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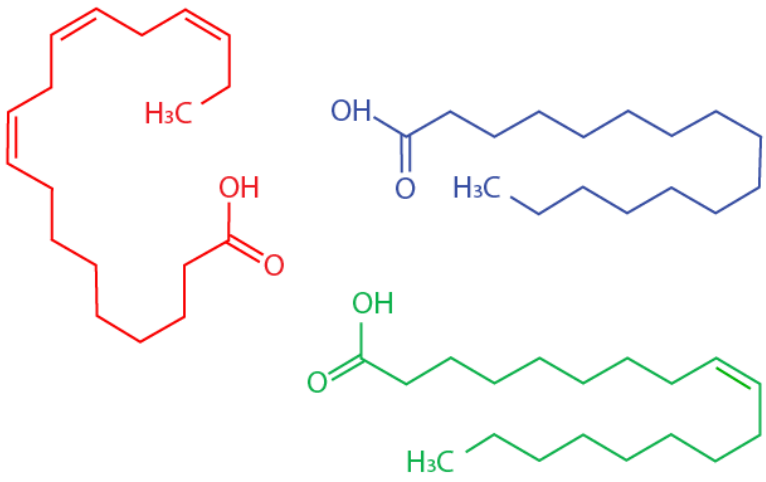
Spinsolve

Characterizing fatty acids with advanced multinuclear NMR methods



Fatty acids consist of long carbon chains ending with a carboxylic acid on one side and a methyl group on the other. Most naturally occurring fatty acids have an even number of carbon atoms and can be either saturated or unsaturated. Unsaturated fatty acids have one or more double bonds between carbon atoms. Typical fatty acids found in vegetable oils are saturated palmitic acid (C16:0) and stearic acid (C18:0), as well as oleic acid (C18:1) with a single double bond starting at carbon nine (omega-9), linoleic acid (C18:2) with two double bonds starting at position 6 (omega-6), and alpha-linolenic acid with three double bonds starting at position 3 (omega-3).

^{1}H spectroscopy

^{1}H NMR spectra of fatty acids show some characteristic signals corresponding to the different chemical groups in the molecules that can be used to quantify the amount of saturated, mono-, double-, and triple-unsaturated chains in the sample. Saturated chains show the methyl group ω_1 (●), α-CH_2 (●), β-CH_2 (●), and the rest of the methylene CH_2 groups overlapping at about 1.3 ppm. Oleic acid has one double bound between carbons 9 and 10, each of which is coupled to a single olefinic hydrogen (●) with a signal at about 5.3 ppm, well separated from the rest of the signals. The presence of the double bond also affects the chemical shift of the first CH_2 neighbours of the olefinic hydrogens, which are known as the allylic CH_2 (●). Linoleic acid has two double bonds, one between carbons 9 and 10 and the other one between carbons 6 and 7. The four olefinic hydrogens (●) generate a peak structure that differs in shape from the one correspondent to the olefinic hydrogens in oleic acid, but appear at the same position in the spectrum. In contrast with the previous structures, the signal of the CH_2 group at position 8, known as the bis-allylic group (●), appears at a position well separated from the rest. Finally, alpha-linolenic acid has three double bonds and a total of six olefinic hydrogens with a signal appearing at the same position as the olefinic hydrogens in oleic and linoleic acid. Moreover, the two bis-allylic CH_2 groups (●) generate a signal at the position of the bis-allylic group in linoleic acid. This molecular structure can only be recognized by the fact that as its first double bond starts at the third carbon (ω_3), the signal of the methyl group appears as a clear triplet generated from the coupling with the CH_2 at position ω_2. These different signals are typically used to quantify the fatty acid profile of oils requiring only minimum sample preparation but no derivatization as is typically needed for gas chromatography.

