Losses of DHA from High Temperatures of Columns During GLC of Methyl Esters of Long-Chain Omega-3 Fatty Acids

Sir:

There are now "official methods" (e.g., AOCS Ce 1b-89) (1) for the determination of long-chain omega-3 (n-3) FA, primarily eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) acid, but possibly including docosapentaenoic (DPA, 22:5n-3) acid. The use of a saturated FA as an internal standard for GLC quantification is common, usually being 23:0. This is very stable and can be related to the polyunsaturated acids, all being used as esters with factors developed from the theoretical FID correction factors (2,3), and thus simplifying conversion of "peak areas" to actual contents of the particular omega-3 FA without considering other components in the sample (methyl esters).

Recently, attention was drawn to results from these methods by persons getting results that were showing at least 2–3% less DHA than was known to be present. These were oral comments by experienced lipid chemists at the time of the AOCS 2006 annual meeting. They were frankly coupled to criticisms of the theoretical response factors developed in 1964 (2,3) and, as far as commonly known, in satisfactory use since then (4), even with modern developments such as open-tubular columns.

Fortuitously, recently published work from France (5) and Norway (6) criticized the use of temperatures higher than 180°C in deodorizing and otherwise refining marine oils. Additional material was shown in detail by Fournier *et al*. (7,8) in two oral presentations at the AOCS 2006 annual meeting. These included both isomerization of the normal *cis* ethylenic bonds to *trans* and, as a separate problem, the formation of singular cyclic molecules from DHA.

Multiple GC peaks were shown, similar to those artifacts recognized and published in 1974 for thermal damage to α-linolenic acid (9), and later for damage to EPA (10,11). The latter led to the adoption in our laboratory of short-path, high-vacuum distillation of esters, originally called molecular distillation. "Pot" conditions were basic, but novel wiped-wall thin-film units were available by 1988 (10). For fish oils themselves, this reduced-temperature technology is also useful in removing organic environmental pollutants (12).

When this activity in the laboratory was under study, it was not possible to access DHA of high quality such as is now available. However, the probability of thermal damage during GLC was amply demonstrated by a paper available in this journal since 1992 (13). Tande *et al*. compared DHA and EPA. They used the following program: 170°C for 0.5 min, then 10°C per minute to 240°C, hold at 240°C for 22 min. This led to a shortfall in DHA of 10%, using ethyl esters and methyl 23:0.

In contrast, North American methods seem to have settled on operating temperatures for GLC column of 225°C; and the European Pharmacopoeia 5.0, for cod liver oil, also stipulates 225°C for column temperatures. Although injection ports and detector equipment may be set somewhat higher than the column temperature, the exposure times are brief. The time of exposure can be minimized by increasing current gas flow rates whenever possible.

The reason that thermal loss was not often noticed with EPA could rest on the exposure time, half that of DHA in most methods, or on the fact that the first ethylenic bond is protected from interaction with the carboxyl group by an extra carbon atom over the short-chain segment of two methylenes in DHA.

The column lining potentially remains an enigma. Most official methods use CARBOWAX-based liquid phases, as these offer a good separation within each even-carbon chain-length and minimal chain-length overlaps. A modest shortfall in DHA methyl ester in Biomedical Test Materials derived from fish oil was also noted using DB 225 (30 m × 0.25 μm) with an oven temperature programmed from 170 to 225°C and a total run time of 55 min, necessitating the use of empirical correction factors (G. Seaborn, personal communication). However, potential pitfalls of their use and the importance of instrument optimization and theoretical correction factors were noted in the analytical methods manual for this program (14).

In short, the theoretical correction factors remain valid, and empirical correction factors must be adapted for accurate EPA and DHA analyses conducted at temperatures causing thermal losses.

REFERENCES

1. Fatty Acid Composition by GLC: Marine Oils, in Official Methods and Recommended Practices of the AOCS, 5th edn., AOCS Press, Champaign, IL, 1997, Method Ce 1b-89.
2. Ackman, R.G., and J.C. Sipos, Application of Specific Response Factors in the Gas Chromatographic Analysis of Methyl Esters of Fatty Acids with Flame Ionization Detectors, *J. Am. Oil Chem. Soc. 41*:377–378 (1964).
3. Ackman, R.G., and J.C. Sipos, Flame Ionization Detector Response for the Carbonyl Carbon Atom in the Carboxyl Group of Fatty Acids and Esters, *J. Chromatogr. 16*:298–305 (1964).
4. Ackman, R.G., The Gas Chromatograph in Practical Analyses of Common and Uncommon Fatty Acids for the 21st Century, *Anal. Chim. Acta 465*:175–192 (2002).
5. Fournier, V., F. Destaillats, P. Juaneda, F. Dionisi, P. Lambelet, J.-L. Sebedio and O. Berdeaux, Thermal Degradation of Long-Chain Polyunsturated Fatty Acids During Deodorization of Fish Oil, *Eur. J. Lipid Sci. Technol. 108*:33–42 (2006).

Paper no. J11433 in *JAOCS 83,* 1069–1070 (December 2006).

6. Mjos, S.A., and M. Solvang, Geometrical Isomerization of Eicosapentaenoic and Docosahexaenoic Acid at High Temperatures, *Ibid. 108*:589–597 (2006).
7. Fournier, V., O. Berdeaux, P. Juaneda, F. Dionisi, P. Lambelet, J.L. Sébédio, and F. Destaillats, Thermal Degradation of Long-Chain Polyunsaturated Fatty Acids (LC-PUFAs) During Deodorization of Fish Oil: Part I, Formation of Geometrical Isomers, presented at the 97th AOCS Annual Meeting & Expo, St. Louis, Missouri, May 3, 2006.
8. Berdeaux, O., V. Fournier, P. Juaneda, F. Dionisi, P. Lambelet, J.L. Sébédio, and F. Destaillats, Thermal Degradation of Long-Chain Polyunsaturated Fatty Acids (LC-PUFAs) During Deodorization of Fish Oil: Part II, Formation of Cyclic Fatty Acid Monomers, presented at the 97th AOCS Annual Meeting & Expo, St. Louis, Missouri, May 3, 2006.
9. Ackman, R.G., S.N. Hooper, and D.L. Hooper, Linolenic Acid Artifacts from the Deodorization of Oils*, J. Am. Oil Chem. Soc. 51*:42–49 (1974).
10. Ackman, R.G., The Year of the Fish Oils, *Chem. Ind. (7 March)*:139–145 (1988) .
11. Wijesundara, R.C., W.M.N. Ratnayake, and R.G. Ackman, Eicosapentaenoic Acid Geometrical Isomer Artifacts in Heated Fish Oil Esters, *Ibid. 66*:1822–1830 (1989).
12. Breivik, H., and O. Thorstad, Removal of Organic Environmental Pollutants from Fish Oil by Short-Path Distillation, *Lipid Technol. 7*:55–58 (2005).
13. Tande, T., H. Breivik, and T. Aasoldsen, Validation of a Method for Gas Chromatographic Analysis of Eicosapentaenoic Acid and Docosahexaenoic Acid as Active Ingredients in Medicinal Products, *J. Am. Oil Chem. Soc. 69*:1124–1130 (1992).
14. Seaborn, G.T., J.A. Gooch, F.M. Van Dolah, and S.B. Galloway (eds.), *Biomedical Test Materials Program: Analytical Methods for the Quality Assurance of Fish Oil: Second Edition*, NOAA Tech. Mem. *NMFS-SEFSC-329*, 1993, 123 pp.

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