Determination of ω-3 fatty acids by ^{1}H and ^{13}C NMR spectroscopy

^{1}H- and ^{13}C NMR spectra are sensitive for chemical structures, different subgroups of similar chemical surroundings are separated in regions of similar chemical shifts (see Fig. 1).

Any common fatty acid consists of a terminal methyl group. Saturated and unsaturated fatty acids show different chemical shifts of these methyl groups. The difference in the chemical shift depends on the distance between the terminal methyl group and the position of the next double bond.

In comparison to the chemical shift of a saturated fatty acid w-3 fatty acids show a downfield shift od approx. 0.1 ppm and therefor it is baseline separated from all other fatty acid terminal methyl groups. A differentiation of ω-6 and ω-9 fatty acids is not so easy (see Fig. 2).

Total measuring Time: ^{1}H NMR approx. 2 minutes, ^{13}C NMR approx. 25 minutes
100 mg sample needed, the method is non-destructible!

Fig. 1: ^{1}H NMR spectrum of a vegetable TAG

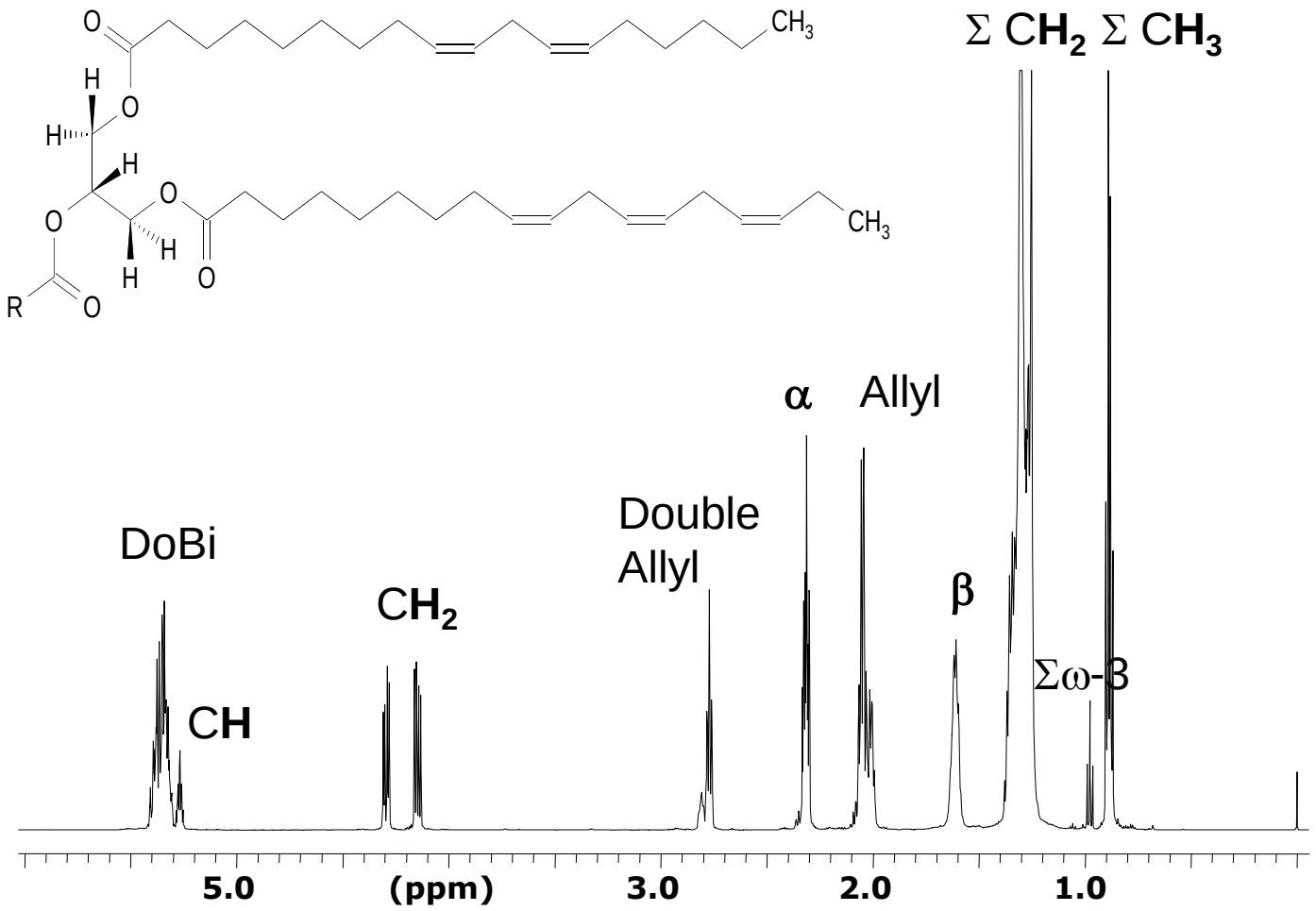


Fig. 2: Separation of ω-3 from ω-6, ω-9 and saturated fatty acids in ^{1}H NMR

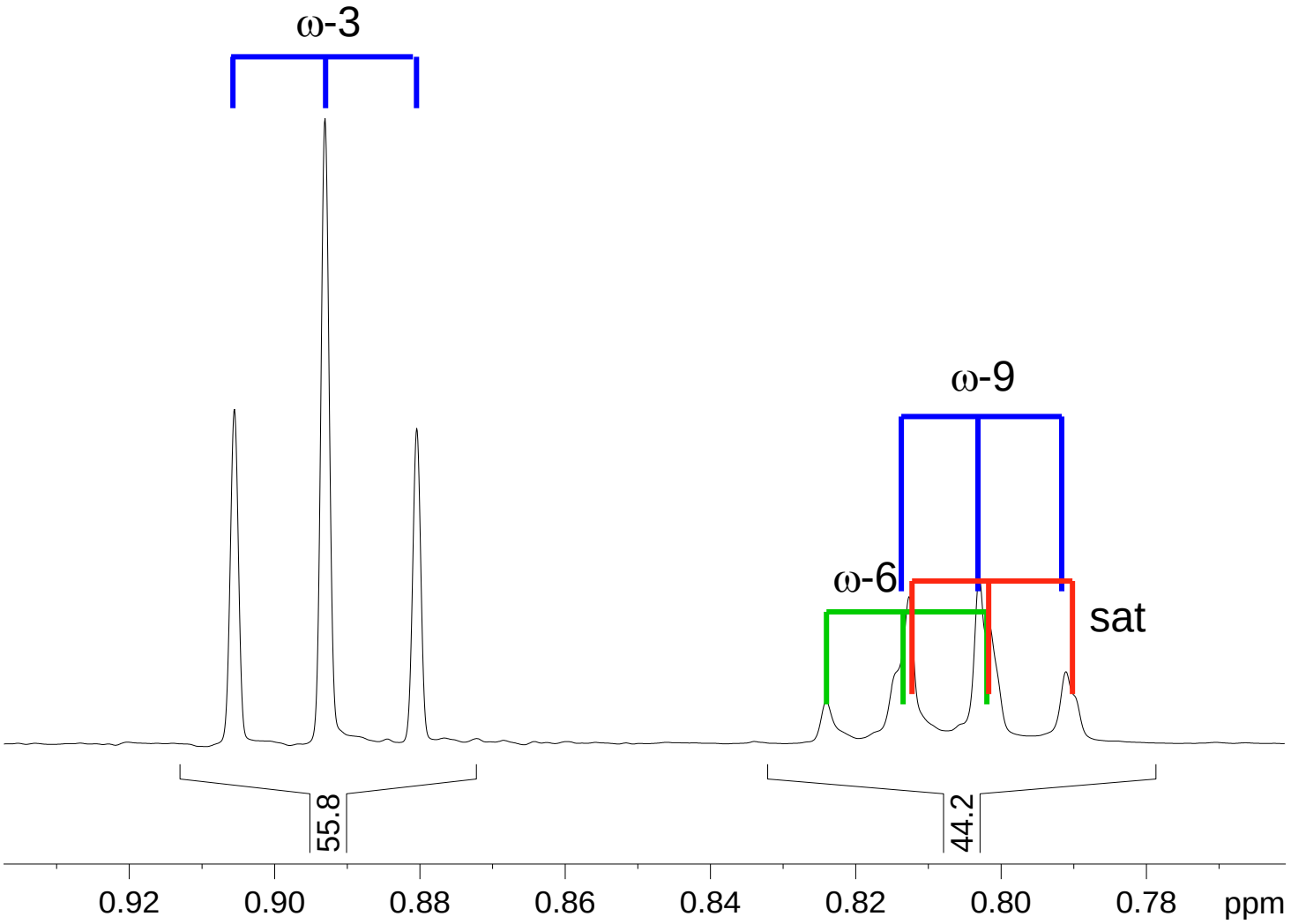
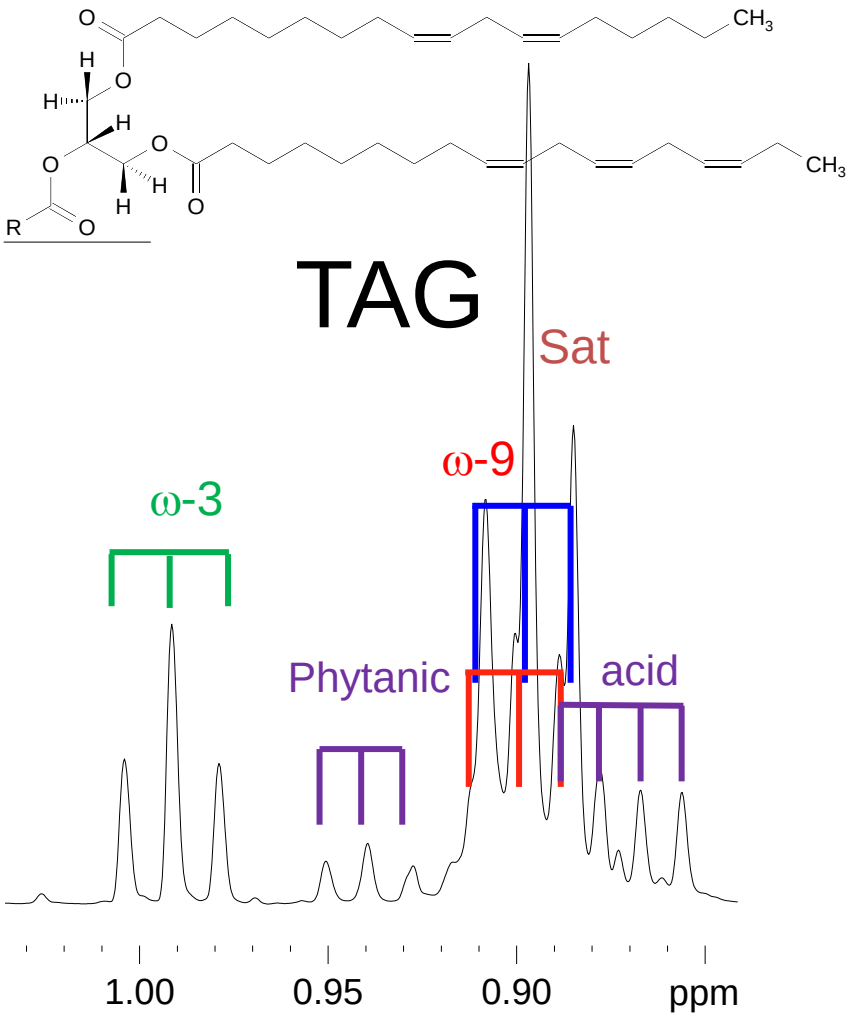