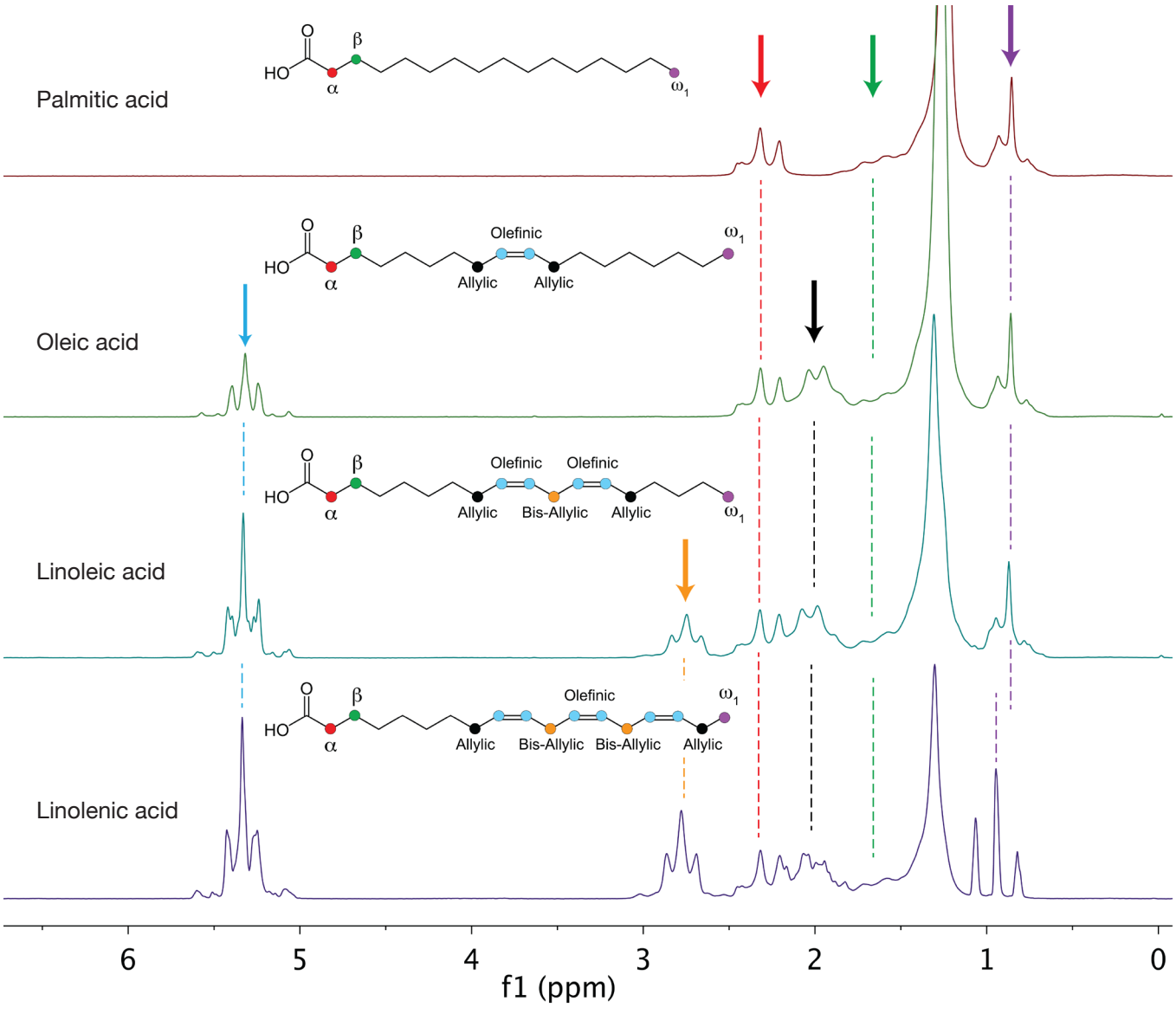


Figure 1: ^{1}H NMR spectra of palmitic, oleic, linoleic, and linolenic acid measured with a Spinsolve 60 MHz in a single scan. The samples were dissolved in $CDCl_3$ at a concentration of 0.5 M for palmitic acid (close to the solubility limit) and 1 M for the rest.

