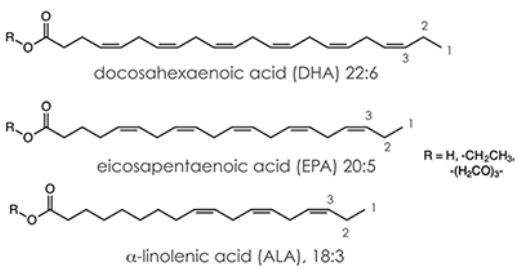
NMReady-60e Benchtop NMR Spectra of Fish Oil Supplements

Nature is a constant source of inspiration for scientific advancement. Examples are too numerous to count:

- solar cells to mimic photosynthesis
- aspirin from willow bark
- penicillin from bread mould

The ocean is no exception; both vertebrates and invertebrates are exploited in the quest for new medicinal compounds.

Phytoplankton and marine algae, for example, are capable of doing what mammals cannot - producing a variety of omega-3 (ω-3) polyunsaturated fatty acids (PUFAs). This natural product is vital to basic metabolic processes, including basic brain and heart activity. PUFAs are also thought to decrease inflammation and have anti-cancer activity.



Humans' main source of PUFAs is a diet that includes the fish that feed on these ω-3 rich microorganisms. The designation 'ω-3' refers to a double bond originating from the third carbon from the chain terminus. There are a variety of molecules in the ω-3 class, differing in chain length and degree of saturation. The three most important are shown here: DHA, EPA and ALA.

EPA acts as a precursor to a series of eicosanoids that are involved in important feedback loops in blood regulation, including platelet aggregation and white blood cells activation.

DHA is the primary component of the cerebral cortex, skin, and retina. It can be metabolized directly or synthesized *in vivo* from ALA. This is a vital fatty acid and has been shown to:

- maintain proper brain function
- slow the progression of Alzheimer's
- improve cardiovascular health
- improve the efficiency of chemotherapy
- inhibit the growth of some type of tumors

As many human diets lack sufficient ω-3 and typically injest too high of a ω-6/ω-3 ratio, a variety of supplements are available. Herein, we describe the differences between four of these alternatives using both standard 400 MHz and benchtop 60 MHz ^{1}H NMR Spectroscopy.

- ω-3 supplements
- wild salmon oil
- cod liver oil
- shark liver oil

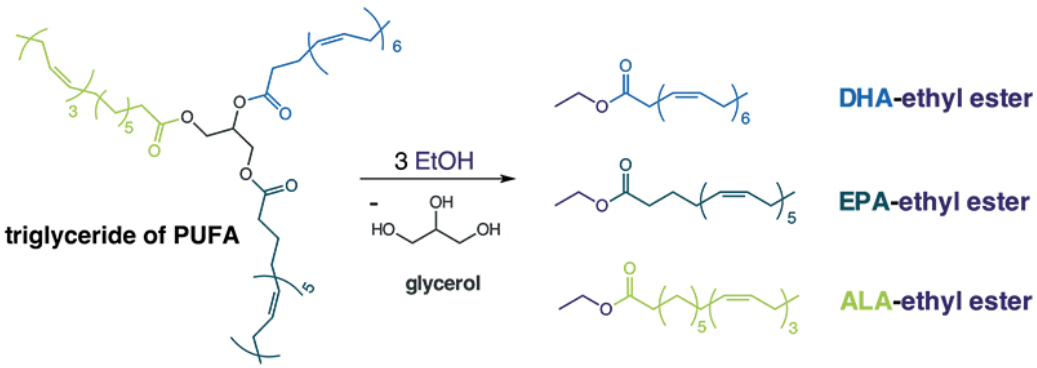
Omega-3 Supplements

The label of a typical ω-3 supplement will usually list:

- **Fish Oil Concentrate** (PUFA-EE)
- **Eicosapentaenoic Acid** (EPA)
- **Omega-3 Fatty Acids** (PUFA)
- **Docosahexaenoic Acid** (DHA)