Fig. 3: Separation of ω-3 from ω-6, ω-9 and saturated fatty acids in ^{1}H NMR, comparison of TAG and Phospholipids from krill oil after preparative separation. The neutral lipids fraction contains the branched phytanic acid which also can be quantified.



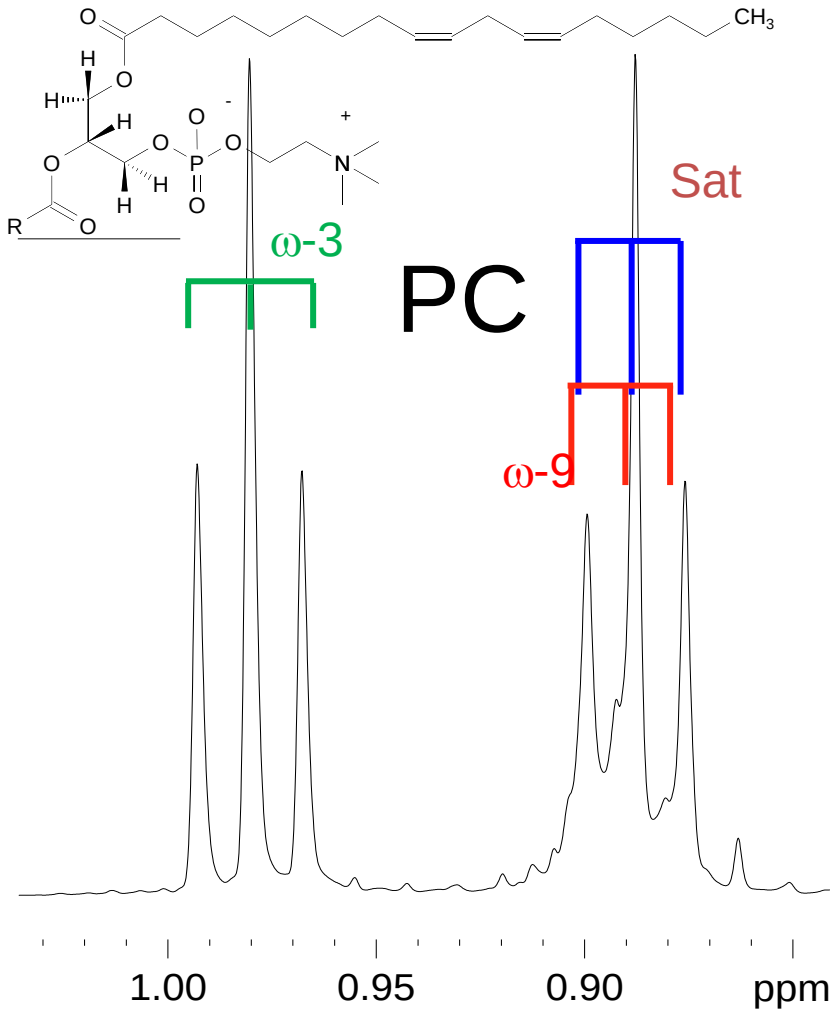
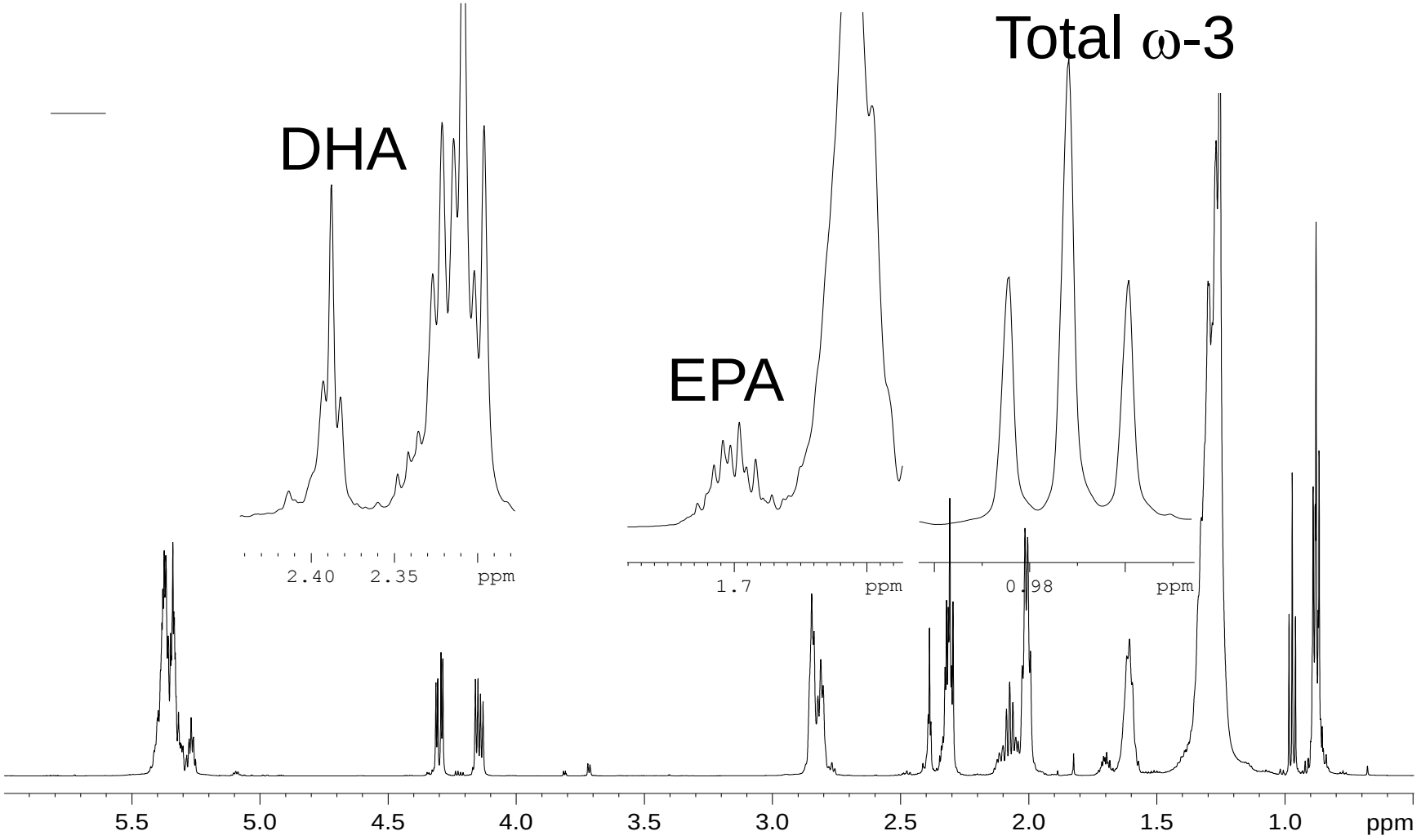


Fig. 4: Total ω-3 consists mainly of EPA and DHA. Other regions of chemical shift enable the simultaneously detection of these types. The ^{1}H NMR spectrum belong to salmon oil.