^{13}C spectroscopy

The larger chemical shift of ^{13}C NMR makes it possible to resolve additional chemical groups compared to ^{1}H NMR. For example, for palmitic acid, not only the signals of alpha (●), beta (●), and ω_1 (●) groups are resolved but also the ones of ω_2 (●) and ω_3 (●) can be clearly identified. The rest of the carbons along the chain (methylene CH_2 groups), which are farther away from the ends, appear close to each other but still with a structure that allows some of them to be identified. For oleic acid, the two olefinic carbons (●) appear as two clearly resolved peaks and the two allylic carbons (●) as a single line. The spectrum of linoleic acid shows the four olefinic (●) and the bis-allylic carbons (●) well resolved. It can be noted that as the first olefinic carbon is at position ω_6 the position of the ω_3 line shifts towards lower ppm values, compared to the spectrum of oleic acid. Finally, for linolenic acid, the six olefinic carbons give 5 signals (two overlap) and the two bis-allylic ones are slightly resolved. The ω_3 is in this structure a olefinic group so it shifts to the olefinic region, the ω_2 becomes an allylic carbon and shifts to lower ppm values (●), and the ω_1 shifts its position to a slightly larger ppm value. It is also interesting to note how the structure of the signals between 28 and 30 ppm, which correspond to the methylene carbons in the center of the chain, varies as the double bonds are incorporated into the molecules. While for palmitic acid there are 10 carbons contributing to this group, there are only four for linolenic acid.

