

Separations of Fatty Acid Methyl Esters

General separation technologies, Inc.

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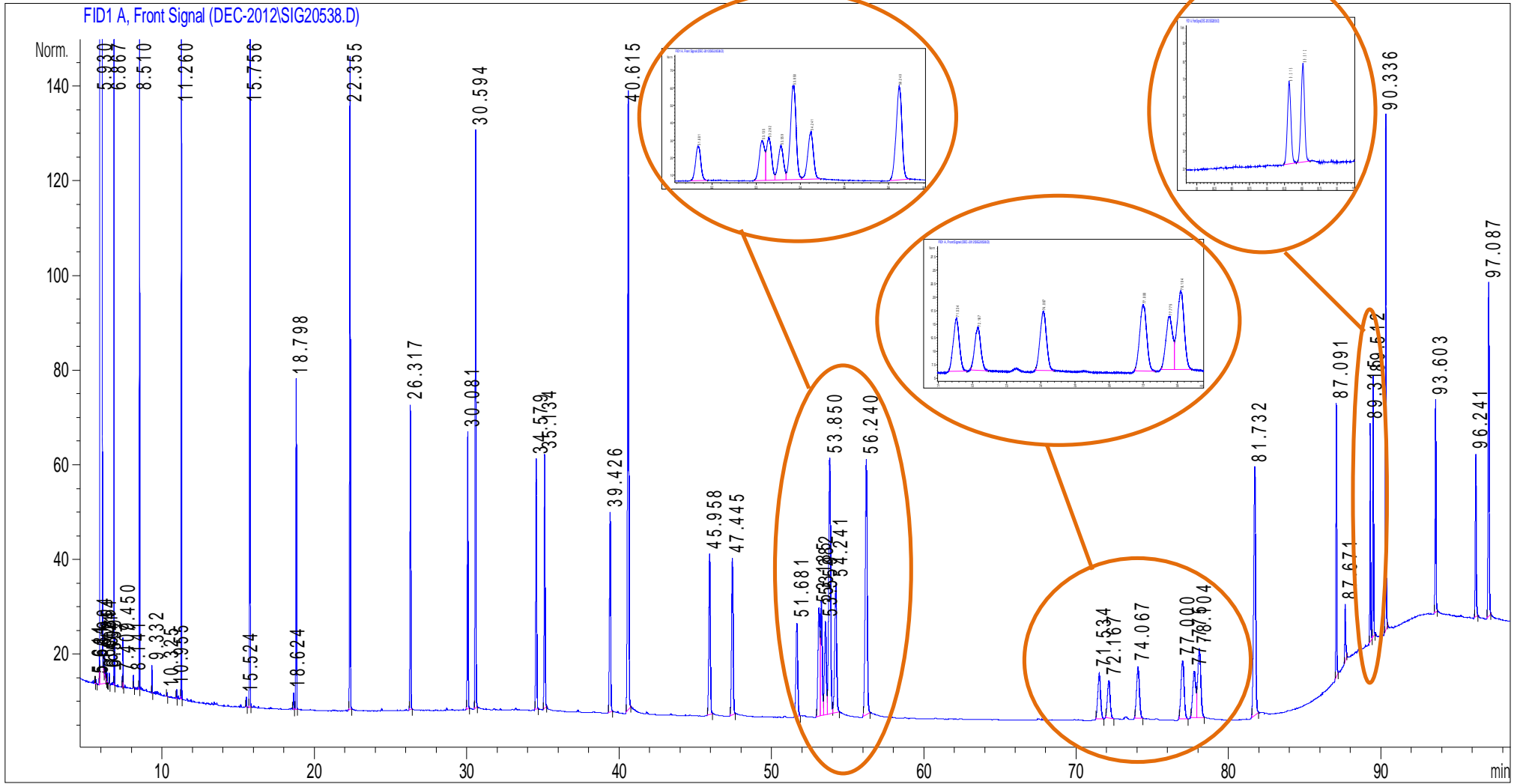
Background

- Fatty acid methyl esters (FAMES) analysis is very important in food inspection for nutritional labeling purposes. In recent years, trans fatty acid have become predominant synthetic food additives to improve flavor stability though they can interfere with natural metabolic process, resulting in an imbalance of the LDL:HDL ratio, and increasing lipoprotein levels.
- Separating and analyzing FAMES become more necessary. But they are also challenging especially for complex profiles, such as the cis and trans forms of polyunsaturated fatty acids. Gas chromatography is a good technique due to higher efficiencies that are needed to resolve the individual components.
- Either non-polar columns such as GsBP-5MS or polar columns such as Inowax columns is usually used. However, separations always have low resolution in a few FAMES. Though the baseline resolution was achieved on the 100m column, the time is very long. The problem could be solved in two-dimensional chromatography system where the components elute from the first column, then to a second column which has a different separation mechanism from the first one.

