Supporting Information

Localization of Double Bonds in Wax Esters by High-Performance Liquid Chromatography/Atmospheric Pressure Chemical Ionization Mass Spectrometry Utilizing Fragmentation of Acetonitrile-Related Adducts

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Experimental details

LCQ Fleet ion-trap

The liquid chromatograph consisted of a Rheos Alegro UHPLC pump, an AS 3000 autosampler and an LCQ Fleet ion-trap mass spectrometer; the system was controlled by an SN 4000 control unit and Xcalibur software (all by Thermo Fisher Scientific, San Jose, CA, USA). Nova-Pak C18 (150 and 300 × 3.9 mm, particle size: 4 μm; Waters, Milford, MA, USA) stainless-steel columns were used. A Labio RT 04 column thermostat (Labio, Prague, Czech Republic) maintained the column temperature at 30°C or 40°C. The gradient was programmed from acetonitrile (A) and ethyl acetate (B) as follows: 0 min – 100 % of A; 167 min – 100 % of B. The flow rate of the mobile phase was 0.7 mL/min and the injected volume of the samples was 10 μL. The WE standards were dissolved in chloroform (0.4 μmol/mL), the WE mixtures isolated from beeswax and jojoba were prepared in ethyl acetate/chloroform (1:1, by vol.) at concentrations of 10 mg/mL and 15 mg/mL, respectively. The APCI vaporizer and heated capillary temperatures were set to 425°C and 250°C, respectively; the corona discharge current was 3.5 μA. Nitrogen served both as the sheath and auxiliary gas at a flow rate of eight-two and forty-eight arbitrary units, respectively. The CID MS/MS spectra were recorded using helium as the collision gas in the ion trap. The data were collected using data-dependent analysis with three scan events. The full-scan spectra of the positively-charged ions were recorded in the range of m/z 450–1000. The most intense precursor ions ($[M+H]^+$) were fragmented in the first data-dependent scan (second scan event). The $[M+55]^{+\bullet}$ adducts were selected using Mass Tag (with a mass delta of fifty-four units) and fragmented in the second data-dependent scan (third scan event). The precursors were selected with an isolation width of two mass units and a normalized collision energy of 24–32%. Using these settings, roughly 40 MS and 80 MS/MS spectra were recorded across the chromatographic peaks (the peak width at half-height ~20 sec; the peak width at the base ~75 sec). To confirm the positions of the double bonds in very low-abundance WEs, the data dependent analysis with only two scan events was performed (the MS/MS of $[M+H]^+$ was skipped). The spectra presented in this work were averaged across the entire peaks. Dynamic exclusion was not applied, as good chromatographic separation of the WEs differing in molecular weight was achieved. The coeluting peaks were almost exclusively isomers of WEs with the same molecular weight. Scanning without dynamic exclusion made it possible to characterize all of the coeluting isomers. The direct infusion experiments were performed with samples dissolved in the mobile phase. The sample solution (10–100 μg/mL in acetonitrile/ethyl acetate 1:1, a flow rate of 5–30 μL/min) was mixed in a low-dead-volume T-piece with the mobile phase (acetonitrile/ethyl acetate 1:1, by vol.; 0.7 mL/min) and introduced into the APCI source.

LTQ Orbitrap XL

The high-resolution MS data were recorded using an LTQ Orbitrap XL hybrid mass spectrometer (Thermo Fisher Scientific) equipped with an APCI ion source. The chromatographic and ionization conditions were the same as for low-resolution experiments; the spectra were acquired at a resolution of 100,000.

Q-Tof micro

The q-tof experiments were performed with a quadrupole orthogonal acceleration time-of-flight tandem mass spectrometer Q-Tof micro (Waters) equipped with a Z-spray and an APCI probe. The instrument was operated in the positive ion mode with the probe and source temperatures at 400°C and 100°C, respectively, and sample and extraction cone voltages of 20V and 2V, respectively. The collision energies for CID experiments were 15–25V (WEs) or 35–50V (TGs).

