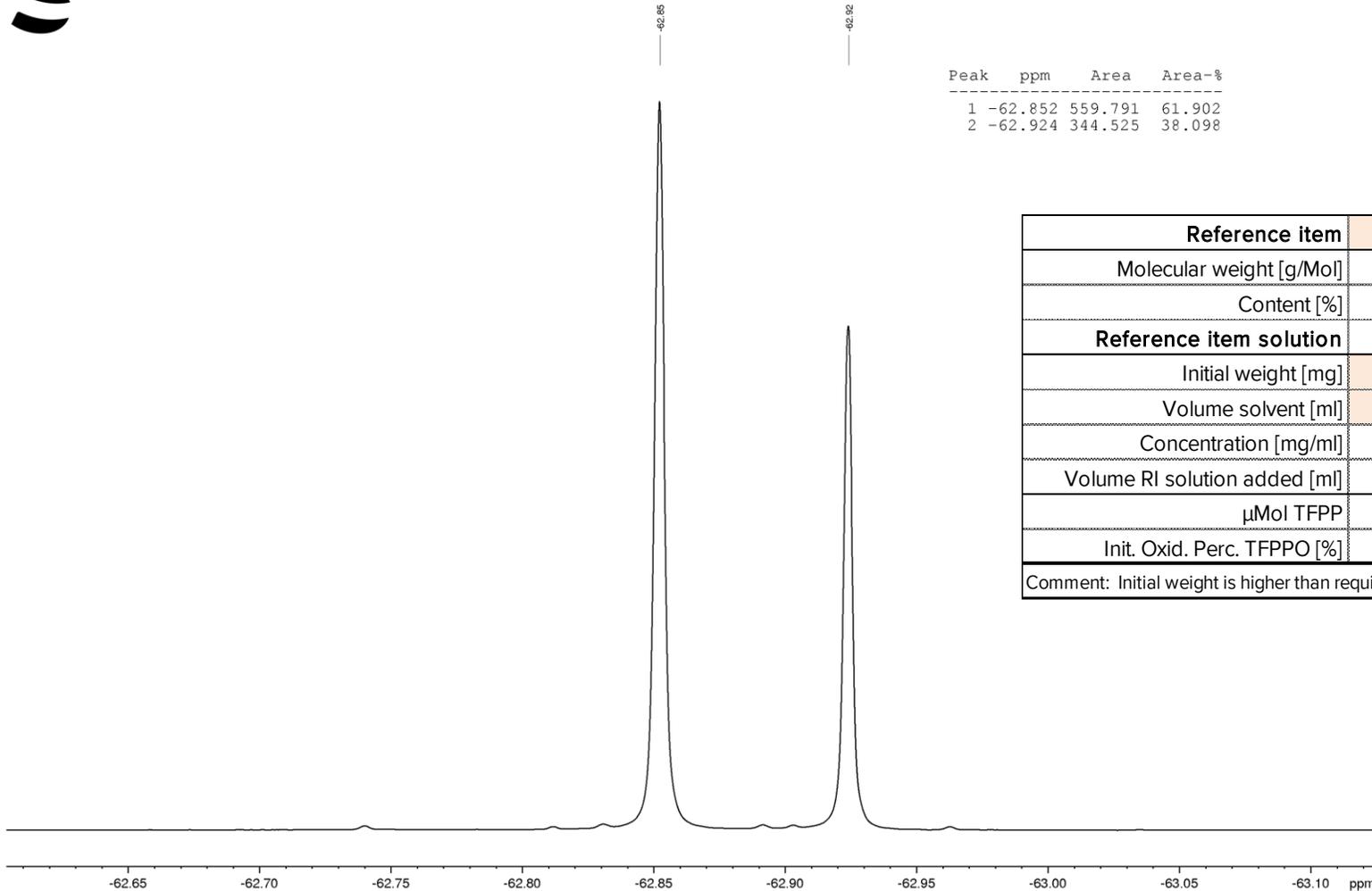
Reference item		Test Item	
Molecular weight [g/Mol]		Test Item Weight [mg]	314.90
Content [%]		Final Oxid. Perc. [%]	69.28
Reference item solution		O ₂ [μMol]	2.0512
Initial weight [mg]		POV [mMol O₂/kg]	6.51
Volume solvent [ml]		Rounding	2
Concentration [mg/ml]			
Volume RI solution added [ml]	1.00		
μMol TFPP	1.4963		
Init. Oxid. Perc. TFPPPO [%]	0.74	Balance	Mettler-Toledo XPR206DR
Comment: Initial weight is higher than required MinWeigh of 10 mg.			XPR206DR

