

# Determination of $\omega$ -3 fatty acids by $^1\text{H}$ and $^{13}\text{C}$ NMR spectroscopy

$^1\text{H}$ - and  $^{13}\text{C}$  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  $\omega$ -3 fatty acids show a downfield shift of approx. 0.1 ppm and therefore it is baseline separated from all other fatty acid terminal methyl groups. A differentiation of  $\omega$ -6 and  $\omega$ -9 fatty acids is not so easy (see Fig. 2).

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

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

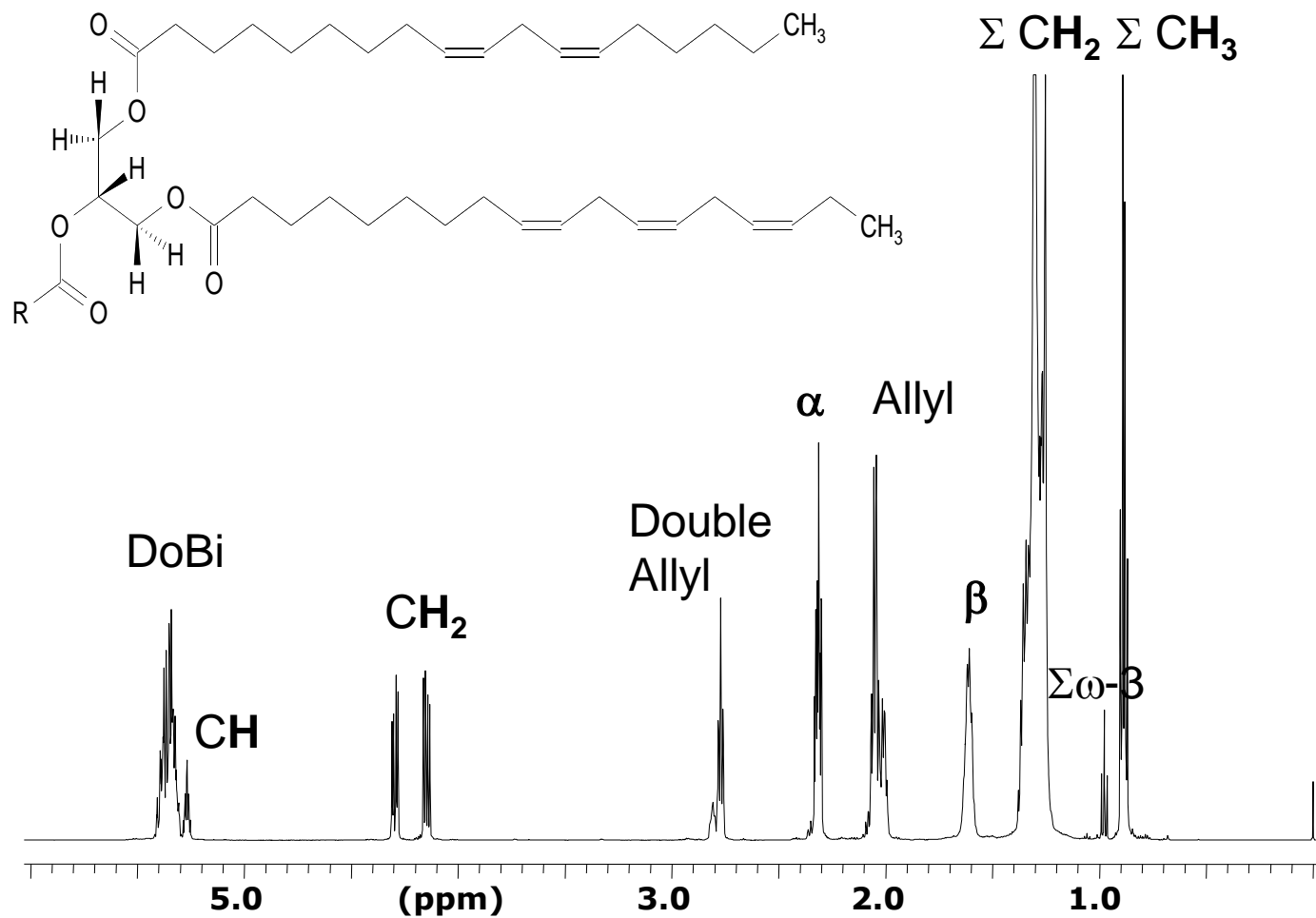


Fig. 2: Separation of  $\omega$ -3 from  $\omega$ -6,  $\omega$ -9 and saturated fatty acids in  $^1\text{H}$  NMR

