



^{31}P -NMR – Krill and PS methods

Developed at Spectral Service AG,
Implemented into USP methods



Why should the USP support NMR methods?

NMR methods are well suited as USP methods because they are inherently quantitative, highly reproducible, and require minimal method-specific calibration.

Signal intensities in NMR are directly proportional to the number of nuclei, enabling accurate quantification—especially when using an internal standard—without relying on compound-specific response factors. In addition, NMR is non-destructive, requires relatively simple sample preparation, and provides structurally specific information, ensuring high specificity and robustness. These features make NMR methods reliable, transferable between laboratories, and well aligned with regulatory expectations for validated, standardized analytical procedures.

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1 Phospholipid analysis in krill oil

- ^{31}P -NMR method was **developed** at Spectral Service AG.
- In 2012 the USP **adopted** our ^{31}P NMR method as the official USP monograph [1].
- Further investigations at Spectral Service were performed demonstrating robustness of the method. Method at Spectral Service AG is **validated** [3] **verified** [5] **established as a standard operating procedure (SOP)** [7]

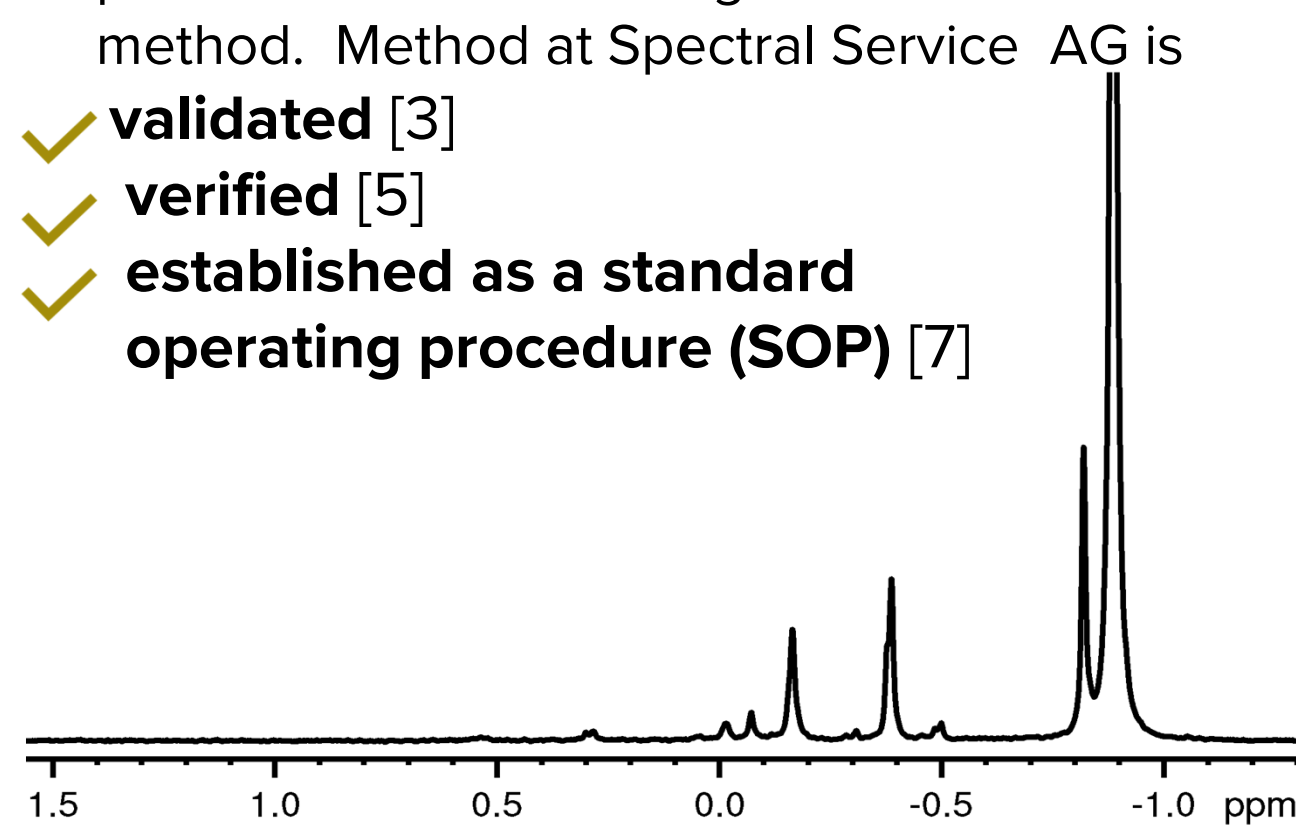


Figure: ^{31}P -NMR spectrum of a krill oil sample

3 Phospholipid analysis in PS enriched powder

- ^{31}P -NMR method at Spectral Service AG is **implemented** and already in use for many years at Spectral Service AG.
- We are actually **working together with the USP** to become this method an official USP method as well
- Validations** were performed at Spectral Service AG [2] and from our daughter company Steelyard Analytics (Gathersburg; US) [6]