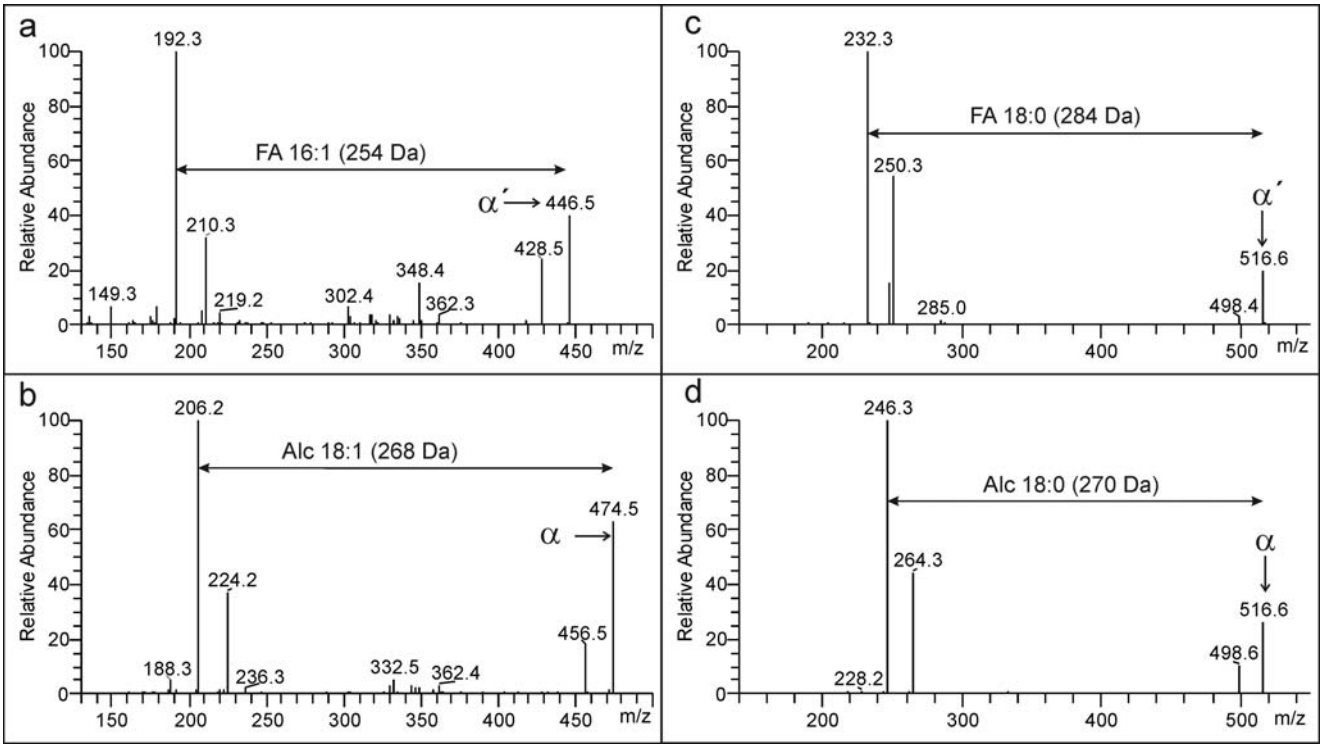

Figure S-1. The APCI MS^3 spectra of the α'-fragment (*m/z* 446) (a) and α-fragment (*m/z* 474) (b) generated by CID of the $[M+55]^{+\bullet}$ adduct of oleyl palmitoleate (18:1(n-9)-16:1(n-7)). The APCI MS^3 spectrum of the α'-fragment (*m/z* 516) (c) generated by CID of the $[M+55]^{+\bullet}$ adduct of linoleyl stearate (WE 18:2(n-6)-18:0). The APCI MS^3 spectrum of the α-fragment (*m/z* 516) (d) generated by CID of the $[M+55]^{+\bullet}$ adduct of stearyl linoleate (WE 18:0-18:2(n-6)). The samples was prepared in acetonitrile/ethyl acetate (1:1, by vol.) and directly infused into the ion source of LCQ Fleet.