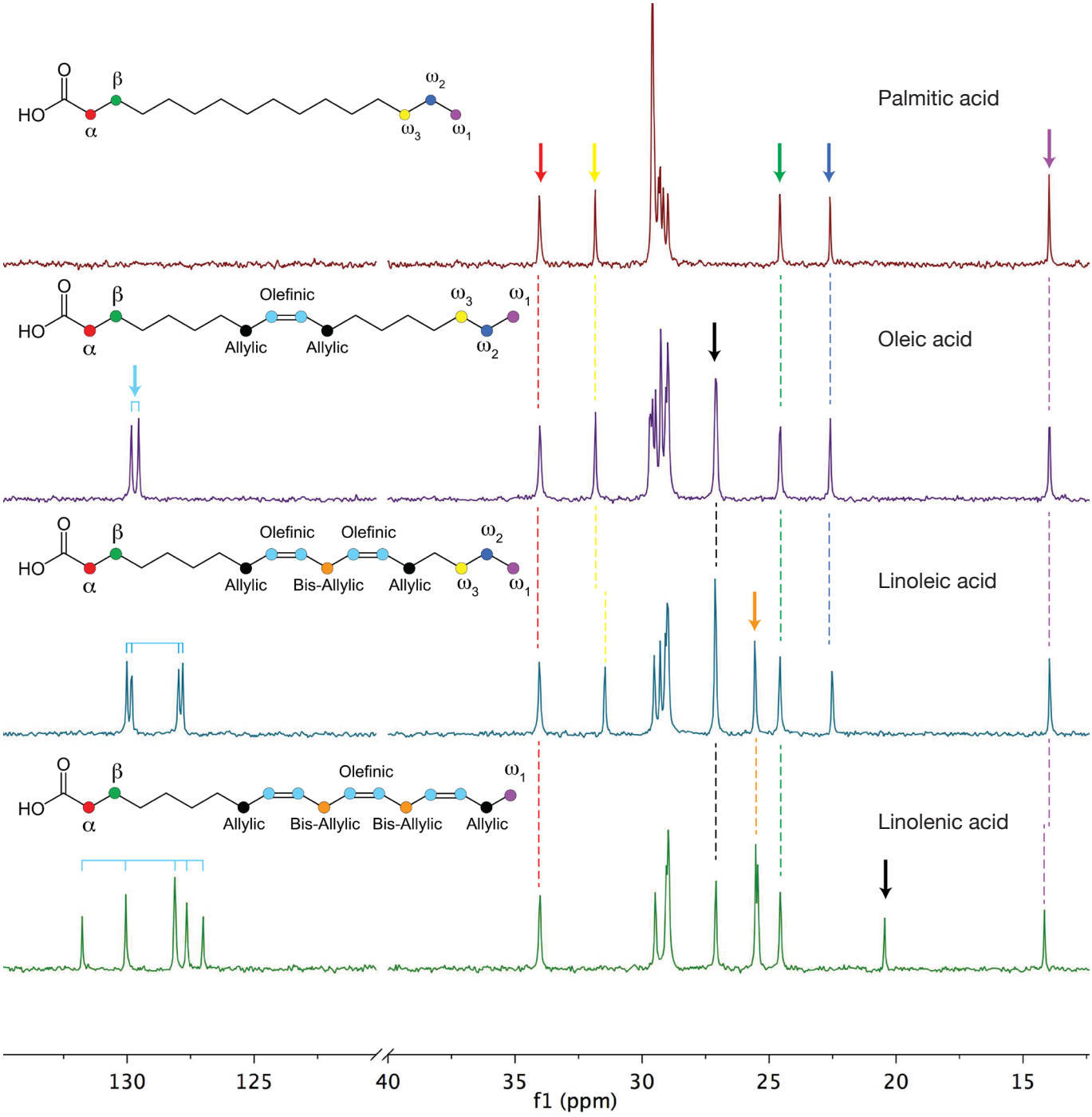


Figure 2: Dept-45 spectra measured for palmitic (0.5 Molar), oleic, linoleic, and linolenic acid (1 Molar) with a Spinsolve 60 Carbon.

Homonuclear correlation spectroscopy (COSY)

The COSY sequence is a two-dimensional homonuclear experiment that shows correlations between chemical groups that belong to the same molecule. In general, first neighbours are easily identified but also longer range couplings can be observed depending on the coupling constants between groups.