Instrumentation Conditions

- GC: Agilent 7890 w/ FID
- Cat no: *0125-A010 100m x 0.25mm x 1.0um*
- Oven: 80°C 35 °C /min to 180°C 2 °C /min to 240°C(47min) to 320 °C(10min)
- Carrier: Hydrogen, column flow 1.5ml/min
- Inlet: Split, 275 °C, split flow 60ml/min
- Detector: FID 325 °C
- Sample: Food Industry FAME Mix (37 components)

Chromatography



Peak Identifications and Resolutions:

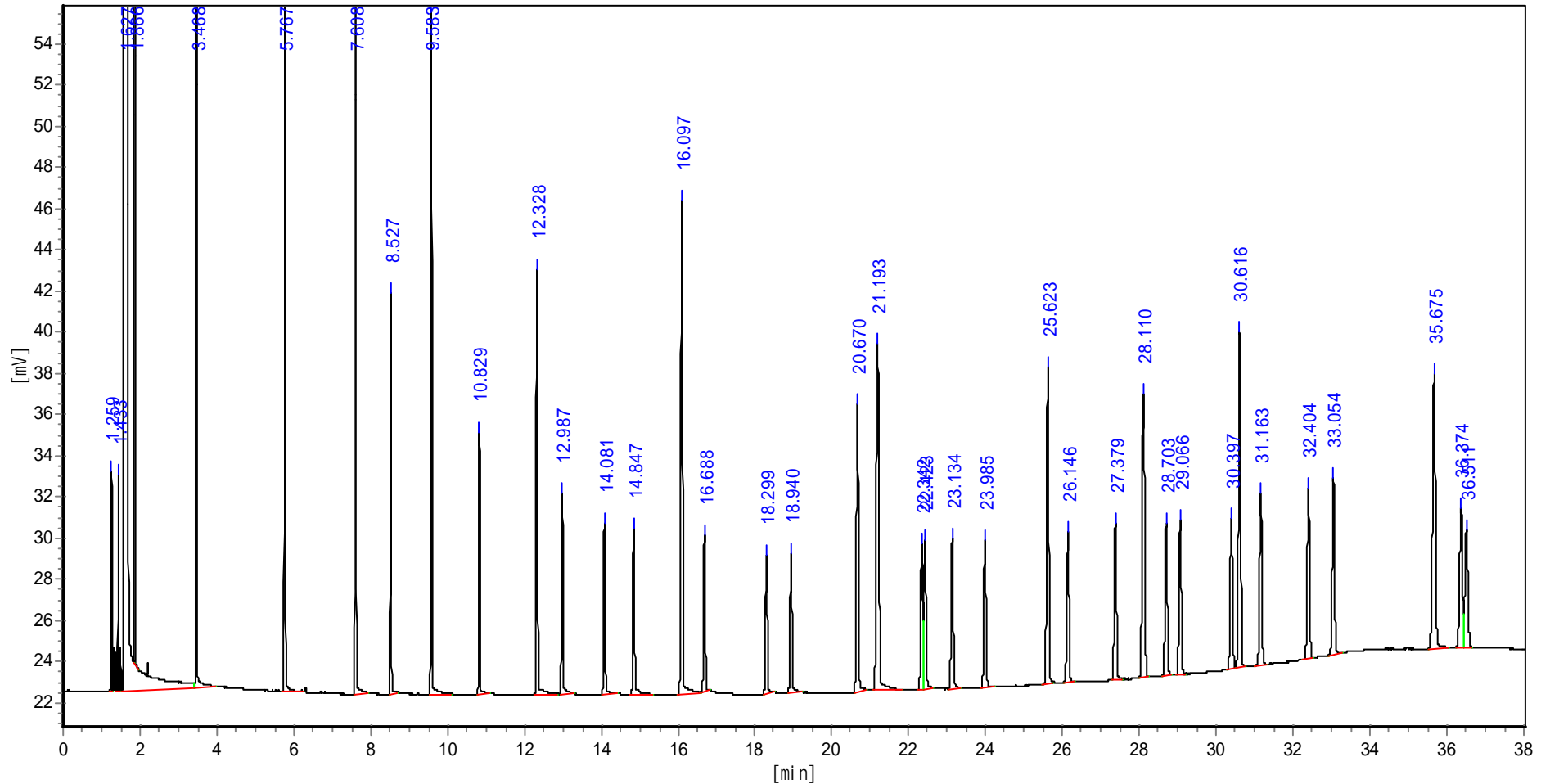
Peak#	Compound	Retention Time	Resolution
0	DCM	5.930	
1	C4:0 Methyl butyrate	6.867	
2	C6:0 Methyl hexanoate	8.510	
3	C8:0 Methyl octanoate	11.260	
4	C10:0 Methyl decanoate	15.756	
5	C11:0 Methyl undecanoate	18.798	
6	C12:0 Methyl laurate	22.355	
7	C13:0 Methyl tridecanoate	26.317	
8	C14:1 Methyl myristoleate (cis-9)	30.081	
9	C14:0 Methyl myristate	30.594	
10	C15:1 Methyl pentadecanoate (cis-10)	34.579	
11	C15:0 Methyl pentadecanoate	35.134	
12	C16:1 Methyl palmitoleate (cis-9)	39.426	
13	C16:0 Methyl palmitate	40.615	
14	C17:1 Methyl heptadecenoate (cis-10)	45.958	
15	C17:0 Methyl heptadecanoate	47.445	
16	C18:3 Methyl linolenate (cis-6,9,12)	51.681	
17	C18:2 Methyl linoleaidate (trans-9,12)	53.135	
18	C18:2 Methyl linoleate (cis-9,12)	53.282	1.45

