ELSEVIER

Food Chemistry 70 (2000) 425–426

Food Chemistry

www.elsevier.com/locate/foodchem

Research Letter

Direct use of methyl tricosanoate as an internal standard and overcoming a potential error in the quantitation of the omega-3 long-chain polyunsaturated fatty acids of marine oils as ethyl esters

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Received 12 November 1999; received in revised form 30 January 2000; accepted 30 January 2000

Two trends are apparent in the use of fish oils as nutritional supplements (Garcia, 1998; Lambertsen, 1999). Those in foods will essentially be based on highly refined triacylglycerol fish oils (Newton, 1996), but in encapsulated "omega-3" nutraceuticals many concentrates prepared from fish oils are offered in the form of ethyl esters. The latter are totally digested and absorbed in vivo, according to thorough studies (Krokan, Bjerve & Mørk, 1993). The accurate analysis of omega-3 fatty acids in oils, methyl esters or free acids by AOCS (Firestone, 1996) or AOAC (1998) official methods in terms of mg/g sample could be effected by analyses as methyl esters, but the operating conditions described herein show that methyl tricosanoate, the internal standard commonly available and employed for methyl ester analysis, can also be conveniently applied directly to the ethyl ester sample without the need for conversion of the ethyl esters to methyl esters.

A concentrate of ethyl esters of fish oil prepared by urea complexing (Ratnayake, Olsson, Matthews & Ackman, 1988) and enriched by removal of C_{14} and C_{16} fatty acids by distillation was provided by a commercial laboratory. Methyl tricosanoate was purchased from Aldrich Canada, Oakville ON.

All GLC analyses were conducted with a Perkin–Elmer Autosystem Gas Chromatograph (Norwalk, CT) equipped with a flame ionization detector and a split injection system. The analyses were conducted on an Omegawax 320 fused silica capillary column (30 m, 0.32 mm I.D., 0.25 μm film thickness, Supelco, Sigma-Aldrich Canada, Oakville ON). The detector temperature was 270°C and the injector temperature was held at 250°C. The carrier gas was helium at a pressure of 9.2 psi (63 kPa).

On investigation we have found that in analysing the ethyl esters of fish oils with our usual GLC temperature program the peak for methyl 23:0 closely preceded the peak for marine fatty acid 21:5n-3 ethyl ester when a very light on-column load was used. When the on-column load was increased the 21:5n-3 fatty acid ethyl ester unfortunately disappeared under the trailing edge of the methyl 23:0. If this coincidence were not expected or known, then the 21:5n-3 ethyl ester would add additional area to the apparent 23:0 (methyl ester) peak, depressing the calculated contents of other n-3 fatty acids such as 20:5n-3, 22:5n-3 and 22:6n-3 in the sample.

The standard temperature program used in our laboratory for methyl esters of marine oil fatty acids was: inject at 180°C, hold for 8 min, ramp to 220°C at 3°C/min, hold for 14 min. The retention time to 22:6n-3 was 30.3 min for the methyl esters and 31.5 min for the ethyl esters. Initially, altering the final isothermal temperature only was tested at 210 and 230°C, the latter showing some improvement in resolution between 23:0 methyl ester and 21:5n-3 ethyl ester. A further change to a program of: inject at 200°C, hold for 5 min, ramp at $3°C/min^{-1}$ to 230°C, hold, gave baseline separation of methyl 23:0 from ethyl 21:5n-3 with 22:6n-3 ethyl ester emerging in 21 min (Fig. 1), while preserving the necessary detailed resolution among earlier eluting peaks. In practice all analysts will select operating conditions best suited to their column polarity, but the changes made in our program may assist in selecting those conditions.

DPA (22:5n-3) was not included in the calculations for the original two official methods (AOAC, 1998; Firestone, 1996). For practical purposes in correctly

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PII: S0308-8146(00)00090-X

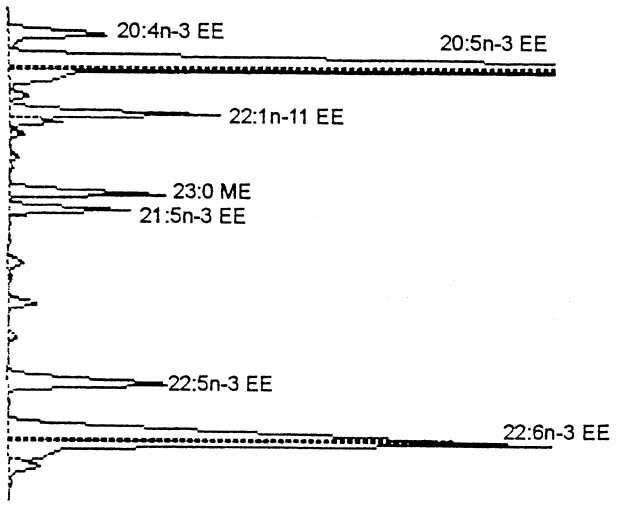

Fig. 1. Improved resolution of 23:0 ME and 21:5n-3 EE shown in a partial chromatogram of ethyl esters of fish oil fatty acids, with added methyl tricosanoate, when operated with the modified temperature program.

quantitating DPA (22:5n-3) in methyl ester analyses the theoretical relative flame ionization detector response factor explained in the two official methods for the methyl esters can be taken as the same as that for EPA (20:5n-3), since Craske and Bannon (1988) show 0.9452 for EPA and 0.9443 for DPA. Similarly, since 21:5n-3 is a minor component, the EPA correction could be applied as that calculated differs by less than 1%. A further correction of 1.04, to incorporate the actual weight of 23:0 acid added, is necessary to permit the results for 20:5n-3, 22:5n-3 and 22:6n-3 to be given as mg/g sample.

Acknowledgements

The ethyl ester concentrate was the gift of Ocean Nutrition Canada, Mulgrave, Nova Scotia. Research support was provided by the Natural Sciences and Engineering Research Council of Canada.

References

AOAC (1998, March). *Method 41.1.30. Official methods of analysis of AOAC International* (16th ed.). Arlington, VA: Association of Official Analytical Chemists.

Craske, J. D., & Bannon, C. D. (1988). Letter to the editor. *Journal of the American Oil Chemists' Society, 65*, 1190–1191.

Firestone, D. (1996). Method Ce 1b-89. *Official methods and recommended practices of the American Oil Chemists' Society* (4th ed.). Champaign, IL: American Oil Chemists Society.

Garcia, D. J. (1998). Omega-3 long-chain PUFA nutraceuticals. *Food Technology 52, 6*, 44–49.

Krokan, H. E., Bjerve, K. S., & Mørk, E. (1993). The enteral bioavailability of eicosapentaenoic acid and docosahexaenoic acid is as good from ethyl esters as from glyceryl esters in spite of lower hydrolytic rates by pancreatic lipase in vitro. *Biochimica Biophysica Acta, 1168*, 59–67.

Lambertsen, G. (1999). Marine lipids: production for food. *INFORM, 10*, 345–346, 348.

Newton, I. S. (1996). Food enrichment with long-chain n-3 PUFA. *INFORM, 7*, 169–177.

Ratnayake, W. M. N., Olsson, B., Matthews, D., & Ackman, R. G. (1988). Preparation of omega-3 PUFA concentrates from fish oils via urea complexation. *Fat Science Technology, 90*, 381–386.