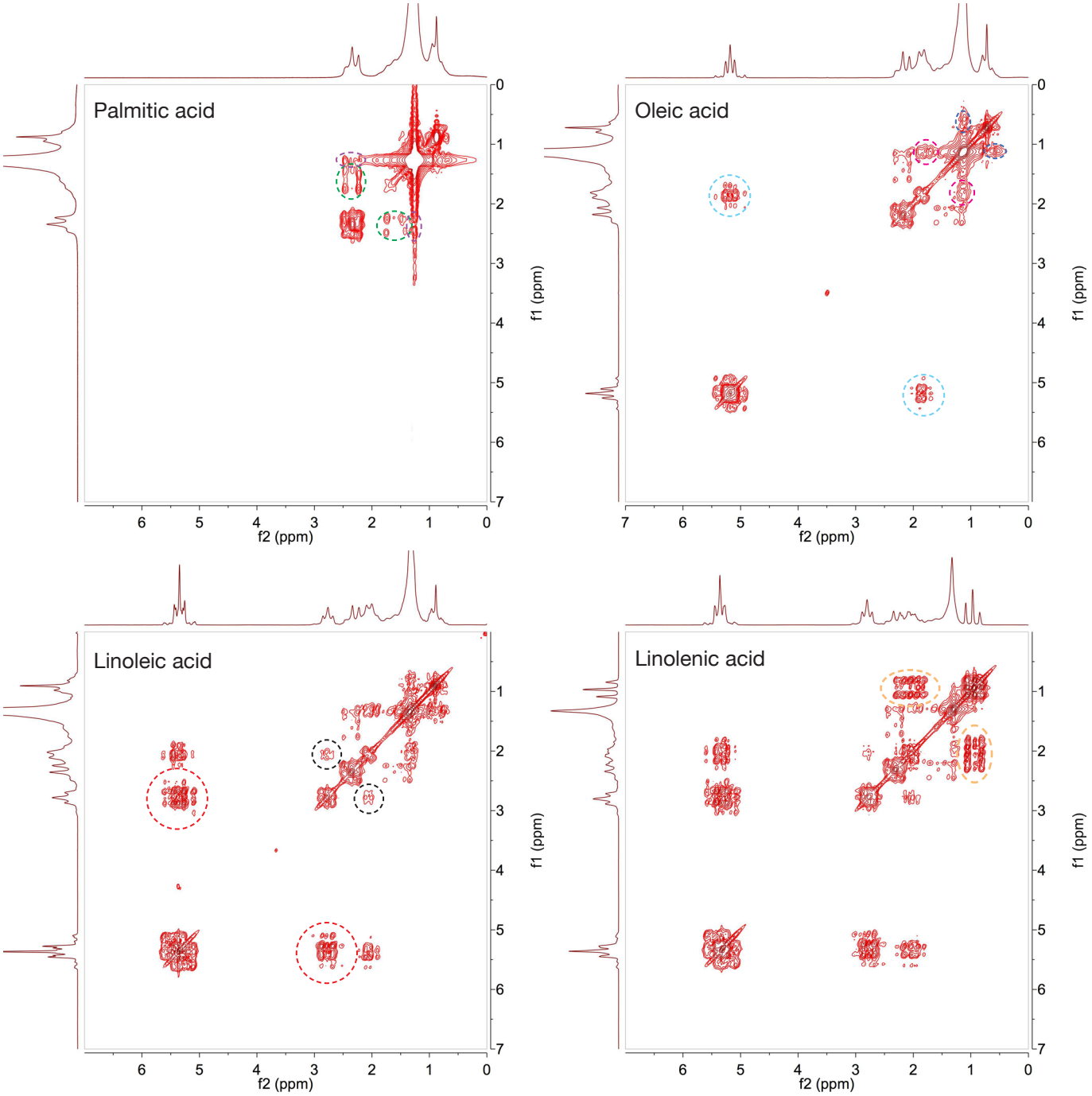


Figure 3: COSY spectra showing the correlations between different chemical groups in palmitic, oleic, linoleic, and linolenic acid. By setting 256 steps along the indirect dimension, 4 scans per step, and a repetition time of 4 seconds, a total experimental time per spectrum of 1 hour was required.

For palmitic acid the cross peaks corresponding to the coupling between α and β hydrogens can be observed marked with circles (●), however, the large star shape of the diagonal peak of the methylene hydrogens overlaps with the cross peaks showing the coupling between the methylene and the beta hydrogens as well as the one corresponding to the coupling between methylene and methyl hydrogens. The coupling of methylene and alpha hydrogens is just visible (●). The spectrum of oleic acid shows cross peaks between the olefinic and the allylic hydrogens (●), as well as the cross peaks between the allylic and the methylene ones (●). In this spectrum the coupling between methylene and methyl hydrogens becomes visible (●). For linoleic acid the bis-allylic hydrogens show correlation with the olefinic ones (●) and also with the allylic ones (●) although the last one is a long range coupling. Finally, for linolenic acid, the large cross peaks between the methyl hydrogens and the allylic ones (●) confirm the fact that they are next to each other. This cross peak can be used as a direct confirmation of the presence of omega-3 in vegetable oils. At the same time the coupling of the methyl group with the methylene ones disappear from the spectrum of linolenic acid as they are no longer neighbours.

Heteronuclear correlation (HSQC-ME)

The HSQC is a powerful sequence widely used to correlate the ^{1}H with the one-bond coupled ^{13}C nuclei. The Spinsolve is equipped with a multiplicity-edited version (HSQC-ME) of this method. It provides the editing power of the DEPT-135 sequence, which is useful to identify the signal of the CH_2 groups (blue) from the CH and CH_3 (red). Figure 4 shows the HSQC-ME spectrum of palmitic, oleic, linoleic, and linolenic acid in $CDCl_3$, where all the expected ^{1}H-^{13}C correlations can be identified. The signals of the methyl and the olefinic groups appear in red, while the rest of the peaks are blue confirming that they correspond to CH_2 groups.