Spectral Service

ILPS International Lecithin & Phospholipids Society

Peak	ppm	Area	Area-%
1	14.113	47.477	16.101
2	13.960	71.342	24.194
3	13.954	114.718	38.904
4	13.945	22.199	7.528
5	13.914	39.140	13.273

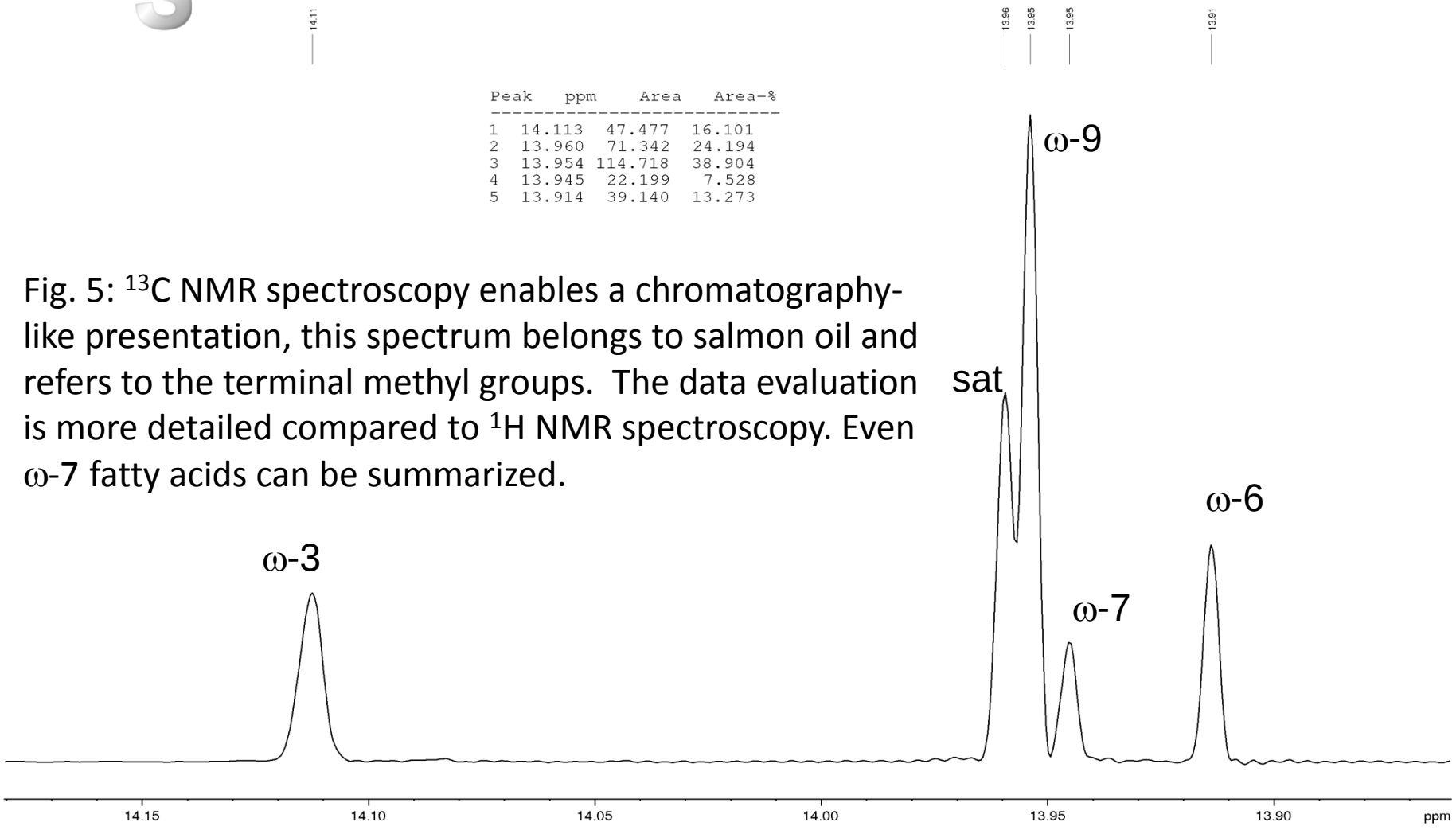


Fig. 5: ^{13}C NMR spectroscopy enables a chromatography-like presentation, this spectrum belongs to salmon oil and refers to the terminal methyl groups. The data evaluation is more detailed compared to ^{1}H NMR spectroscopy. Even ω-7 fatty acids can be summarized.