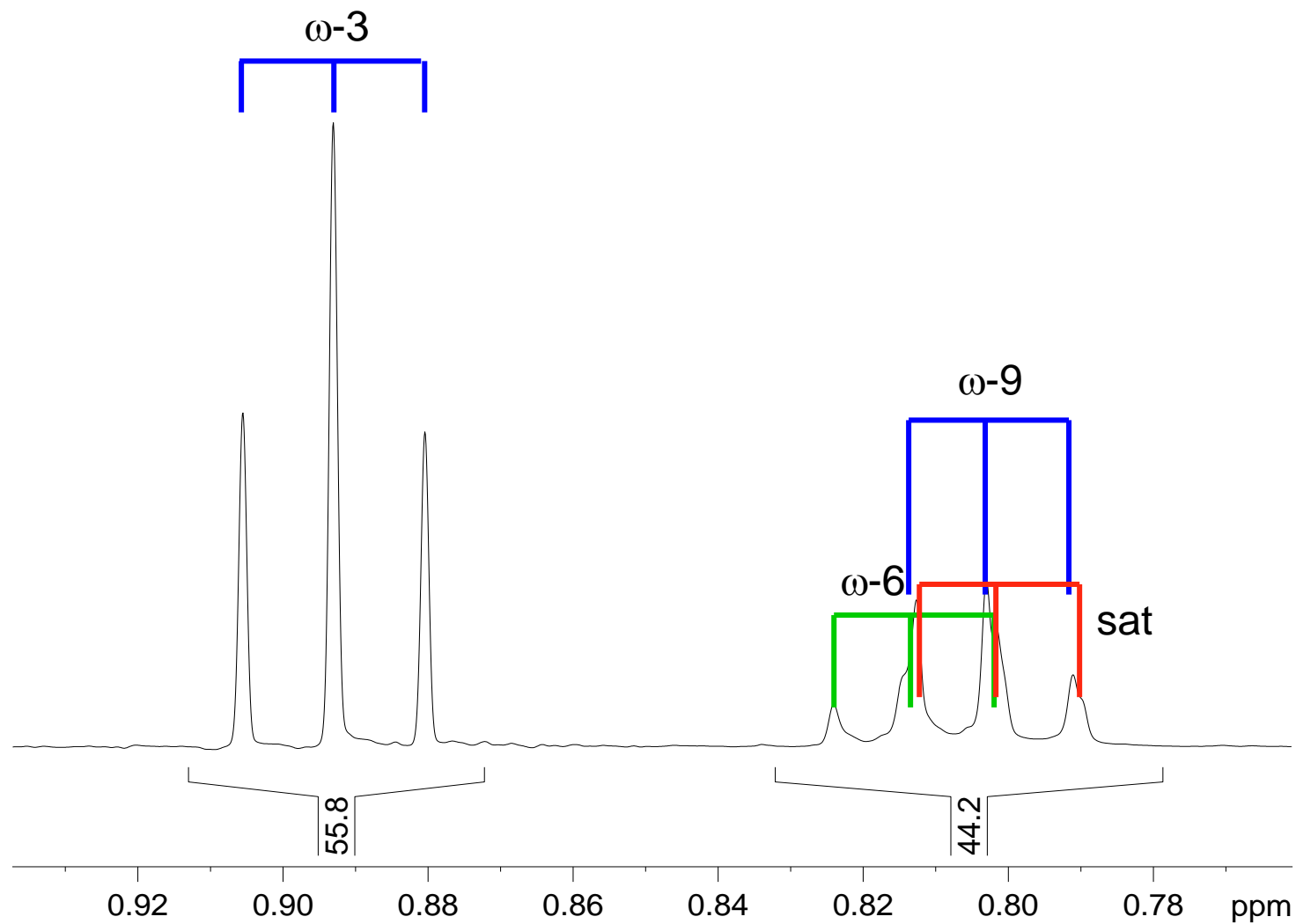


Fig. 3: Separation of  $\omega$ -3 from  $\omega$ -6,  $\omega$ -9 and saturated fatty acids in  $^1\text{H}$  NMR, comparison of TAG and Phospholipids from krill oil after preparative separation. The neutral lipids fraction contains the branched phytanic acid which also can be quantified.