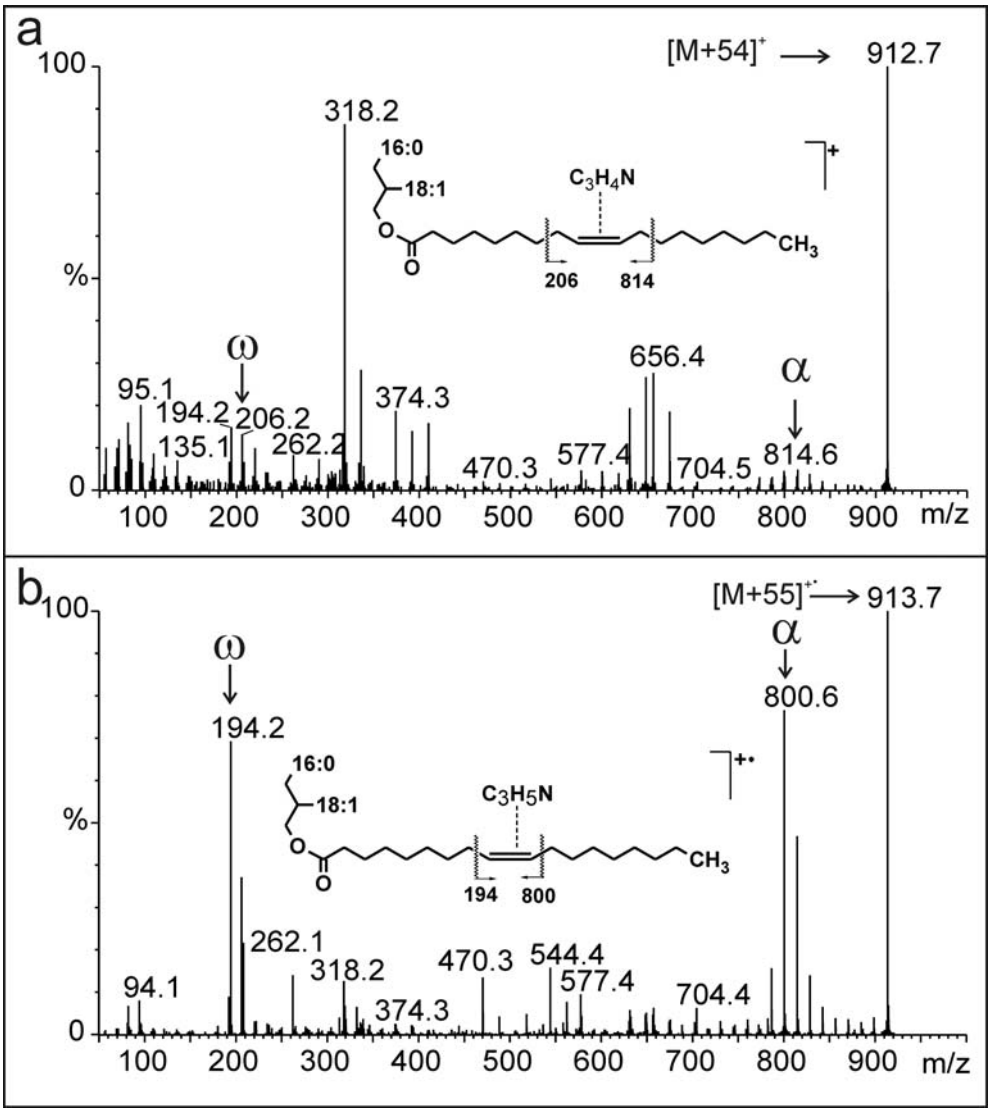

Figure S-2. The APCI MS/MS spectra of the C_3H_4N ($[M+54]^+$) (a) and C_3H_5N ($[M+55]^{+\bullet}$) (b) adducts of 1-palmitoyl-2,3-dioleoyl-*sn*-glycerol (POO). The samples were prepared in acetonitrile/chloroform (9:1, by vol.) at a concentration 100.0 μg/mL and directly infused into the ion source of Q-Tof micro, with a flow rate of 100 μL/min.

