SIGNIFICANCE OF ULTRAVIOLET ABSORPTION DATA OF FISH-OIL FATTY ACIDS

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ABSTRACT

Several ultraviolet absorption characteristics exist that are peculiar to the double-bond character of polyunsaturated fatty compounds and that make ultraviolet absorption measurements a means of evaluating the degree of unsaturation of compounds derived from fish oils. Accordingly, when pure polyunsaturated fatty acids from fish oils become available as analytical standards, a practical quantitative analysis will be possible.

INTRODUCTION

Fish oils are made up largely of triglycerides that contain straight-chain fatty acid groups having both saturated and unsaturated carbon-carbon chain linkages. The unsaturated fatty acids obtained from fish oils may possess from one to six carbon-carbon double bonds per molecule. Experimental results indicate that these double bonds are separated by single methylene ($-CH_2-$) groups. That is, the unsaturated portions of the fatty acids have nonconjugated structures. An example of a nonconjugated unsaturated fatty acid is 9,12,15-octadecatrienoic acid:

$$CH_3CH_2CH{=}CH{-}CH_2{-}CH{=}CH\ \ CH_2{-}CH{=}CH(CH_2)_7COOH \qquad (a)$$

The absorption of ultraviolet light at certain wavelengths is characteristic of definite chemical structures. The ultraviolet absorption caused by carbon-carbon unsaturation is brought about only by a conjugated double-bond system. The fatty acids in fish oils occur naturally with nonconjugated unsaturation. For the ultraviolet absorption method of analysis to be effective with fish-oil fatty acids, the unsaturation in these acids therefore must be converted to the conjugated form. For example, the following equation (b) depicts the isomerization process of nonconjugated 9,12-octadecadienoic acid to the acid possessing conjugated unsaturation:

$$CH_3(CH_2)_4CH{=}CH{-}CH_2{-}CH{=}CH(CH_2)_7COOH \xrightarrow[\text{heat}]{\text{alkali}} \qquad (b)$$
$$CH_3(CH_2)4{-}CH_2{-}CH{=}CH{-}CH{=}CH(CH_2)_7COOH$$

ANALYTICAL METHOD: The double bonds in unsaturated fatty acids are conjugated by alkali-isomerization. The analytical procedure commonly employed is that of the American Oil Chemists' Society Tentative Method Cd 7-48: An 80-milligram[1] sample of fatty material is mixed with 11 grams of 21-percent potassium hydroxide in ethylene glycol that is preheated to 180° C. (356° F.) in a suitable isomerization flask. An atmosphere of nitrogen is passed over the mixture during the entire isomerization process. The mixture is held at 180° C. for exactly 15 minutes, after which time the reaction is stopped by immediately cooling it to room temperature. The cooled mixture is diluted with an appropriate solvent to a known volume such that the concentration of isomerized material permits adquate measurement of the ultraviolet absorption spectra.

Spectral absorption peaks at 233, 268, 315, 346, and 374 millimicrons are the result of the presence within the molecules of two, three, four, five, and six conjugated carbon-carbon double bonds, respectively. From the relative heights of the absorption peaks can be calculated specific extinction coefficients. The extinction coefficients are used to determine the quantity of material present in the analyzed mixture which contribute to the particular absorptions. (This is discussed in the next section.)

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[1] This figure is based on the unsaturation commonly found in commercial fish oils.

The specific extinction coefficients are calculated from the equation

$$K = \frac{A}{c-1} \qquad (c)$$

where k is the specific extinction coefficient, A is the absorbance (or optical density) at a given wavelength, c is the concentration of isomerized substances in grams per liter, and 1 is the cell length in centimeters.

Table 1 - Specific Extinction Coefficients of Pure Natural Polyunsaturated Fatty Acid1/

Fatty Acid			Specific Extinction Coefficient				
Acid	No. of Carbon Atoms	No. of Double Bonds	233 mμ	268 mμ	315 mμ	346 mμ	374 mμ
Linoleic	18	2	91.6	-	-	-	-
Linolenic	18	3	47.5	90.5	-	-	-
Arachidonic	20	4	39.7	48.2	60.6	-	-
Eicosapentaenoic	20	5	39.4	41.2	82.4	87.5	-
Clupanodonic	22	5	43.5	46.0	56.9	50.4	-
Docosahexaenoic	22	6	41.7	52.2	29.6	27.7	29.3

1/Herb and Riemenschneider, *Anal. Chem.* 25, 953 (1953). Isomerization in 21 percent KOH in ethylene glycol at 180° C. (356° F.) for 15 minutes under nitrogen.