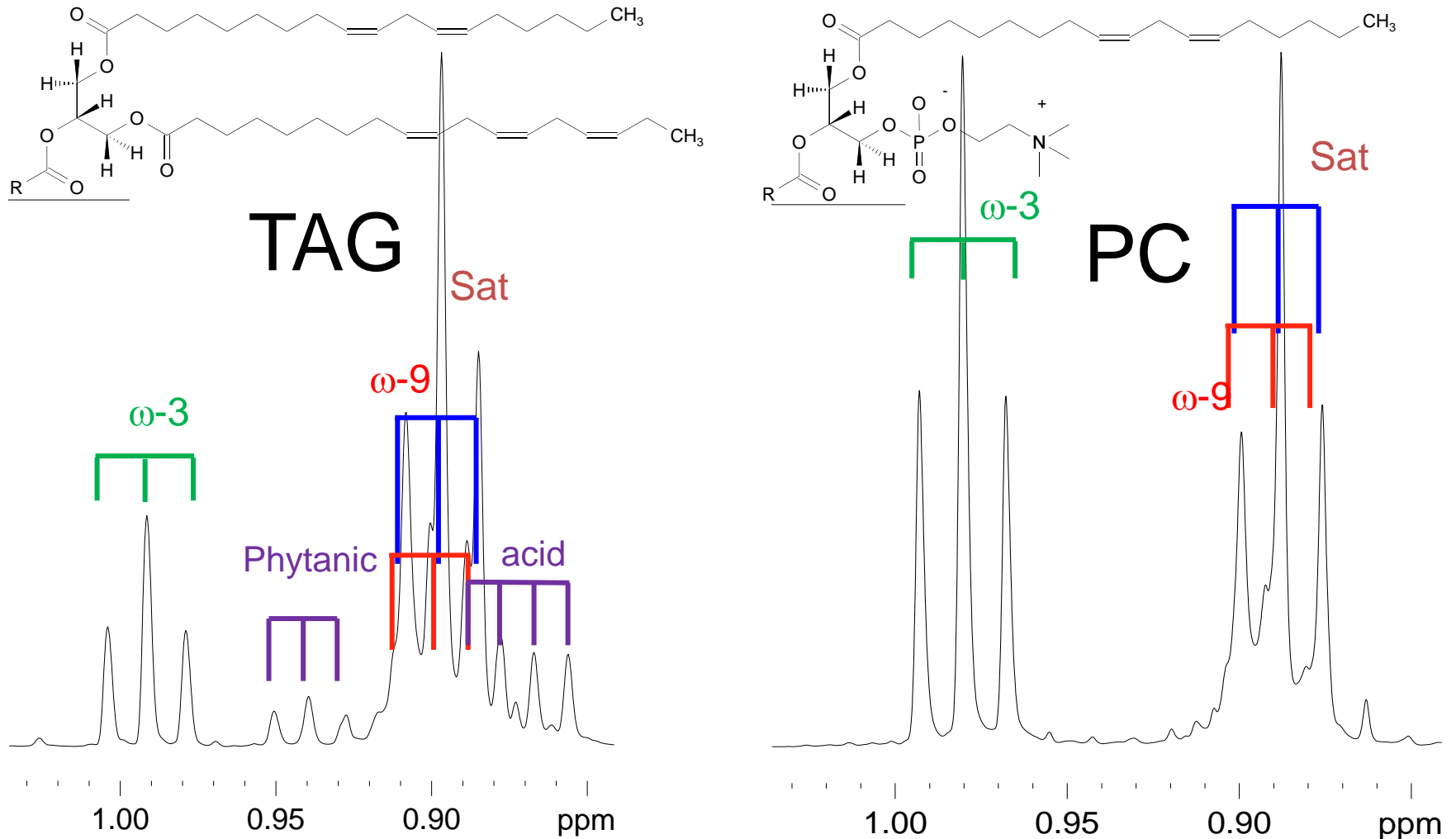