Peak#	Compound	Retention Time	Resolution
19	C18:1 Methyl elaidate (trans-9)	53.559	2.67
20	C18:1 Methyl oleate (cis-9)	53.850	2.68
21	C18:3 Methyl linolenate (cis-9,12,15)	54.241	3.60
22	C18:0 Methyl stearate	56.240	
23	C20:5 Methyl eicosapentaenoate (cis-5,8,11,14,17)	71.534	
24	C20:4 Methyl arachidonate (cis-5,8,11,14)	72.167	
25	C20:3 Methyl eicosatrienoate (cis-8,11,14)	74.067	
26	C20:2 Methyl eicosadienoate (cis-11,14)	77.000	
27	C20:1 Methyl eicosenoate (cis-11)	77.775	
28	C20:3 Methyl eicosatrienoate (cis-11,14,17)	78.104	1.92
29	C20:0 Methyl arachidate	81.732	
30	C21:0 Methyl heneicosanoate	87.091	
31	C22:6 Methyl docosahexaenoate (cis-4,7,10,13,16,19)	87.671	
32	C22:2 Methyl docosadienoate (cis-13,16)	89.315	
33	C22:1 Methyl erucate (cis-13)	89.512	3.82
34	C22:0 Methyl behenate	90.336	
35	C23:0 Methyl tricosanoate	93.603	
36	C24:1 Methyl nervonate (cis-15)	96.241	
37	C24:0 Methyl lignocerate	97.087	

Instrumentation Conditions

- GC: Agilent 7890 w/ FID
- Cat no: 2025-3002 30m x 0.25mm x 0.25um
- or 1525-3002 30m x 0.25mm x 0.25um
- Oven: 80 °C (3min) 20 °C /min 165 °C (1min) 3 °C /min 240 °C (10min)
- Carrier: Hydrogen, column flow 1.3ml/min
- Inlet: Split, 250 °C, split flow 60ml/min
- Detector: FID 325 °C
- Sample: Food Industry FAME Mix (37 components)