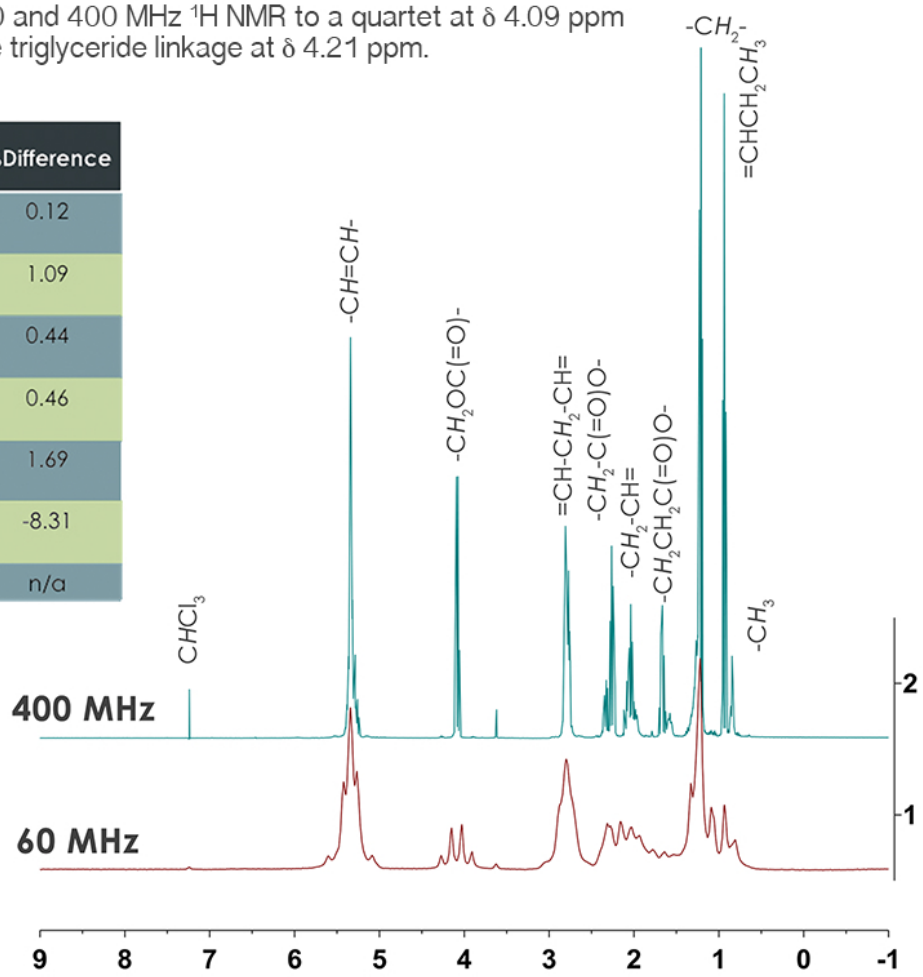
Fish oil concentrate is unique to ω-3 supplements. It is not naturally occuring, but rather it's formed through a simple transesterification reaction. As depicted here, this involves a reaction of a triglyceride of PUFA with ethanol (EtOH) to eliminate the triglyceride linkage as glycerol and afford the ω-3s as ethyl ester derivatives.

This is process is done because it renders the PUFAs easier to refine, purify and concentrate. These derivatives, however, are considered less bioavailable as they have to be re-converted to the triglyceride form *in vivo*.



The ethyl ester derivativegives rise in both 60 and 400 MHz ^{1}H NMR to a quartet at δ 4.09 ppm (-C*H*$_2$-OC(=O)-) and a notable absence of the triglyceride linkage at δ 4.21 ppm.

%	60 MHz	400 MHz	%Difference
Olefin -CH=CH-	22.87	22.84	0.12
ethyl ether -CH_2O-	5.18	5.13	1.09
bis-allylic =CH-CH2-CH=	18.54	18.46	0.44
α and β -CH_2-	21.47	21.37	0.46
alkyl -CH_2-	24.54	24.14	1.69
Terminal methyl –CH_3	7.39	8.06	-8.31
Total %	100.00	100.00	n/a

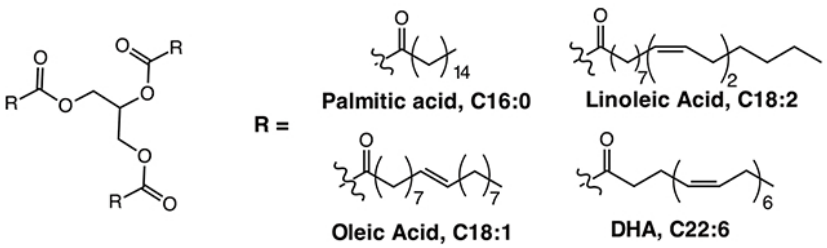


Although the ethyl ester derivative is considered more difficult to absorb, the strength in these supplements is that they are prepared in the absence of excess saturated or monounsaturated fatty acids or toxins that remain from the nutrient source.