Countertop Olive oil



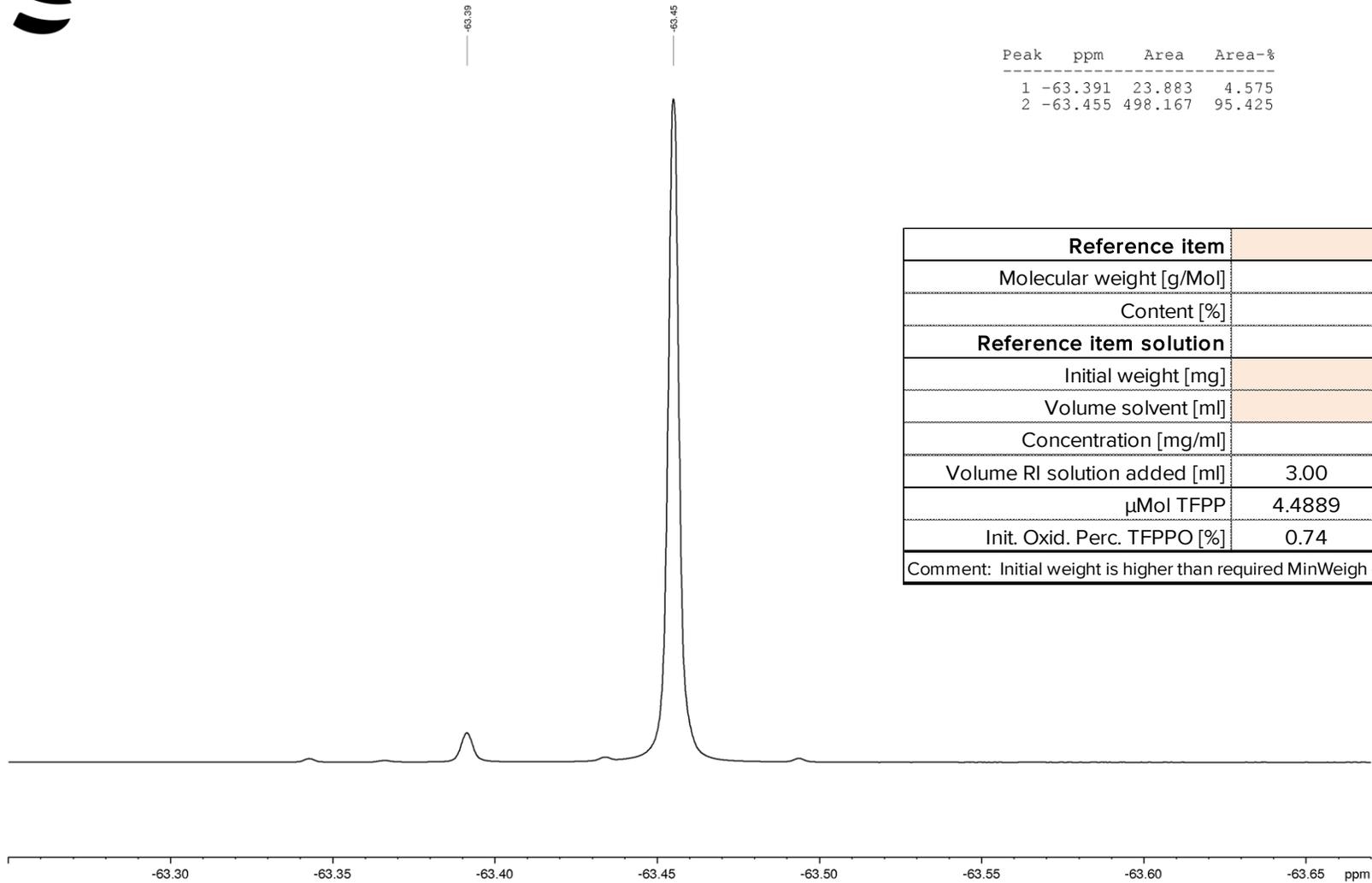
Reference item		Test Item	
Molecular weight [g/Mol]		Test Item Weight [mg]	311.98
Content [%]		Final Oxid. Perc. [%]	99.48
Reference item solution		O ₂ [μMol]	3.1095
Initial weight [mg]		POV [mMol O₂/kg]	9.97
Volume solvent [ml]		Rounding	2
Concentration [mg/ml]			
Volume RI solution added [ml]	1.00		
μMol TFPP	1.5745		
Init. Oxid. Perc. TFPPO [%]	0.74	Balance	Mettler-Toledo XPR206DR
Comment: Initial weight is higher than required MinWeigh of 10 mg.			XPR206DR

