Analysis of OMEGA-3 Fatty Acids Using a Highly Selective GC Capillary Column

Dan DiFeo Jr - SGE, Incorporated. 2007 Kramer Lane, Austin, Texas 78758, USA
Angus Hibberd, Gerard Sharp - SGE International Pty. Ltd. 7 Argent Place, Ringwood 3134, Australia.

Introduction

Fats are contained in a large number of foods that are consumed in the diets of many people. While certain fats are contributors to heart disease, not all fats are detrimental. Omega-3 fatty acids are part of a class of unsaturated fatty acids and are a healthy dietary supplement. Fish is regarded as healthy due to the recognized beneficial attributes of fish oils. These fish oils provide many nutritionally important classes of fatty acids, including Omega-3 fatty acids, which have an important role in retarding heart disease. Omega-3 fatty acids have been found to lower cholesterol in the blood and therefore help lower the risk of heart disease. For this reason, Omega-3 fatty acids are used in enriched foods and health food supplements. An important requirement is to be able to analyse the content of these products for consumer safety and information.

Discussion

The analysis of fatty acids in food is typically as Fatty Acid Methyl Ester (FAME). The free fatty acids are difficult to analyse by gas chromatography (GC) because of their poor peak shape and quantitation. This poor peak shape is due mainly to the acid functionality of the long chain fatty acid and the interaction with the capillary column phase. Esterification of the fatty acid prior to analysis avoids these problems as the esterified compound gives better peak shape, neutral pH, more reproducible quantitation and improved separation of the large number of cis/trans and structural isomers. Figure 1 shows the structure of one of the major Omega-3 fatty acids: alpha-linolenic acid (LNA), and the esterification of the Omega-3 fatty acid to the methyl ester.

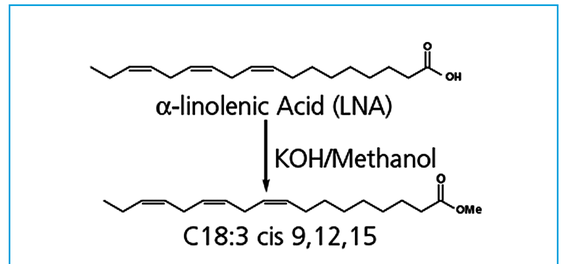


Figure 1: Structure of alpha linolenic acid, a major Omega-3 fatty acid, and its esterification to the methyl ester.

Analysis by GC requires an extremely polar phase capillary column to achieve the desired separation of the numerous isomers of FAME compounds. The SGE BPX70 column is a cyanopropyl phase that is specifically designed for the analysis of FAME compounds. Thermally stable to 260 °C, the BPX70 can easily elute the high boiling FAME compounds without damage to the stationary phase.

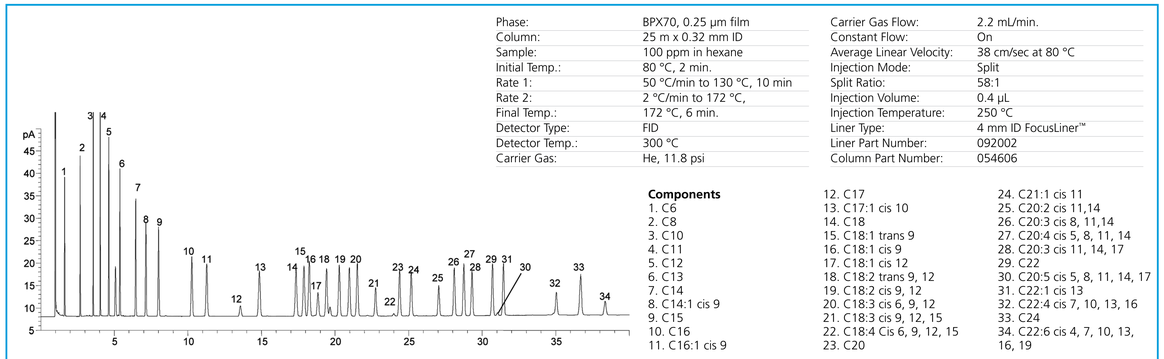
FAME compounds are very sensitive to changes in column polarity resulting in coelutions or complete changes in elution order of some components.

The BPX70 column gives a unique separation of the FAME compounds and can easily distinguish between the complex mixtures of Omega-3 fatty acids that are regularly screened in food laboratories.

Figure 2 shows a chromatogram of a complex mixture of 34 FAME compounds that are regularly screened in many food substances. Note the excellent peak shape and separation with no coelutions present in this complex mixture.

All components are baseline resolved, in particular the C18:1cis/trans, C18:2, C18:3 and C20:3 structural isomers. Note also the excellent separation of C20:5 (peak 30) and C22:1 (31), two of the more difficult to resolve components.

This chromatogram demonstrates the unique separation properties of the SGE BPX70 column for complex FAME compounds.



Phase:	BPX70, 0.25 µm film
Column:	25 m x 0.32 mm ID
Sample:	100 ppm in hexane
Initial Temp.:	80 °C, 2 min.
Rate 1:	50 °C/min to 130 °C, 10 min
Rate 2:	2 °C/min to 172 °C,
Final Temp.:	172 °C, 6 min.
Detector Type:	FID
Detector Temp.:	300 °C
Carrier Gas:	He, 11.8 psi

Carrier Gas Flow:	2.2 mL/min.
Constant Flow:	On
Average Linear Velocity:	38 cm/sec at 80 °C
Injection Mode:	Split
Split Ratio:	58:1
Injection Volume:	0.4 µL
Injection Temperature:	250 °C
Liner Type:	4 mm ID FocusLiner™
Liner Part Number:	092002
Column Part Number:	054606

Components
1. C6
2. C8
3. C10
4. C11
5. C12
6. C13
7. C14
8. C14:1 cis 9
9. C15
10. C16
11. C16:1 cis 9
12. C17
13. C17:1 cis 10
14. C18
15. C18:1 trans 9
16. C18:1 cis 9
17. C18:1 cis 12
18. C18:2 trans 9, 12
19. C18:2 cis 9, 12
20. C18:3 cis 6, 9, 12
21. C18:3 cis 9, 12, 15
22. C18:4 Cis 6, 9, 12, 15
23. C20
24. C21:1 cis 11
25. C20:2 cis 11,14
26. C20:3 cis 8, 11,14
27. C20:4 cis 5, 8, 11, 14
28. C20:3 cis 11, 14, 17
29. C22
30. C20:5 cis 5, 8, 11, 14, 17
31. C22:1 cis 13
32. C22:4 cis 7, 10, 13, 16
33. C24
34. C22:6 cis 4, 7, 10, 13, 16, 19

Figure 2: The chromatogram shows the excellent separation of a complex mixture of FAME compounds. Note the excellent peak shape and separation of the Omega-1,2 and 3 fatty acid isomers both structural and cis and trans.

Summary

The BPX70 capillary column gives unparalleled separation of complex FAME compounds. Difficult to separate cis/trans and structural isomers are easily baseline resolved making quantitation easy and reproducible. Thermally stable, the BPX70 capillary column easily elutes high boiling FAME compounds making it the first choice column for FAME analyses.

Acknowledgement

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