Wild Salmon Oil

Fish oils, like this wild salmon example, are a less processed supplement:

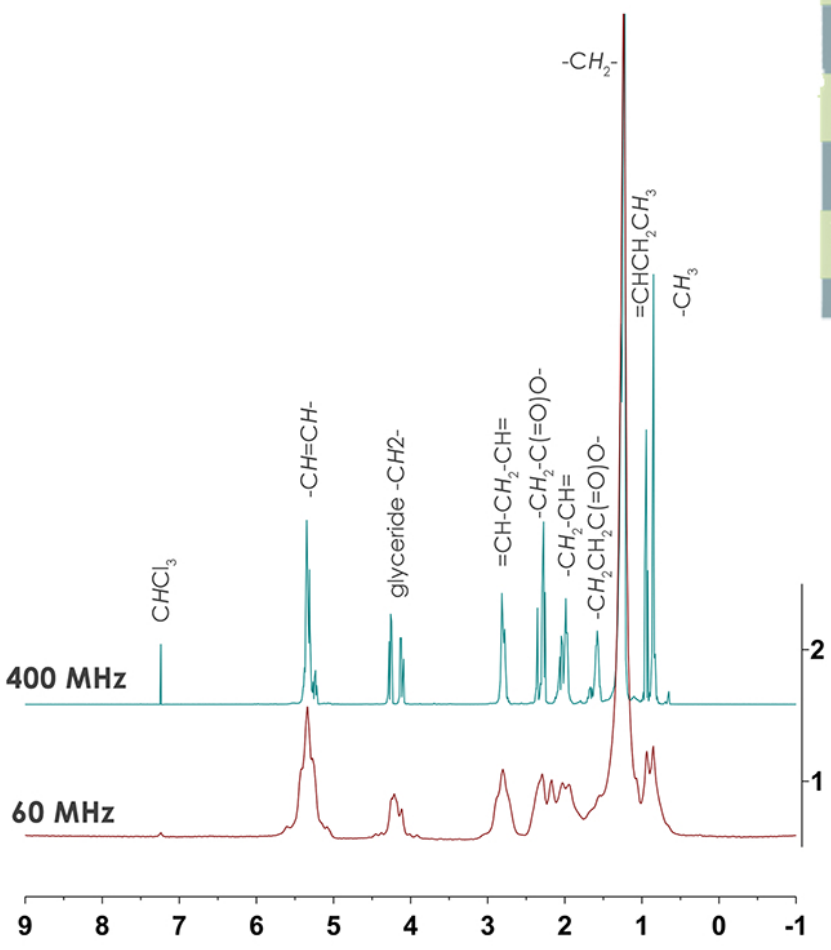
- Wild Alaskan Salmon Oil
- Eicosapentaenoic Acid (EPA)
- Docosahexaenoic Acid (DHA)



Salmon oil is mostly comprised of the natural form of fatty acids, the triglyceride, that is combined with some extra EPA and DHA. As it is less processed, this supplement contains a higher amount of saturated (*e.g.*, palmitic acid) and monounsaturated (*e.g.*, oleic acid) fatty acids in addition to the desired PUFAs (*e.g.*, linoleic acid, DHA). The molecules depicted here are generally found in the highest concentration in salmon oil.[1]

Saturated fats have been shown to increase the risk of cardiovascular diease. Although there is evidence to suggest that monounsaturated fatty acids reduce inflammation and are beneficial for maintaining normal cell growth, most Western diets contain significantly more ω-6 than ω-3. If this ratio is too high, it has been found to be detrimental to human health.[2]

%	60 MHz	400 MHz	%Difference
Olefin -CH=CH-	12.25	12.08	1.47
glyceride $-CH_2-$	3.91	3.79	3.18
bis-allylic =CH-CH2-CH=	7.10	7.00	1.44
α and β $-CH_2-$	20.30	20.50	-1.02
alkyl $-CH_2-$	47.02	47.47	-0.94
Terminal methyl $-CH_3$	9.41	9.16	2.82
Total %	100.00	100.00	n/a



Like the ω-3 supplements, there is good agreement between the 60 and 400 MHz data for percent composition of the nutritional supplement.