Fish Oil Capsules



Reference item		Test Item	
Molecular weight [g/Mol]		Test Item Weight [mg]	311.71
Content [%]		Final Oxid. Perc. [%]	61.90
Reference item solution		O ₂ [μMol]	1.9260
Initial weight [mg]		POV [mMol O₂/kg]	6.18
Volume solvent [ml]		Rounding	2
Concentration [mg/ml]			
Volume RI solution added [ml]	1.00		
μMol TFPP	1.5745		
Init. Oxid. Perc. TFPPO [%]	0.74	Balance	Mettler-Toledo XPR206DR
Comment: Initial weight is higher than required MinWeigh of 10 mg.			XPR206DR

Whole Krill Oil Capsule



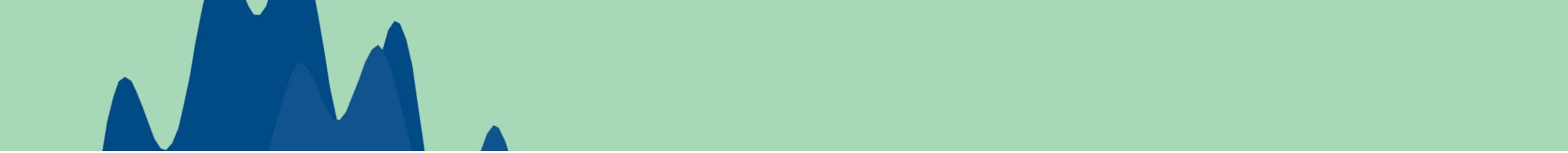
Peak	ppm	Area	Area-%
1	-63.391	23.883	4.575
2	-63.455	498.167	95.425

Reference item		Test Item	
Molecular weight [g/Mol]		Test Item Weight [mg]	1697.04
Content [%]		Final Oxid. Perc. [%]	4.58
Reference item solution		O ₂ [μMol]	0.3443
Initial weight [mg]		POV [mMol O₂/kg]	0.2
Volume solvent [ml]		Rounding	2
Concentration [mg/ml]			
Volume RI solution added [ml]	3.00		
μMol TFPP	4.4889		
Init. Oxid. Perc. TFPO [%]	0.74	Balance	Mettler-Toledo XPR206DR
Comment: Initial weight is higher than required MinWeigh of 10 mg.			XPR206DR



