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Applications note

Using Computer Modeling to Optimize FAME Analysis

Background

Gas chromatography (GC) is an effective means of characterizing fatty acids in food as well as other matrices. The Association of Official Chemists (AOAC) and the American Oil Chemists Society (AOCS) provide several methods for GC analysis of fatty acid methyl esters (FAMEs). AOAC Method 963.22 provides general guidelines and conditions for analyzing a wide range of saturated and unsaturated FAMEs, from C8:0 to C24:0, using packed column GC[1]. If capillary GC is used as an alternative method, specifications can be met or exceeded easily. AOAC Official Method 991.39[1] and AOCS Official Method Ce 1b-89[2] describe the analysis of polyunsaturated FAMEs in fish oils using capillary GC[2]. These methods list conditions for separating all of the FAMEs in complex fish oils on polyethylene glycol (PEG) and cyanopropyl stationary phases. By properly optimizing analytical conditions, you can improve resolution and decrease analysis time.

Unfortunately, the typical optimization process can be extremely time consuming and frustrating. Furthermore, many analysts identify unsaturated fatty acids, including polyunsaturated fatty acids (PUFAs) by equivalent chain length (ECL) values rather than by retention time[3]. Changing conditions may alter the elution order, and require peak re-identification. Trial and error with different column configurations and parameters, along with the necessary re-identifications, can waste additional analyst and instrument time. A more efficient approach to optimizing analytical parameters is to use computer modeling software. Programs such as *Pro ezGC*™ use thermodynamic retention indices (TRIs) to model GC analyses and provide optimized conditions in minutes. Additionally, this program can recalculate the ECL values for the new set of run conditions. *Pro ezGC*™ can provide optimized FAME analyses that meet or exceed the resolution and ECL specifications stated in the official methods.

How Well Does Computer Modeling Work?

To illustrate the accuracy and efficiency of the modeling software, a mixture of 21 saturated FAMEs were analyzed on a 60m, 0.25mm ID, 0.25µm Rtx®-Wax column (cat.# 12426). The FAMEs ranged from methyl butanoate (C4:0) to methyl tetracosanoate (C24:0). TRIs were generated by analyzing the mixture with two different temperature programs. The first program was a relatively slow ramp, while the second was a fast ramp. The resulting retention times for each component were entered into the program, which automatically calculated the TRIs. The software then was able to evaluate a wide range of run conditions and predict the resulting retention times under each set of conditions. Once the predictions are made, the software selects the set of conditions that provides the best separation in the fastest analysis time. To demonstrate the accuracy of this

Table I

Experimental retention times versus predicted retention times.

FAMEs MSD Data
60m, 0.25mm ID, 0.25µm Rtx®-Wax column (cat.# 12426)

Oven temp.:
45°C @ 6°C/min. to 265°C (hold 7 min.)

Carrier gas:
hydrogen (constant pressure)

Linear velocity:
53.5cm/sec. @ 45°C

Dead time:
1.980 min. @ 45°C

	Component	Exp. tR (min.)	Calc. tR (min.)	Exp. -Calc. Error (min.)	(Exp. -Calc.)/ Exp. % Error
1.	me butanoate	3.918	3.924	-0.006	-0.1
2.	me pentanoate	5.315	5.327	-0.012	-0.2
3.	me hexanoate	7.158	7.171	-0.013	-0.2
4.	me heptanoate	9.293	9.299	-0.006	-0.1
5.	me octanoate	11.573	11.577	-0.004	-0.0
6.	me nonanoate	13.855	13.854	0.001	0.0
7.	me decanoate	16.083	16.075	0.008	0.0
8.	me undecanoate	18.228	18.205	0.023	0.1
9.	me dodecanoate	20.282	20.253	0.029	0.1
10.	me tridecanoate	22.247	22.191	0.056	0.3
11.	me tetradecanoate	24.127	24.078	0.049	0.2
12.	me pentadecanoate	25.927	25.855	0.072	0.3
13.	me hexadecanoate	27.653	27.567	0.086	0.3
14.	me heptadecanoate	29.310	29.238	0.072	0.2
15.	me octadecanoate	30.902	30.856	0.046	0.1
16.	me nonadecanoate	32.432	32.382	0.050	0.2
17.	me eicosanoate	33.907	33.824	0.083	0.2
18.	me heneicosanoate	35.330	35.205	0.125	0.4
19.	me docosanoate	36.702	36.542	0.160	0.4
20.	me tricosanoate	38.120	37.872	0.248	0.6
21.	me tetracosanoate	39.710	39.396	0.314	0.8
			Avg. error:	*0.070*	*0.2*

software, the FAMEs were analyzed using an optimized temperature program chosen by the software. Table I shows the percent error between the predicted and the actual retention times. The program's average prediction error was only 0.2%, with a maximum error less than 1%!