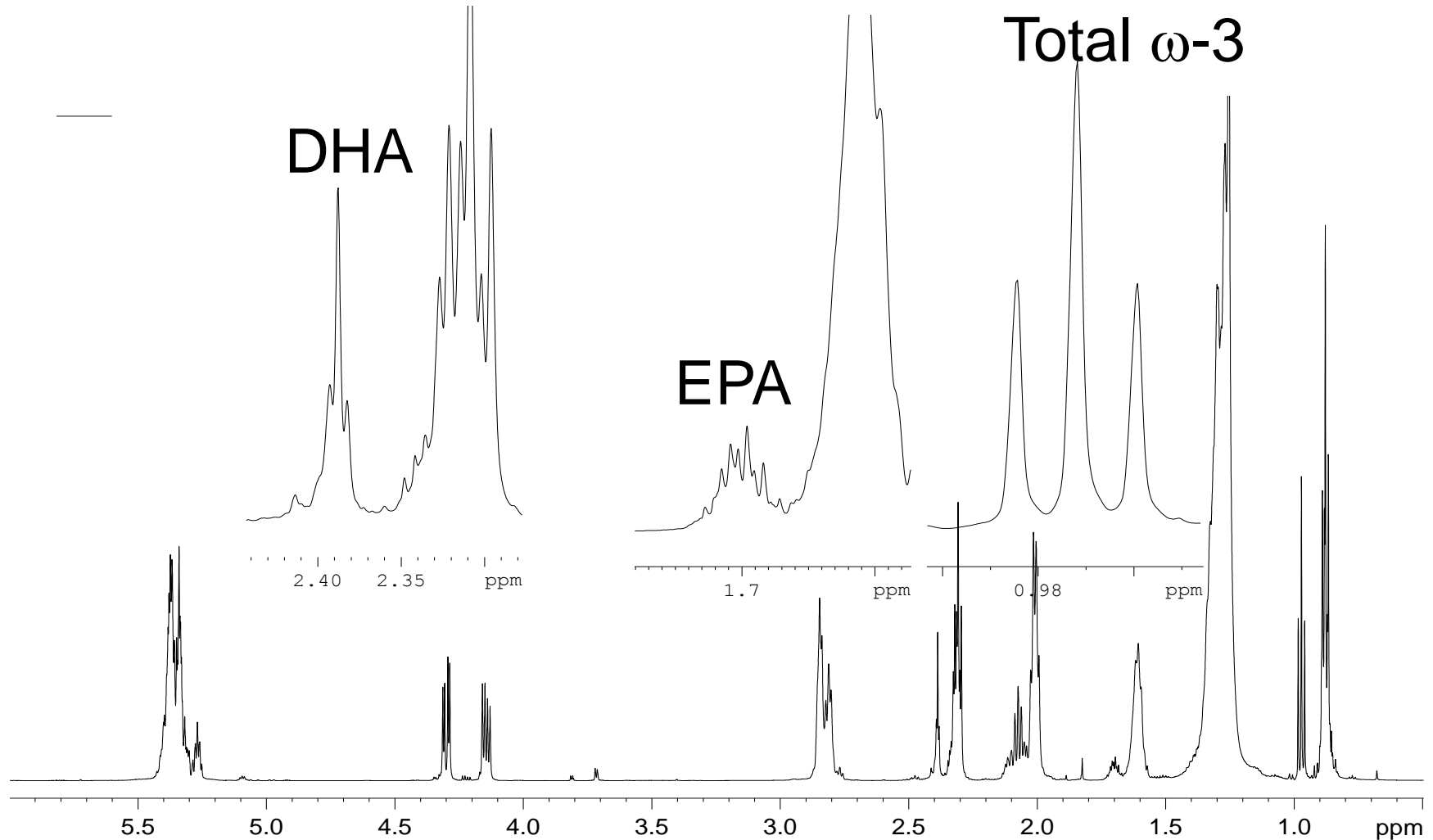