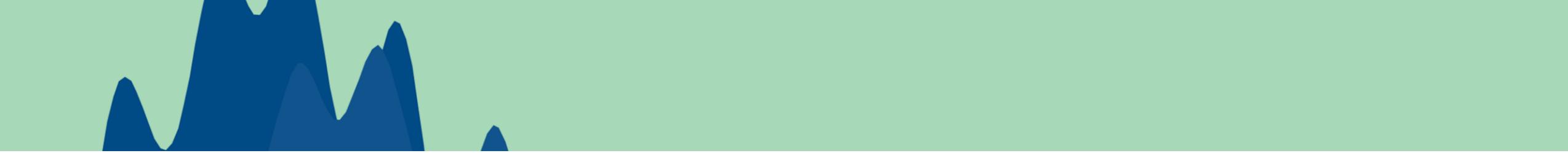
Summary and Conclusion

- Our ^{19}F POV method demonstrates the full power of NMR spectroscopy with sensitivity ranges that at least match or are better than traditional POV methods
- Our patented reference item selectively reacts with active oxygen species giving great reproducibility and robustness
- The ability to combine POV sample preparation with phospholipid analysis allows our customers to cut testing costs

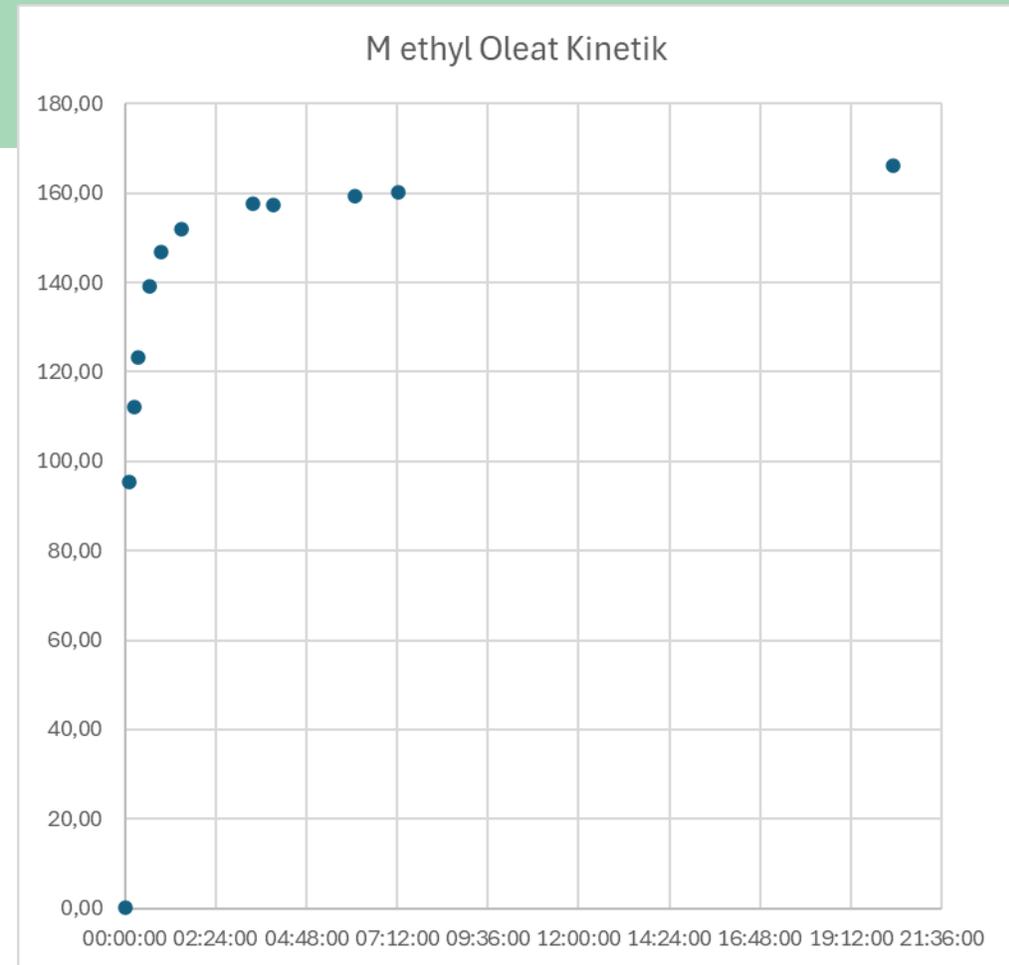
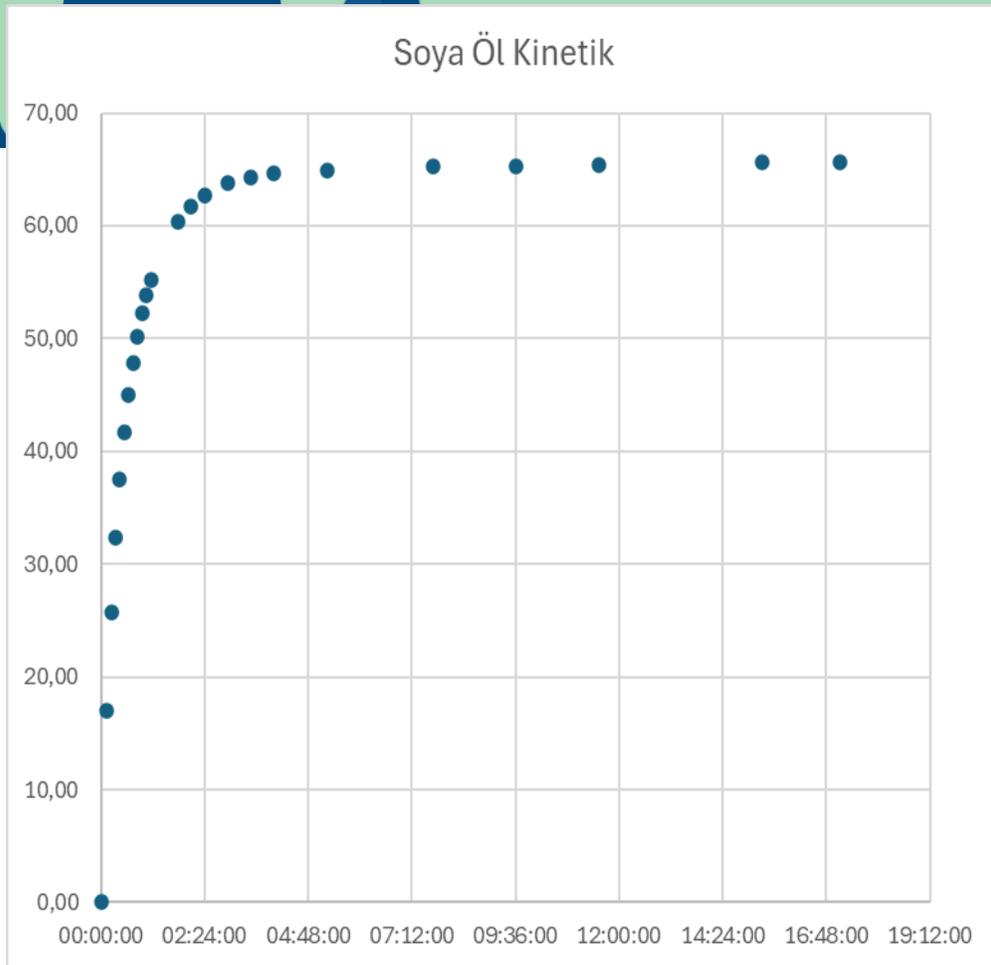


