Electronic Supplementary Information

Scheme S1. Workflow of GC-MS method development in analysing TPN components

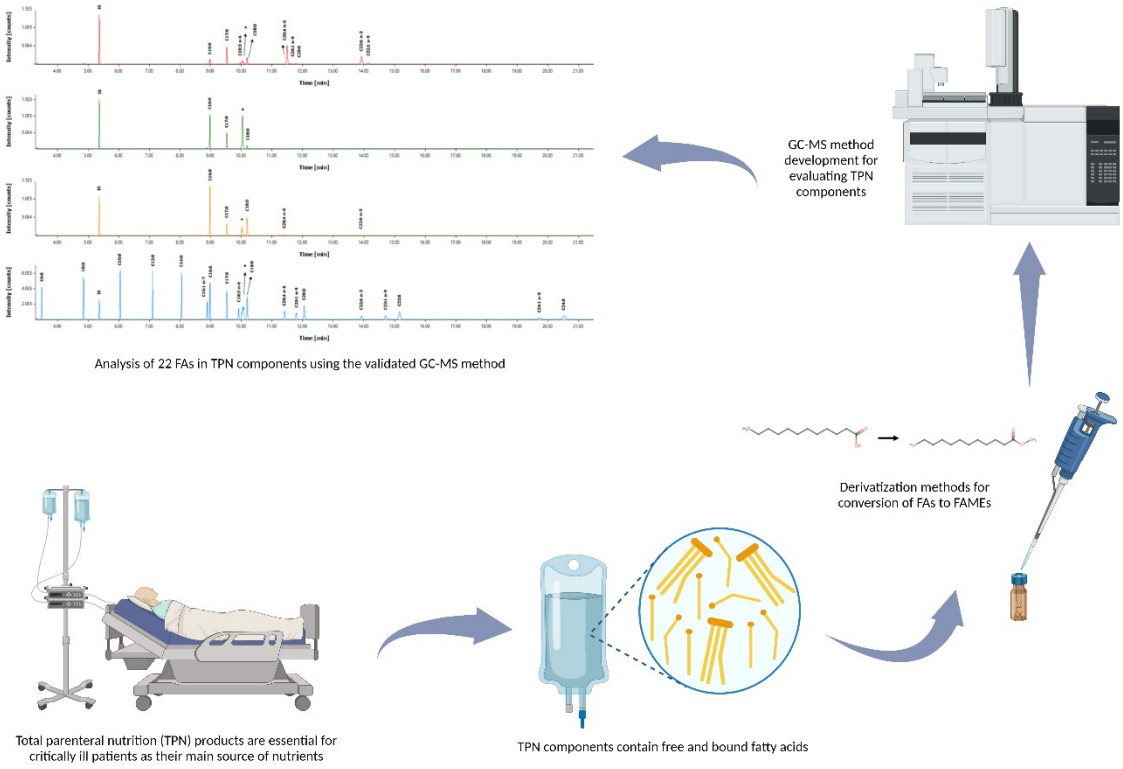


Table S1. The retention times and selected ion monitoring (SIM) parameters for 22 FAMEs and internal standard (IS) naphthalene in GC-MS.

No.	FAMEs	Retention time (min)	Start time of time window (min)	Selected ions, m/z	Total dwell Time (ms)
1	C6:0	3.482	3.20	43.1, 74.0, 87.0	150
2	C8:0	4.848	4.70	43.0, 74.0, 87.0	150
3	Naphthalene	5.362	5.20	128.0	50
4	C10:0	6.051	5.80	74.0, 87.0, 143.1	150
5	C12:0	7.105	6.90	43.1, 74.0, 87.0	150
6	C14:0	8.046	7.90	43.1, 74.0, 87.0	150
7	C16:1 n-7	8.888	8.70	55.1, 69.1, 74.0, 87.0	200
8	C16:0	8.977			
9	C17:0	9.527	9.30	43.1, 74.0, 87.0	150
10	C18:3 n-6	9.911	9.65	67.1, 79.1, 80.0	150
11	C18:2 n-6	10.009	9.97	55.1, 67.0, 222.0, 292, 294	200
12	C18:1 n-9	10.039			
13	C18:3 n-3	10.065			
14	C18:1 n-7	10.073			
15	C18:0	10.192	10.15	43.0, 74.0, 87.0	150
16	C20:4 n-6	11.413	11.20	79.0, 80.0, 91.0	150
17	C20:1 n-9	11.794	11.65	55.0, 69.0, 83.0	150
18	C20:0	12.052	11.96	43.0, 74.0, 87.0	150
19	C22:6 n-3	13.922	13.60	67.0, 79.0, 91.0	150
20	C22:1 n-9	14.713	14.50	55.0, 69.0, 83.0	150
21	C22:0	15.166	15.00	43.0, 74.0, 87.0	150
22	C24:1 n-9	19.762	19.40	55.0, 69.0, 83.0	150
23	C24:0	20.553	20.30	43.0, 74.0, 87.0	150

Table S2. Freeze-thaw stability (-80℃ / room temperature) testing of FAME standards

		2 cycles			3 cycles			4 cycles		
	FAME	Mean, μg/mL	SD, μg/mL	CV, %	Mean, μg/mL	SD, μg/mL	CV, %	Mean, μg/mL	SD, μg/mL	CV, %
low	C18:3 n-6	4.98	0.38	7.56	5.23	0.17	3.31	5.62	0.50	8.92
	C20:1 n-9	4.89	0.40	8.18	4.91	0.16	3.29	5.47	0.60	10.92
	C20:0	4.75	0.44	9.35	4.74	0.20	4.19	5.32	0.67	12.52
medium	C18:3 n-6	44.19	1.30	2.95	42.21	1.65	3.91	45.94	0.12	0.25
	C20:1 n-9	43.77	1.12	2.56	40.74	3.87	9.50	46.77	0.65	1.39
	C20:0	43.11	1.25	2.89	39.93	3.97	9.95	45.03	0.42	0.94
high	C18:3 n-6	129.58	5.47	4.22	131.03	5.01	3.82	135.71	3.73	2.75
	C20:1 n-9	128.55	6.91	5.38	128.44	6.35	4.94	138.35	8.53	6.17
	C20:0	122.49	6.71	5.48	122.25	6.08	4.97	129.82	8.66	6.67

Table S3. Ambient stability testing of FAME standards

		24h			48h			72h		
	FAME	Mean, μg/mL	SD, μg/mL	CV, %	Mean, μg/mL	SD, μg/mL	CV, %	Mean, μg/mL	SD, μg/mL	CV, %
low	C18:3 n-6	5.62	0.50	8.92	5.17	0.25	4.81	5.11	0.26	5.03
	C20:1 n-9	5.47	0.60	10.92	4.81	0.22	4.59	4.69	0.19	4.12
	C20:0	5.32	0.67	12.52	4.64	0.22	4.73	4.51	0.19	4.19
medium	C18:3 n-6	45.94	0.12	0.25	46.63	0.58	1.25	46.35	1.83	3.94
	C20:1 n-9	46.77	0.65	1.39	46.96	0.26	0.55	46.29	2.57	5.55
	C20:0	45.03	0.42	0.94	44.96	0.32	0.70	44.03	2.58	5.86
high	C18:3 n-6	135.71	3.73	2.75	148.45	4.22	2.84	151.25	20.50	13.55
	C20:1 n-9	138.35	8.53	6.17	151.13	9.24	6.11	151.97	28.42	18.70
	C20:0	129.82	8.66	6.67	140.18	9.25	6.60	139.62	26.81	19.20

Table S4. The four main FA contents in the different TPN component matrices (n=3) detected by the five derivatization methods (mg/g)

Unknown lipid sample					
FA	Acetyl chloride method	Sulfuric acid method	Potassium hydroxide method	Potassium hydroxide-sulfuric acid method	Sodium methoxide method
C16:0	143.64	141.73	<LOQ	185.42	137.12
C18:2 n-6	119.60	119.76	<LOQ	157.94	90.33
C18:1 n-9	115.37	177.30	<LOQ	229.88	140.93
C18:0	95.82	96.20	<LOQ	121.35	85.42
Fish oil					
C16:0	8.93	9.27	<LOQ	13.67	10.33
C18:1 n-9	74.08	70.85	6.07	93.73	60.65
C18:1 n-7	30.85	26.21	<LOQ	32.38	19.84
C18:0	26.06	26.77	<LOQ	35.52	26.59
Olive oil					
C16:0	113.54	99.30	<LOQ	126.67	95.43
C18:2 n-6	118.93	94.24	7.95	118.94	77.05
C18:1 n-9	817.21	614.81	13.05	803.91	553.46
C18:0	15.23	11.66	<LOQ	19.79	12.29

Note: <LOQ, analyte was detected, but its concentration was lower than LOQ

Table S5. Methyl esterification yield of five derivatization methods in different TPN component matrices (n=3).

