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Automated Analysis and Quantitation of Fish Oil Supplements using the MIDI Sherlock™ Marine Oil Analysis Package

Application Note – Food, Beverages | Omega-3 Fatty Acid Analysis

Abstract

Omega-3 fatty acids from marine oils are a group of polyunsaturated fatty acids (PUFAs) that are essential for human health. Analyzing these fatty acids (as well as other fatty acids in marine oil is a tedious process). Using the automated Sherlock Marine Oil analysis package reduces turnaround time and cost, while limiting potential errors that can occur with "manual" analysis approaches.

Introduction

Marine oils and concentrates contain a wide variety of polyunsaturated fatty acids (PUFAs), including the two most important long-chain Omega-3 fatty acids, eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3). These two fatty acids are recognized for their cardiovascular benefits and may help with a number of other diseases. Proper identification and quantification of these Omega-3s, as well as other fatty acids contained in marine oils, is critical to dietary supplement labeling and quality control testing. In addition, the levels and ratios of key fatty acids are important biomarkers for certain health indicators, including the HS-Omega-3 Index®[1].

In fatty acid analysis, laboratory methods have a large impact on results. In one study, when Omega-3-containing samples were sent to five independent laboratories offering determination of an Omega-3 Index, results differed by a factor of 3.5.[2] While results may be internally valid in one laboratory, a difference by a factor of 3.5 makes it impossible to compare results among laboratories.[1] Part of the problem is that multiple different Gas Chromatography (GC) and Gas Chromatography-Mass Spectroscopy (GC-MS) methods and instrument types have been used to determine marine oil fatty acid profiles. However, most of these methods are performed manually, and the analysis process is laborious and potentially error-prone.

MIDI's Sherlock Analysis software has been used for over 25 years to standardize fatty acid analysis across laboratories and has been cited more than 4,000 times.[3] The software has traditionally been used to analyze the fatty acids from microbes, but was recently extended to the automation of the complex fatty acid profiles found in marine oils.

Experimental

Two different marine oil products were extracted in triplicate and analyzed on three different instruments using the Sherlock Marine Oil analysis package: an Agilent 6890N GC, an Agilent 7890B GC, and a Shimadzu 2010 Plus GC. The first product ("NF") was a fish oil supplement that contained the natural "triglyceride" (TAG) form and the second product ("MN") was a concentrated fish oil supplement containing the "ethyl ester" form of the fatty acids.

The extraction procedure used was based on AOCS Official Method Ce 1b-89.[4] A known number of moles of the internal standard (ISTD), 1,2-dinonadecanoyl-sn-glycero-3-phosphocholine (19:0 PC, Avanti Polar Lipids p/n 850367), were added at the beginning of the process to assist in the quantitation calculations.

The MIDI Sherlock software automates the process of running batches of samples, communicating with the GC instrument controller software (LabSolutions or ChemStation). The MIDI Calibration Standard (MIDI p/n 1208), is processed first in the batch; the results from the calibration are used to determine the expected retention times (RT) of each fatty acid in the sample. The calibration run was followed by a hexane blank and individual sample runs.