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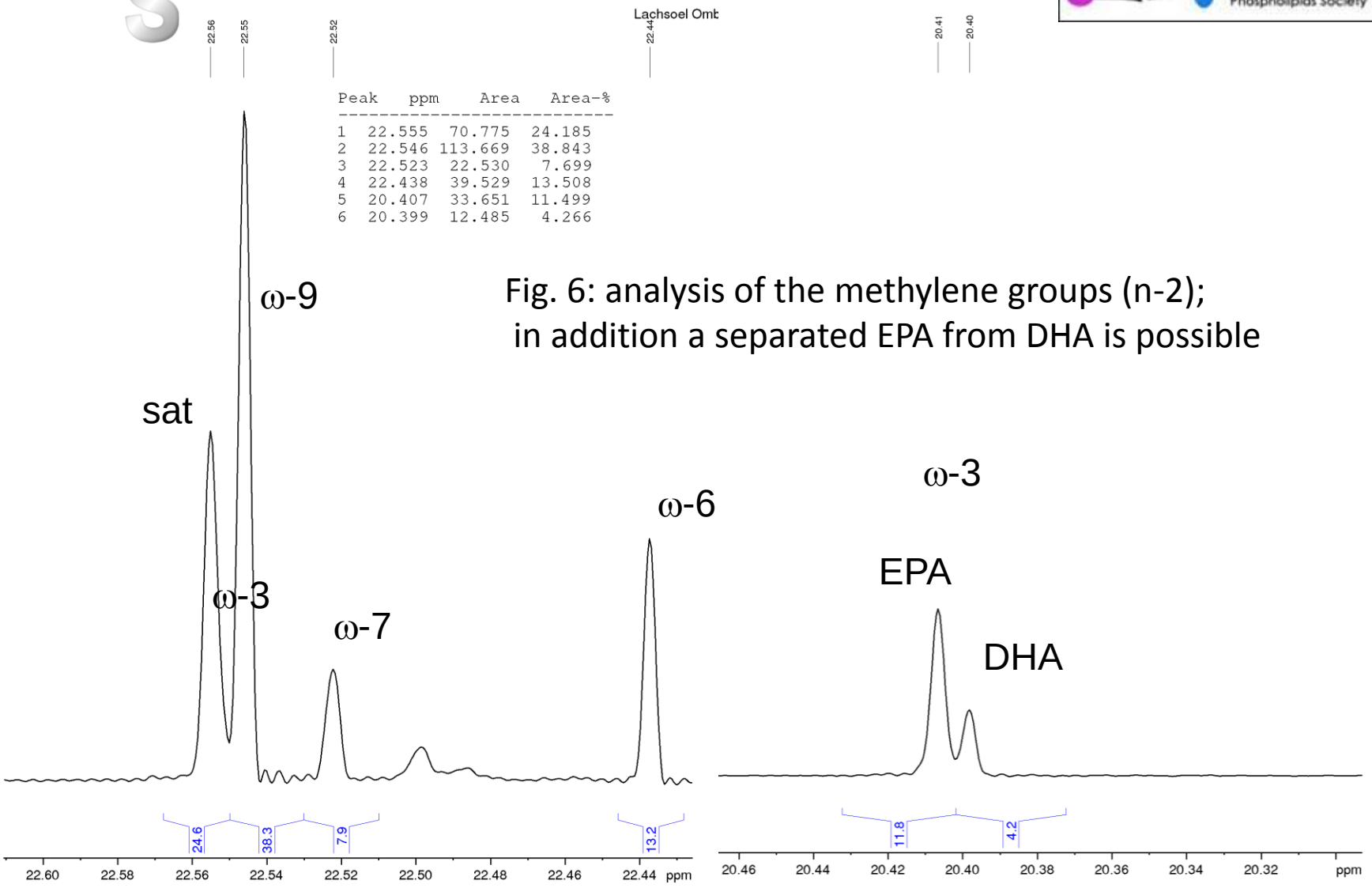
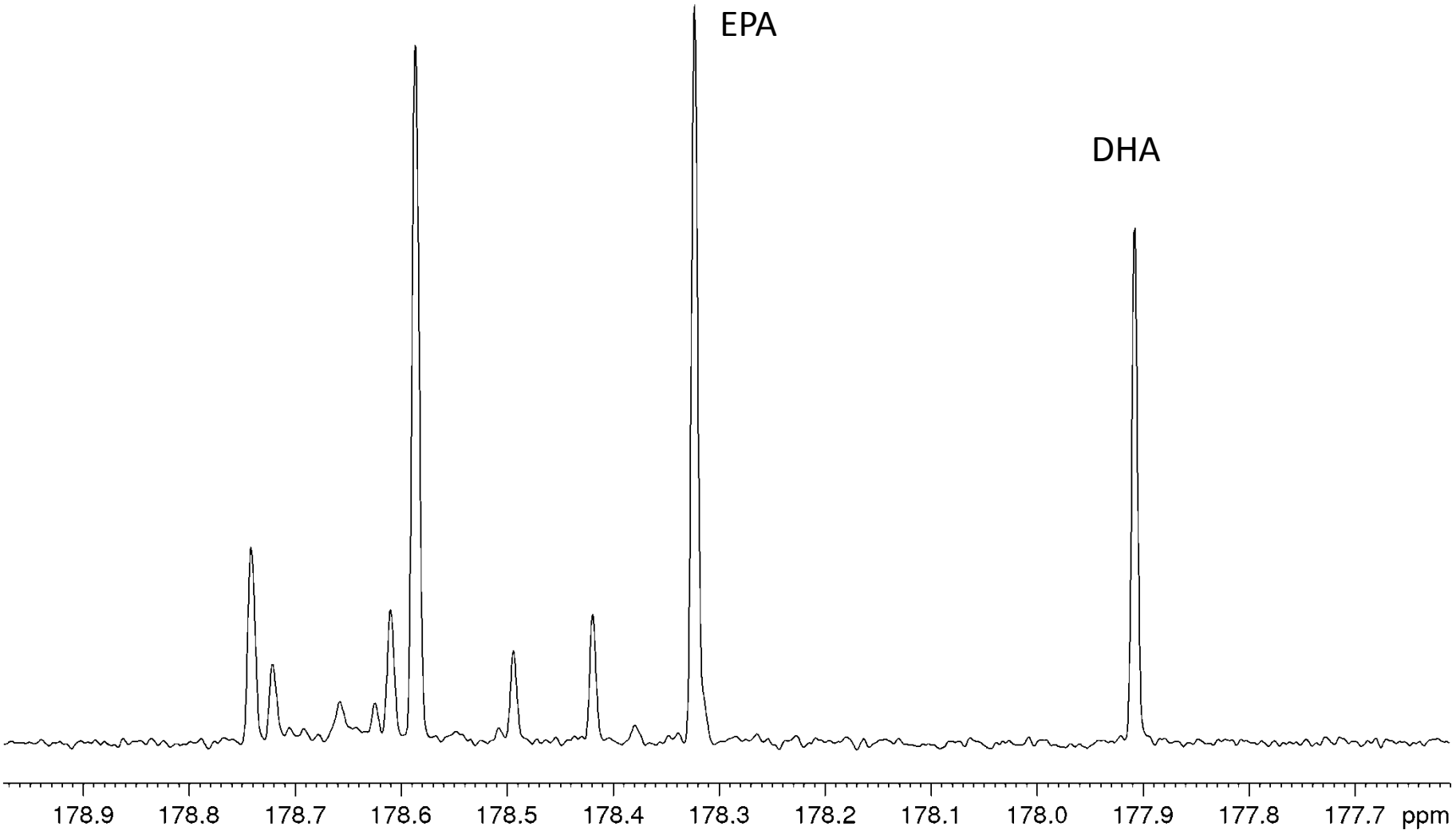