The ratio of ω-3:ω-6 acids can be determined by 400 MHz NMR, but there is insufficient resolution at 60 MHz to determine the ratio precisely. Regardless, the saturates and monounsaturates can be obseved through:

- significantly greater alkyl $-CH_2-$ component (~47% *cf.*, 24% in ω-3 supplement)
- a higher olefin to *bis*-allylic ratio (1.7 *cf.*, 1.2 in ω-3 supplement)

The triglyceride linkage is easily observable via the multiplet centered at δ 4.21 ppm.

[1]Aursand, M. *et. al. JAOCS*, **1993**, *70*(10), 971
[2]Simopoulous, A. P., *Biomed Pharmacother*, **2002**, *56*(8), 365

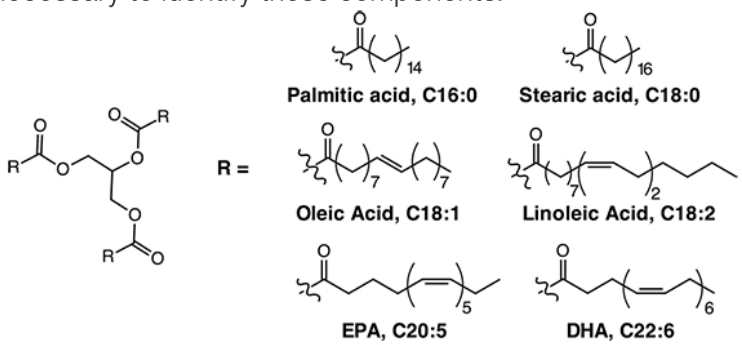
Cod Liver Oil

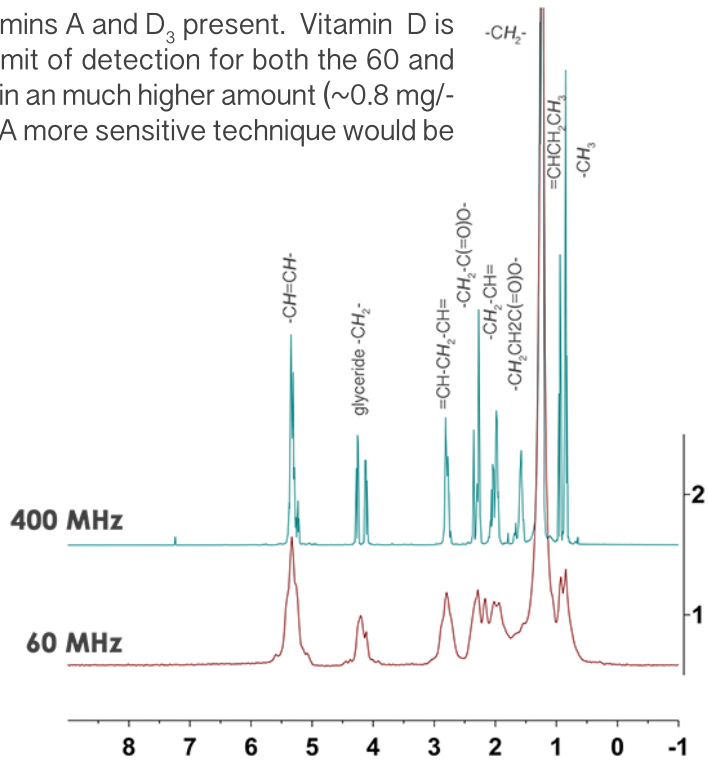
A cod liver oil supplement typically includes:

- Cod Liver Oil
- Vitamin A (palmitate)
- Vitamin D (cholecalciferol)

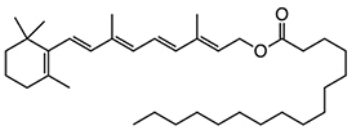
Like the previous two supplements, cod liver oil is an excellent source of ω-3, particularly EPA and DHA. These free fatty acids are in their naturally occuring triglyceride form. The relative composition is also very similar to that of salmon oil - containing a large amount of saturated fatty acids (*e.g.,* palmitic and stearic), monounsaturated ω-9 (*e.g.,* oleic acid), and ω-6 (*e.g.,* linoleic acid).[1]

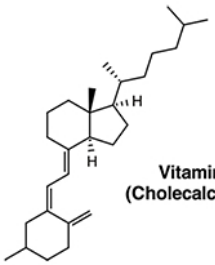
The distinguishing feature of cod liver oil is the amount of vitamins A and D_3 present. Vitamin D is in lower concentration (~0.01 mg/capsule) and is below the limit of detection for both the 60 and 400 MHz 1D ^{1}H NMR spectrum. Although vitamin A is present in an much higher amount (~0.8 mg/-capsule) resonances are not readily evident in either spectra. A more sensitive technique would be necessary to identify these components.

