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

COSHH ASSESSMENT

Session 1 **Assessment date:**

Chemicals- Hazardous in quantities used in this experiment	
Hexane	**HIGHLY FLAMMABLE**. Can cause serious damage if splashed into eyes. May cause impaired fertility. Harmful by inhalation **IRRITANT**. Prolonged exposure may cause serious health damage.
First aid for above chemicals	
Eyes	Irrigate thoroughly with water for at least 10 minutes. **OBTAIN MEDICAL ATTENTION**.
Lungs	Remove from exposure, rest and keep warm. In severe cases **OBTAIN MEDICAL ATTENTION**.
Skin	Wash off thoroughly with water. Remove contaminated clothing and wash before re-use. In severe cases **OBTAIN MEDICAL ATTENTION**.
Mouth	Wash out mouth thoroughly with water and give plenty of water to drink. **OBTAIN MEDICAL ATTENTION**.

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Session 1

Background

Cod liver oil has long been recognised as a beneficial supplement to our diet. Western civilisation, through development, has gradually removed itself from the natural environment and therefore one major source of essential unsaturated fatty acids, fish. The introduction of food supplements, such as cod liver oil, aims to combat this potential deficiency.

Cod liver oil is highly effective at delivering omega-3 fatty acids such as eicosapentaenoic acid (EPA) and omega-6 fatty acids which include linoleic acid. It is also a valuable source of vitamins A and D. The fatty acid lipids are commonly believed to help joint stiffness associated with arthritis, together with the promotion of healthy heart, bone and brain tissue. The oils are extracted by cooking and pressing cod livers, whereas fish oil is extracted from the whole fish and contains fewer essential unsaturated fatty acids.

Current Concerns

The Department for Sustainable Development (DSD) has recently reported a number of concerns surrounding the use of cod as a source of omega-3 and omega-6 fatty acids, and these are summarised as follows:

1. Cod stocks are on the decline, so alternative sources of fatty acids are being considered.
2. Some cod stocks may not yield such high quantities of fatty acids as a proportion of total lipids (e.g. farm-produced versus wild cod)
3. It is thought that the correct balance of omega-3 and omega-6 fatty acids is at least as important as total abundances, but is often overlooked
4. Fatty acids in food supplements may not be in their 'free' or bioavailable form, but present as 'complexes'

Aims and objectives

Five Oceans, is an analytical laboratory specialising in the analysis of 'natural' and genetically modified foodstuffs. Working for *Five Oceans*, your aim is to analyse various commercial sources of omega-3 and omega-6 fatty acids and report back to the DSD on your findings. You will be supplied with various samples of capsules containing fatty acids, together with labelling information. Over the next few weeks, you will need to carry out a series of investigations to see which of the commercial sources of fatty acids aligns best with the overall aim. Create a series of objectives that will form the basis for your experimental approach. Use the **Current Concerns** to guide you.

Agreed Objectives

For *each* source of fatty acids:

- Objective 1: Design and carry out procedure(s) to extract the lipids from the capsules (3 methods)

- Objective 2: Determine whether the fatty acids are 'free' or as 'complexes' and obtain masses of fatty acids in capsules.

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

- Objective 4: Compare the quantities and distributions of fatty acids against labelling information

- Objective 5: Evaluate the capsules against each other with respect to the overall aims

To what extent can you take the labelling information to address these objectives and the overall aim ?

Experimental work

The aim of this first session is for you to extract and quantify the total lipid content of a range of cod liver oil capsules and of some alternatives. Working in groups, discuss the aims of the experiment and suggest general approaches that should enable you to achieve these aims. Use the list of individual steps provided to create 3 specific methods that represent each of the following:

1. A chemical method
2. A physical method
3. A combination of physical and chemical methods

Create your 3 methods from the following individual steps:

1. Dissolve a capsule in 5 mL water in a vial or small conical flask and warm on a water-bath

2. Dissolve a capsule in 5 mL warm water in a vial or small conical flask

3. Dissolve a capsule in 5 mL water in a round bottomed flask and warm on a water bath

4. Dissolve a capsule in 5 mL warm water in a round bottomed flask

5. Add 5 mL hexane to the dissolved capsule, shake the mixture and transfer the hexane phase to a round bottomed flask using a pipette.

6. Add 5 mL hexane to the dissolved capsule, shake, and transfer the hexane solution to a round bottomed flask using a pipette. Repeat this twice more.

7. Cut a capsule in a small beaker using a scalpel blade

8. Squeeze the contents of the pierced capsule into a vial

9. Withdraw the contents of the pierced capsule into a vial using a pipette.

10. Dissolve contents of pierced capsule using up to 5 mL hexane and transfer the resulting solution to a vial or small conical flask

11. Dissolve contents of capsule using 5 mL hexane and transfer the resulting solution to a round-bottomed flask

12. Dissolve contents of capsule using 50 mL hexane and transfer to a round-bottomed flask

13. Remove the hexane using a rotary evaporator

14. Remove the hexane using a heating block and a slow stream of nitrogen

15. Determine the mass of the extracted fatty acids in the vial/conical flask/round-bottomed flask.

Other:

The vials or small conical flasks must be weighed before use

The round-bottomed flasks must be weighed before use

How will you know when all of the hexane has been removed ?

Carry out each extraction method for as many of the different capsules as you can in the allotted time. Record your findings in the summary table.

Summary of extraction data

	Sample name	Pre-weighed vessel (g)	Vessel plus fatty acids (g)	Lipid extract (g)	% Lipid in capsule
Chemical method					
Physical method					
Combination method					

Session 1 Post Laboratory Exercise

Having collated the combined data for the whole group, answer the following questions before the end of the laboratory session (refer to the objectives).

1. Which of the three extraction methods would you consider to be the 'best' and why ?
2. Can this best method be considered to be quantitative in terms of the extraction (i.e. 100% effective) ?
3. What are the main reasons for the losses associated with the two least efficient methods ?
4. Why might the extraction efficiency by greater than 100% ?
5. Summarise how the extraction data (masses of lipid extracts) compare with the labelling information
6. What does the data reveal/not reveal about the composition of the capsules ?

Session 2 Pre-Laboratory Exercise

"DSD are particularly concerned that some commercial sources of omega fatty acids may be providing them in their native or 'complexed' form rather than as the 'free' fatty acids . . . ."

Using this statement in conjunction with Objective 2, research the native forms of omega fatty acids and propose a method for converting them into their 'free' form. In addition, investigate analytical methods for quantifying the individual fatty acids in order that the remaining objectives (3-5) can be met.

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