Method	Unidentified lipid sample	Fish oil	Olive oil
Acetyl chloride method	29.95-31.97%	29.11-30.94%	32.63-32.65%
Sulfuric acid method	29.72-30.66%	27.30-29.75%	22.52-24.70%
Potassium hydroxide method	7.40-10.45%	1.34-1.49%	0.08-0.24%
Potassium hydroxide - sulfuric acid method	29.19-41.49%	31.80-32.45%	21.95-30.96%
Sodium methoxide method	26.12-29.62%	23.79-28.42%	23.00-25.30%

Table S6. Recovery measurements in three study targets—C8:0, C14:0, and C18:0 in different TPN component matrices (n=3).

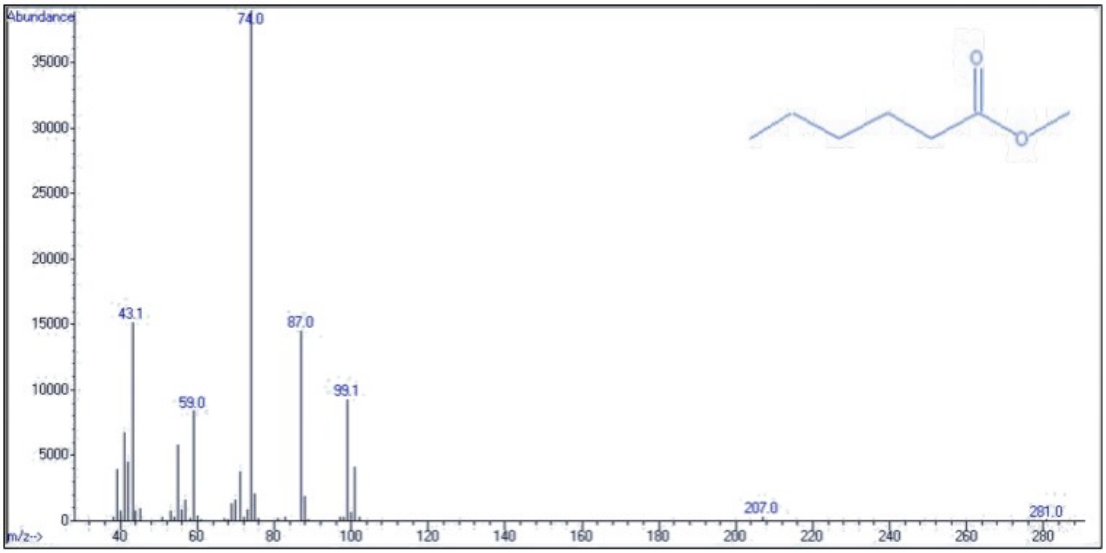
Fatty acid	Unidentified lipid sample		Olive oil		Fish oil	
	Mean ± SD, µg/mL	Recovery	Mean ± SD, µg/mL	Recovery	Mean ± SD, µg/mL	Recovery
C8:0	28.40 ± 1.02	95.89 %	28.37 ± 0.55	112.48 %	25.66 ± 4.00	95.69 %
C14:0	25.33 ± 1.53	89.62 %	22.94 ± 1.48	95.20 %	24.01 ± 1.39	93.75 %
C18:0	86.32 ± 5.85	98.40 %	32.52 ± 3.12	108.94 %	39.32 ± 2.80	105.20 %

Table S7. Quantification of fatty acids expressed in mg/g present in different TPN component matrices (n=3) using the validated GC-MS method and optimized sodium methoxide FAME derivatization technique.

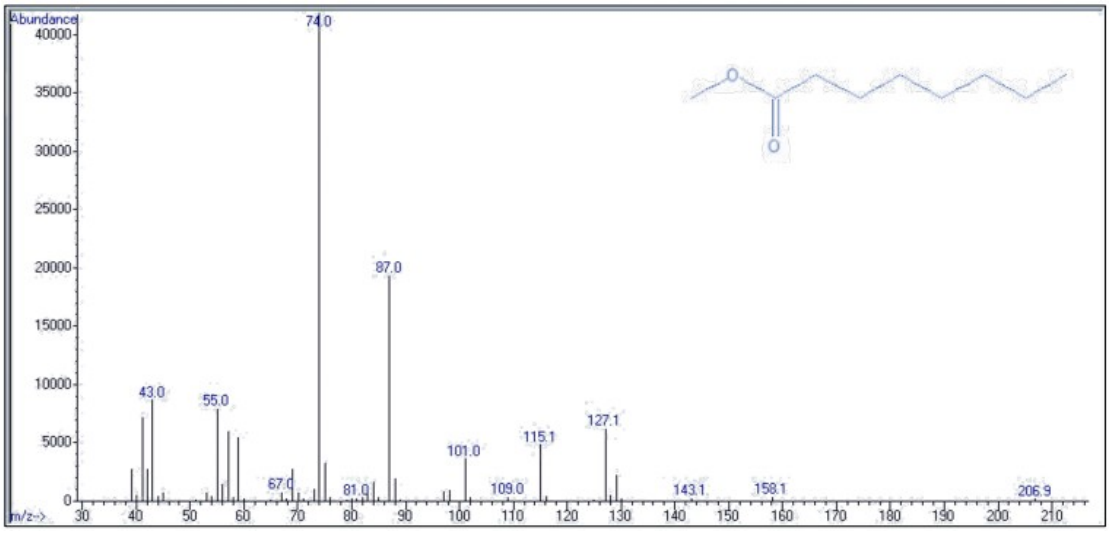
Fatty acids	Unidentified lipid sample, mg/g	Olive oil, mg/g	Fish oil, mg/g
C6:0	ND	ND	ND
C8:0	ND	< LOQ	< LOQ
C10:0	ND	ND	< LOQ
C12:0	ND	ND	ND
C14:0	< LOQ	< LOQ	< LOQ
C16:1 n-7	2.80 ± 0.32	10.18 ± 0.30	3.27 ± 0.12
C16:0	144.03 ± 16.13	122.38 ± 1.75	10.70 ± 0.39
C18:3 n-6	3.80 ± 0.39	ND	6.43 ± 0.26
C18:2 n-6	125.20 ± 12.83	146.27 ± 4.94	19.44 ± 0.93
C18:1 n-9	185.48 ± 18.89	921.80 ± 12.36	87.86 ± 3.44
C18:3 n-3	6.28 ± 0.65	13.56 ± 0.19	13.76 ± 0.61
C18:1 n-7	ND	ND	25.45 ± 1.04
C18:0	90.82 ± 9.19	15.25 ± 0.60	30.29 ± 0.83
C20:4 n-6	32.31 ± 3.06	ND	14.81 ± 0.53
C20:1 n-9	ND	7.58 ± 0.18	24.13 ± 1.02
C20:0	3.67 ± 0.34	8.01 ± 0.19	8.80 ± 0.23
C22:6 n-3	36.69 ± 3.23	ND	342.14 ± 23.61
C22:1 n-9	ND	ND	ND
C22:0	5.80 ± 0.49	9.33 ± 0.21	9.04 ± 0.28
C24:1 n-9	18.27 ± 1.44	ND	27.39 ± 0.99
C24:0	12.80 ± 1.01	ND	ND

Note: ND, Analyte was not detected in the sample; < LOQ, analyte was detected, but its concentration was lower than LOQ.