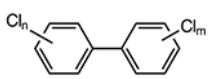


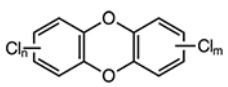


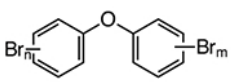
%	60 MHz	400 MHz	%Difference
Olefin -CH=CH-	11.46	12.08	-2.60
glyceride $-CH_2-$	4.00	4.00	-0.12
bis-allylic =CH-CH2-CH=	6.80	6.84	-0.50
α and β $-CH_2-$	20.53	20.90	-1.75
alkyl $-CH_2-$	48.40	47.60	1.67
Terminal methyl $-CH_3$	8.81	8.89	-0.95
Total %	100.00	100.00	n/a

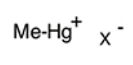

Vitamin A (Retinyl palmitate)


Vitamin D (Cholecalciferol)


Polychlorinated biphenyl (PCB)


Polychlorinated dibenzodioxins (PCDD)


Polybrominated diphenyl ethers (PBDE)


Methylmercury

Nevertheless, the amount of retinyl palmitate present can be cause for concern because it approaches the upper tolerable level for humans. Vitamin A, although vital for many functions including vision, is a fat-soluble vitamin and is known to bioaccumulate in fat tissue. People taking cod liver oil generally are required to regulate and limit other sources of vitamin A in their diets, and it is recommended that they do so under guidance of a physician.

Additionally, as the liver is used to remove toxins, there is also the potential for trace amounts of contaminants (*e.g.,* PCBs, PCDDs, PBDEs, MeHg). If present in these samples, they are at too low a concentration to be detected at either 60 or 400 MHz.

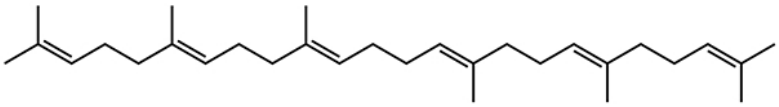
[1]Galarraga, B. *et. al. Rheumatology*, **2008**, *47*, 665

Shark Liver Oil

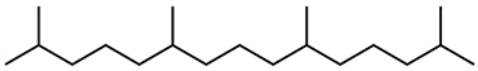
Shark liver oil, procured from certain species of deep sea sharks,[1] is often incorrectly classified as a PUFA supplement when there are virtually no ω-3 PUFAs present. A shark liver has a unique composition because it acts as a swim bladder. It is filled with oils that effectively increase the buoyancy of the shark. This oil is almost entirely squalene.

Squalene is an essential terpenoid substance that is a precursor to *in vivo* development of all steroids. Although there are other natural sources of squalene (*e.g.*, vegetable oil, amaranth), shark liver is a main source.

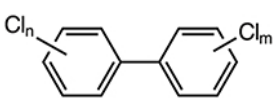
Although squalene possess a very different bioactivity, its health benefits are similar to that of PUFAs. Squalene is thought to have anti-carcinogenic activity, reducing risk of cancer and shriking existing tumors. Additionally it has been found to potentiate chemotherapy drugs.[2]