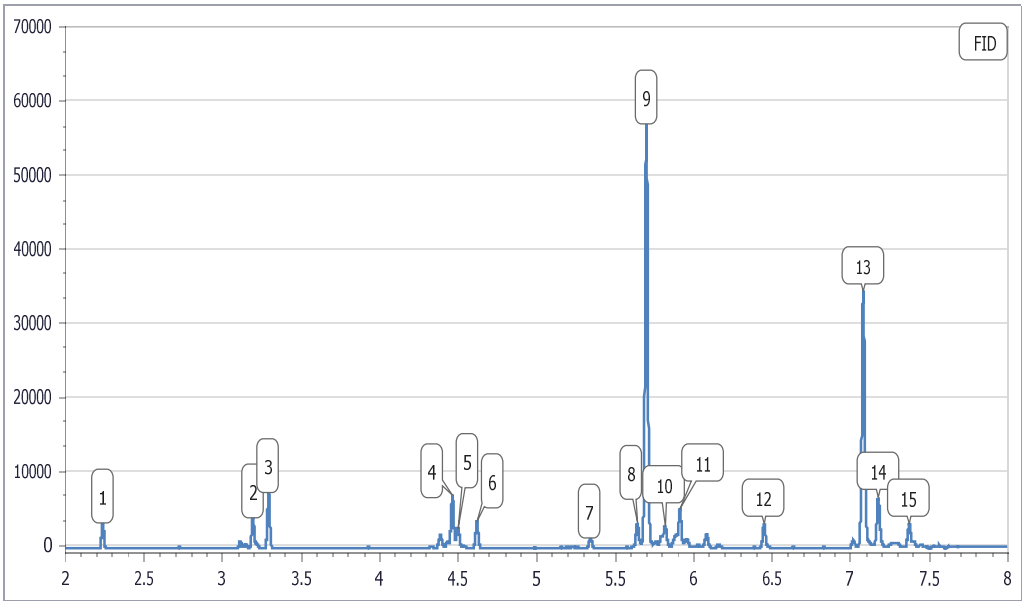
The Sherlock software automatically named between 20 and 35 fatty acids for the two different fish oils. The software then used the internal standard added at the beginning of the extraction procedure to quantitate the fatty acids.

An example result for fish oil "NF" is shown in the Table 1. The highlighted rows show the Omega-3s that the Sherlock software identified in the sample.

Table 1: Automated Results for a Fish Oil "NF" Sample

Compound	Amount (ug)		Compound	Amount (ug)
C14:0	14,922		C20:1n9	37,807
C15:0	885		C20:1n7	7,544
C16:1n7	22,438		C20:1n4	3,345
C16:0	32,675		C20:0	9,337
C17:0	938		C21:5n3	16,445
C18:3n6	1,310		C22:5n6	6,189
C18:4n3	9,599		**C22:6n3 (DHA)**	**183,761**
C18:1n9	38,654		C22:5n3	38,552
C18:1n7	13,525		C22:1n11	22,581
C18:1n6	1,679		C22:1n6	3,061
C18:0	16,512		C22:1n3	1,467
C19:1n6	1200		C22:0	1,255
C20:4n6	17,834		C24:4n6	1,840
C20:5n3 (EPA)	**275,472**		C24:1n9	8,139
C20:4n3	24,092			
Total	**813,058**			

An annotated chromatogram with the primary compounds identified is shown in Figure 1.



1. C14:0
2. C16:1n7
3. C16:0
4. C18:1n9
5. C18:1n7
6. C18:0
7. (C19:0 ISTD)
8. C20:4n6
9. C20:5n3 (EPA)
10. C20:4n3
11. C20:1n9
12. C21:5n3
13. C22:6n3 (DHA)
14. C22:5n3
15. C22:2n6

The MIDI Sherlock enhanced analysis software can automatically categorize compounds by type. Based on the label of the two fish oils analyzed, the samples passed the specification for EPA, DHA and total Omega-3 fatty acids, as shown in Table 2.

Table 2: Categorized Results for a Fish Oil "NF" Sample

	Fish-MN (n=9)		Fish-NF (n=9)	
	Range (mg/g)	Label (mg/g)	Range (mg/g)	Label (mg/g)
EPA	357 – 430	300	235 – 275	270
DHA	271 – 324	245	158 – 183	180
Total Omega-3	763 - 899	599	471 - 553	500

For each of these ranges, all nine samples (three runs on three instruments) were within 10% of the average. The method can be customized to return a variety of information, including total Omega-6 and total Omega-9, or the ratio of EPA to DHA.