Modeling Saturated and Unsaturated FAMEs in Cocoa Butter

AOAC Method 963.22, entitled "Methyl Esters of Fatty Acid in Oils and Fats," describes the GC analysis of FAMEs ranging from 8 to 24 hydrocarbons in chain length. Although the method describes GC using packed columns, capillary columns coated with Carbowax® PEG 20M can easily exceed the required specifications. The minimum separation requirements are: 1) a resolution value (R) of at least 1.25 between methyl stearate (C18:0) and methyl oleate (C18:1n9); and 2) baseline resolution of methyl linolenate (C18:3n3), methyl arachidate (C20:0), and methyl gadoleate (C20:1). Methyl stearate should elute 15 minutes after the solvent peak, and the analytical column should have at least 2000 theoretical plates.

GC operating conditions were entered into the computer program to obtain the optimum analysis of a reference standard of cocoa butter FAMEs. Then, a very broad range of temperature conditions including the initial temperature, the program rate, and the final temperature were entered into the software. Based upon these selection criteria, over 1000 possible temperature programs were evaluated by the software in only a few minutes. The solutions were automatically ranked according to the best separations and fastest analysis times. In this instance, the default resolution factor was set at 1.5 to provide baseline resolution. Although this value is typical, it may be increased or decreased to meet the unique needs of a specific analysis.

The software predicted that all of the cocoa butter FAMEs could be separated adequately in less than 10 minutes on a 30m, 0.25mm ID, 0.25µm Famewax™ column (cat.# 12497). Using this optimized capillary method, total analysis is completed in less time than methyl stearate elutes under the original AOAC recommended conditions! This capillary column provides approximately 2700 plates-per-meter, which exceeds the efficiency requirement specified in AOAC Method 963.22. The new analytical conditions not only provide baseline resolution of the required components, but of all components (R>1.5), thereby easily exceeding the method's resolution requirement. Figure 1 shows the predicted chromatogram provided by the software and the actual analysis of the cocoa butter FAMEs standard reference mixture. The actual chromatogram shows excellent correlation to the chromatogram predicted by the software.

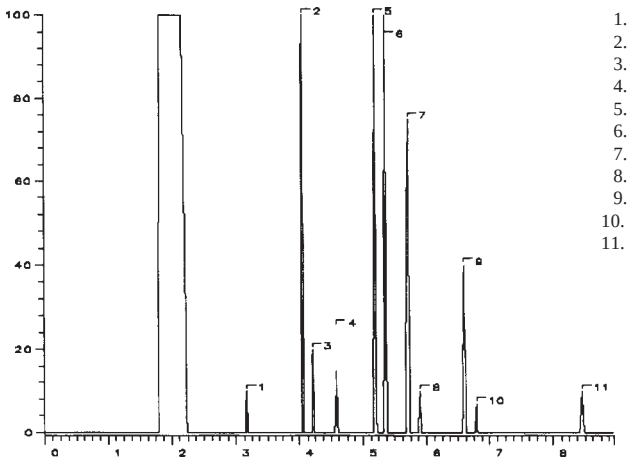
Reducing Analysis Time of Menhaden Oil PUFAs

GC analysis of menhaden oil is described in AOAC Method 991.39, entitled "Fatty Acids in Encapsulated Fish Oils and Fish Oil Methyl and Ethyl Esters," and in AOCS Official Method Ce 1b-89, entitled "Fatty Acid Composition by GLC." These methods illustrate chromatograms of polyunsaturated fatty acids (PUFAs) on Carbowax®-20M capillary columns. Any column providing the same elution pattern of FAMEs and baseline

