Determination of Fatty Acid Methyl Esters (FAMEs) in Salmon Oil Using Automated Sample Preparation

Application Note

Area

Authors

Norbert Helle and Monika Bzduch,
TeLA GmbH Bremerhaven
Rebecca Veeneman,
Agilent Technologies, Inc.

Introduction

The automated derivatization of fatty acids (FAs) was performed with the Agilent 7696A Sample Prep WorkBench. Since free fatty acids show tailing in gas chromatography, transformation of fatty acids into fatty acid methyl esters (FAMEs) is widely used. Manual sample derivatization is time-consuming and may lead to poor repeatability. Automated derivatization shows significant enhancement of reproducibility and saves time. Especially for highly unsaturated fatty acids, slight variations in reaction temperature and time can negatively affect repeatability when using manual procedures.

Salmon oil is an excellent source of polyunsaturated omega-3 fatty acids. The two main fatty acids–eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) - have been identified as important health factors and are correlated with a normal function of the heart. The concentration of EPA and DHA is the crucial quality factor for salmon oil capsules. This application note demonstrates the use of the Agilent 7696A Sample Prep WorkBench for derivatization and subsequent determination of both EPA and DHA from salmon oil capsules.

Agilent Technologies

Materials and Methods

For sample preparation, 10 mg of salmon oil was weighed into a 2-mL autosampler vial. The sample was diluted in 500 µL of tert-butyl methyl ether (TBME), using the liquid dispensing module of the Agilent 7696A Sample Prep WorkBench and mixed for 90 seconds with the onboard vortex mixer. A 250-µL aliquot of the prepared sample was tranferred to an empty vial and 125 µL of a Trimethylsulfoniumhydroxide (TMSH) derivatization solution [MachereyNagel, Düren] was added and the mixture was again mixed using the vortex mixer of the WorkBench. The mixture was heated for 5 minutes at 80 °C in the single vial heater. The flow diagram for the automated procedure on the Agilent 7696A Sample Prep WorkBench is in shown in Figure 1.

The gas chromatographic conditions were chosen as shown in Table 1.

Table 1. GC/FID Conditions

Peak identification

C14:0	Myristic acid
C16:0	Palmitic acid
C16:1	Palmitoleic acid
C18:0	Stearic acid
C18:1	Oleic acid
C18:2	Linoleic acid
C20:0	Arachidic acid
C18:3	γ-Linolenic acid
C20:1	Gadoleic acid
C18:3	Linolenic acid
C22:1	Erucic acid
C20:4	Arachidonic acid
C20:5	Eicosapentaenoic acid
C24:1	Nervonic acid
C22:6	Docosahexaenoic acid

GC Conditions

Instrument	Agilent 6890 Series GC
Column	HP 88, 100 m × 250 µm, 0.20 µm
Injection volume	2 µL
Injector	Split/Splitless, Split 50:1
Carrier gas	H_2
Temperature-program	70 °C–260 °C
Flow	1.4 mL/min
Detector	250 °C, FID
	H_2 flow: 40 mL/min
	Air flow: 450 mL/min
	Makeup flow, N_2: 45 mL/min

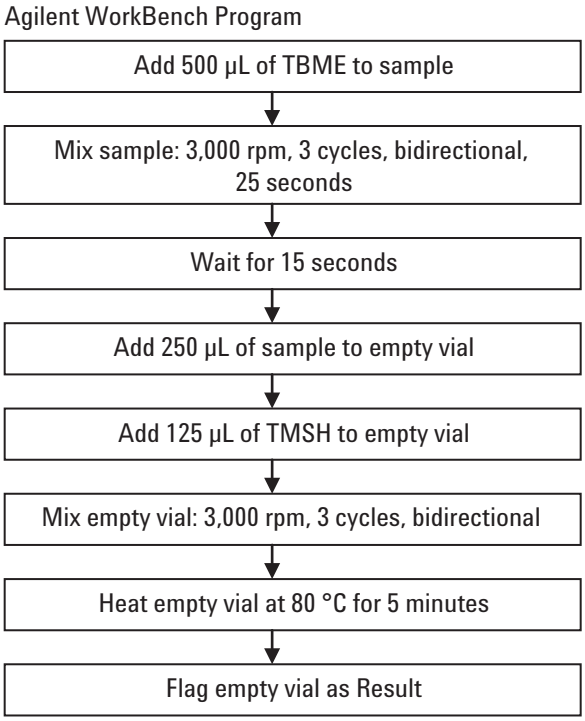

Figure 1. Flow diagram of FAME sample preparation with the Agilent 7696A Sample Prep WorkBench.

Results and Discussion

Figure 2 shows the separation of FAMEs from salmon oil on an Agilent 7696A WorkBench. The separation allows the unequivocal identification of all FAMEs. The two compounds of main interest show retention times of 35.07 minutes (EPA) and 40.55 minutes (DHA). Besides EPA (23.7%) and DHA (20.0%) salmon oil further consists of unsaturated fatty acids oleic (12%), linoic (11%) and palmitoleic (8%) acid. The content of saturated fatty acids, palmitic and stearic acid, is low, 4% and 5% respectively.

For the repeatability test, 10 individual salmon oil samples were derivatized and analyzed to determinate the reproducibility of the automatic sample preparation and chromatography. As shown in Figure 3, excellent repeatability was obtained.