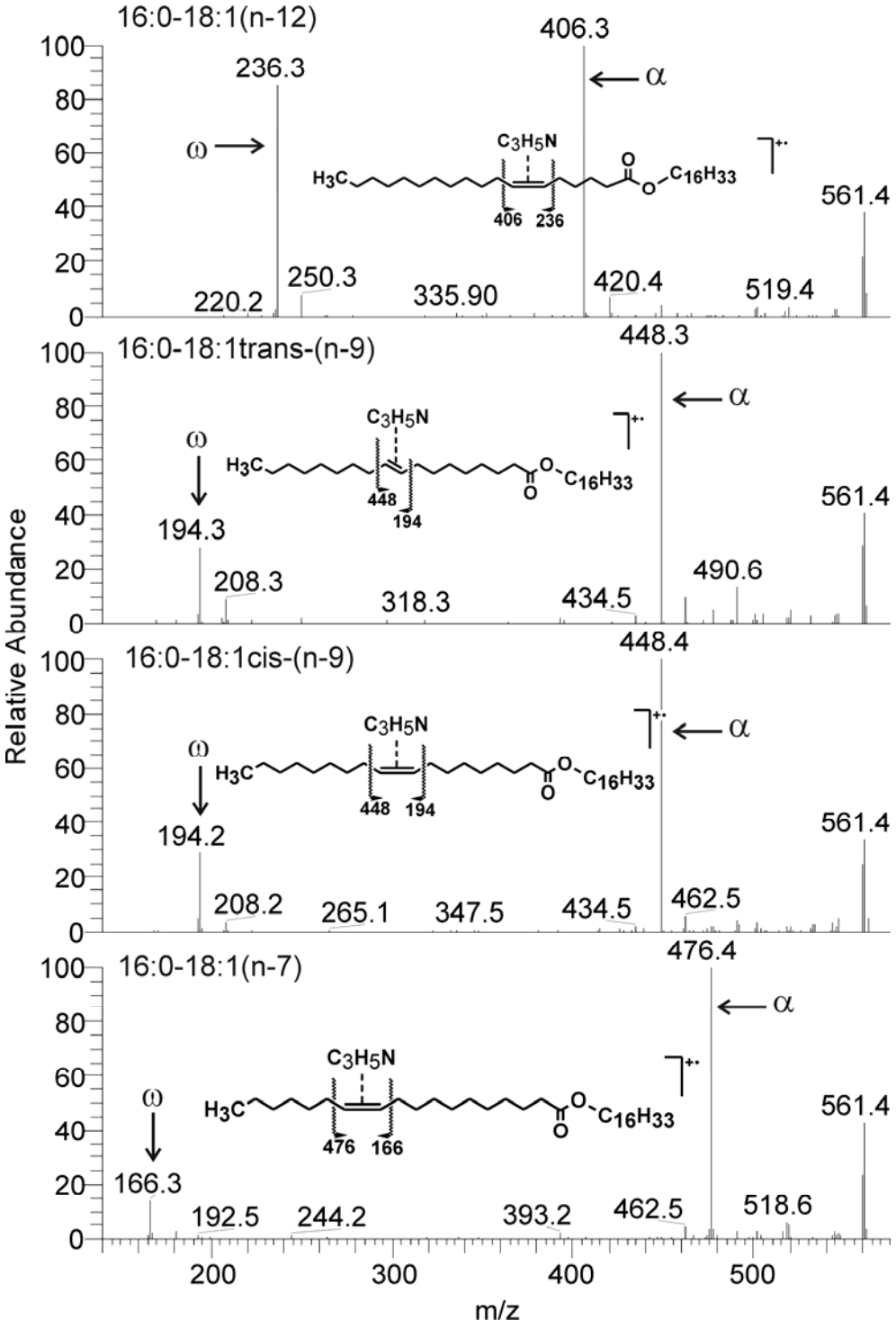

Figure S-3. The APCI MS/MS spectra of the $[M+55]^{+\bullet}$ adducts of WE 16:0-18:1(n-12), WE 16:0-18:1(*trans*-(n-9)), WE 16:0-18:1(*cis*-(n-9)), and WE 16:0-18:1(n-7) recorded under optimized chromatographic conditions using a (30+15)cm-column at 30°C.