Questions

- Thank you for Listening!



Extra Information Slides for Questions



Formation of TFMTPPPO as a reaction product. 2nd order kinetics, with good dosage about 4 hours to 99%
 If necessary, heat to 50 or 60° C for 30 minutes

Das Reagenz gehört zur patentierten Gruppe der Swiss-Army-Knife NMR Standards

Spezifische Redoxreaktion mit Hydroperoxiden

Detection mittels Multikern NMR

^1H , ^{31}P und ^{19}F

Messzeit 2 Min

Ab initio Bestimmung der Reagenzqualität mittels multikern NMR

SI/NO 5 mg/ml 500 MHz Prodigy, NS 16 = 15500:1

SI/NO 5 mg/ml 500 MHz Prodigy, Blanc (2.5%) NS 16 = 350:1

SI/NO 1 mg/ml 500 MHz Prodigy, NS 16 = 3200:1

SI/NO 1 mg/ml 500 MHz Prodigy, Blanc (2.5%) NS 16 = 73:1

Bei Verwendung von 600 MHz cryo Systemen und bei entsprechend höherer Messzeit sind Verbesserungen um den Faktor 10 – 50 möglich. Die Methode ist zur Spurenanalytik und Diagnostik geeignet.

Klassische NMR-Lösungsmittel, CDCl_3 , MeOD oder DMSO-d_6 geeignet

Bestimmung für alle Lipide, Vegetable oils, marine und vegetable Lecithine, modifizierte und hydrolysierte Lecithine,

Bestimmung von POZ bis 0.1 also 50-mal tiefer als für gutes Material definiert

Standardabweichung bei Krill Öl (Lecithin) POZ 20 \pm 0,2,

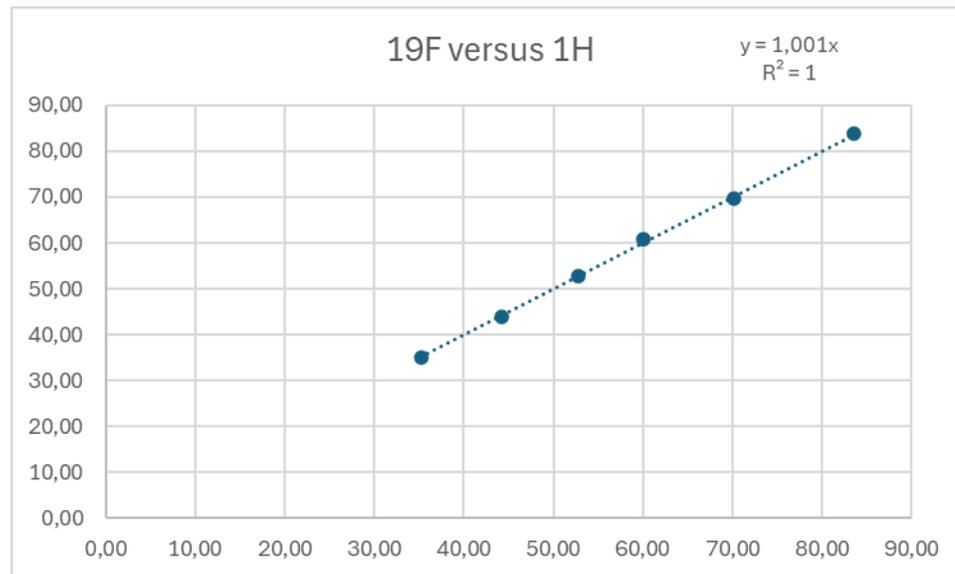
Standardabweichung bei Krill Öl (Lecithin) POZ 2,5 \pm 0,1

Standardabweichung durch Integration aber auch durch inhomogene Proben, Oxidation an der Oberfläche

