Trans Fatty acid Content Analysis By Gas Chromatography

1. Experiment

1) Sample

* Hydrolysis of fat : Specimen 25mg => Add 1.5ml of 0.5N Sodium Hydroxide Methanolic solution 1.5ml Then mix together=> Heating at 100 ℃ for 5min => Cooling at 30-40 ℃

* Fatty acid Derivatization : Add 2ml of 14% Trifluoro borane Methanol solution then mix. => Heating at 100 ℃ for 2min => after cooling at 30-40 ℃, add 1ml of isooctane and then stir for 30sec => stir again after adding 5ml of saturated NaCl solution => seperate layer at room temperature => use upper layer as Sample

2) Standard : Fatty methyl ester 37 species
cis, trans isomer reference material of 18:2, 18:3

2. Analytical Condition

Capillary Column : SP-2560(100m*0.25mm*0.2um)
Injector : Capillary 280 ℃ Column flow 1ml/min
Detector : FID 280 ℃
Oven program : 180 ℃(40min)-> temp-programed 3℃/min -> 230℃(10min)
Split ratio 50:1
Injection Volume : 1ul

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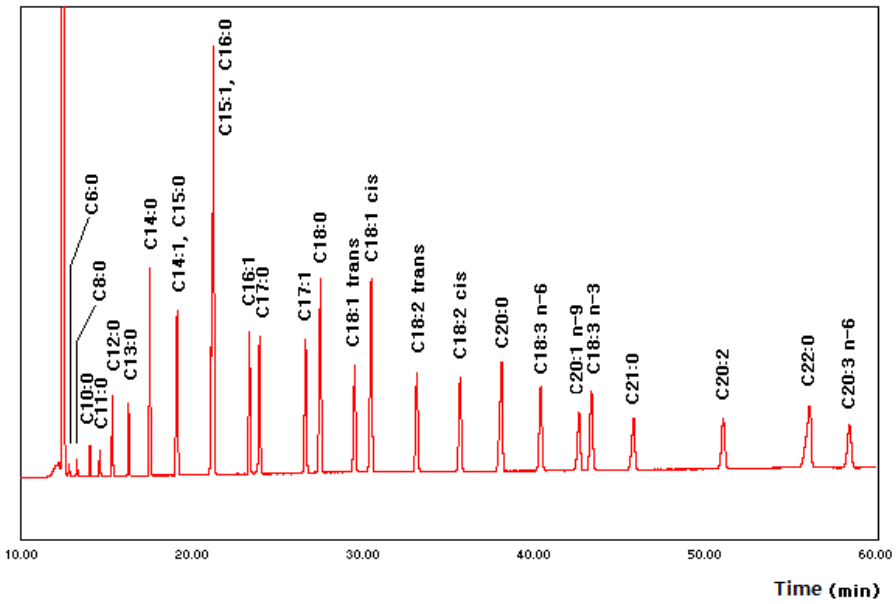
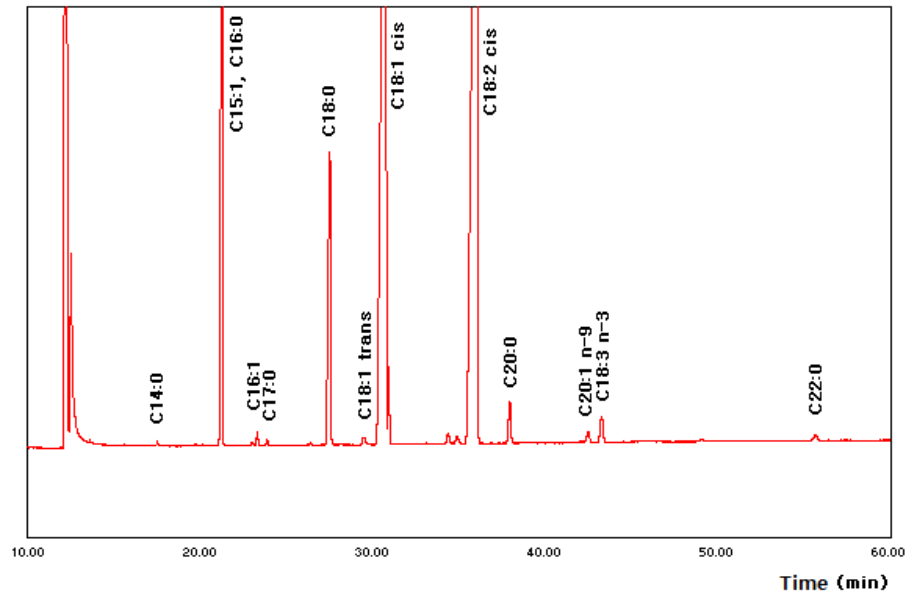
3. Chromatogram