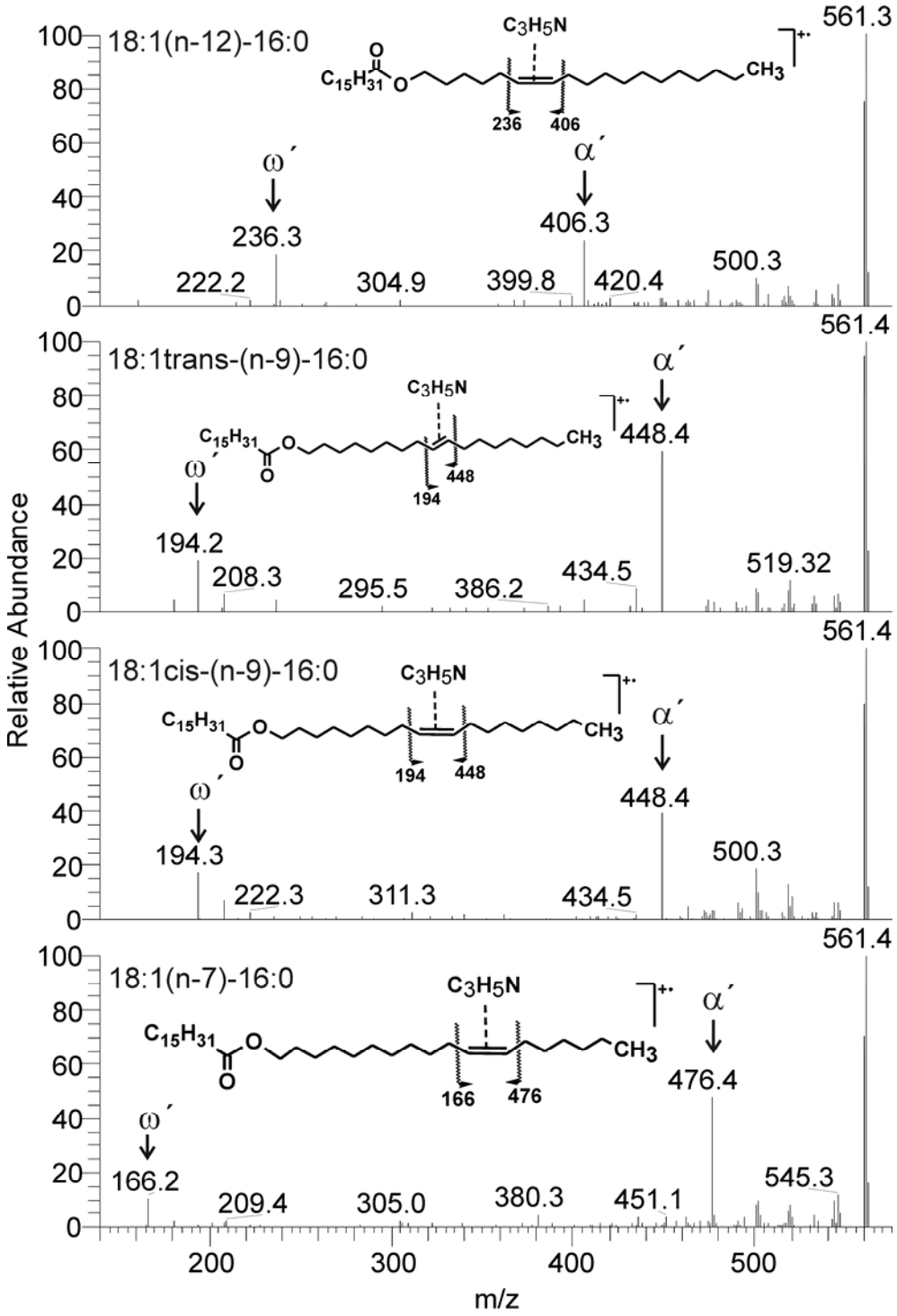

Figure S-4. The APCI MS/MS spectra of $[M+55]^{+\bullet}$ adducts of WE 18:1(n-12)-16:0, WE 18:1(*trans*-(n-9))-16:0, WE 18:1(*cis*-(n-9))-16:0, WE 18:1(n-7)-16:0 recorded under optimized chromatographic conditions using a (30+15)cm-column at 30°C.

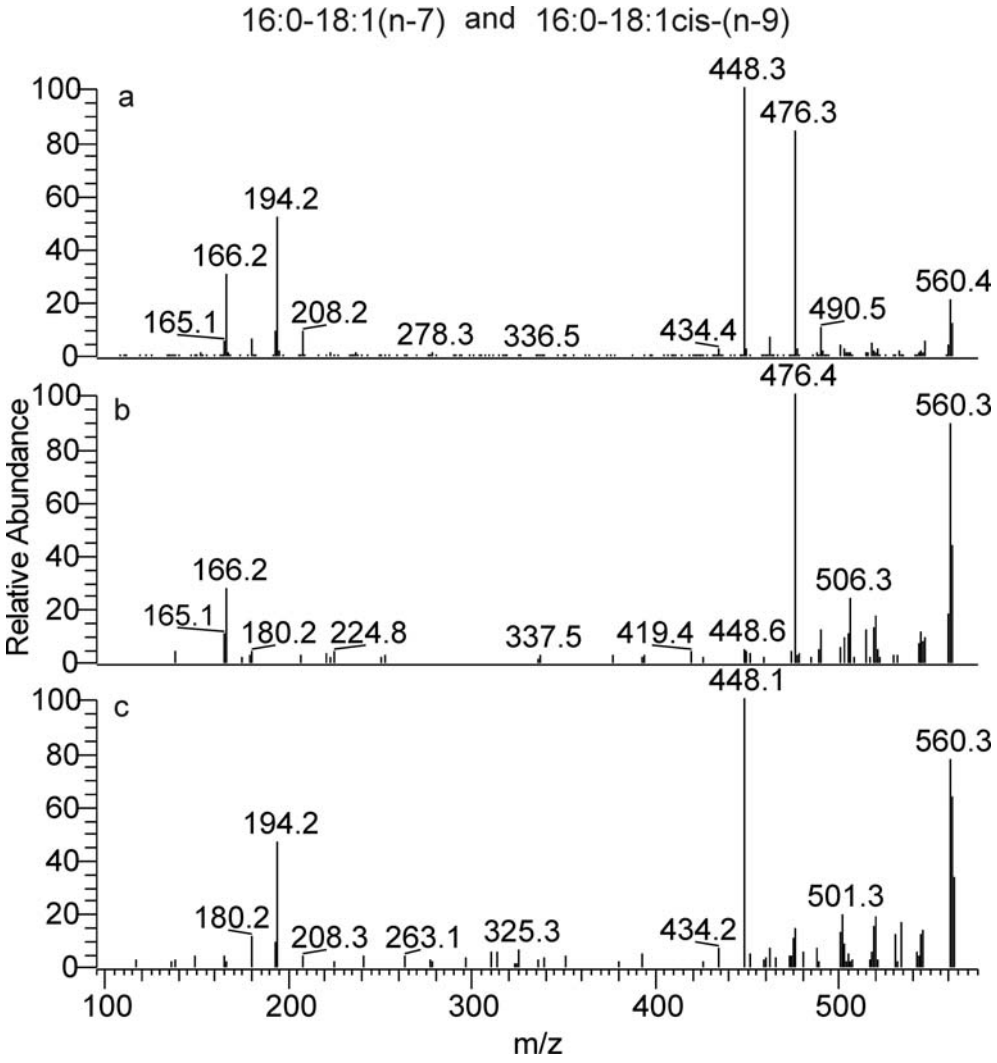

Figure S-5. The APCI MS/MS spectra of the $[M+55]^{+\bullet}$ adducts of two closely eluting esters WE 16:0-18:1(n-7) and WE 16:0-18:1(n-9) (peaks 1 and 2 in Figure 8) taken across the whole unresolved peak (a), in the ascending (b) and the descending (c) part of the peak. The spectra were recorded under optimized chromatographic conditions using a (30+15)cm-column at 30°C and data dependent scanning.