Figure 1

Cocoa Butter FAME Reference Standard

Predicted

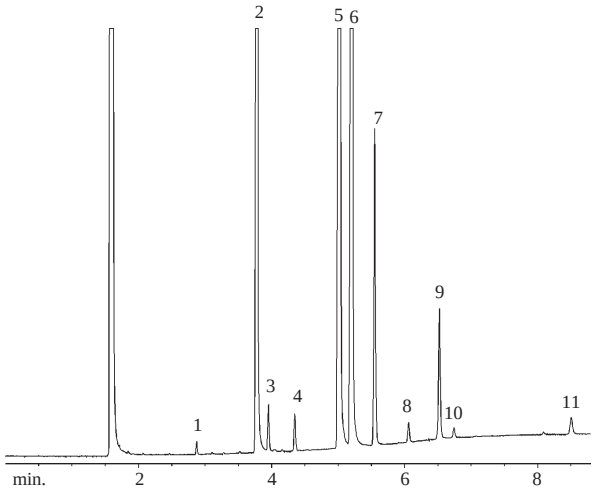


1. C14:0
2. C16:0
3. C16:1n7
4. C17:0
5. C18:0
6. C18:1n9
7. C18:2n6
8. C18:3n3
9. C20:0
10. C20:1n9
11. C22:0

30m, 0.25mm ID, 0.25µm Famewax™ (cat.# 12497).

Oven temp.:	200°C to 250°C @ 8°C/min. (hold 3 min.)
Carrier gas:	hydrogen
Linear velocity:	31cm/sec.

Actual



30m, 0.25mm ID, 0.25µm Famewax™ (cat.# 12497).
1.0µL split injection of #14 FAME Standard (cat.# 35035).

Oven temp.:	200°C to 250°C @ 8°C/min. (hold 3 min.)
Inj./det. temp.:	250°C
Det. sensitivity:	8 x 10^{-11} AFS
Carrier gas:	hydrogen
Linear velocity:	31.4cm/sec. @ 200°C
Split ratio:	45:1

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separation of C21:5n3, C23:0, and C22:4n6 can be used for these methods. The peak height of the internal standard, methyl tricosanoate (C23:0), must be greater than 50% of the peak heights of methyl eicosapentaenoate (EPA) and docosahexanoate (DHA)[1]. In addition, Method Ce 1b-89 specifies that methyl tetracoanoate (C24:0) must be baseline resolved from DHA (C22:6n3)[2].

Pro ezGC™ software and a Famewax™ column were used to optimize the analysis of menhaden oil. Using the GC conditions suggested in these methods results in baseline resolution of all critical components, but with an analysis time of more than 45 minutes. The computer program was able to determine a faster, more efficient analysis time that still resulted in the required separations. A program with a single temperature ramp rate and a higher column flow rate allowed baseline separation of all critical components in only 30 minutes. Figure 2 is the actual optimized analysis of menhaden oil. Analysis time was reduced by 23 minutes, a greater than 50% savings in analysis time!

Calculating ECL Values for Polyunsaturated FAMEs Under Optimized Conditions

Since there are many mono-, di-, and polyunsaturated FAMEs in a menhaden oil sample, identification by ECL is useful. ECLs are similar to linear temperature-program indices, but are calculated relative to saturated FAMEs instead of hydrocarbons. Although the elution order did not change with the optimized chromatogram of menhaden oil, there could still be slight changes in the ECL values due to changes in retention of the individual FAMEs. To ensure the accuracy of the identifications, the *Pro ezGC*™ software can recalculate the ECL values from the actual retention times of the analyses. After entering a few marker retention times, such as the saturated FAMEs in the oil, the program calculates ECLs for all of the components in the sample. Table II illustrates the retention times and ECL values for the FAMEs in menhaden oil using the optimized conditions.

Summary

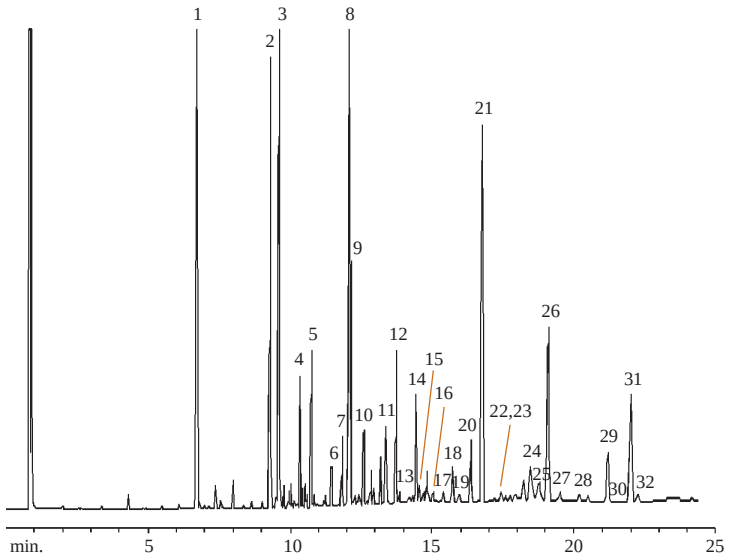
Computer modeling on the basis of TRIs is a powerful, accurate, and effective tool for optimizing the analysis of FAMEs. The predicted analysis can be illustrated clearly in the form of a list of components and retention times or by a simulated chromatogram. Comparisons between actual retention times of C4-C24 saturated FAMEs and predicted vs. actual chromatograms of cocoa butter FAMEs demonstrate the precision and accuracy of computer modeling. In addition, ECL values can be calculated for the actual column and conditions used.