Chromatography of Polar Column



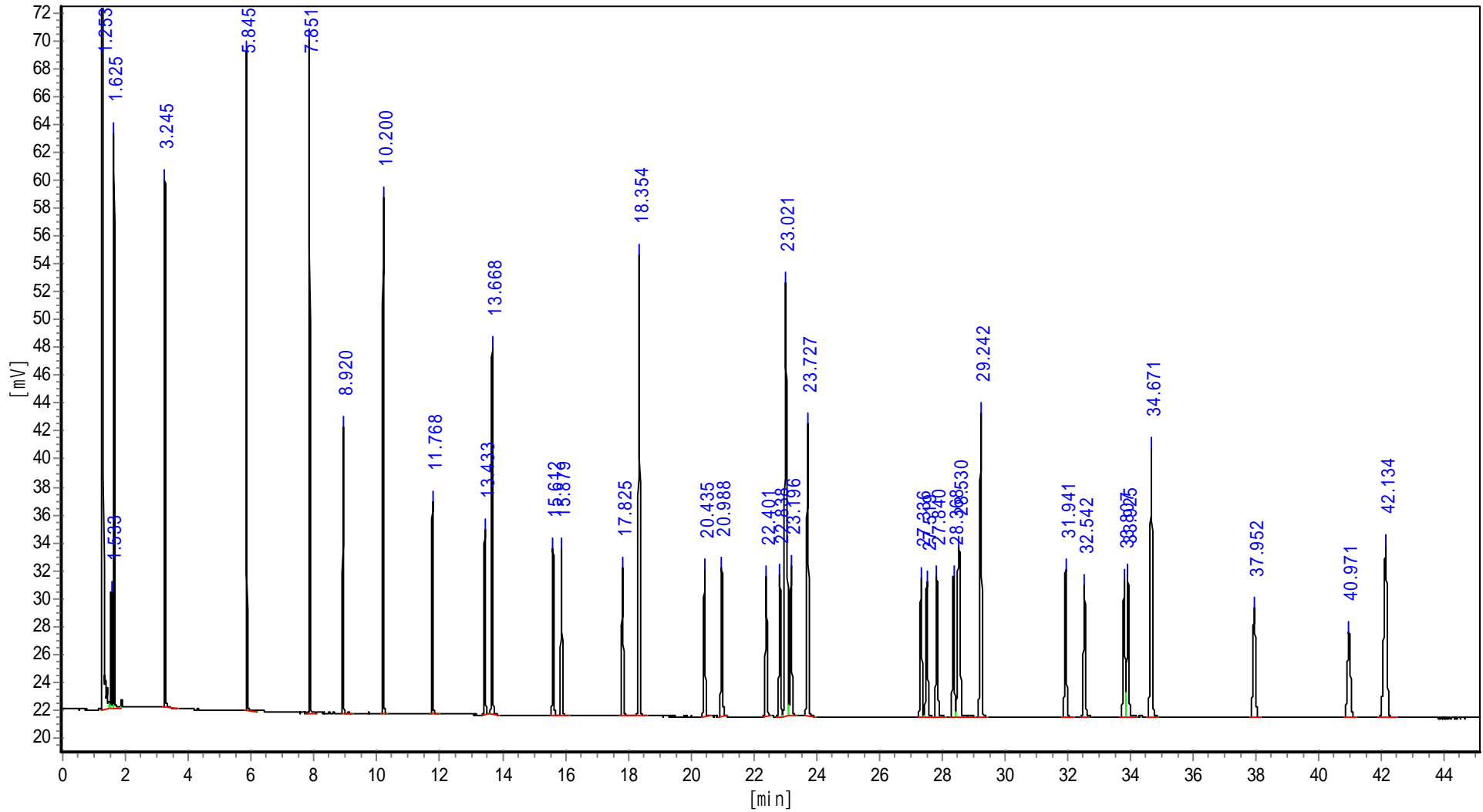
Column: 2025-3002 30m x 0.25mm x 0.25um

Peak Identifications and Resolutions:

Peak #	Compound	Retention time	Resolution
0	DCM	1.627	
1	C4:0 Methyl butyrate	1.866	
2	C6:0 Methyl hexanoate	3.468	
3	C8:0 Methyl octanoate	5.767	
4	C10:0 Methyl decanoate	7.608	
5	C11:0 Methyl undecanoate	8.527	
6	C12:0 Methyl laurate	9.583	
7	C13:0 Methyl tridecanoate	10.829	
8	C14:0 Methyl myristate	12.328	
9	C14:1 Methyl myristoleate (cis-9)	12.987	
10	C15:0 Methyl pentadecanoate	14.081	
11	C15:1 Methyl pentadecanoate (cis-10)	14.847	
12	C16:0 Methyl palmitate	16.097	
13	C16:1 Methyl palmitoleate (cis-9)	16.688	
14	C17:0 Methyl heptadecanoate	18.299	
15	C17:1 Methyl heptadecenoate (cis-10)	18.94	
16	C18:0 Methyl stearate	20.67	
17	C18:1 Methyl elaidate (trans-9) C18:1 Methyl oleate (cis-9)(n9)	21.193	