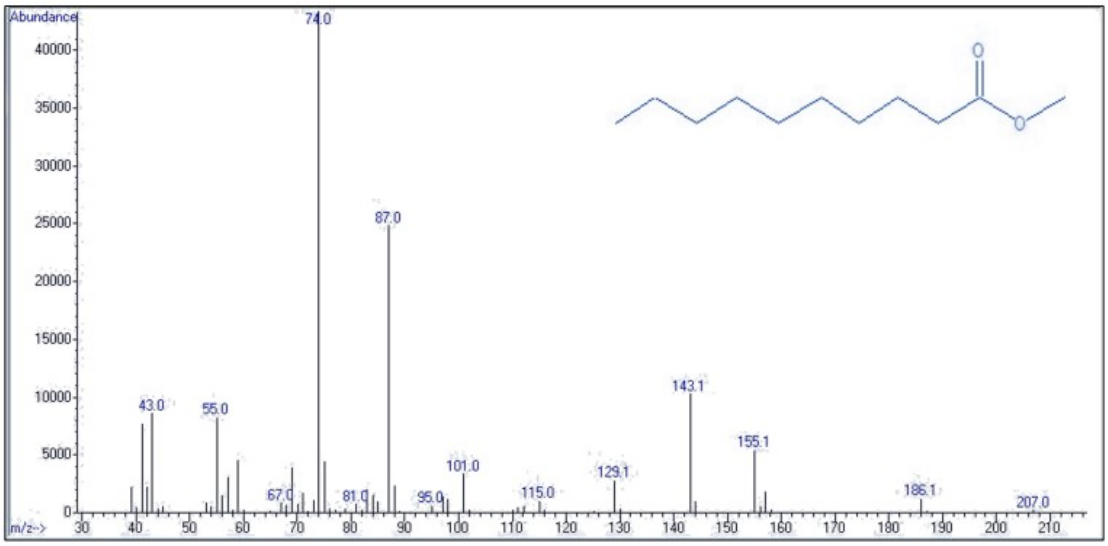
Figure S1. Standard mass spectra and chemical structure of FAMEs.



(a) C6:0



(b) C8:0


Abundance
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87.0
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55.0
67.0
81.0
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115.0
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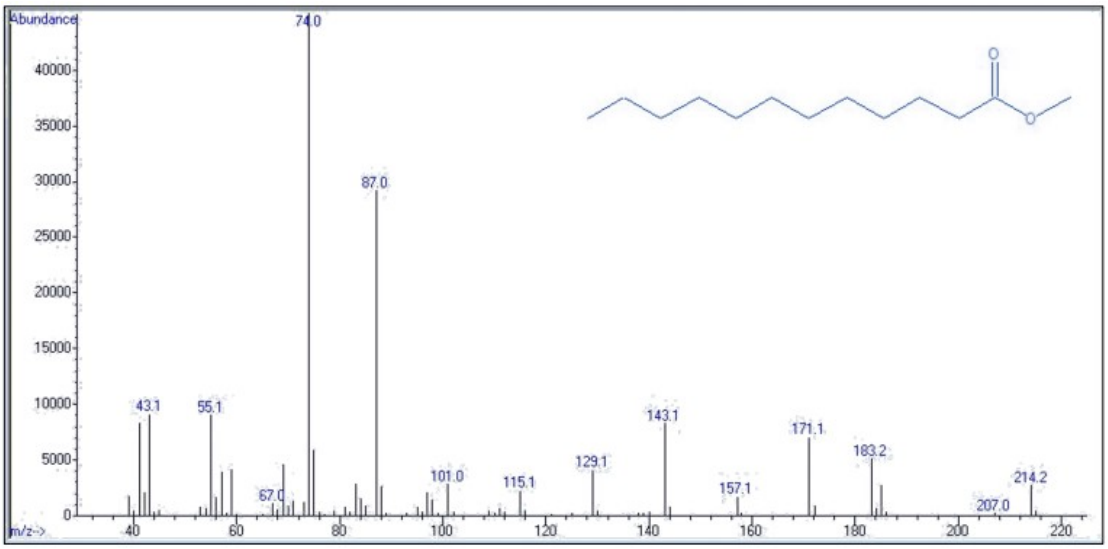
143.1
155.1
186.1
207.0
m/z-->

(c) C10:0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67.0
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143.1
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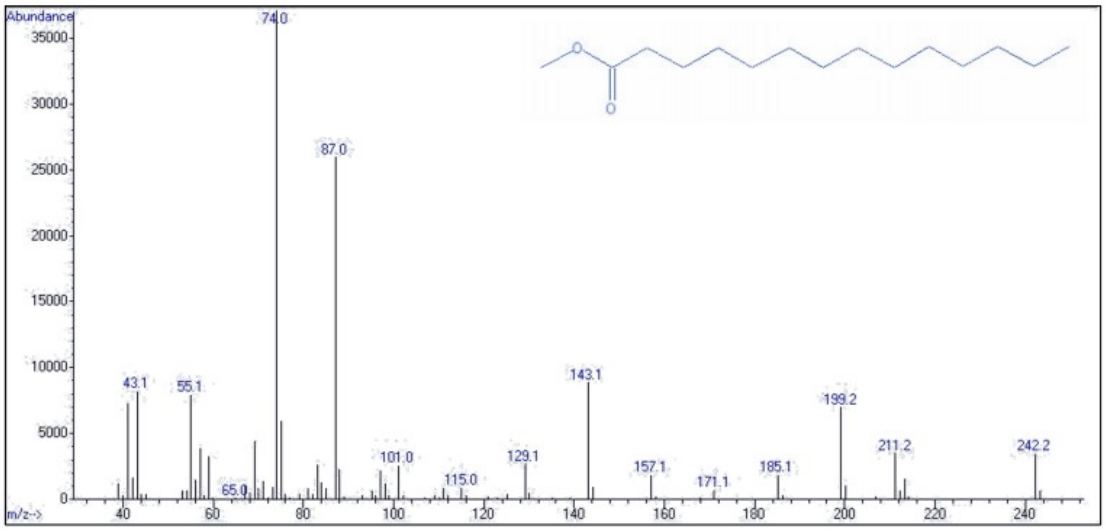
171.1
183.2
207.0
214.2
m/z-->

(d) C12:0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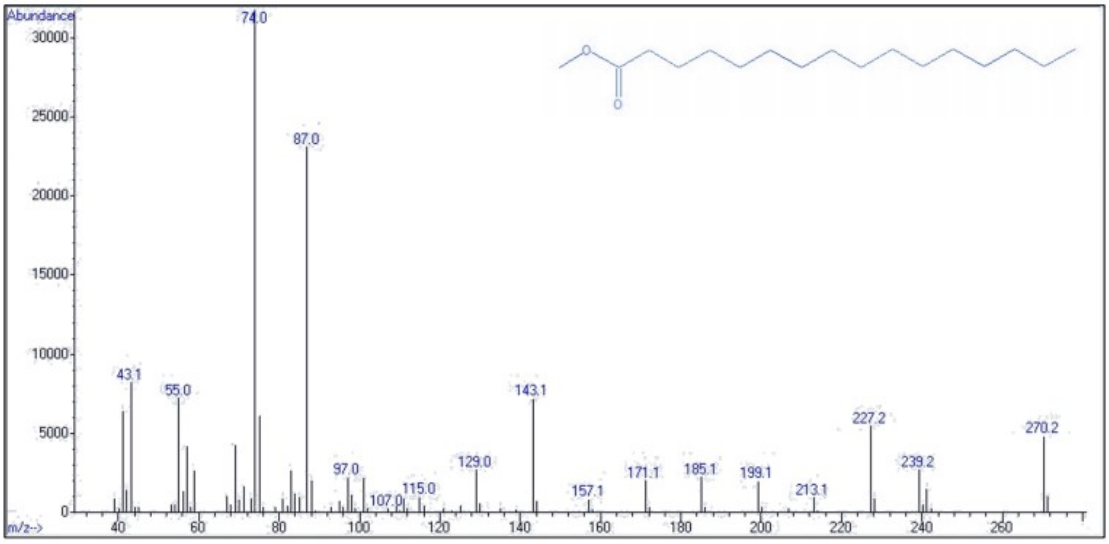
199.2
211.2
242.2
m/z-->