References

1. *Association of Analytical Chemists, International AOAC Official Methods of Analysis. 15th Ed.: 3rd Supplement, 1992, pp 140-142.*
2. *American Oil Chemists Society, Official Methods and Recommended Practices of the American Oil Chemists Society, 1994. 8.*
3. *Christie, W.W., Gas Chromatography and Lipids, 1989, pp 92-96.*

Figure 2

Menhaden Oil PUFA Analysis (Actual Chromatogram)



1. C14:0	12. C18:4n3	23. C22:1n9
2. C16:0	13. C20:0	24. C22:2n6
3. C16:1n7	14. C20:1n9	25. C21:5n3
4. C16:2n4	15. C20:1n7	26. C23:0
5. C16:3n4	16. C20:2n6	27. C22:4n6
6. C16:4n1	17. C20:3n6	28. C22:5n6
7. C18:0	18. C20:4n6	29. C22:5n3
8. C18:1n9	19. C20:3n3	30. C24:0
9. C18:1n7	20. C20:4n3	31. C22:6n3
10. C18:2n6	21. C20:5n3	32. C24:1n9
11. C18:3n3	22. C22:0	

30m, 0.25mm ID, 0.25µm Famewax™ (cat.# 12497).
0.8µL split injection of menhaden oil PUFA with C23:0 (IS).
On-column concentration 100-150ng.

Oven temp.:	120°C to 220°C @ 7°C/min. (hold 20 min.).
Inj. & det. temp.:	220°C
Carrier gas:	hydrogen
Linear velocity:	60 cm/sec. set @ 120°C
FID sensitivity:	8 x 10^{-11} AFS
Split ratio:	50:1

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Table II

ECL values for FAMEs in Menhaden Oil

Retention Indices & Corrected Calculated Retention Times

Column:
30m, 0.25mm ID, 0.25µm Famewax™ column (cat.# 12497)

Oven temp.:
120°C to 220°C @ 7°C/min. (hold 11 min.)

Carrier gas:
hydrogen (constant pressure)

Linear velocity:
60 cm/sec. @ 120°C

Dead time:
0.833 min. @ 120°C

Component	Calc. tR (min.)	ECLs	Component	Calc. tR (min.)	ECLs
1. C14:0	6.630	14.00	17. C20:3n6	15.375	20.85
2. C16:0	9.230	16.00	18. C20:4n6	15.685	21.07
3. C16:1n7	9.539	16.27	19. C20:3n3	15.928	21.24
4. C16:2n4	10.260	16.87	20. C20:4n3	16.342	21.52
5. C16:3n4	10.669	17.19	21. C20:5n3	16.714	21.77
6. C16:4n1	11.358	17.70	22. C22:0	17.076	22.00
7. C18:0	11.775	18.00	23. C22:1n9	17.393	22.16
8. C18:1n9	12.023	18.22	24. C22:2n6	18.286	22.60
9. C18:1n7	12.098	18.29	25. C21:5n3	18.752	22.83
10. C18:2n6	12.567	18.69	26. C23:0	19.124	23.00
11. C18:3n3	13.347	19.33	27. C22:4n6	19.419	23.13
12. C18:4n3	13.702	19.60	28. C22:5n6	20.186	23.45
13. C20:0	14.224	20.00	29. C22:5n3	21.035	23.80
14. C20:1n9	14.437	20.16	30. C24:0	21.551	24.00
15. C20:1n7	14.536	20.24	31. C22:6n3	21.848	24.11
16. C20:2n6	15.019	20.60	32. C24:1n9	22.142	24.23

Product Listing

FAMEWAX™ Columns

ID	df (µm)	Temp. Limits	30m
0.25mm	0.25	20 to 250°C	11120
0.32mm	0.25	20 to 250°C	11136
0.53mm	0.50	20 to 250°C	11137

Methods Development Software

Description	cat.#
Pro ezGC™ (V1.0 for Windows®)	21487
Master Library Set	21460

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