Peak #	Compound	Retention Time	Resolution
18	C18:2 Methyl linoleaidate (trans-9,12)	22.342	
19	C18:2 Methyl linoleate (cis-9,12)	22.423	1.37
20	C18:3 Methyl -linolenate (cis-6,9,12)	23.134	
21	C18:3 Methyl linolenate (cis-9,12,15)	23.985	
22	C20:0 Methyl arachidate	25.623	
23	C20:1 Methyl eicosenoate (cis-11)	26.146	
24	C20:2 Methyl eicosadienoate (cis-11,14)	27.379	
25	C20:3 Methyl eicosatrienoate (cis-8,11,14) C21:0 Methyl heneicosanoate	28.11	
26	C20:3 Methyl eicosatrienoate (cis-11,14,17)	28.703	
27	C20:4 Methyl arachidonate (cis-5,8,11,14)	29.066	
28	C20:5 Methyl eicosapentaenoate (cis-5,8,11,14,17)	30.397	
29	C22:0 Methyl behenate	30.616	
30	C22:1 Methyl erucate (cis-13)	31.163	
31	C22:2 Methyl docosadienoate (cis-13,16)	32.404	
32	C23:0 Methyl tricosanoate	33.054	
33	C24:0 Methyl lignocerate	35.675	
34	C22:6 Methyl docosahexaenoate (cis-4,7,10,13,16,19)	36.374	
35	C24:1 Methyl nervonate (cis-15)	36.511	1.75