(e) C14:0


Abundance
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43.1
55.0
97.0
107.0
115.0
129.0
143.1
157.1
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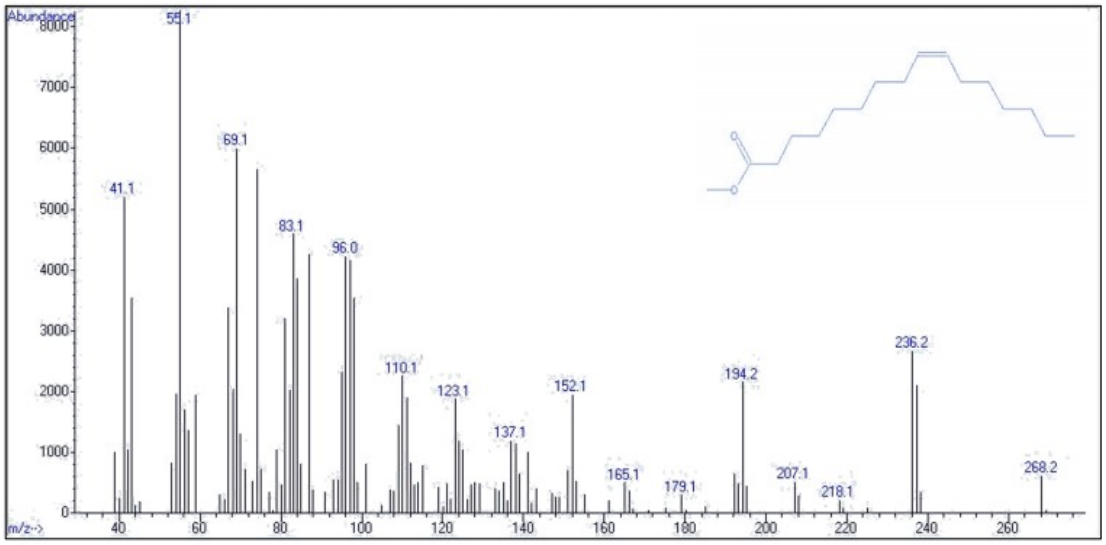
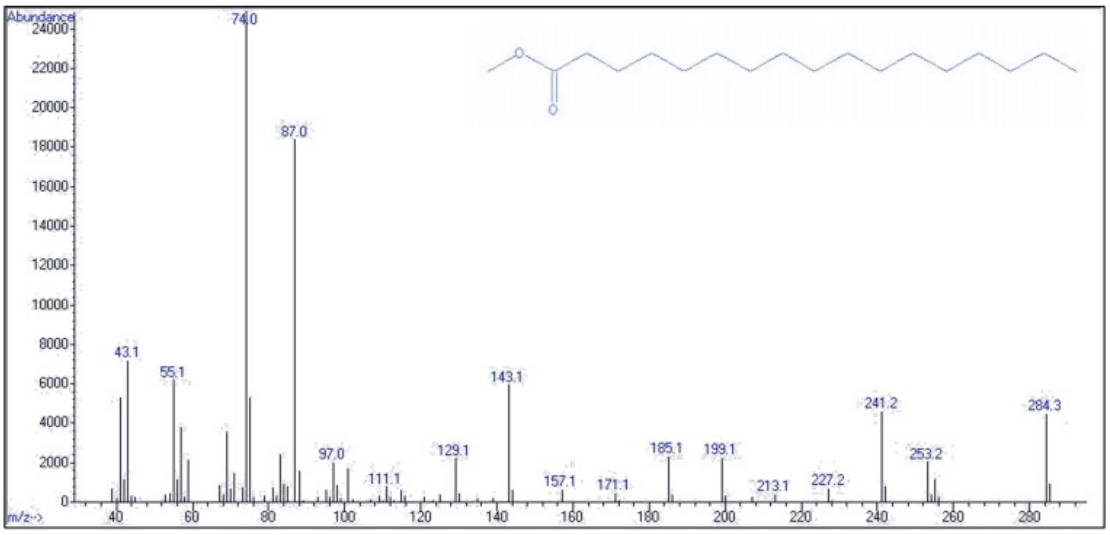
227.2
239.2
270.2
m/z-->