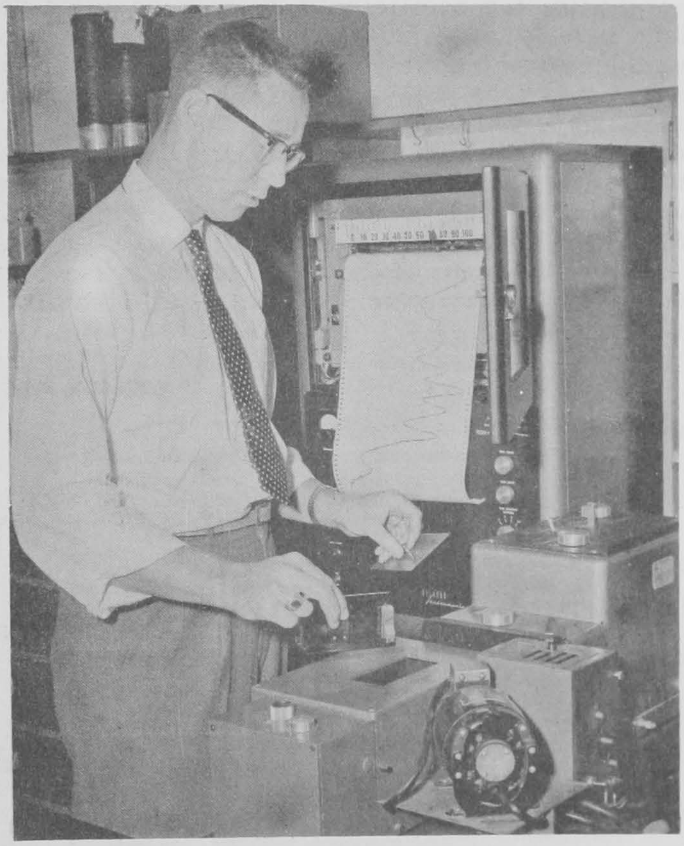

Fig. 1 - Chemist using an automatic-recording spectrophotometer to measure the ultraviolet absorption characteristics of alkali-isomerized fatty acids from fish oil.

Listed in table 1 are specific extinction coefficients of pure natural polyunsaturated fatty acids. These fatty acids were prepared and studied by Herb and Riemenschneider (1953) in their work on microanalytical determinations of animal and vegetable oils at the Eastern Regional Research Laboratory of the U. S. Department of Agriculture.

CONSIDERATIONS: Fish oils contain fatty acid groups having from 18 to 24 carbon atoms and one to six double bonds. From table 1, it would be expected that a C_{24} fatty acid with six double bonds would have a lower extinction coefficient at 374 millimicrons than would a C_{22} fatty acid with six double bonds. This reasoning is based on the fact that a C_{22} fatty acid with five double bonds has a lower extinction coefficient at 346 millimicrons than has a C_{20} fatty acid with five double bonds. Also, this decrease in extinction coefficient can be explained by the effect of increasing chain length, which in turn increases the molecular weight. An increase in molecular weight causes a lowering of the molar concentration of double bonds for a given weight of sample. This then lowers the spectral absorption due to the double bonds and consequently lowers the specific extinction coefficient. These changes can be seen by examining equation (c).

With mixtures of highly unsaturated compounds from fish oils, such as fatty acids and fatty alcohols, the specific extinction coefficient calculated for a given wavelength is an additive value resulting from each compound in the mixture. Also, if six double bonds is the maximum number found in fish oils, there are no other ultraviolet absorptions at higher wavelengths contributing to the absorption at 374 millimicrons. One would expect, therefore, that a comparison of extinction coefficients at 374 millimicrons to the value of 29.3 (in table 1) will give a fair quantitative approximation of the content of heraenoic acids present. A pure C_{24} fatty acid of high unsaturation has not as yet been reported, thus the more complete analysis is not possible at this time.

One further matter to consider is the possibility of the existence of fatty acids of C_{20} to C_{24} chain lengths having only two and three carbon-carbon double bonds. Until the contrary has been unquestionably proven and reported, complete fatty acid analysis by ultraviolet absorption will not be possible.

APPLICATIONS: The ultraviolet absorption data are valuable as a means of determining the success of methods of separating fish-oil fatty acids or their derivatives. The data also can be used to determine the effect of storage conditions on the high degrees of unsaturation; that is, whether or not storage treatment has affected the pentaene and hexaene content.

Extinction coefficients, from absorption data of the type described above, are used in a set of simultaneous equations for solving quantitatively the percentage of each compound contributing to the particular absorptions. For quantitative analyses of this type to be accurate, however, pure compounds (the pure fatty acids in the case of fish oils) must be available for use as reference standards. Contract work being carried out by Dr. Orville Privett at the Hormel Institute, University of Minnesota, Austin, Minn., is designed to prepare the necessary "standard" fatty acids and to analyze the major commercial fish oils for their fatty acid composition. When this contract is completed, it will be possible to obtain a practical quantitative measurement of the relative proportions of the different fatty acids of various degrees of unsaturation present in all fish oils by the use of ultraviolet absorption measurements.

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