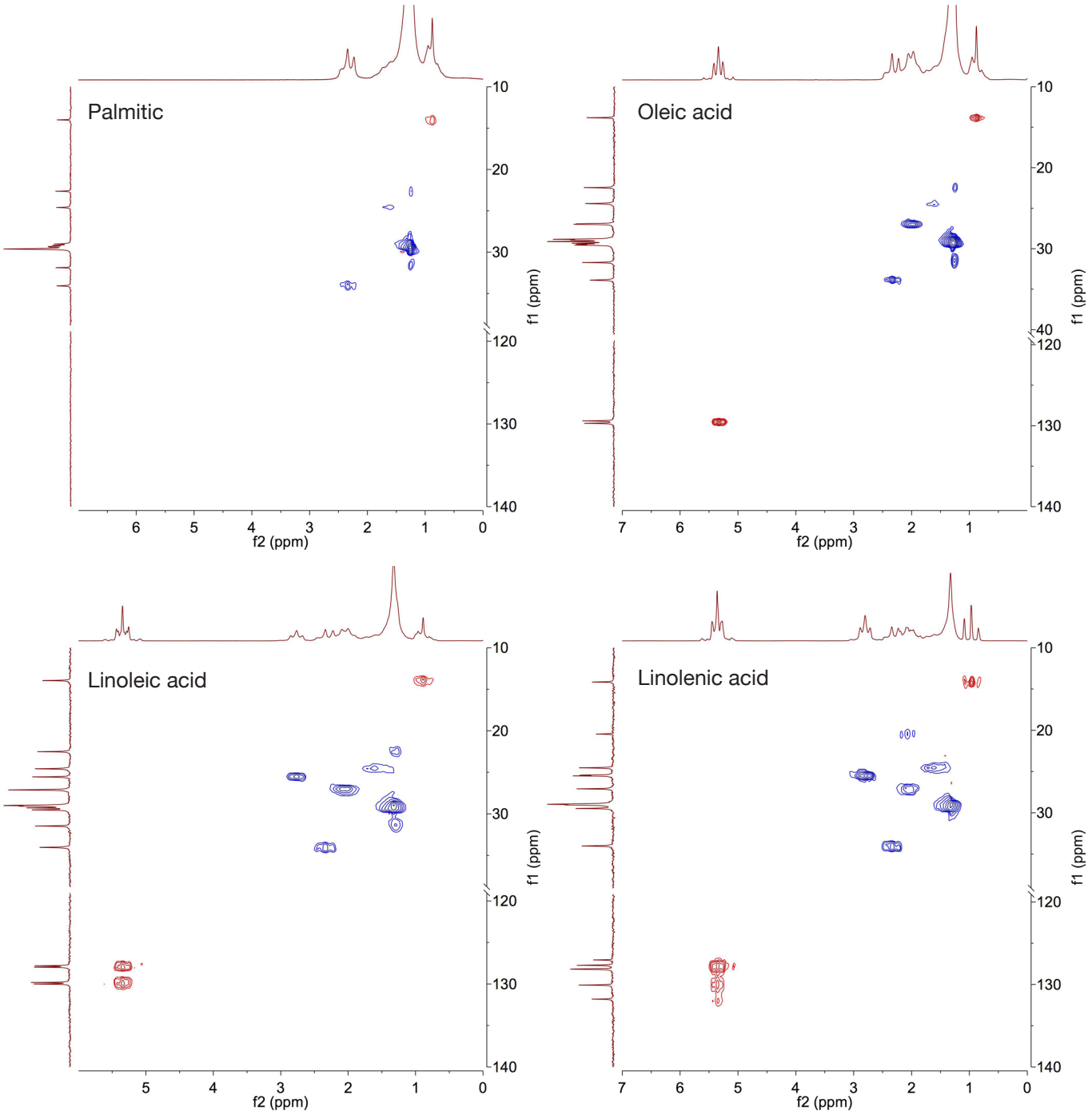


Figure 4: HSQC-ME spectra of palmitic, oleic, linoleic, and linolenic acid in $CDCl_3$ measured on a Spinsolve 60 Carbon. The data was acquired using 128 steps along the indirect dimension and 8 scans per step. Setting a repetition time of 3 seconds a total measurement time of 50 minutes was required per spectrum.