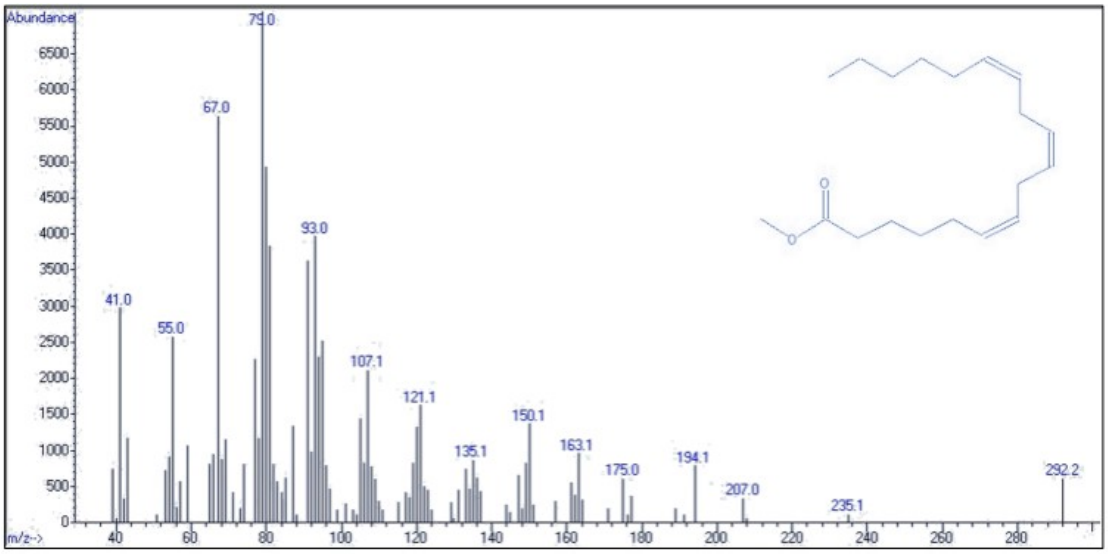
(f) C16:0



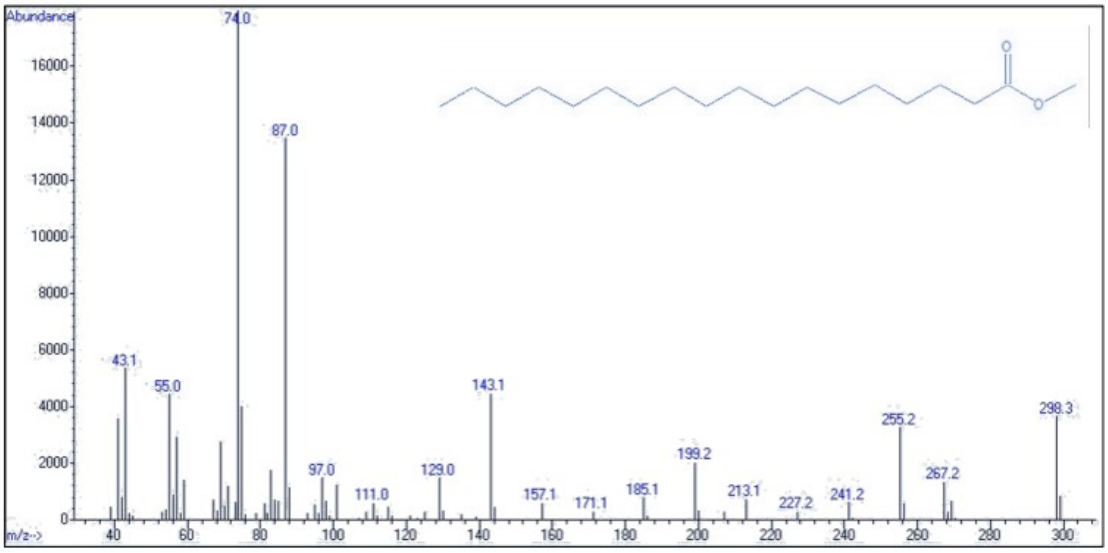
(g) C16:1 n-7



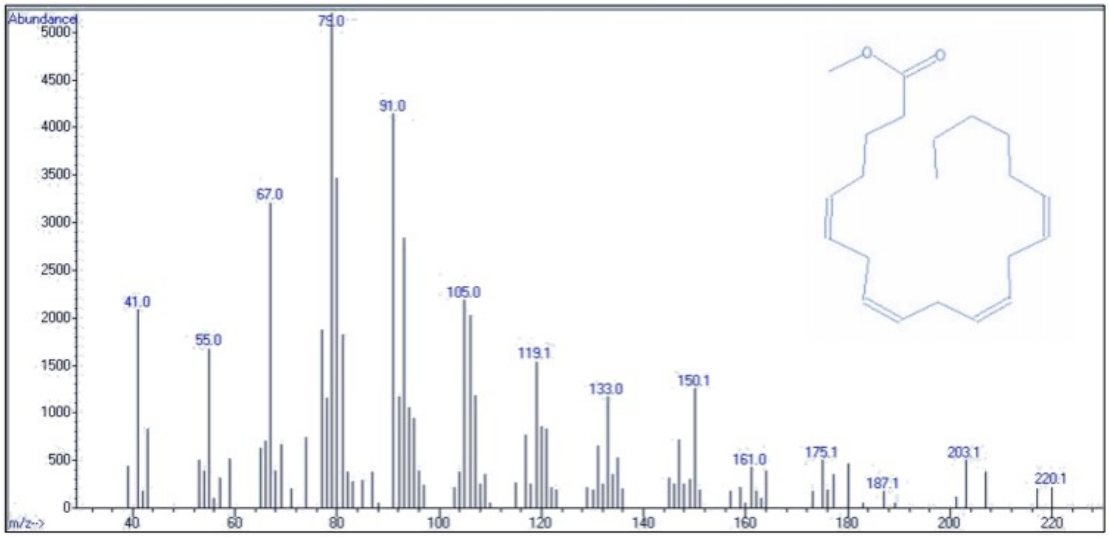
(h) C17:0



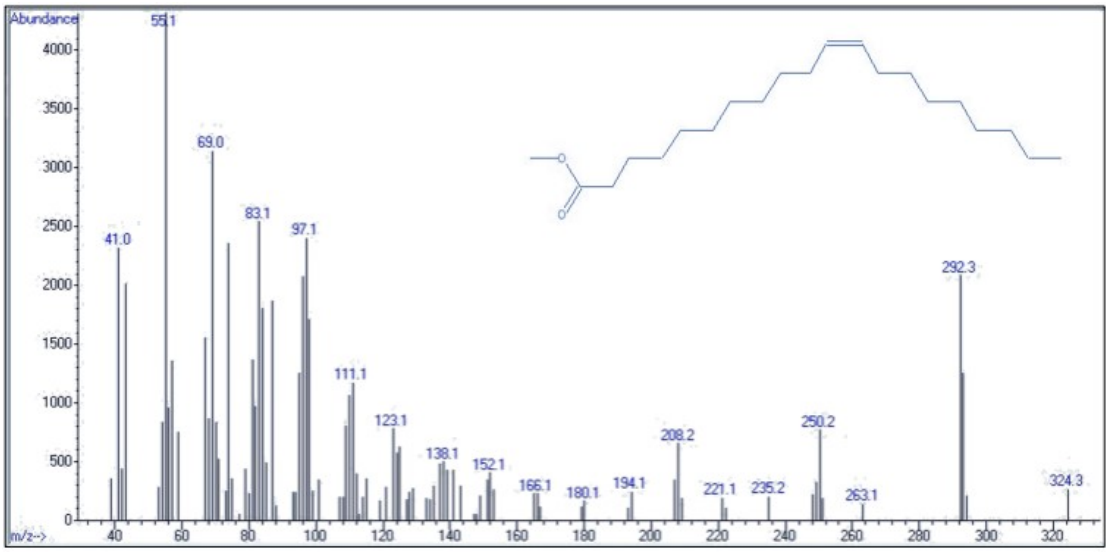
(i) C18:3 n-6