Chromatography of Non-polar Column



Column: 1525-3002 30m x 0.25mm x 0.25um

Peak Identifications and Resolutions

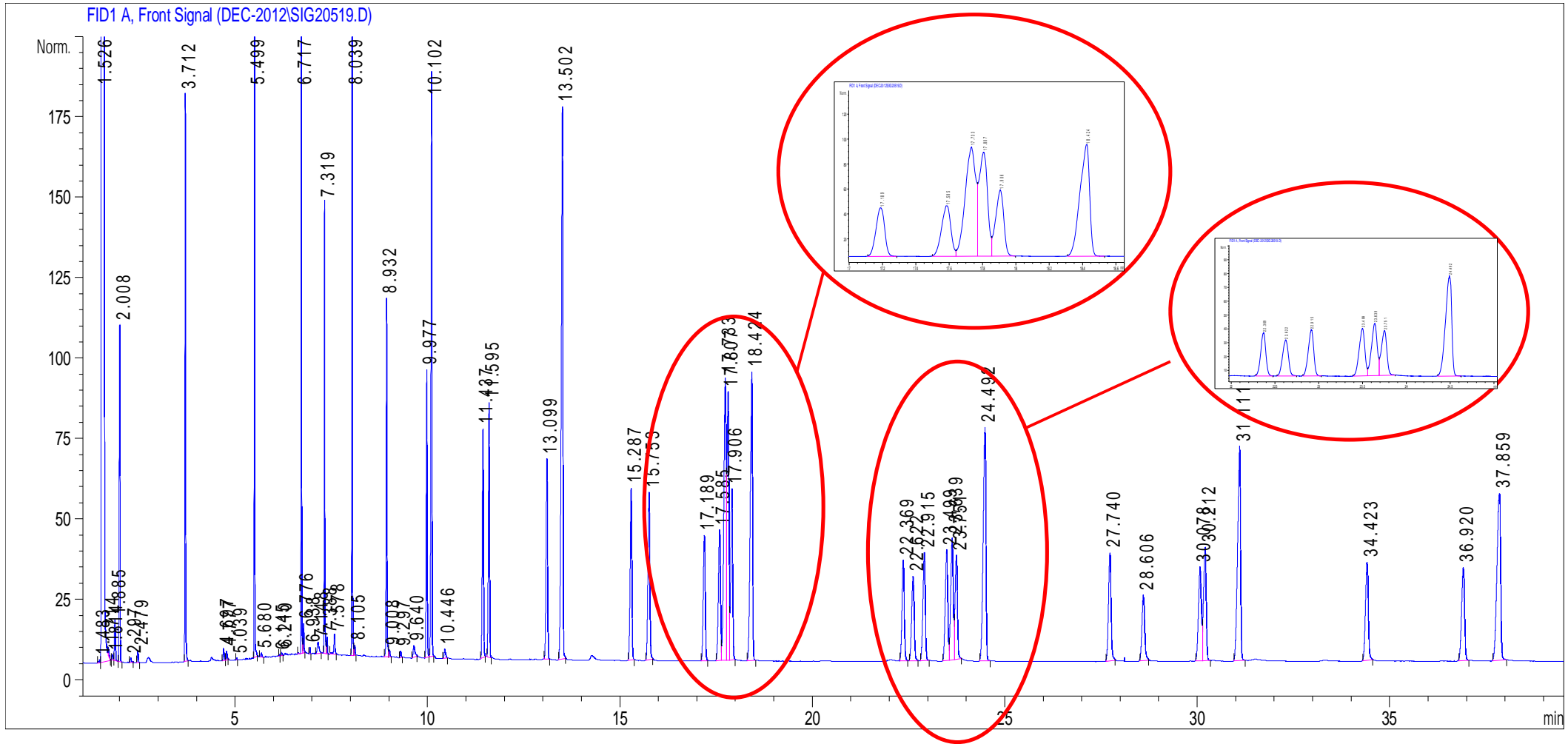
Peak #	Compound	Retention Time	Resolution
0	DCM	1.253	
1	C4:0 Methyl butyrate	1.625	
2	C6:0 Methyl hexanoate	3.245	
3	C8:0 Methyl octanoate	5.845	
4	C10:0 Methyl decanoate	7.851	
5	C11:0 Methyl undecanoate	8.92	
6	C12:0 Methyl laurate	10.2	
7	C13:0 Methyl tridecanoate	11.768	
8	C14:1 Methyl myristoleate (cis-9)	13.433	
9	C14:0 Methyl myristate	13.668	
10	C15:1 Methyl pentadecanoate (cis-10)	15.612	
11	C15:0 Methyl pentadecanoate	15.879	
12	C16:1 Methyl palmitoleate (cis-9)	17.825	
13	C16:0 Methyl palmitate	18.354	
14	C17:1 Methyl heptadecenoate (cis-10)	20.435	
15	C17:0 Methyl heptadecanoate	20.988	
16	C18:3 Methyl ??-linolenate (cis-6,9,12)	22.401	
17	C18:2 Methyl linoleaidate (trans-9,12) C18:2 Methyl linoleate (cis-9,12)	22.838	

Peak #	Compound	Retention Time	Resolution
18	C18:1 Methyl elaidate (trans-9) C18:1 Methyl oleate (cis-9)(n9)	23.021	3.13
19	C18:3 Methyl linolenate (cis-9,12,15)	23.196	3.02
20	C18:0 Methyl stearate	23.727	
21	C20:5 Methyl eicosapentaenoate (cis-5,8,11,14,17)	27.336	
22	C20:4 Methyl arachidonate (cis-5,8,11,14)	27.519	
23	C20:3 Methyl eicosatrienoate (cis-8,11,14)	27.84	
24	C20:2 Methyl eicosadienoate (cis-11,14)	28.368	
25	C20:1 Methyl eicosenoate (cis-11) C20:3 Methyl eicosatrienoate (cis-11,14,17)	28.53	2.12
26	C20:0 Methyl arachidate	29.242	
27	C21:0 Methyl heneicosanoate	31.941	
28	C22:6 Methyl docosahexaenoate (cis-4,7,10,13,16,19)	32.542	
29	C22:2 Methyl docosadienoate (cis-13,16)	33.807	
30	C22:1 Methyl erucate (cis-13)	33.925	1.98
31	C22:0 Methyl behenate	34.671	3.13
32	C23:0 Methyl tricosanoate	37.952	3.02
33	C24:1 Methyl nervonate (cis-15)	40.971	
34	C24:0 Methyl lignocerate	42.134	