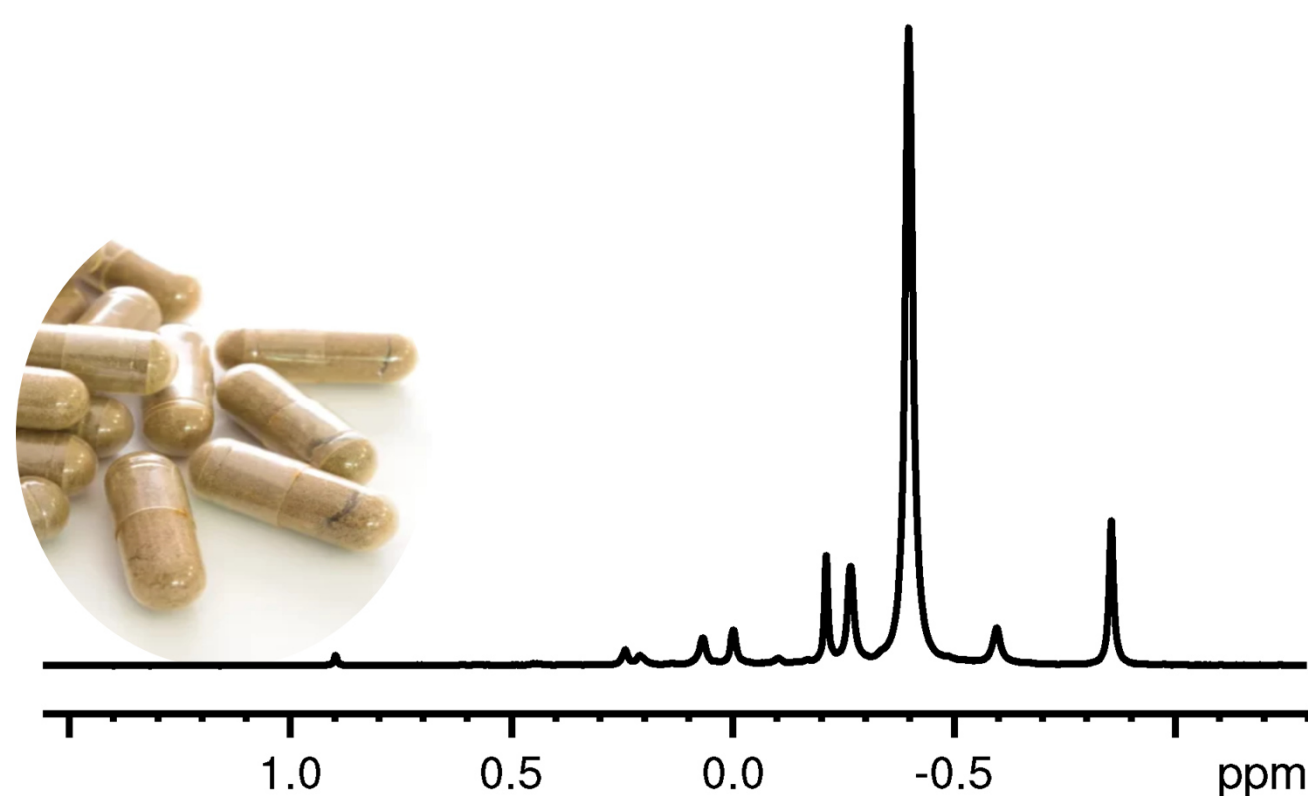


Figure: ^{31}P -NMR spectrum of Phosphatidylserine (PS)

2 Robustness of method (Krill oil)

	USP method	Spectral Service adjustments	Comments/Evidence
Initial weight of test item	300-350 mg	50-450 mg	shown in validation report [3]
Initial weight of internal standard (TPP)	25 mg	5-35mg	shown in method verification report [5]
Volumes of solvents (chloroform/methanol/CsEDTA solution)	1/1/1 mL	1.5/3/3 mL	shown in verification report [3] validation report [5]
D Use of deuterated solvents	Fully deuterated	Only deuterated chloroform	no solvent related signals in ^{31}P -NMR spectrum
reference standard	USP reference standard	No reference standard needed	^{31}P -NMR is robust regarding chemical shift, signal intensity, signal overlapping
Stability of CsEDTA solution	pH 7.2-7.5, no information about stability	could be used at pH 7.2 - 8.5 for minimum 3 weeks	Stability was examined within a specific study [4]

4 Suggestions to make USP methods for Phospholipid analysis MORE PRACTICABLE



Always use ranges of initial weights



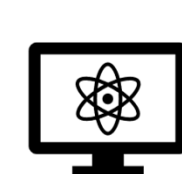
The volumes of solvents do not need to be extremely precise (e.g. 3 mL instead of 3.0 mL)



Measurement of reference standards are not needed for NMR analysis (if an official method exists)



Using full deuterated solvents are cost consuming and is not always necessary



The additional analyses of ^1H -NMR spectra (incl. ^1H line shape and sensitivity test) is not needed

→ Spectral Service AG uses customized USP methods, ensuring analysis to be:



COST-EFFICIENT



USER FRIENDLY



PRACTICAL



FAST

Collaboration with the USP is important:

- ensures that a chemical laboratory follows internationally recognized quality standards.
- improves the accuracy and reliability of results,
- supports compliance with regulators like the FDA and strengthens the lab's credibility.

Take-Home Message



Spectral Service will promote cooperation with the USP



Ideas for the future: UPS methods for more Phospholipid containing matrices like lecithin or milk



Hand in Hand with the USP: expanding NMR based USP methods – Diving implementation – Shaping the future of standardized processes

[1] Krill oil monograph (USP); [2] VAL00106: Phosphatidyl-Serine (PS) - Validation of ^{31}P -NMR Quantification Method (Spectral Service AG); [3] VAL00312-01: Lecithin from Soybean and Krill - Validation of ^{31}P -NMR Quantification Method (Spectral Service AG) [4] QRM CsEDTA-H₂O 01: Risikobewertung zur Haltbarkeit der CsEDTA-H₂O-Lösung (Spectral Service AG); [5] Ver011-01: Determination of phospholipids in krill oil samples (Spectral Service AG); [6] Val23001-02: Phosphatidylserine Powder (Steelyard Analytics, Inc.); [7] SAA-MET002 (Quantitative Bestimmung von Phospholipiden)