Quantitative Analysis of Furanoid Fatty Acids in Crude and Refined Cod Liver Oil

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ABSTRACT AND SUMMARY

A method is described for determining the furanoid fatty acids F_1-F_6 in cod liver oil using squalane as an internal standard added prior to isolation of the F esters by silver ion chromatography (Ag^+ TLC) and urea crystallization. The F acids amount to about 1% of the oil and can be determined with an accuracy of ± 15%. There is no significant change in the amount of F acids after refining.

INTRODUCTION

Furanoid fatty acids (F acids—see Table II for structure) have now been reported from a number of fish sources (1-3). In special circumstances these acids form a large proportion of the fatty acids in fish lipids, but usually they are present at rather low levels. In commercial fish oils such as cod liver, dogfish liver, and capelin oils, the F acids amount to 1% or less of the fatty acids (3). Thus far, the biosynthetic origin and the biological and nutritional properties of the F acids are unknown, but this promises to be a field of growing interest. Studies of these topics will require analytical data on the amount of F acids present in lipid samples.

When the F acids are present in large amounts (>10%) they can be quantified directly from a gas liquid chromatographic (GLC) analysis of the total methyl esters obtained by base catalyzed transesterification of the oil. The assignment of a particular peak in the total ester trace can be checked by subsequent isolation of the F esters. When the F acids are present at levels of only a few percent it is not possible to identify peaks in the total ester trace as being exclusively F esters, nor can such peaks be quantified with accuracy. Then, a quantitative analysis requires the isolation of the F esters for certain identification and the addition of an internal standard to quantify the analysis.

DISCUSSION AND RESULTS

The F esters can be separated from saturated and unsaturated esters by a combination of silver ion chromatography (Ag^+ TLC) and urea crystallization (2,3). On Ag^+ TLC the F esters migrate just behind the saturates and can be separated easily from polyenes. When crystallized with urea the F esters do not form adducts and so can be separated from saturates and monoenes. Combination of these methods gives a mixture of the F esters along with a number of methyl branched saturated and possibly monoenoic esters. An alternative to the Ag^+ TLC step is hydrogenation of the polyene to saturated acids followed by urea crystallization. However, the furan system is not completely resistant to hydrogenation (2), so this method is less suitable for an analytical procedure.

To quantify the analysis a known amount of an internal standard must be added to the sample prior to the separation of the F esters, and this standard must behave in the same way as the F esters during the separation process. The most obvious choice for a standard is a synthetic furan ester of odd chain length, which will behave identically to the natural furans, but be distinguishable on GLC. Unfortunately such compounds are not easily synthesized from readily available starting materials, and a more accessible compound would be an advantage for a general analytical method. Squalane (2,6,10,15,19,23-hexamethyltetracosane) resembles the F esters in general—as a saturated hydrocarbon it is very mobile on Ag^+ TLC and being highly branched it does not form a urea adduct. As a commonly used GLC stationary phase it is readily available in high purity. The suitability of squalene as an internal standard was therefore investigated.

Squalane is more mobile than the F esters on Ag^+ TLC. With 10% ether in petrol as the developing solvent, squalane migrates to the solvent front while the F esters lie just behind the saturates (Rf ca. 0.6-0.7) but are separated from the monoenes (Rf ca. 0.4-0.5). The F esters can not be detected on the plate at low levels, but the front of the monoene band can be used as a convenient marker (monoenes are abundant in most fish oils). A band removed from the plate from just below the front of the monoene band to above the solvent front contains all the squalane, saturates, and F esters, along with some monoenes. Urea crystallization leaves only the squalane, methyl branched esters, and F esters in the filtrate or "furan extract."

The procedure was tested with cod liver oil. The amounts of the major fatty acids in the oil and the composition of a typical furan extract are shown in Table I. F_1-F_6 make up 40-50% of the furan extract, the remainder (apart from the squalane) being mainly components of equivalent chain length (ECL) 18 or less. The major components of these are presumably isoprenoid acids which are found in most fish oils (4). There are no components above 0.5% which could be $C_{16\text{-}18}$ saturates or monoenes so it is most unlikely that significant amounts of $C_{20\text{-}24}$ saturates or monoenes overlap the F ester peaks (ECL 20.5-24.0). When the isolation was repeated without added squalane the furan extract contained no components between ECL 18.96-21.16. The ECL of squalane is temperature dependent, and its position in the trace can be adjusted a little to ensure separation from other components. With the DEGS column used, the ECL decreased by 0.04 per °C and the optimum temperature was 195 C.

For squalane to be a satisfactory internal standard there must be no differential loss of either squalane or the F esters during the isolation procedure. To check this point a furan extract from an analysis was resubjected to the Ag^+ TLC and urea crystallization steps. The ratios of squalane to each of the F esters were compared before and after the reisolation and found to be the same within ± 10%. Thus there is no systematic loss of either squalane or the F esters, and the variability observed in the reisolation experiment gives an indication of the accuracy which can be expected from the method. When complete analyses were carried out in duplicate or triplicate the standard deviation for the total F acid content was ± 15%. Standard mixtures of squalane and C_{21} ester were analyzed and the weight and area ratios compared to correct for differences in the detector response and the slight tailing observed on the squalane peak. However the correction factor is small (<5%) compared with the other experimental errors in the analysis, and the results given are uncorrected.