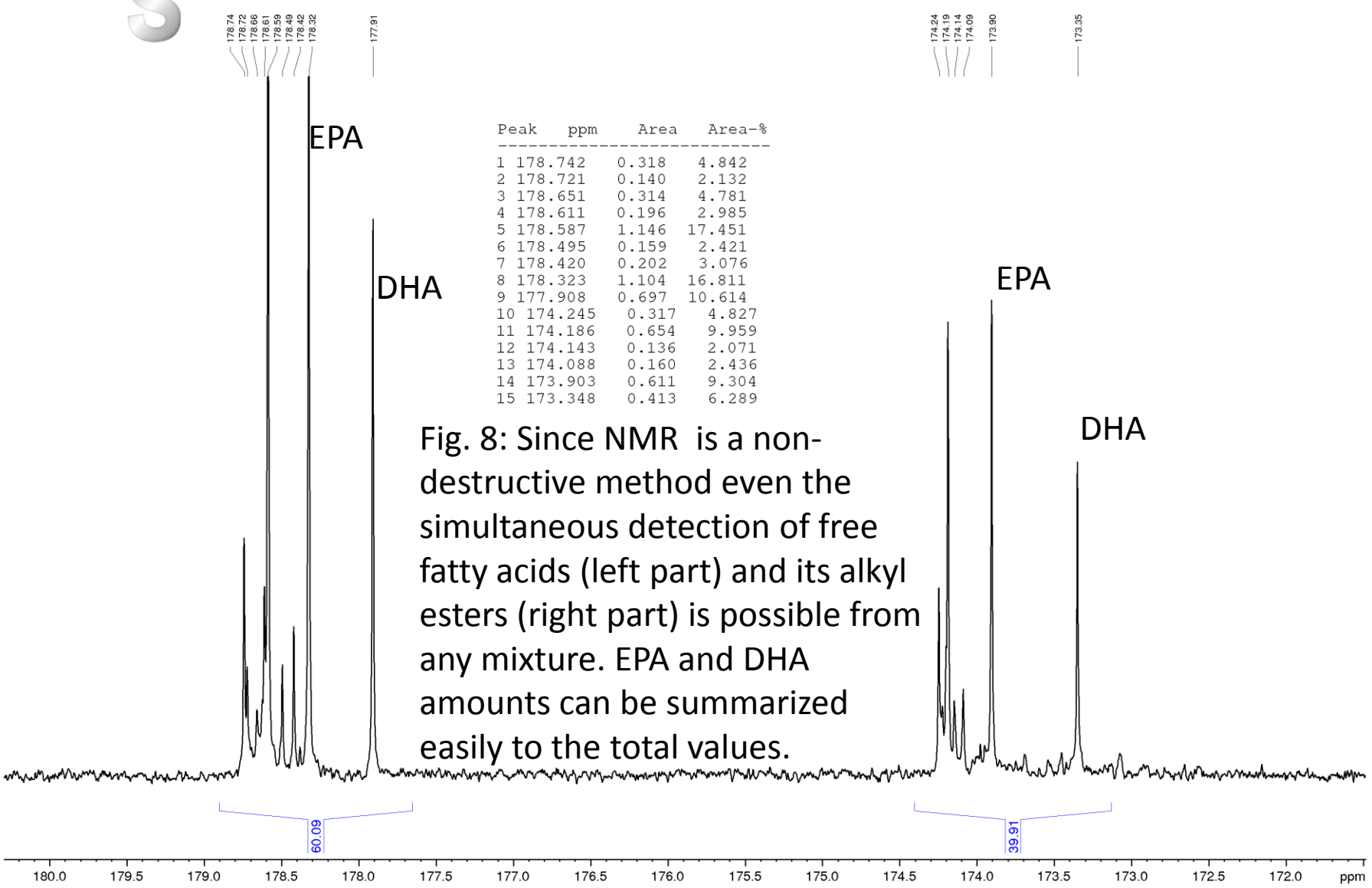
Fig. 6: analysis of the methylene groups (n-2);
in addition a separated EPA from DHA is possible

Fig. 7: ^{13}C NMR of the carbonyl signals enable a selective analysis of the different Δ types of fatty acids, DHA is a Δ-4 and EPA a Δ-5 fatty acid



Free Fatty Acids

Ethyl Esters



Peak	ppm	Area	Area-%
1	178.742	0.318	4.842
2	178.721	0.140	2.132
3	178.651	0.314	4.781
4	178.611	0.196	2.985
5	178.587	1.146	17.451
6	178.495	0.159	2.421
7	178.420	0.202	3.076
8	178.323	1.104	16.811
9	177.908	0.697	10.614
10	174.245	0.317	4.827
11	174.186	0.654	9.959
12	174.143	0.136	2.071
13	174.088	0.160	2.436
14	173.903	0.611	9.304
15	173.348	0.413	6.289

Fig. 8: Since NMR is a non-destructive method even the simultaneous detection of free fatty acids (left part) and its alkyl esters (right part) is possible from any mixture. EPA and DHA amounts can be summarized easily to the total values.

Fig. 9: The observation of EPA and DHA amounts of fatty ester mixtures can be detected on the ester group, too. The spectrum belongs to the ethyl ester (-CO-O-**CH_2**-CH_3).