Instrumentation Conditions

- GC: Agilent 7890 w/ FID
- Cat no: 2025-3002 , *0.5m x 0.25mm x 0.25um* 1525-3002 *30m x 0.25mm x 0.25um*
- Oven: 80 °C (3min) 20 °C /min 180 °C 1C/min 200 °C (10min) 2 °C /min 240 °C (20min)
- Carrier: Hydrogen, column flow 1.3ml/min
- Inlet: Split, 250 °C, split flow 60ml/min
- Detector: FID 325 °C
- Sample: Food Industry FAME Mix (37 components)

Two-dimensional Chromatography



Peak Identifications and Resolutions

Peak #	Compound	Retention Time	Resolution
0	DCM	1.527	
1	C4:0 Methyl butyrate	2.008	
2	C6:0 Methyl hexanoate	3.712	
3	C8:0 Methyl octanoate	5.499	
4	C10:0 Methyl decanoate	6.717	
5	C11:0 Methyl undecanoate	7.319	
6	C12:0 Methyl laurate	8.039	
7	C13:0 Methyl tridecanoate	8.932	
8	C14:1 Methyl myristoleate (cis-9)	9.976	
9	C14:0 Methyl myristate	10.102	
10	C15:1 Methyl pentadecanoate (cis-10)	11.437	
11	C15:0 Methyl pentadecanoate	11.595	
12	C16:1 Methyl palmitoleate (cis-9)	13.099	
13	C16:0 Methyl palmitate	13.502	
14	C17:1 Methyl heptadecenoate (cis-10)	15.287	
15	C17:0 Methyl heptadecanoate	15.753	
16	C18:3 Methyl linolenate (cis-6,9,12)	17.189	
17	C18:2 Methyl linoleaidate (trans-9,12)	17.585	

Peak #	Compound	Retention Time	Resolution
18	C18:2 Methyl linoleate (cis-9,12)	17.733	2.58
19	C18:1 Methyl elaidate (trans-9) C18:1 Methyl oleate (cis-9)	17.806	1.31
20	C18:3 Methyl linolenate (cis-9,12,15)	17.906	1.89
21	C18:0 Methyl stearate	18.424	
22	C20:5 Methyl eicosapentaenoate (cis-5,8,11,14,17)	22.369	
23	C20:4 Methyl arachidonate (cis-5,8,11,14)	22.622	
24	C20:3 Methyl eicosatrienoate (cis-8,11,14)	22.915	
25	C20:2 Methyl eicosadienoate (cis-11,14)	23.499	
26	C20:3 Methyl eicosatrienoate (cis-11,14,17)	23.639	2.17
27	C20:1 Methyl eicosenoate (cis-11)	23.750	1.70
28	C20:0 Methyl arachidate	24.492	
29	C21:0 Methyl heneicosanoate	27.740	
30	C22:6 Methyl docosahexaenoate (cis-4,7,10,13,16,19)	28.606	
31	C22:2 Methyl docosadienoate (cis-13,16)	30.078	
32	C22:1 Methyl erucate (cis-13)	30.212	1.96
33	C22:0 Methyl behenate	31.111	
34	C23:0 Methyl tricosanoate	34.422	
35	C24:1 Methyl nervonate (cis-15)	36.920	
36	C24:0 Methyl lignocerate	37.858	

Conclusion

- Though the baseline separations of 37 FAMES are achieved on a 0125-A010 column, the separation time was long. The baseline separations of 37 FAMES were achieved on a 2025-3002 × 1525-3002 column (there are two FAMES C18:1 Methyl elaidate and C18:1 Methyl oleate could not be separated.) with a short period of time.
- In 2D GC, The bands that are poorly resolved from the first column may be completely separated in the second column.
- The resolutions of critical separated pairs of FAMES: C18:2 Methyl linoleate (cis-9,12) ,C18:1 Methyl elaidate (trans-9) , C18:1 Methyl oleate (cis-9) and C18:3 Methyl linolenate (cis-9,12,15) ; C20:2 Methyl eicosadienoate (cis-11,14), C20:3 Methyl eicosatrienoate (cis-11,14,17), and C20:1 Methyl eicosenoate (cis-11); C22:2 Methyl docosadienoate (cis-13,16) and C22:1 Methyl erucate (cis-13),are improved in 2D system.