This analytical method was applied to three grades of cod liver oil: crude, cold filtered at 0 C, and a fully refined oil, all of which contained similar amounts of the normal fatty acids. Table II shows the composition of the F acid

mixture and the amount of each F acid in the three oils. Within the experimental error there is no significant difference in either the amount or distribution of the F acids, suggesting that they are not affected by the refining process.

EXPERIMENTAL PROCEDURES

All reagents and solvents were "Analar" grade and a petroleum fraction bp 40-60 C was used. The gas chromatograph used was a Perkin Elmer F11, fitted with a flame ionization detector and a stainless steel column (2 m x 2 mm ID) packed with 15% DEGS on 80/100 Chromosorb W AW. The optimum column temperature was 195 C (see above), with a nitrogen carrier flow rate of 40 ml/min and an injection port temperature of 240 C. For Ag^+ TLC, 20 x 20 cm plates were coated with a 1 mm (wet thickness) layer of Silica Gel G containing 10% w/w $AgNO_3$, and activated at 110 C for 2 hr immediately before use. For transesterification a stock solution of two volumes of 0.5 N NaOMe in methanol, and one volume of benzene was used. The standard squalane solution was about 0.25 mg/ml in benzene.

The following method is recommended for an oil containing about 1% of furanoid fatty acids: Weight 150-180 mg of oil into a quickfit test tube and add 1 ml of the standard squalane solution and 6 ml of the stock methoxide solution. Stopper the tube and heat it at 50 C for 20 min, cool the contents, and transfer them to a separating funnel. Add 10 ml 5% brine and 0.5 ml glacial acetic acid, then extract twice with 10 ml petroleum. Combine the petroleum extracts, wash once with 5 ml water and dry over Na_2SO_4 before evaporating the solution to about 0.5 ml. Apply this solution as a streak to one Ag^+ TLC plate and develop the plate with 10% ether in petroleum, before spraying with fluorescein and visualizing under UV light. The monoene band is prominent at Rf 0.4-0.5. Mark the plate just below the front of this band and scrape off all the silica above it, extract the silica with ether and evaporate the extract to dryness. Transfer the extract to a small tube (1 x 5 cm) with 3 ml methanol, add 0.5 g urea, dissolve it by warming, and leave the solution to crystallize at -15 C for 3 hr. Filter the solution through a glass sinter into a small quickfit test tube (without washing the precipitate) and add 6 ml 5% brine to the filtrate. Shake this twice with 0.5 ml petroleum, drawing the petroleum extract off with a Pasteur pipette each time. Dry the extract over Na_2SO_4 and concentrate as required before GLC analysis.

TABLE I

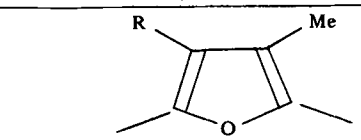
Equivalent Chain Length (ECL) and Composition Data for Cod Liver Oil Esters and Furan Extract from Cod Liver Oil

Cod liver oil Me esters			Furan extract		
ECL	wt %	Assignment	ECL	wt %	Assignment
14.00	3	14:0	13.51	.65	
16.00	9	16:0	14.00	8.68	
16.69	8	16:1	14.63	1.61	Methyl
18.00	2	18:0	15.48	6.28	branched
18.63	22	18:1	16.30	.35	esters
19.36	2	18:2	16.76	18.06	cf. ref. 4
20.49	10	20:1	17.07	10.26	
20.95	4	18:4	18.25	.34	
22.40	5	22:1	18.59	.11	18:1?
23.30	13	20:5	18.96	2.80	?
25.73	18	22:6	19.41	6.33	Squalane
	4	Other	20.16	.25	?
			20.56	1.48	F_1[a]
			20.86	.10	?
			21.53	6.26	F_2
			22.07	2.23	F_3
			22.57	6.23	F_4
			22.90	.44	?
			23.46	3.67	F_5
			24.04	23.87	F_6

[a]F esters were identified by comparison with an F ester extract obtained from pike liver and testes as described in ref. (1). Small amounts of F_7 and F_8 may also be present but these were not usually observed. The structures of the F acids are given in Table II.

TABLE II

Furanoid Fatty Acids in Cod Liver Oil



$Me(CH_2)_n$ $(CH_2)_mCOOH$

				Crude oil		Cold filtered		Refined	
Furan[a] acid	m	n	R	A[b]	B[b]	A	B	A	B
F_1	8	2	Me	3.3	.03	3.4	.04	3.0	.03
F_2	8	4	H	14.3	.14	15.0	.16	18.4	.19
F_3	8	4	Me	5.2	.05	5.7	.06	5.9	.06
F_4	10	2	Me	14.3	.14	13.6	.15	14.0	.14
F_5	10	4	H	8.4	.08	8.0	.09	8.1	.08
F_6	10	4	Me	54.5	.52	54.3	.59	50.6	.52
ΣF_1-F_6					.96 ± .15		1.09 ± .15		1.02 ± .15

[a]Named as in ref. (2).

[b]A = % wt composition of furan mixture; B = % wt of furans in whole oil.

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