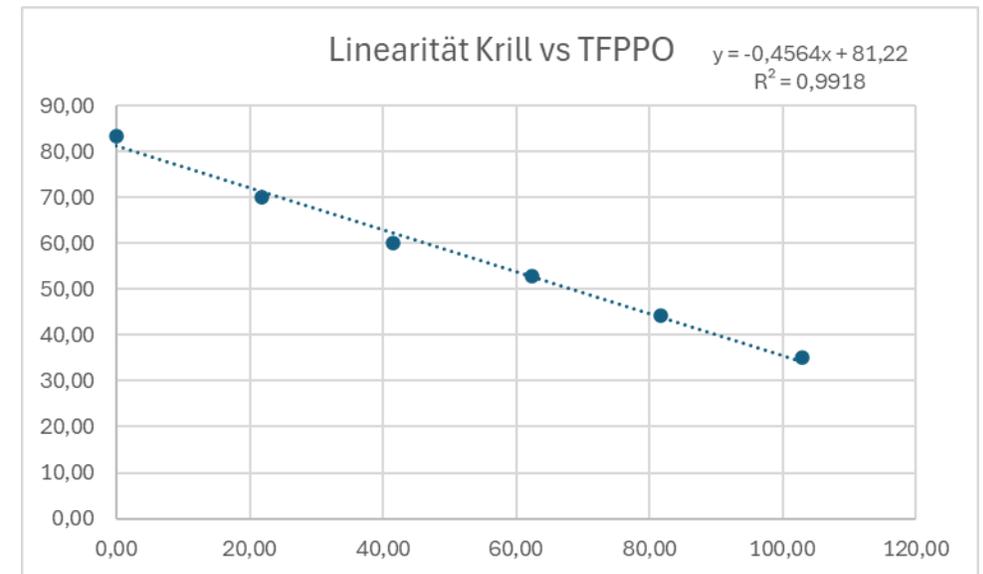
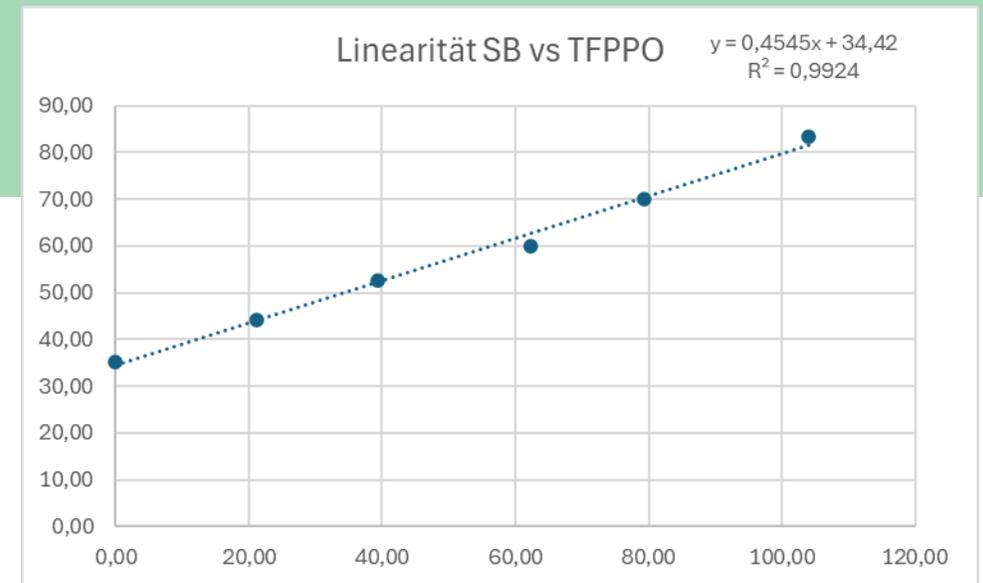
Mischung von Sonnenblumenöl und Krill Lecithin

SB	Krill	19F	1H	1H
Einwaagen		Integral % TFPPO		
104,06	0,00	83,46	83,75	9,37
79,36	21,86	70,09	69,60	9,21
62,32	41,49	59,98	60,74	10,20
39,27	62,32	52,75	52,75	9,00
21,07	81,77	44,17	44,01	9,60
0,00	103,00	35,19	34,97	9,30

Die Bestimmung des TFPPO Gehaltes mit 1H bzw. 19F-NMR ist identisch



Mischung von Sonnenblumenöl und Krill Lecithin



Stabilität der POZ-Messungen bei Variation der Probenmenge an Sonnenblumenöl über mehrere Tage

Stabilität Sonnenblumenöl an drei Tagen			
Einwaage	12.10.	17.10.	22.10.
0,0	2,8	2,8	2,8
10,0	9,2	9,5	10,0
25,0	20,4	21,5	21,8
49,9	37,5	38,5	39,2
75,3	54,7	56,0	56,7
100,5	71,9	73,1	74,0

The stability of the reaction is given for 10 days with pure vegetable oils. A slight secondary process takes place depending on the weight.

It is recommended to take the measurements within one day of preparation.