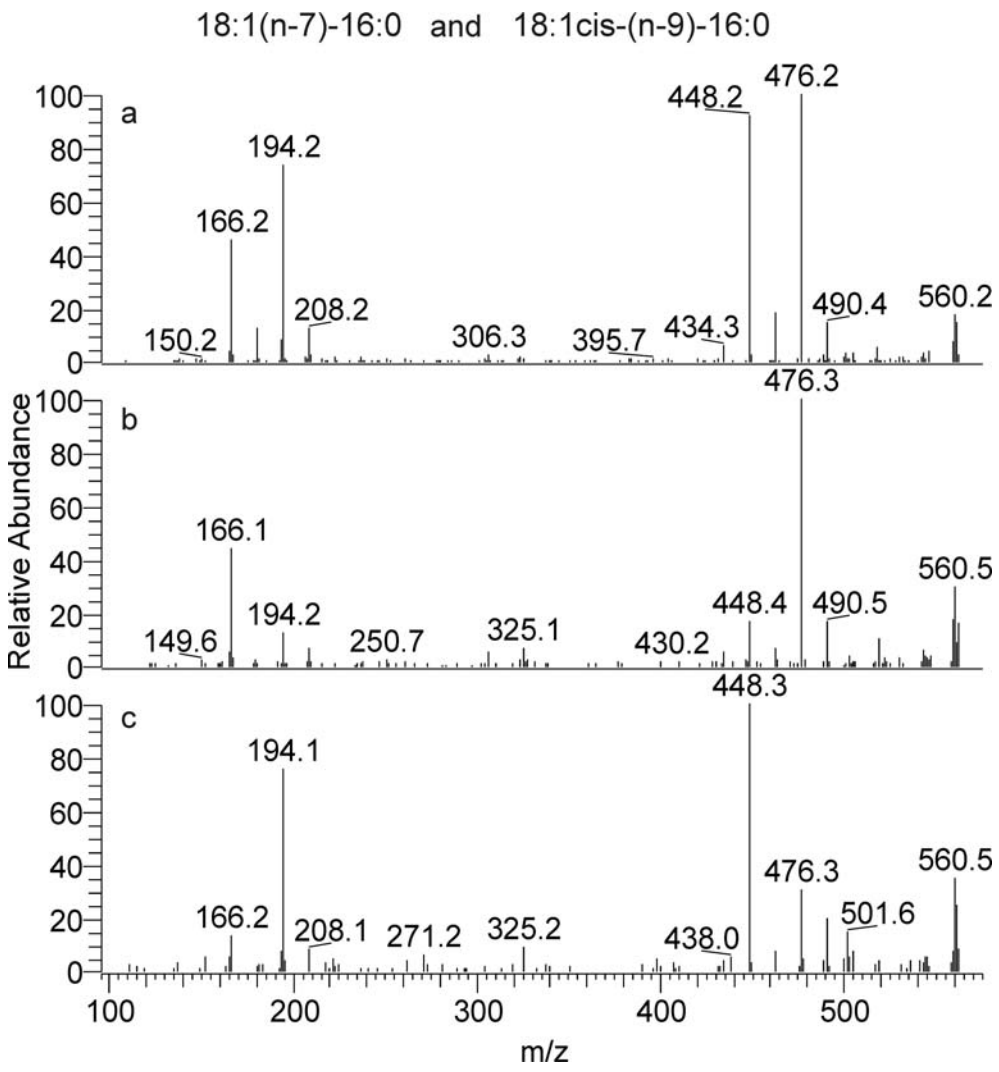

Figure S-6. The APCI MS/MS spectra of the $[M+55]^{+\bullet}$ adducts of two closely eluting esters WE 18:1(n-7)-16:0 and WE 18:1(n-9)-16:0 (peaks 5 and 6 in Figure 8) taken across the whole unresolved peak (a), in the ascending (b) and the descending (c) part of the peak. The spectra were recorded under optimized chromatographic conditions using a (30+15)cm-column at 30°C and data dependent scanning.

Table S-1. WEs identified in jojoba oil using HPLC/APCI-MS[2].