Standard



Sample



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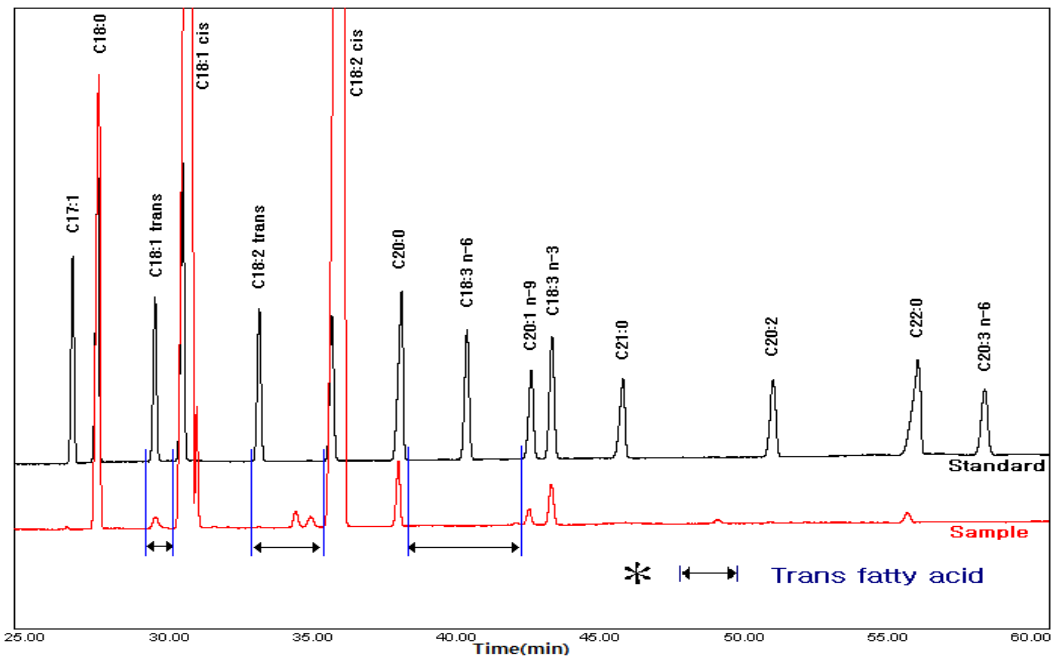
4. Result Analysis

<Trans Fatty acid Distinguishment in Sample>



<Trans Fatty acid Content Calculation>

1. Input the values of area and Retention Time(RT) of Fatty acid standard as **Figure 1.**(next page)
2. Input the value of area as applicable Fatty acid peak in sample as **Figure 2.**
   (Input the value of area after sample integration result converting to excel form.
   Distinguish trans fatty peak carefully.)
3. Trans fatty acid content is able to be calculated in **Figure 3.**

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<Example>

[Figure 1.] Input the values of Standard Retention Time(RT) and Area

The calculation of FID conversion factor in fatty acid determination

Peak No.	FFA	RT	wt %	Area	Area/wt	Me-->FA	R.
15	17:1	26.6450	2	291.1480	145.57	0.9503	1.219
16	18:0	27.5183	4	456.8850	114.22	0.9530	0.956
17	18:1 trans	29.5117	2	256.2285	128.11	0.9527	1.073
18	18:1 cis	30.4817	4	489.9968	122.50	0.9527	1.026
19	18:2 trans	33.1325	2	265.0443	132.52	0.9524	1.110
20	18:2 cis	35.6567	2	269.4878	134.74	0.9524	1.128
21	20:0	38.0850	4	375.5219	93.88	0.9570	0.786
22	18:3 n-6	40.3542	2	266.8227	133.41	0.9520	1.117
23	20:1 n-9	42.5883	2	197.2178	98.61	0.9568	0.826
24	18:3 n-3	43.3192	2	268.6953	134.35	0.9520	1.125
25	21:0	45.7825	2	191.3588	97.63	0.9588	0.818
26	20:2	50.9967	2	203.5307	101.77	0.9565	0.852
27	22:0	56.0083	4	353.5496	88.39	0.9604	0.740
28	20:3, n-6	58.3558	2	198.6657	99.33	0.9562	0.832

[Figure 2.] Input the value of area of sample

			1. Spl		
Peak No.	FFA	RT	Area	FFA	
				R.peak	ratio,%
7	13:0	16.3		0.0	0.0
8	14:0	17.6	5.5697	8.9	0.1
9	14:1	19.2		0.0	0.0
10	15:0			0.0	0.0
11	15:1	21.3		1198.1	10.8
12	16:0		1267.9562	0.0	0.0
13	16:1	23.4	24.1758	20.5	0.2
14	17:0	24.0	10.1529	8.8	0.1
15	17:1	26.6		0.0	0.0
16	18:0	27.5	851.9145	848.9	7.6
17	18:1 trans	29.5	29.1672	25.9	0.2
18	18:1 cis	30.5	4575.7647	4249.9	38.1
19	18:2 trans	33.1	59.3938	55.2	0.5
20	18:2 cis	35.7	5254.2141	4435.2	39.8
21	20:0	38.1	115.7874	141.0	1.3
22	18:3 trans	40.4		0.0	0.0
23	20:1 n-9	42.6	35.9979	41.7	0.4
24	18:3 n-3	43.3	88.1929	74.6	0.7
25	21:0	45.8		0.0	0.0
26	20:2	51.0		0.0	0.0
27	22:0	56.0	25.2707	32.8	0.3
28	20:3, n-6	58.4		0.0	0.0

[Figure 3.] Automatic calculation of Trans fatty acid content

FFA	1. Spl
14:1	0.0
15:0	0.0
15:1	10.8
16:0	0.0
16:1	0.2
17:0	0.1
17:1	0.0
18:0	7.6
18:1 trans	0.2
18:1 cis	38.1
18:2 trans	0.5
18:2 cis	39.8
20:0	1.3
18:3 trans	0.0
20:1 n-9	0.4
18:3 n-3	0.7
21:0	0.0
20:2	0.0
22:0	0.3
Total trans FA	0.7