IAFMM FISH OIL BULLETIN
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RECOMMENDED METHOD FOR DETERMINATION OF HYDROCARBONS IN FISH OILS

1. General

This method is applicable to the determination of saturated hydrocarbons in glycerides.

2. Principle

The oil is dissolved in petroleum ether and chromatographed on a column of activated alumina. The hydrocarbons are recovered from the eluate by evaporation of the solvent and determined gravimetrically.

3. Reagents

Aluminium oxide; activated alumina, grade F-20, 80-200 mesh. Alcoa, Aluminium Company of America, or equivalent. (Note: Dry the alumina for at least 4 hours at 200°C before using. Store in sealed bottles in a desiccator. The alumina must have a moisture content of less than 3% to retain glycerides).

Sodium sulphate; anhydrous, analytical reagent grade.

Petroleum ether; specific gravity at 15.5°C is 0.630 to 0.660, water white colour, initial boiling temperature between 35°C and 38°C, with not more than 60% distilling under 40°C and at least 95% distilling under 54°C (see AOCS specification H2-41).

4. Apparatus

Erlenmeyer flasks (125 and 500ml).

Soxhlet flasks (250 ml).

Glass wool.

Chromatographic column, fitted with Teflon stopcock (25 to 35 mm diameter; 400 mm long).

The chromatographic column is prepared by tamping a plug of glass wool into the bottom of the column in such a manner that some of the glass wool is in the constricted portion above the stopcock. Petroleum ether is poured in to a level 2/3 up the column and 200 g of alumina slowly added through a powder funnel. It is essential to aid packing by tapping the side of the column. The alumina is covered with about 1 cm of anhydrous sodium sulphate.

5. Method

A 10 g portion of the melted, well mixed sample is weighed (to nearest 10 mg) into a 125 ml Erlenmeyer flask and dissolved in 50 to 100 ml of petroleum ether, with gentle warming if necessary.

The elution rate from the column is fixed at 3.0 to 3.5 ml/min using the stopcock for control. When the solvent level is reduced to about 1 cm above the packing, the sample solution is added. The solution is allowed to flow, and the eluate collected in a 500 ml Erlenmeyer flask, until about 1 cm of head remains. The sample flask is rinsed four times with 50 ml of petroleum ether and the rinsings are added in turn to the column when the column head has been reduced to 1 cm following the previous addition. The sides of the column are washed down on transferring. More petroleum ether is added to the column until a total of 400 ml has passed through. (An inverted volumetric flask can serve as a convenient reservoir for this final addition of solvent).

The volume of the eluate is reduced to 50 to 75 ml by evaporation on a steam bath. A stirring rod placed in the flask will prevent superheating and subsequent boiling over. A gentle stream of air will aid in solvent removal.

The concentrate is transferred to a tarred 250 ml Soxhlet flask; three 20 ml portions of petroleum ether are used to rinse the flask.

The solvent remaining is removed by evaporation on a warm surface employing a gentle stream of air and keeping the solution below the boiling point. The flask is cooled to room temperature in a desiccator and the residue weighed on an analytical balance.

The heating, cooling and weighing operations are repeated, with additional heating periods of 20 min each, until the change in residue weight is less than 0.5 mg.

A blank determination is carried out by repeating the procedure using the same volume of solvent at each stage. A high blank value would indicate the presence of unacceptable quantities of high boiling point hydrocarbons in the batch of solvents used in which case an alternative batch should be tested and used.

6. Calculation

Hydrocarbons content (%)

$$= \frac{\text{Weight of sample residue (g) - Weight of blank residue (g) x 100}}{\text{Weight of sample (g)}}$$

7. Repeatability

The difference between the results of two determinations carried out simultaneously or in rapid succession, by the same analyst should not exceed 0.09, 0.14 and 0.33 for hydrocarbons contents of 0.05, 0.5 and 5.0% respectively.