Peak no.	t_R (min)	Rel. peak area (%)	Adducts masses (*m/z*)	CN:DB [ECN][1]	Alcohol/acid composition	Double bond(s) related ions (*m/z*)		Identification
						α-related ions	ω-related ions	
1	54.2	0.7	557.4 / 611.3	38:4 [30]	20:1-18:3	582.5 (α), 543.0 (α_{n-6}), 503.0 (α_{n-9}), 498.5 (α')	190.3 (ω), 194.1 (ω')	20:1(n-9)-18:3(n-3)
2	60.9	0.1	559.3 / 613.3	38:3 [32]	20:0-18:3; 20:1-18:2	542.6 (α), 584.3 (α)	-	20:0-18:3(n-3)[2]; 20:1(n-9)-18:2(n-6)[2]
3	61.0	0.4	585.4 / 639.3	40:4 [32]	unknown	526.4, 610.4	194.2, 190.2	unknown 40:4, (n-9), (n-3)[2]
4	61.4	0.4	585.4 / 639.3	40:4 [32]	22:1-18:3	-	-	unknown
5	61.7	0.4	533.2 / 587.3	36:2 [32]	20:1-16:1; 18:1-18:1	-	-	unknown
6	67.7	1.9	613.5 / 667.3	42:4 [34]	unknown	554.6, 638.6, 598.7	-	unknown 42:4 (n-9), (n-3)[2]
7	67.9	0.4	587.3 / 641.3	40:3 [34]	22:1-18:2	528.5 (α'), 570.5 (α), 530.7(α_{n-6})	192.1 (ω)	22:1(n-9)-18:2(n-6)
8	68.7	**4.3**	561.2 / 615.3	38:2 [34]	20:1-18:1; 18:1-20:1	502.3 (α,α')	194.2 (ω, ω')	**20:1(n-9)-18:1(n-9); 18:1(n-9)-20:1(n-9)**
9	70.5	0.3	535.1 / 589.3	36:1 [34]	16:0-20:1; 20:0-16:1[3]	476.3 (α)	194.2 (ω)	16:0-20:1(n-9); 20:0-16:1(n-9)[3]
10	74.0	1.4	641.4 / 695.4	44:4 [36]	unknown	582.4, 666.6, 626.4, 586.6	194.4	unknown 44:4 (n-9), (n-3)[2]
11	74.2	1.1	615.3 / 669.3	42:3 [36]	unknown	556.5, 598.6, 558.4, 518.7	192.2, 194.4	unknown 42:3 (n-9), (n-6)[2]
12	75.3	**20.6**	589.2 / 643.3	40:2 [36]	20:1-20:1; 22:1-18:1	530.4 (α,α')	194.2 (ω, ω')	**20:1(n-9)-20:1(n-9); 22:1(n-9)-18:1(n-9)**
13	77.4	0.1	563.3 / 617.3	38:1 [36]	20:0-18:1; 18:0-20:1	504.3 (α)	194.3 (ω)	20:0-18:1(n-9); 18:0-20:1(n-9)
14	79.9	0.3	669.4 / 723.4	46:4 [38]	unknown	610.6, 694.7	-	unknown 46:4, (n-9), (n-3)[2]
15	80.3	0.4	643.3 / 697.3	44:3 [38]	unknown	584.5, 626.6, 586.4	-	unknown 44:3, (n-9), (n-6)[2]
16	81.3	**52.3**	617.2 / 671.3	42:2 [38]	22:1-20:1; 20:1-22:1	558.4 (α,α')	194.2 (ω, ω')	**22:1(n-9)-20:1(n-9); 20:1(n-9)-22:1(n-9)**
17	83.8	0.2	591.2 / 645.3	40:1 [38]	20:0-20:1; 22:0-18:1	532.4 (α)	194.2 (ω)	20:0-20:1(n-9); 22:0-18:1(n-9)
18	84.4	0.1	631.3 / 685.4	43:2 [39]	23:1-20:1	572.5 (α,α')	-	23:1(n-9)-20:1(n-9)
19	87.1	**12.0**	645.3 / 699.4	44:2 [40]	24:1-20:1; 22:1-22:1	586.4 (α,α')	194.2 (ω, ω')	**24:1(n-9)-20:1(n-9); 22:1(n-9)-22:1(n-9)**
20	89.4	0.7	619.3 / 673.3	42:1 [40]	22:0-20:1; 20:0-22:1	560.4 (α)	194.2 (ω)	22:0-20:1(n-9); 20:0-22:1(n-9)
21	92.3	1.6	673.3 / 727.4	46:2 [42]	24:1-22:1; 22:1-24:1	614.4 (α,α')	-	24:1(n-9)-22:1(n-9); 22:1(n-9)-24:1(n-9)
22	94.6	0.2	647.3 / 701.4	44:1 [42]	24:0-20:1; 22:0-22:1	588.4 (α)	-	24:0-20:1(n-9); 22:0-22:1(n-9)
23	97.1	0.2	701.3 / 755.4	48:2 [44]	24:1-24:1	642.5 (α,α')	-	24:1(n-9)-24:1(n-9)

[1]CN - number of carbons, DB - number of double bonds, ECN - equivalent carbon number (ECN=CN-2DB); [2] tentative identification; [3] minority component; The most abundant WEs are printed in bold

Table S-2. WEs identified in beeswax using HPLC/APCI-MS[2].