Heteronuclear Multiple Bond Correlation (HMBC)

To observe long-range ^{1}H-^{13}C correlations through two or three bond couplings, the Heteronuclear Multiple Bond Correlation (HMBC) experiment can be used. In contrast to HSQC, HMBC shows correlations between hydrogens and carbons that are farther than one bond away and no signal for the single bond neighbours. It should be noted that coupling with quaternary carbons also generate correlation signals in this sequence.

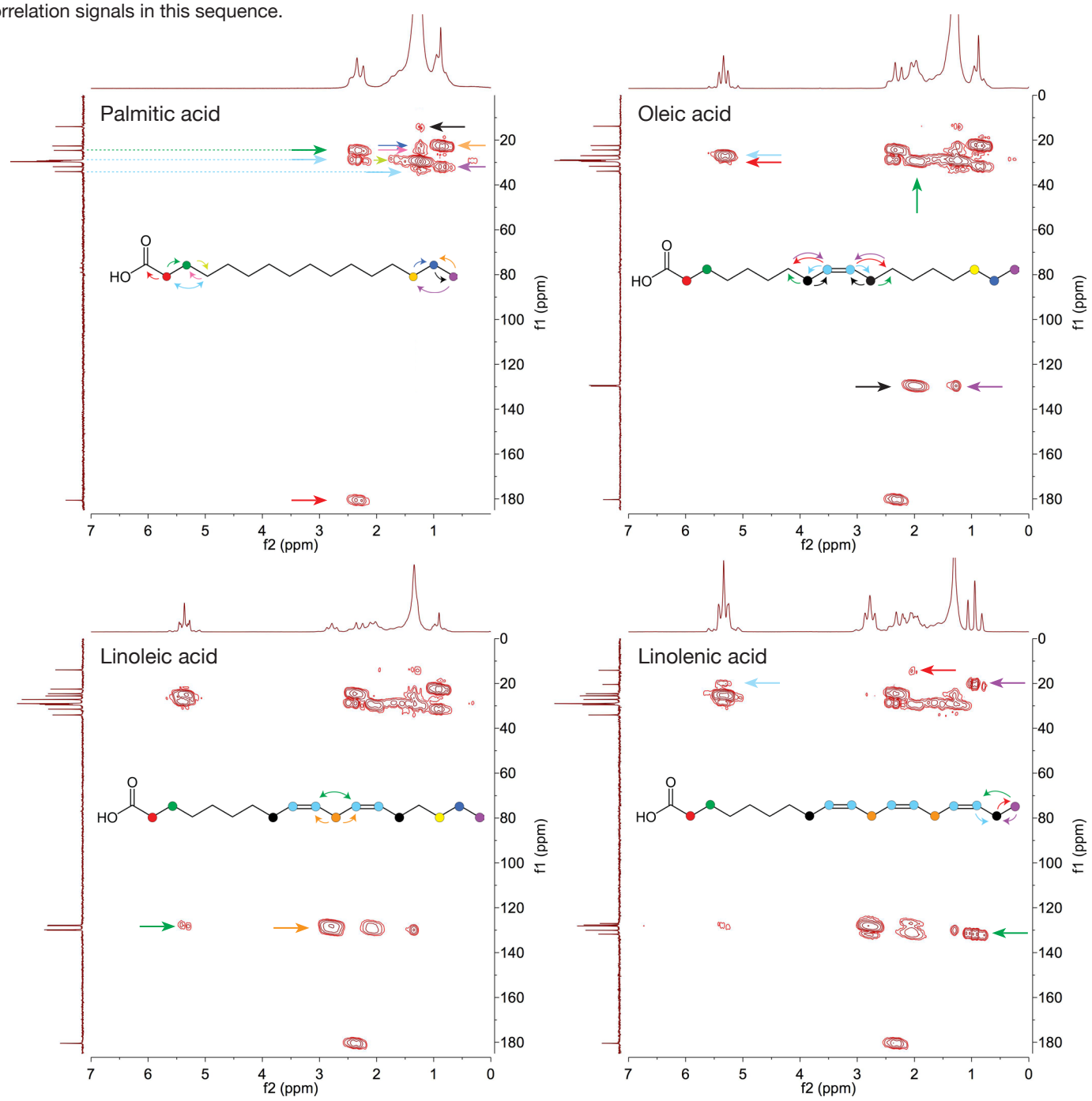


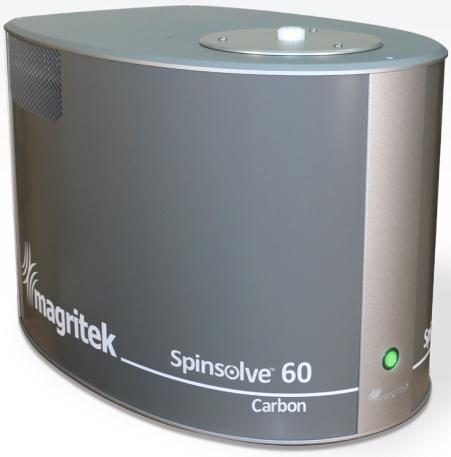
Figure 5: HMBC spectra of palmitic, oleic, linoleic, and linolenic acid in $CDCl_3$ measured on a Spinsolve 60 Carbon.

To read the HMBC spectra let's select first the position of a given ^{1}H group along the horizontal axis and lets then move along the vertical direction to confirm the correlation with the different carbons. For example, for palmitic acid, starting at the α hydrogens we find peaks at the position of the β (●), γ (●), and the quaternary carbon (●) -the colors refer to the arrows shown in the spectra and molecules-. The methyl hydrogens show correlation with the ω_2 (●) and ω_3 (●) carbons. The β hydrogens only show coupling with the methylene carbons (●) (the coupling with the α carbon generates a signal that is not visible with the scale set on this plot). Moving vertically along the position of the methylene protons we can confirm the coupling between the following ^{1}H and ^{13}C: ω_2 - ω_1 (●), ω_3 - ω_2 (●), γ - β (●), and γ – α (●). The spectra of oleic acid shows in addition the coupling of the allylic ^{1}H with both the methylene ^{13}C (●) and