

**Fatty acids in food supplements:  
Can you assume what you consume ?**

**COSHH ASSESSMENT**

**Session 2**

**Assessment Date:**

<b>Chemicals- Hazardous in quantities used in experiment</b>	
Hydrochloric acid	<b>CORROSIVE.</b> Causes severe burns to skin and eyes. Extremely irritating harmful vapour. Dispense in fume cupboard. <b>WEAR GLOVES.</b>
Hexane	<b>HIGHLY FLAMMABLE.</b> Can cause serious damage if splashed into eyes. May cause impaired fertility. Harmful by inhalation <b>IRRITANT.</b> Prolonged exposure may cause serious health damage.
Sodium hydroxide	<b>CORROSIVE.</b> May cause serious burns. Harmful by ingestion, inhalation and contact with skin. Eye contact may result in serious damage.
Methanol	<b>HIGHLY FLAMMABLE. TOXIC</b> by ingestion. Damaging if splashed into eyes. High concentrations of vapour may cause dizziness, stupor, cramps and digestive disturbance. Lower levels may cause headache and nausea. Chronic effects-damages the central nervous system, particularly the optic nerve and internal organs.
<b>Chemicals- hazardous in other circumstances</b>	
Methanol	<b>HIGHLY FLAMMABLE.</b> Evacuate laboratory if spillage exceeds 80 mL.
<b>First aid for above chemicals</b>	
Eyes	Irrigate thoroughly with water for at least 10 minutes. <b>OBTAIN MEDICAL ATTENTION.</b>
Lungs	Remove from exposure, rest and keep warm. In severe cases <b>OBTAIN MEDICAL ATTENTION.</b>
Skin	Wash off thoroughly with water. Remove contaminated clothing and wash before re-use. In severe cases <b>OBTAIN MEDICAL ATTENTION.</b>
Mouth	Wash out mouth thoroughly with water and give plenty of water to drink. <b>OBTAIN MEDICAL ATTENTION.</b>

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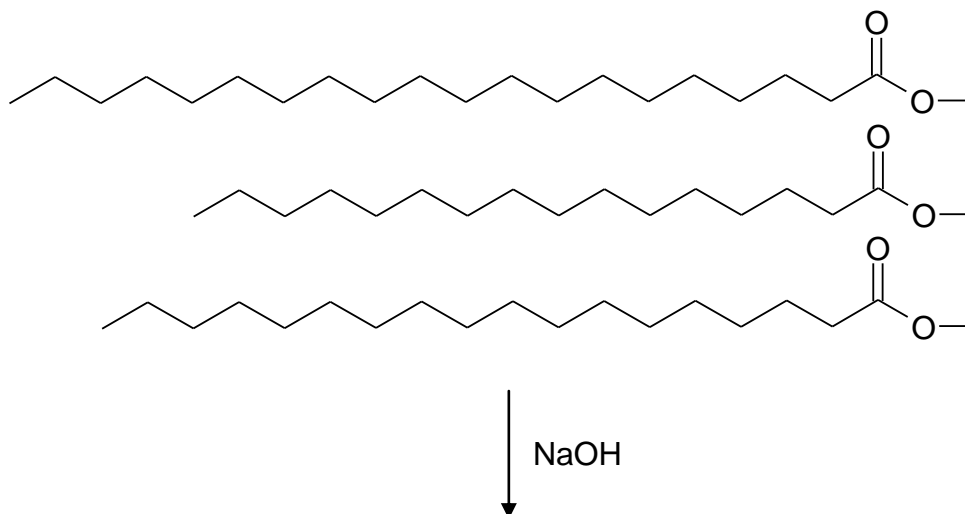
**Introduction**

During Session 1, the advantages and disadvantages of using different methods for extracting fatty acids from food supplement capsules were determined by simple gravimetric (by mass) analysis.

From the Session 2 pre-laboratory exercise, you will have discovered that in nature, fatty acids are biosynthesised as their triglycerides. A triglyceride consists of three fatty acids (often of varying chain length) linked by a glycerol 'backbone' (Figure 1). Since it is possible that the fatty acids in the food supplement capsules are present in 'free' or 'complexed form' (or both), it is necessary to ensure that they are all in the 'free form to simplify the analysis. This is achieved by hydrolysing any triglycerides using sodium hydroxide (saponification), followed by re-acidification of the resulting carboxylate salts to produce the free acids.

Complete Figure 1 to illustrate these transformations.

Figure 1. Fatty acid triglyceride



## **Method**

In order to evaluate the composition of the sources of fatty acids under investigation, *Five Oceans* have been asked by DSD to follow an experimental procedure used by another laboratory. This should guarantee consistency between analyses and provide the opportunity for an inter-laboratory comparison. Unfortunately, the details of this procedure have not been well documented, but the following notes have been extracted from a set of laboratory records. Use these notes to create an experimental procedure that will achieve the session objectives.