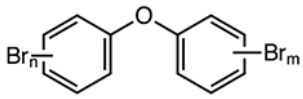
Squalene



Pristane



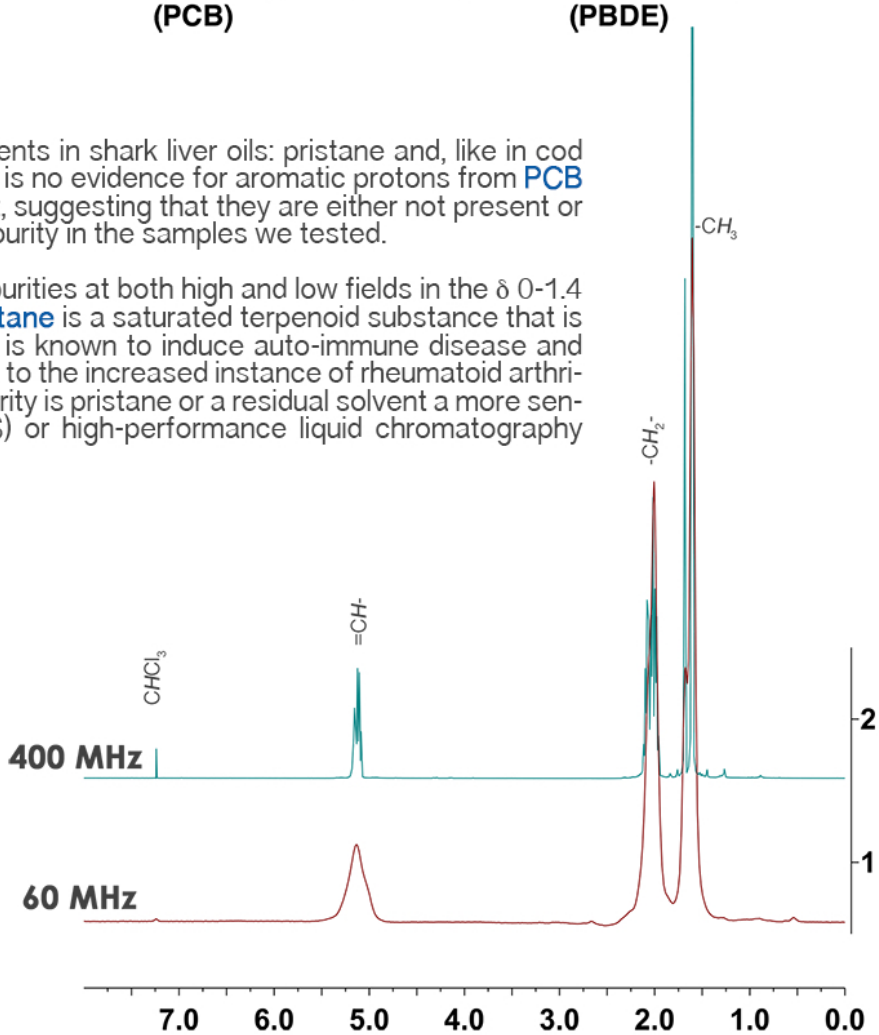
Polychlorinated biphenyl (PCB)



Polybrominated diphenyl ether (PBDE)

There are two potentially problematic components in shark liver oils: pristane and, like in cod liver oil, toxins (*e.g.*, PCBs, PBDEs). While there is no evidence for aromatic protons from PCB or PBDE contaminants at either 60 or 400 MHz, suggesting that they are either not present or below the limit of detection, there is an alkyl impurity in the samples we tested.

The spectra below exhibit evidence of alkyl impurities at both high and low fields in the δ 0-1.4 ppm range. This is likely residual pristane. Pristane is a saturated terpenoid substance that is the second most prevalent oil in shark liver. It is known to induce auto-immune disease and plasmacytosis in mice, and has also been linked to the increased instance of rheumatoid arthritis in humans.[3] To determine whether this impurity is pristane or a residual solvent a more sensitive technique like mass spectrometery (MS) or high-performance liquid chromatography (HPLC) may be required.



[1] Tsujirnoto, M. *J. Ind. Eng. Chem.* **1920**, 63
[2] Deiana, M. *Magn. Reson. Chem.* **2001**, *39*, 29
[3] Carlsson, H.; Grimvall, E. *Occup Environ Med.* **1997**, *54*, 66

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- **Frequency:** ^{1}H 60 MHz
- **Magnet:** permanent, cryogen-free
- **Stray field:** confined within enclosure
- **Sample:** Standard 5 mm NMR Tubes
- **Spectral resolution:** LW at 50% 1.2 Hz
- **Power:** 100-240 VAC, 50-60 Hz
- **Weight:** 55 lbs (25 kg)
- **Dimensions:** 11.8x11.0x19.2" (30x28x49cm)
- **User Interface:** Built-in Touchscreen
- **Connectivity:** USB, Ethernet, VNC
- **File Format:** JCAMP-DX
- **Compatibility:** Mnova, Topspin, Spinworks, LabVIEW

specifications subject to change without notice

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