The absolute areas of the EPA and DHA signals showed standard deviations of less than 1% (EPA 0.51%, DHA 0.78%). Moreover, variations of the EPA and DHA relative concentrations were stable. Relative standard deviations of 0.85% for EPA and 1.22% for DHA were achieved. No outliers were observed over the 10 samples.

The total runtime for sample preparation on the Agilent 7696A Sample Prep WorkBench was only 20 minutes per sample, whereas the time for the manual derivatization depends on the skills of the laboratory technician and can take up to 2 hours.

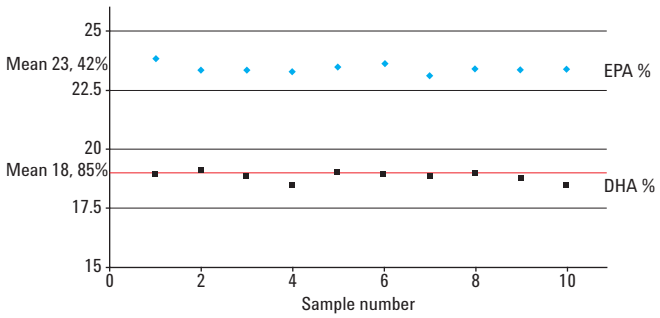

Figure 3. Repeatability data of of automated FAME determination in salmon oil.

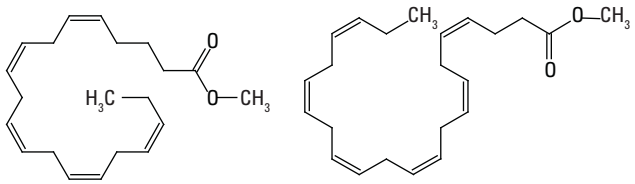

Figure 4. Structure of EPA methyl ester (left) and DHA methyl ester (right).

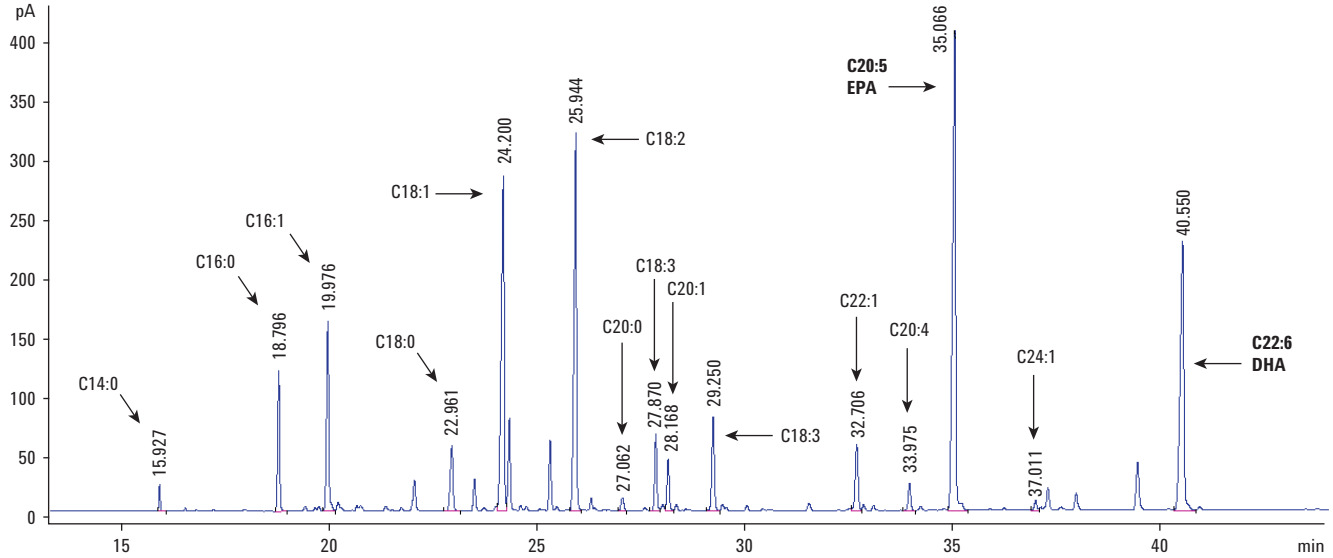

Figure 2. GC/FID chromatogram of a salmon oil sample, prepared using Agilent WorkBench 7696A.

Table 2. Percent Distribution of Fatty Acids in Salmon Oil Sample

C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C20:0	C18:3	C20:1	C18:3	C22:1	C20:4	C20:5	C24:1	C22:6
0.71	4.68	7.95	3.54	12.95	13.86	0.36	2.61	1.71	3.33	3.35	0.87	23.79	0.36	19.93

Conclusion

The automated sample derivatization is easy, fast, and reliable. For samples with high relative concentrations of polyunsaturated fatty acids especially, the automation is significantly more reliable than manual procedures.

Reference

1. Animal and vegetable fats and oils – Gas chromatography of fatty acid methyl esters – Part 3: Preparation of methyl esters using trimethylsulfonium hydroxide (TMSH) (ISO 12966-3:2009)

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
January 23, 2012
5990-9799EN

Agilent Technologies