Fig. 4: Total  $\omega$ -3 consists mainly of EPA and DHA. Other regions of chemical shift enable the simultaneous detection of these types. The  $^1\text{H}$  NMR spectrum belong to salmon oil.



14.11

13.96

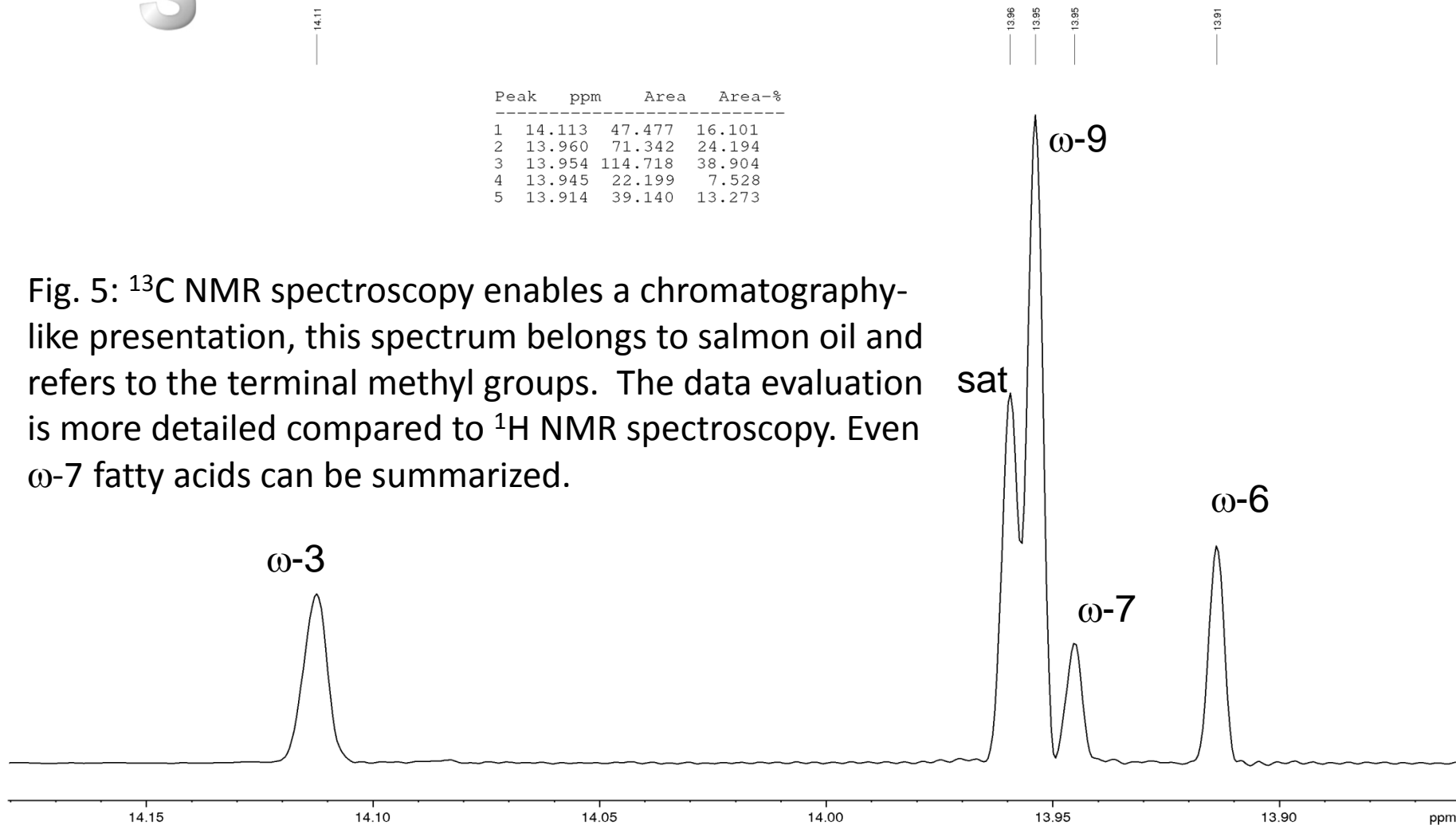
13.95

13.95

13.91

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:  $^{13}\text{C}$  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  $^1\text{H}$  NMR spectroscopy. Even  $\omega$ -7 fatty acids can be summarized.