the olefinic ^{13}C (●). It also shows the coupling of the olefinic ^{1}H with the allylic (●) and methylene ^{13}C (●), however as the separation between these carbons is small a single broad peak is observed. The coupling of the methylene ^{1}H with the olefinic ^{13}C is easier to see (●). The spectrum of linoleic acid is similar to the one of oleic acid, but on top, it shows the correlation between the bis-allylic ^{1}H and olefinic ^{13}C (●) and between one pair olefinic ^{1}H with the ^{13}C of the other pair (●). Finally, the spectrum of linolenic acid shows the correlation of the methyl ^{1}H with both the allylic ^{13}C (●) and the olefinic ^{13}C (●), as well as the allylic ^{1}H with the methyl ^{13}C (●) and olefinic ^{1}H with the allylic ^{13}C (●).

Spinsolve® 60 Carbon

Specifications

- Nuclei: ^{1}H, ^{19}F, ^{13}C
- Operating frequency: 60 MHz (^{1}H)
- ^{1}H 50% Linewidth: < 0.5 Hz
- ^{1}H 0.55% Linewidth: < 20 Hz
- ^{1}H Sensitivity: >120:1 for 1% Ethyl Benzene
- Operating Temperature Range: 20° C to 25° C (68° F to 77° F)
- Dimensions: 58 x 43 x 40 cm (23” x 17” x16”)
- Weight: 60 kg (120 lb)
- Stray Field: < 2 G all around system
- Power requirement: 110-240V AC



Pulse sequences available on the Spinsolve 60 Carbon spectrometer

1D	1D	1D
Paramagnetic	Paramagnetic	DEPT
2D COSY	2D F - COSY	HETCOR
2D TOCSY	2D F - JRES	HMBC
2D JRES	2D FH - COSY	HMQC
T_1, T_2	Reaction Monitoring	HSQC
Reaction Monitoring		HSQC-ME

Other sequences may be available, contact Magritek for details.

Contact us now for a quote, to request a demo or to measure your samples

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