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GC Analysis of a Fish Oil Supplement using the Agilent Select FAME Column and MIDI's Sherlock Analysis Software

Application Note – Fish Oil

Introduction

MIDI's Sherlock Analysis software has been used for over 25 years to automatically name fatty acids and mycolic acids by GC and/or HPLC. The software was recently expanded with the use of the Agilent Select FAME column to automate the analysis of edible oils with complex FAME patterns (e.g. fish oil, those with 18:1 isomers and cis/trans).

The software is able to accurately name fatty acids by using a calibration standard containing a mixture of saturated fatty acids from 4 to 25 carbons in length with each batch of samples. These known fatty acids act as a "fence-post" of small naming windows, allowing precise determination of the expected times of all fatty acids for a batch of samples run on this particular GC. Along with the enhanced resolution provided by the Select FAME column, the software is able to name fatty acids down to 0.05% percent.

Because the MIDI calibration mixture contains known amounts of each fatty acid, the system can determine whether and by how much the GC gives preference to specific peaks. These computed "response factor corrections" (*RFact* column) are more accurate than a fixed table of corrections since they are uniquely calculated for each particular instrument.

The software detects any peak "drift" by tracking systematic variations in peak positions of saturated fatty acids in the samples. It triggers automatic recalibration if the drift exceeds a pre-set limit thereby allowing unattended operation.

This note details the automated analysis of a "pharmaceutical grade" fish oil supplement by MIDI's Sherlock Analysis software. Once the Sherlock user selects "SEL-FAME" analysis from the list of available methods within the Sherlock Sample Processor, the correct method parameters are loaded into the Agilent GC. The software automatically calculates the name, weight percent, mole percent and various other parameters (user-defined) that may be useful. A total of 43 fatty acids were detected in the sample.

Sherlock Sample Analysis

Method: SEL-FAME – GC
Sample ID: OMEGA PHARM GRADE

File: E148115.68T
Created: 8/11/2014 5:38:25 PM

RT	Response	RFact	Peak Name	Wgt %	Mol %
3.2274	6.83E+8	----	SOLVENT PEAK	----	----
9.4366	1077	0.963	12:0 (lauric)	0.10	0.14
11.2229	82632	0.899	14:0 (myristic)	7.14	8.67
11.7909	2435	0.891	15:0 iso	0.21	0.24
11.9316	761	0.890	14:1 n-5 (myristoleic)	0.07	0.08
12.0322	636	0.889	15:0 anteiso	0.05	0.06
12.4179	6206	0.885	15:0 (pentadecylic)	0.53	0.61
13.1304	886	0.882	16:0 iso	0.08	0.08
13.9342	187471	0.880	16:0 (palmitic)	15.84	17.25
14.3886	4836	0.879	16:1 n-11	0.41	0.45
14.6571	2865	0.879	16:1 n-9	0.24	0.27
14.8341	110046	0.879	16:1 n-7 (palmitoleic)	9.29	10.19
15.0817	3043	0.879	16:1 n-5	0.26	0.28
15.2186	1013	0.879	17:0 anteiso	0.09	0.09
15.4682	1347	0.879	16:2 n-6	0.11	0.13
15.7413	3671	0.880	16:2 n-4	0.31	0.34
15.8248	4211	0.880	17:0 (margaric)	0.36	0.37
15.9123	2482	0.880	18:3 n-4	0.21	0.21
16.5414	12385	0.881	17:1 n-7t (t-heptadecenoic)	1.05	1.09
16.8660	3746	0.881	17:0 iso	0.32	0.33
17.7566	15497	0.883	17:1 n-5	1.31	1.37
18.2102	32748	0.883	18:0 (stearic)	2.78	2.74
19.2104	5349	0.885	18:1 n-11	0.45	0.45
19.3847	127609	0.885	18:1 n-9 (oleic)	10.85	10.77
19.6248	49227	0.885	18:1 n-7 (vaccenic)	4.19	4.16
20.0384	4347	0.886	17:3 n6	0.37	0.39
21.5519	15372	0.888	18:2 n-6 (LA)	1.31	1.31
22.2641	2780	----		----	----
22.4945	1789	0.889	19:1 n9	0.15	0.14
23.1655	2617	0.890	18:3 n-6 (GLA)	0.22	0.23
24.0524	3582	0.890	19:1 n5	0.31	0.29
24.4910	9402	0.891	18:3 n-3 (ALA)	0.80	0.81
26.1009	33529	0.891	20:1 n-11 (gadoleic)	2.87	2.60
26.3558	48656	0.891	20:1 n-9 (gondoic)	4.16	3.78
26.7380	7080	0.891	20:1 n-7	0.61	0.55
29.4244	2843	0.889	20:2 n-6 (eicosadienoic)	0.24	0.22

RT	Response	RFact	Peak Name	Wgt %	Mol %
33.1359	11321	0.883	20:4 n-6 (AA)	0.96	0.89
35.0989	41209	0.876	20:4 n-3 (ETA)	3.47	3.20
35.4483	20644	0.874	22:1 n-9 (erucic)	1.73	1.45
36.6160	163785	0.868	20:5 n-3 (EPA)	13.66	12.71
39.6497	7647	----		----	----
39.8972	1854	0.832	22:4 n-6 (docosatetraenoic)	0.15	0.13
40.4944	4900	0.819	22:5 n-6 (docosapentaenoic)	0.39	0.33
40.5986	5731	0.817	24:1n-9 (nervonic)	0.45	0.35
41.6245	26991	0.793	22:5 n-3 (DPA)	2.06	1.76
42.1624	132666	0.776	22:6 n-3 (DHA)	9.88	8.49

Total Response: 1210921

Percent Named: 99.14%

Summary

Fatty Acids Detected	**43**
Omega Type	**Mole Percent**
Omega-3	26.97
Omega-6	3.63
Omega-9	16.76
EPA:DHA Ratio	**3:2**
Fatty Acid Type	
Saturated	29.78
MUFA	37.18
PUFA	31.15
Branched	0.80
Trans	1.09

GC Conditions

GC instrument	Agilent 6890N Series
Autosampler	Agilent 7683 Injector and sample tray
Software	MIDI Sherlock Software v.6.2B Agilent OpenLab CDS ChemStation
Column	Agilent Select FAME, 50 m x 0.25 mm x 0.25 µm film thickness (p/n CP7419)
GC inlet	250 °C
Carrier gas	Hydrogen, constant flow, 1.1 mL/min
Oven program	80 °C (3.0 min), 20 °C/min to 160 °C (4.0 min), 0.6 °C/min to 176 °C (26.7 min), 5 °C/min to 233 °C (11.4 min)
Split ratio	20:1
Detector type	FID
Injection volume	2.0 µL

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