Lachsoel Omt

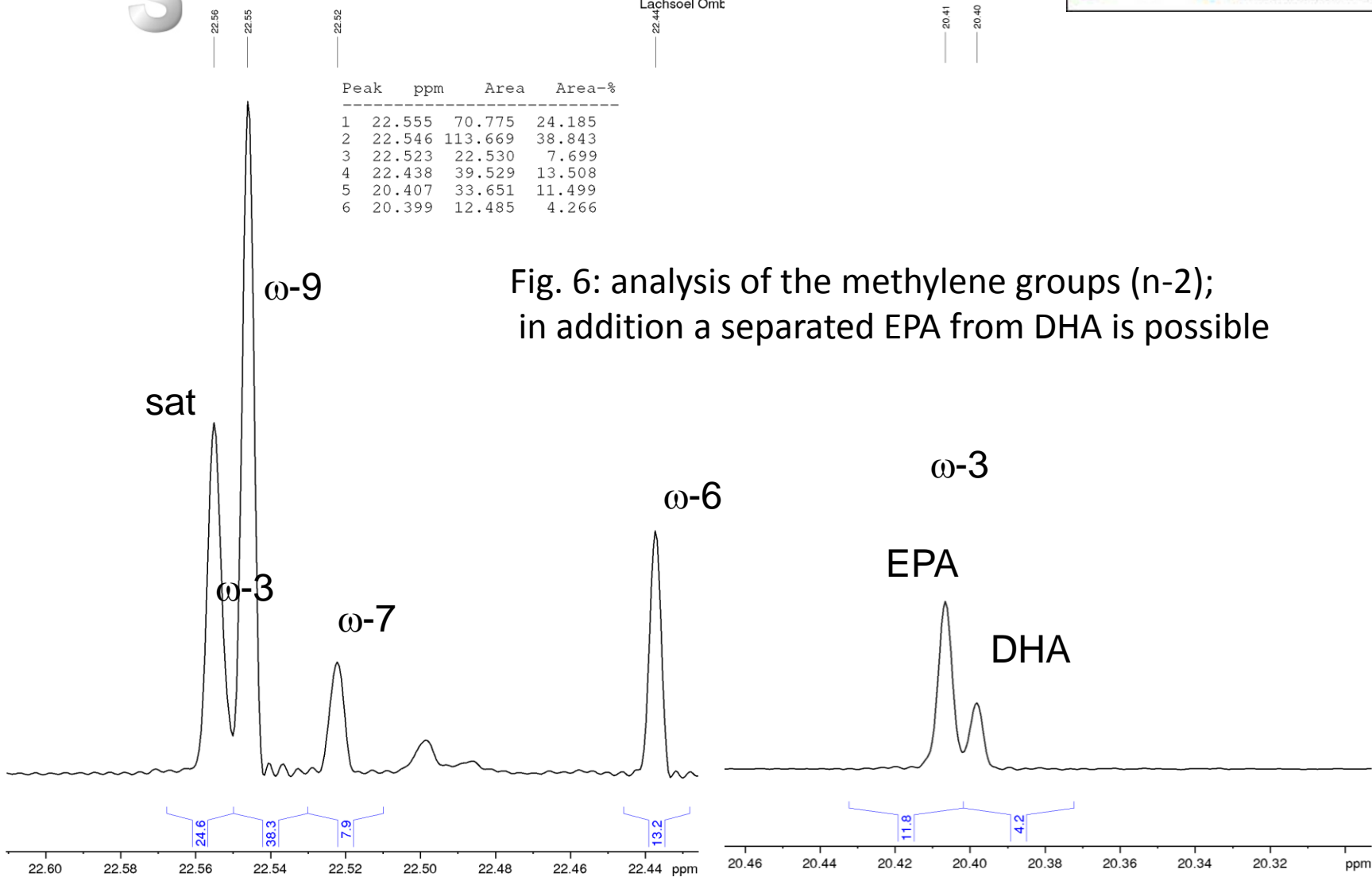
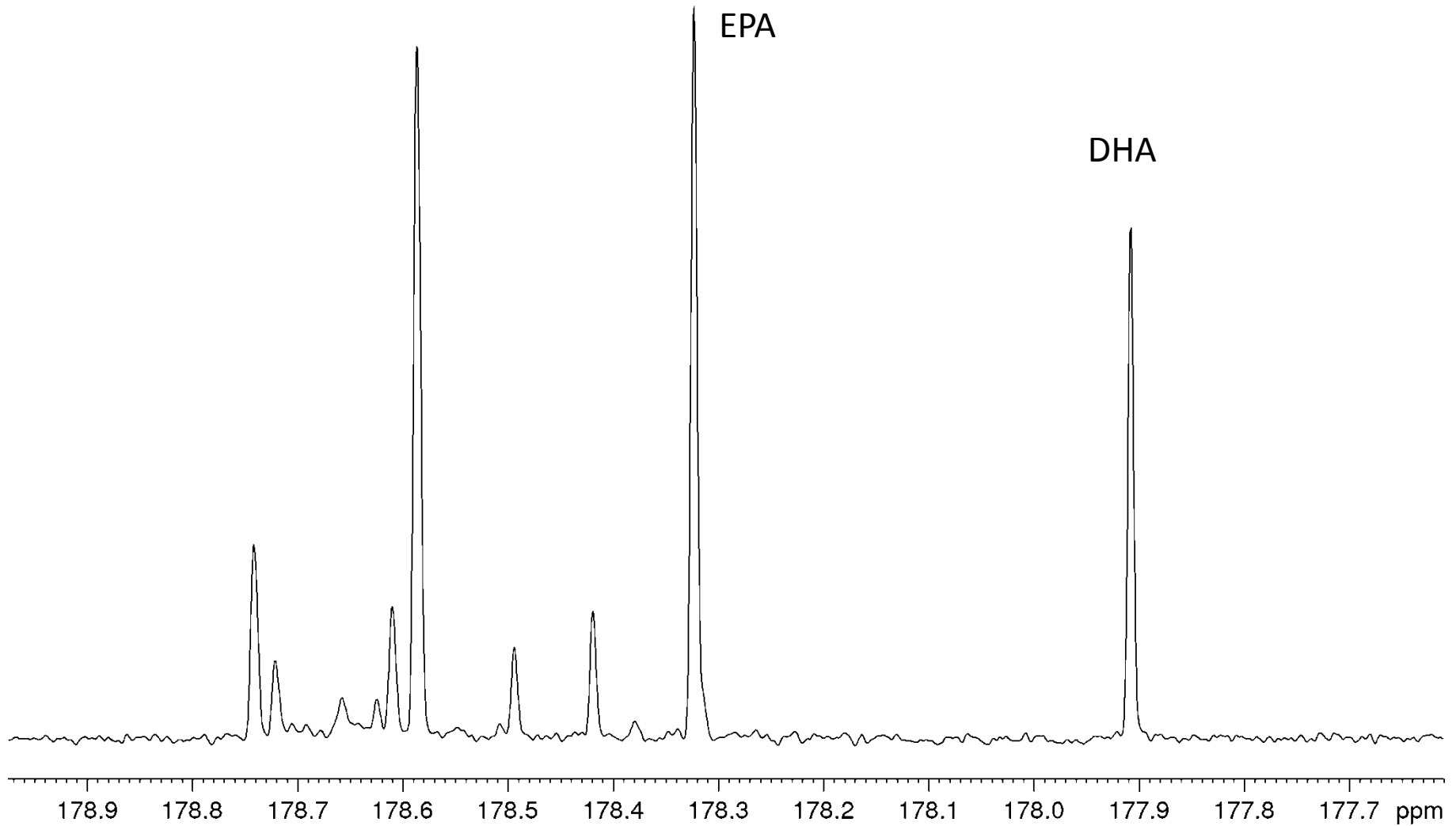


Fig. 6: analysis of the methylene groups (n-2); in addition a separated EPA from DHA is possible