## **Procedures**

Saponification  
Reflux (30 min)  
Procedural blank  
Drying  
Extraction  
Re-acidification

## **Substrates/Chemicals**

Lipid extract from capsule  
50 mL methanol  
1 mL 5M potassium hydroxide  
50 mL water  
1M hydrochloric acid (? mL)  
10 mL hexane (x 3)  
Anhydrous sodium sulphate  
Tristearin (0.5 g)  
Stearic acid (0.5 g)

## **Equipment**

Separating funnel  
Round bottomed flasks  
Condenser/Water supply  
Heating mantle  
Rotary evaporator  
Funnel/Filter paper

**SUGGESTED METHOD:** Dissolve the lipid extract in 50 mL MeOH/1mL 5M KOH in a RBF. Reflux for 30 min. After cooling add 50 mL water. Neutralise the solution with 10 mL 1M HCl. Extract the free fatty acids using hexane (3 x 10 cm<sup>3</sup> hexane). Dry the combined hexane extract with sodium sulphate. Filter the dried solution into a pre-weighed RBF, remove the solvent by rotary evaporation (until constant weight) and determine the yield of fatty acids. Repeat the procedure twice more using 0.5 g of stearic acid and 0.5 g tristearin.

### Summary of saponification of extracts and standards

Sample	Round bottomed flask (g)	Round bottomed flask plus lipid (g)	Lipids (g)	Recovery (%)

### Mid-session conclusions

Using the gravimetric data that you have compiled following saponification and re-extraction of free fatty acids from your extract and of standards, consider the following statements and decide whether you 'agree' or 'disagree' with them, or whether you 'cannot be sure'.

1. The recovery of the lipids after saponification and re-extraction was semi-quantitative (> 90%) **(PROBABLY)**
2. The recovery of the lipids after saponification and re-extraction was about the same as the recovery of stearic acid **(PROBABLY)**
3. The recovery of the lipids after saponification and re-extraction was about the same as the recovery of tristearin **(PROBABLY, SINCE GLYCEROL HAS SUCH A SMALL CONTRIBUTION TO MASS)**
4. The lipids in the capsules are mainly present as free fatty acids **(AGREE/CANNOT BE SURE)**
5. The lipids in the capsules are mainly present as triglycerides of fatty acids **(CANNOT BE SURE)**

## **Analysis of individual free fatty acids**

*Reminder:*

Objective 3: Determine the molecular structures of the fatty acids and their distributions

In order to determine the distributions of individual fatty acids in your saponified extract, you will need to use a technique that combines a separation component with a detection method. From your Session 2 pre-laboratory exercise, you should have found that two suitable methods for achieving this for fatty acids are gas chromatography (GC) and gas chromatography – mass spectrometry (GC-MS).

Before your samples can be analysed by GC or GC-MS, it is necessary to *derivatise* the fatty acids by conversion to their methyl esters. The resulting fatty acid methyl esters (FAMES) are more volatile than their free acid counterparts and, in general, give better resolved peaks when using non-polar GC stationary phases.

### **Procedure**

Transfer some of your saponified fatty acid sample (approximately 3 drops) to a pre-weighed 7 ml vial and re-weigh the vial to determine the mass of the fatty acids. Add 4 mL boron trifluoride / methanol ( $\text{BF}_3$  / MeOH) and place the capped vial in a heating block ( $60^\circ\text{C}$ ) for 10 mins (shake the vial every 2 mins). Allow the vial to cool and transfer the contents to a separating funnel using a total of 30 mL hexane to rinse the contents of the vial. Wash the hexane solution with saturated sodium chloride solution (20 mL x 2) and dry the resulting hexane solution using anhydrous sodium sulphate. When dry, filter the solution and remove the hexane using a rotary evaporator. Determine the masses of the fatty acid methyl esters (FAMES) and dilute your samples (approx.  $0.05 \text{ mg mL}^{-1}$ ) in preparation for analysis by GC or GC-MS.

## **Session 2 Post Laboratory Exercise**

1. By considering the session objectives, explain why it was necessary to carry out the saponification step quantitatively on your fatty acid extract and on two 'standards' (stearic acid and tristearin), but not necessary to use all of your saponified extract during the methylation stage (formation of FAMES).
2. What quantitative and qualitative information can be obtained from these various gravimetric analyses ?

### **Session 3 Pre-Laboratory Exercise**

1. Draw the structures of some commonly occurring fatty acids (below), calculate their molar masses and predict their GC elution order. (Note: the fatty acid nomenclature refers to the carbon chain length and the number of double bonds – e.g. C<sub>18:2</sub> has 18 carbon atoms and 2 double bonds)
2. “. . . . and it is thought that too much emphasis has been placed on achieving a diet with high levels of omega-6 acids. What is currently believed to be more important is the correct balance between omega-3 and omega-6 fatty acids”

Investigate this ‘extract’ further, and write your own summary statement describing recommendations for optimal intake of omega fatty acids in the diet, based on what you can find in the recent literature. Keep notes of your information sources.

#### **Saturated**

C<sub>14:0</sub> – Myristic acid  
C<sub>16:0</sub> – Palmitic acid  
C<sub>18:0</sub> – Stearic acid

#### **ω-3**

C<sub>18:3</sub> – Alpha-Linolenic acid  
C<sub>20:5</sub> – Eicosapentaenoic acid

#### **ω-6**

C<sub>18:2</sub> – Linoleic acid

#### **ω-9**

C<sub>18:1</sub> – Oleic acid  
C<sub>20:1</sub> – Eicosenoic acid

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