Results and Discussion

Traditional methods of analysis require time-consuming manual work, including but not limited to, naming of the compounds, calculations based on the internal standard used, and validation of the methodology. With manual compound identification and calculations, there exists the possibility of an error propagating through the analysis which may lead to inaccurate claims. Using automated compound naming eliminates the possibility of error, as well as increasing the number of compounds which can be identified. Often an analyst only identifies the larger peaks and not the smaller unsaturated fatty acids. As can be seen in Table 1, there are four Omega-3 peaks below 25,000 micrograms per gram of oil which, while small, add to approximately 6% of the total profile and 10% of the Omega-3 component.

Current technology also requires different analytical methods when looking for specific compounds and may cause some peaks to co-elute. Verifying the various analytical methods is very time consuming as each analysis must be compared to a different standard. Any shifts in the peaks due to variations in the instrument can only be corrected by the expertise of the analyst, requiring extended training to ensure accurate decision for the peak identification. The Sherlock system automatically corrects for any shifts in the peak positions by the use of the MIDI external calibration mixture run with every batch. The external calibration mixture is also manufactured to exacting standards to correct for the selectivity of the FID due to the relative size of the fatty acids.

The new extraction procedure used in this study eliminates the use of BF_3 or BCl_3, both of which are extremely hazardous, using $NaOCH_3$ instead. The extraction process that was used for these analyses was compared against the traditional BF_3 / BCl_3 extraction, and yielded equivalent results. The extraction method was found to be extremely robust, allowing for a variation of ±10°C in the heating step without compromising the data.

Conclusion

The Sherlock Marine Oil analysis package automatically names the fatty acids in a sample, quantitates and categorizes them based on the user-defined parameters. This automated process yields consistent and easy-to-interpret results with less chance of errors. The system includes visualization tools and data export capabilities for further study and ease of publication. The sensitivity of the Sherlock method ensures that all discernible fatty acids are measured.

References

1. von Shacky, C. Omega-3 Index and cardiovascular health. *Nutrients.* 2014, *6*, 799-814.
2. Harris, W. S., von Schacky C., Park Y. Standardizing Methods for Assessing Omega-3 Biostatus. In: The *Omega-3 Fatty Acid Deficiency Syndrome: Opportunities for Disease Prevention*. McNamara R.K., editor. Nova Publishers; Hauppauge, NY, USA, 2013 pp 385–398.
3. Sasser, M. *Identification of bacteria by gas chromatography of cellular fatty acids*; Technical Note # 101 for Microbial ID, Inc.: Newark, DE, May 1990.
4. AOCS Official Method Ce 1b-89. Fatty Acid Composition of Marine Oils by GLC. In: Firestone D (ed). Official methods and recommended practices of the AOCS. 6th edn. AOCS, Champaign, IL, 2016.

GC Conditions		
GC instrument	Shimadzu GC-2010 Plus	Agilent 6890N / 7890B
Autosampler	Shimadzu AOC-20i Autoinjector and AOC-20S Autosampler	Agilent 7683 Injector and sample tray
Software	MIDI Sherlock Software v.6.3B with the Marine Oil Analysis Package Shimadzu LabSolutions v.5.85	MIDI Sherlock Software v.6.3B with the Marine Oil Analysis Package Agilent ChemStation B.04.03
Column	J&W Ultra 2, 25m x 0.2mm x 0.33µm film thickness	J&W Ultra 2, 25m x 0.2mm x 0.33µm film thickness
Liner	Split liner for focusing	Split liner, silanized
Syringe	10µL syringe, fixed needle	10µL syringe, fixed needle
Inlet temperature	250°C	250°C
Carrier gas	Hydrogen, constant velocity, 47.7 cm/min	Hydrogen, constant flow, 1.3 mL/min
Oven program	190°C, 10°C/min to 285°C (9.5 min), 60°C/min to 310 °C (0.42 min)	190°C, 10°C/min to 285°C (9.5 min), 60°C/min to 310 °C (0.42 min)
Split ratio	30:1	30:1
Injection volume	2.0µL	2.0µL
FID Temperature	300°C	300°C

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