(j) C18:0



(k) C20:4 n-6



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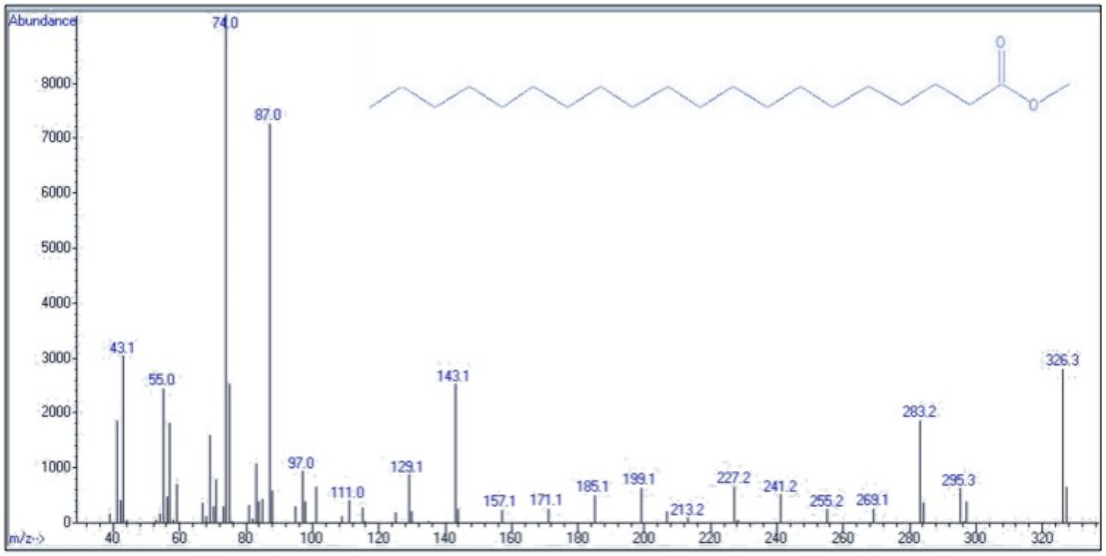
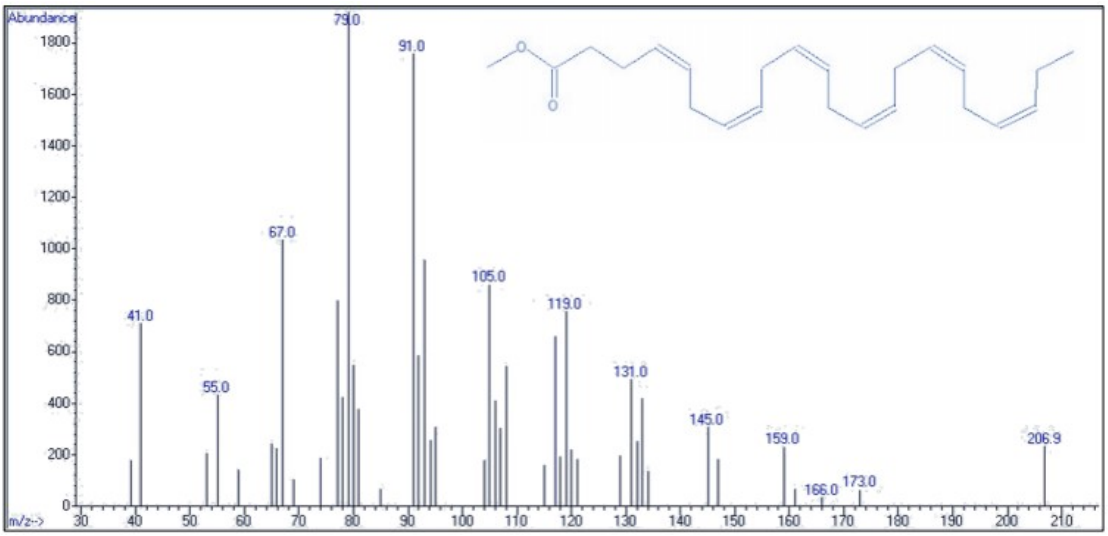
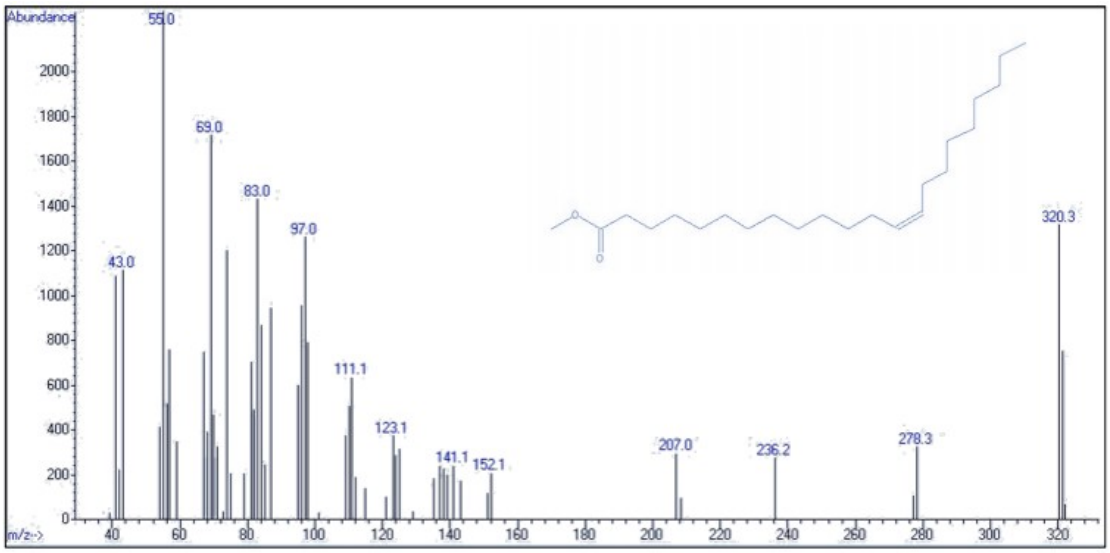