Peak no.	t_R (min)	Rel. peak area (%)	Adducts masses (*m/z*)	CN:DB [ECN][1]	Alcohol/acid composition	Double bond(s) related ions (*m/z*)		Identification
						α-related ions	ω-related ions	
1	77.2	0.2	671.4 / 725.3	46:3 [40]	unknown	696.4 (α)	190.2 (ω)	unknown 46:3 (n-3, n-9)
2	78.6	0.5	671.4 / 725.3	46:3 [40]	28:0-18:3	696.5 (α), 656.6 (α_{n-6})	190.2 (ω)	28:0-18:3(n-3)
3	79.2	**3.6**	619.3 / 673.3	42:1 [40]	24:0-18:1	560.4 (α)	194.2 (ω)	**24:0-18:1(n-9)**
4	80.8	**11.6**	593.2 / -	40:0 [40]	24:0-16:0	-	-	**24:0-16:0**
5	82.5	0.4	699.4 / 753.4	48:3 [42]	32:3-16:0	-	-	32:3-16:0
6	84.0	2.0	699.5 / 753.4	48:3 [42]	30:0-18:3	724.6.5 (α), 684.6 (α_{n-6}), 644.3 (α_{n-9})	190.2 (ω), 150.3 (ω_{n-6})	30:0-18:3(n-3)
7	84.7	**4.8**	647.4 / 701.4	44:1 [42]	26:0-18:1	588.4 (α)	194.2 (ω)	**26:0-18:1(n-9)**
8	86.3	**6.5**	621.1 / -	42:0 [42]	26:0-16:0; 24:0-18:0[3]	-	-	**26:0-16:0**; 24:0-18:0[3]
9	87.5	0.1	727.4 / 781.4	50:3 [44]	unknown	-	-	unknown 50:3
10	88.9	2.1	727.5 / 781.4	50:3 [44]	32:0-18:3	752.6 (α), 712.8 (α_{n-6}), 672.8 (α_{n-9})	190.1 (ω)	32:0-18:3(n-3)
11	89.7	**8.2**	675.4 / 729.5	46:1 [44]	28:0-18:1; 26:0-20:1; 30:0-16:1	616.4 (α)	194.2 (ω)	**28:0-18:1(n-9); 26:0-20:1(n-9); 30:0-16:1(n-9)**
12	91.3	**3.3**	649.2 / -	44:0 [44]	28:0-16:0, 26:0-18:0[3]	-	-	**28:0-16:0**, 26:0 18:0[3]
13	92.2	0.3	729.4 / 783.4	50:2 [46]	22:1-18:1	-	-	22:1-18:1
14	93.4	0.4	755.4 / 809.4	52:3 [46]	34:0-18:3	725.7 (α)		34:0-18:3(n-9)[2]
15	94.3	**27.3**	703.4 / 757.4	48:1 [46]	30:0-18:1; 28:0-20:1[3]; 32:1-16:0[3]	644.5 (α,α')	194.1 (ω,ω')	**30:0-18:1(n-9)**; 28:0-20:1(n-9)[3]; 32:1(n-9)-16:0[3]
16	95.9	**4.8**	677.2 / -	46:0 [46]	30:0-16:0	-	-	**30:0-16:0**
17	98.7	**18.7**	731.5 / 785.4	50:1 [48]	32:0-18:1, 30:0-20:1[3]	672.5 (α)	194.2 (ω)	**32:0-18:1(n-9)**; 30:0-20:1(n-9)[3]
18	100.2	2.2	705.3 / -	48:0 [48]	32:0-16:0	-	-	32:0-16:0
19	102.5	2.6	759.5 / 813.4	52:1 [50]	34:0-18:1; 32:0-20:1, 30:0-22:1[3]	700.5 (α)	194.2 (ω)	34:0-18:1(n-9); 32:0-20:1(n-9); 30:0-22:1 (n-9)[3]
20	104.1	0.2	733.2 / -	50:0 [50]	34:0-16:0	-	-	34:0-16:0
21	105.9	0.2	787.5 / 841.5	54:1 [52]	34:0-20:1; 32:0-22:1	728.5 (α)	194.2 (ω)	34:0-20:1(n-9); 32:0-22:1(n-9)

[1] CN - number of carbons, DB - number of double bonds, ECN - equivalent carbon number (ECN=CN-2DB); [2] tentative identification; [3] minority component; The most abundant WEs are printed in bold