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





## Free Fatty Acids

## Ethyl Esters

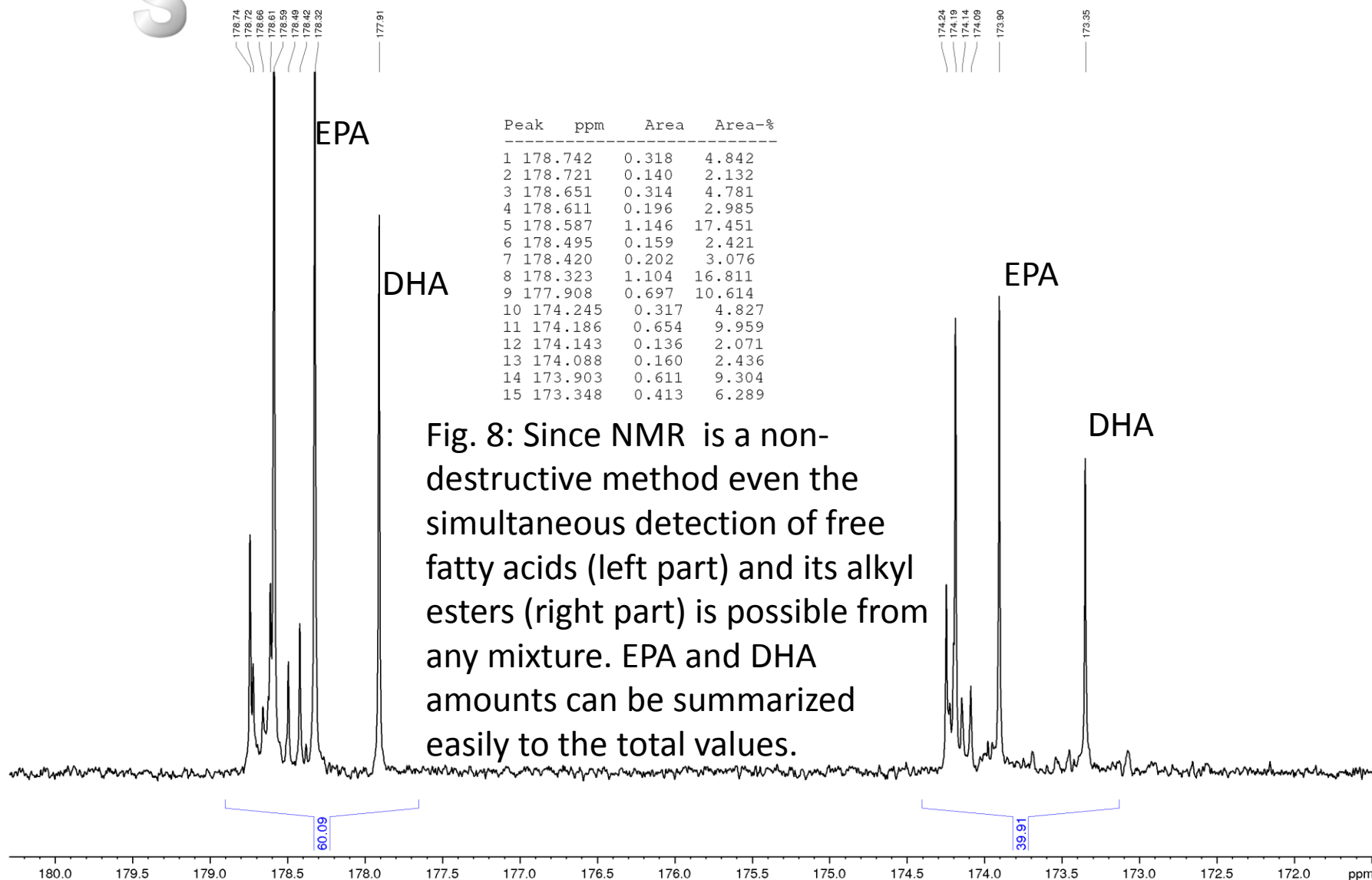


Fig. 8: Since NMR is a non-destructive method even the simultaneous detection of free fatty acids (left part) and its alkyl esters (right part) is possible from any mixture. EPA and DHA amounts can be summarized easily to the total values.