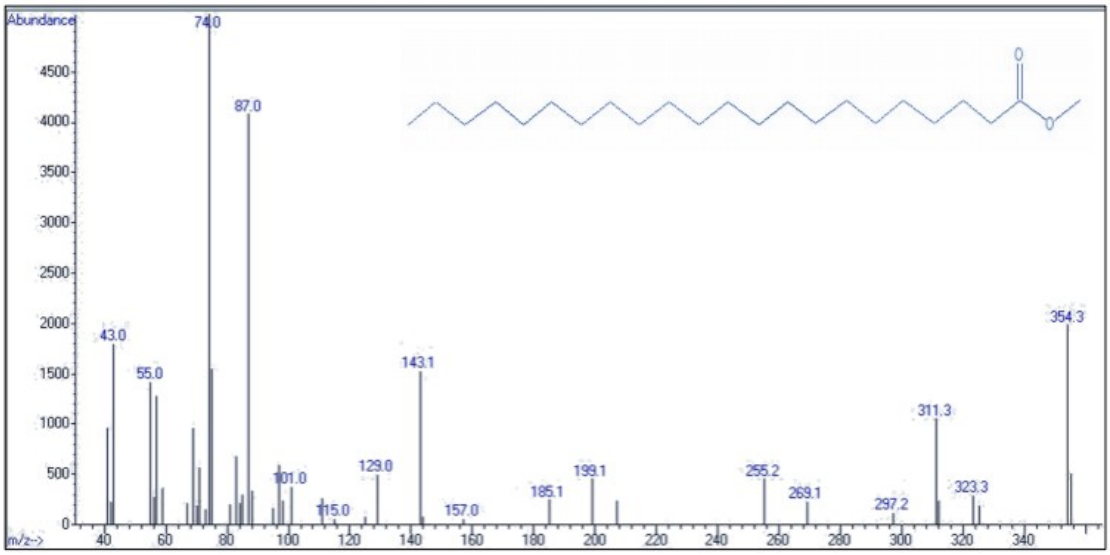
(m) C20:0



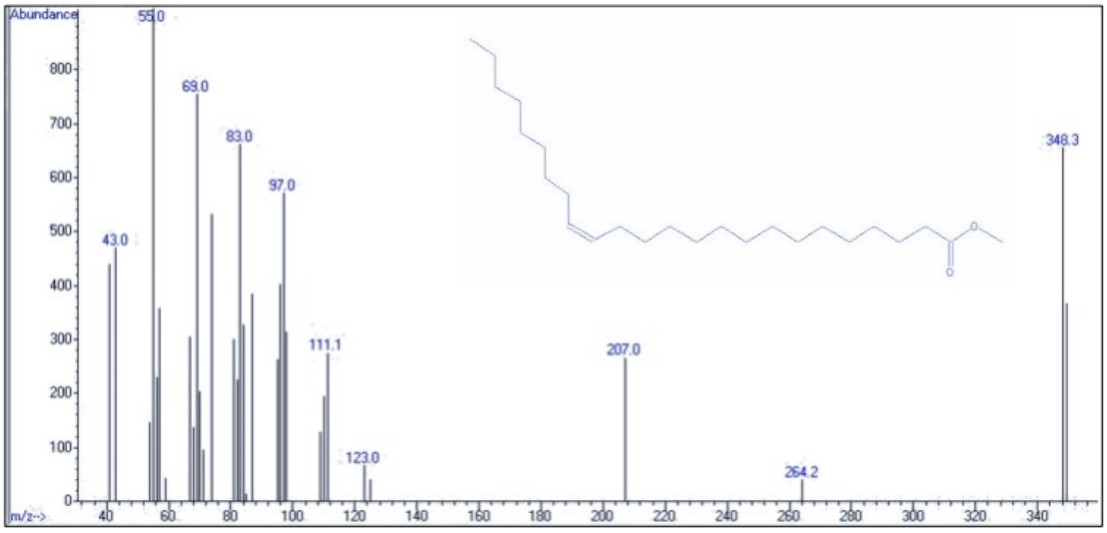
(n) C22:6 n-3



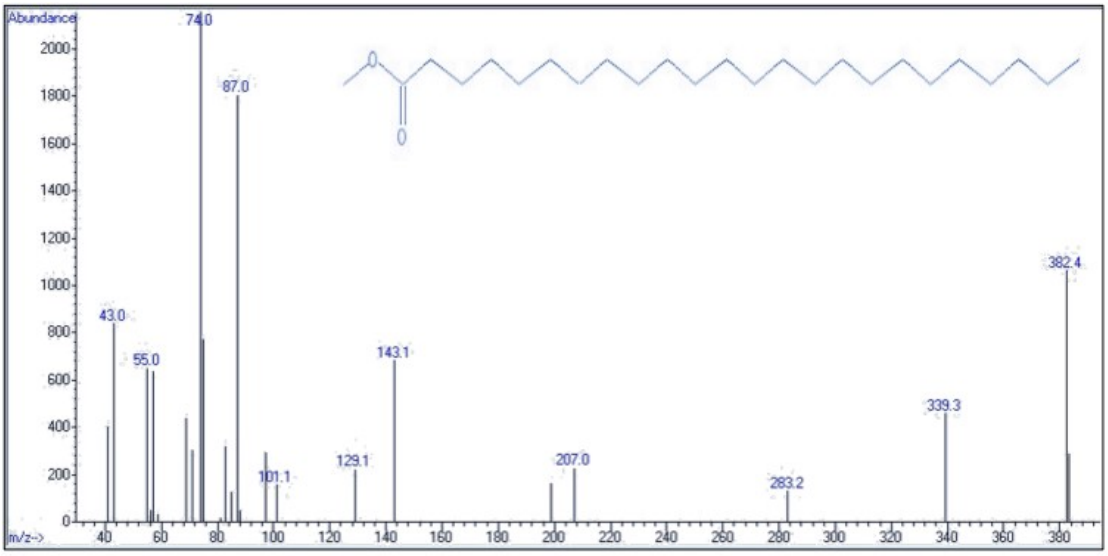
(o) C22:1 n-9



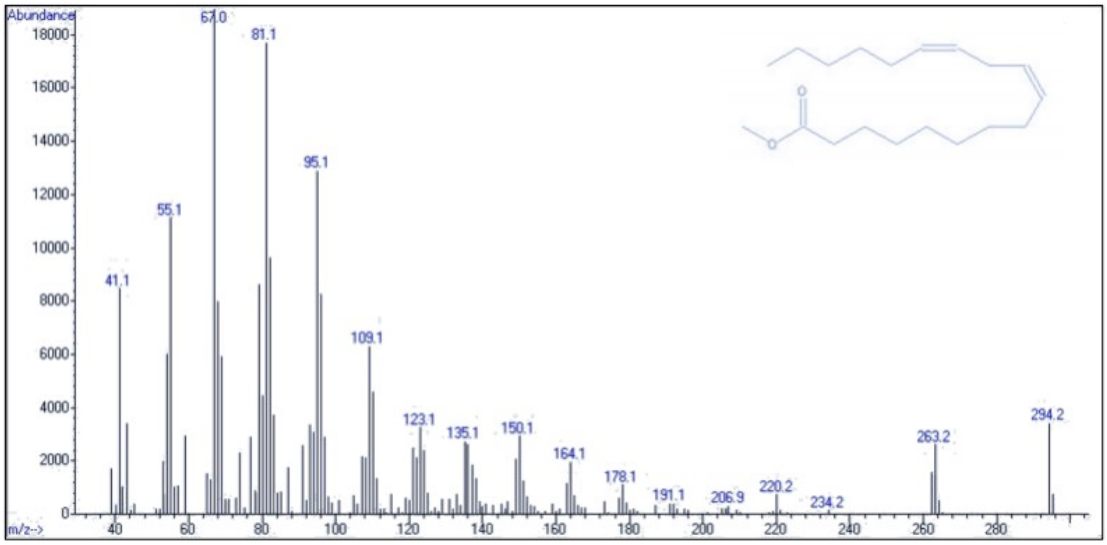
(p) C22:0



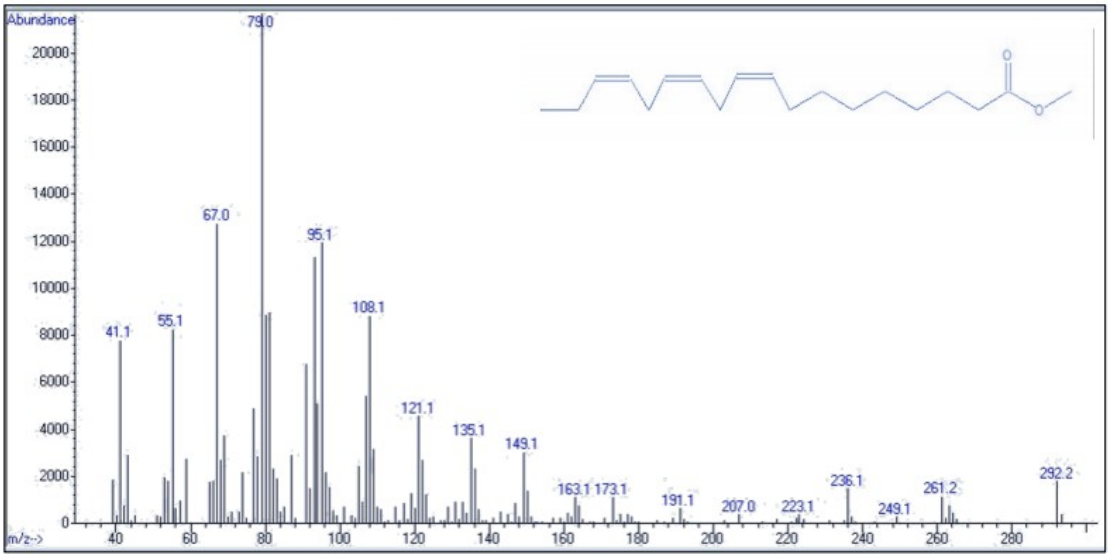
(q) C24:1 n-9



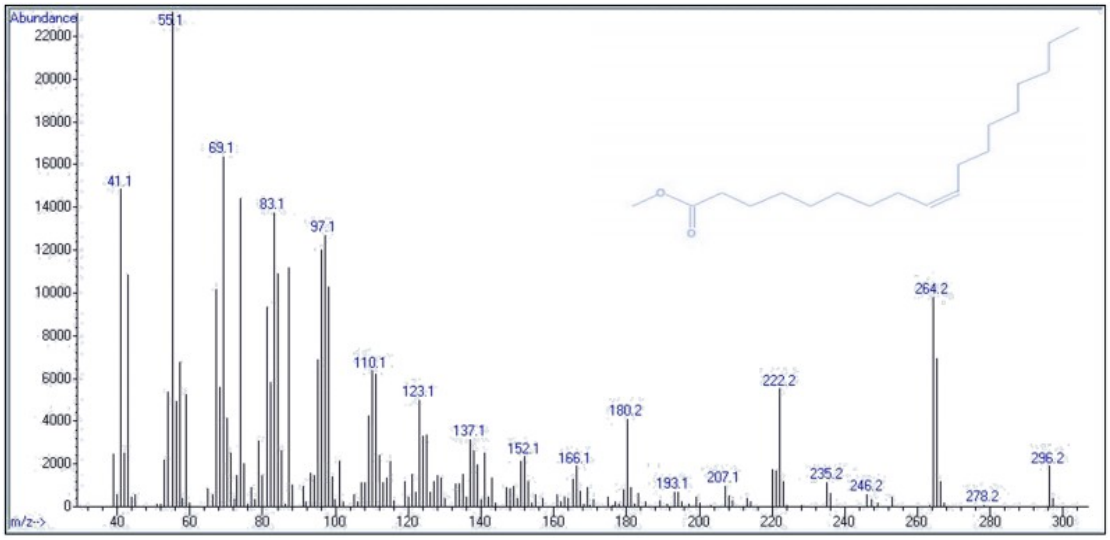
(r) C24:0



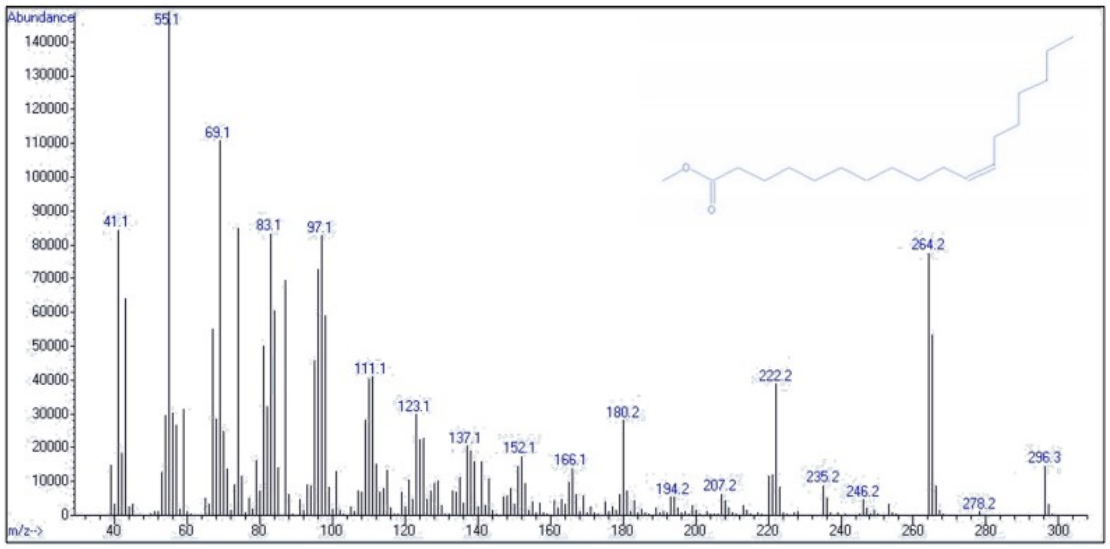
(s) C18:2 n-6



(t) C18:3 n-3



(u) C18:1 n-9



(v) C18:1 n-7

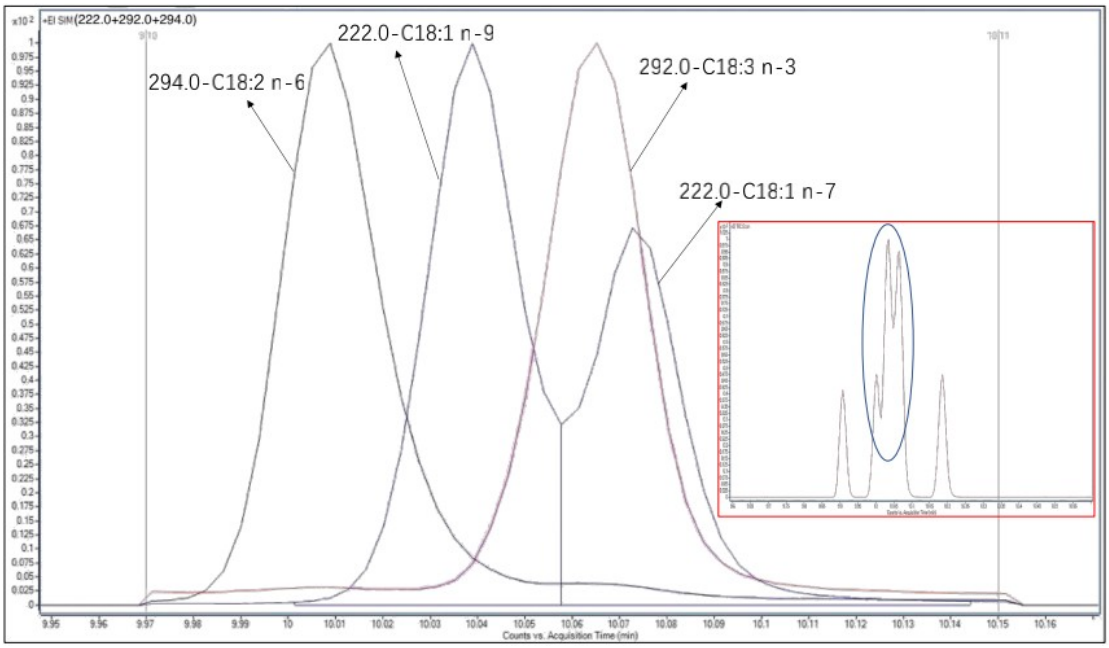
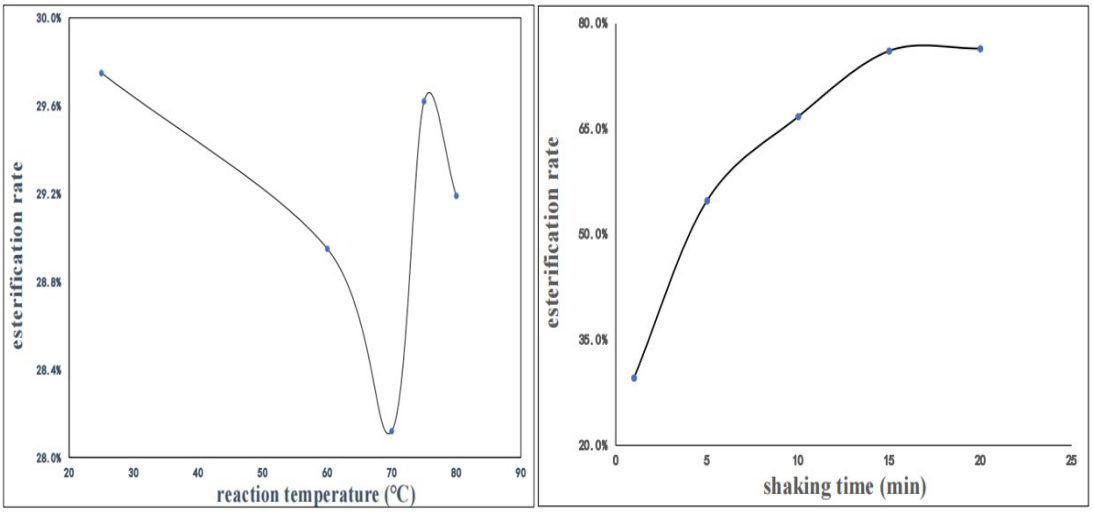


Figure S2. Extraction chromatogram of ions 222, 292 and 294 featuring C 18:x species



(a) *(b)*

Figure S3. Optimization of the sodium methoxide FAME derivatization technique: (a) Effect of temperature on the methylation yield; (b) Effect of shaking time on the methylation yield

Equation S1. Equation for calculating the FAME concentration

$$C_{FAME_i} = \frac{\frac{Area_{FAME_i}}{Area_{IS}} - b}{k}$$

Where b is the intercept of calibration curve

k is the slope of calibration curve

$Area_{FAME_i}$ is the area of FAME

$Area_{IS}$ is the area of internal standard naphthalene

C_{FAME_i} is the actual measured FAME i concentration (μg/mL).

Equation S2. Equation for evaluating the methylation rate

$$F = \frac{C_{FAME_{C17:0}}}{\frac{c_{TAG\ 17:0} \times 1.0048 \times V_{TAG\ 17:0}}{V_{heptane}} \times \frac{V_{supernatant}}{V_{final}}} \times 100\%$$

Where F is the methylation rate

$C_{FAME_{C17:0}}$ is the actual measured FAME-C17:0 concentration (μg/mL)

$c_{TAG\ 17:0}$ is the concentration of triglycerides of heptadecanoic acid (mg/mL)

1.0048 is the conversion factor of triglycerides of heptadecanoic acid to methyl heptadecanoate[47]

$V_{TAG\ 17:0}$ is the volume of triglycerides of heptadecanoic acid added to the sample (μL)

$V_{heptane}$ is the volume of heptane used to dissolve the sample (μL)

$V_{supernatant}$ is the volume of supernatant after centrifugation used for the determination (μL)

V_{final} is the volume after mixing the supernatant with IS (μL).

Equation S3. Equation for determining fatty acid content in sample (mg/g)

$$X_i = \frac{(C_{FAME_i} \div F) \times F_{FAME_i - FA_i}}{\frac{c_{Sample} \times V_{sample}}{V_{heptane}} \times \frac{V_{supernatant}}{V_{final}}} \times 100\%$$

Where X_i is the content of fatty acid i in the sample (mg/g)

$F_{FAME_i - FA_i}$ is the conversion factor of fatty acid methyl ester to fatty acid

c_{Sample} is the concentration of sample (mg/mL)

V_{sample} is the volume of sample used (μL).