Extraction and measurement of total lipids in fish

Suitable methods should be used for determination of lipid content in fish samples collected from bioaccumulation studies according to OECD TG 305 to ensure the correct lipid normalization of bioconcentration factors. Chloroform/methanol extraction technique (**Bligh & Dyer Method**) has been recommended by OECD TG 305 as standard method for lipid extraction; however, the use of alternative techniques based on non-chlorinated solvents (**Smedes Method**) should be considered. Both methods are equally suitable to estimate the lipid content of samples from flow-through fish tests according to OECD TG 305.

Bligh & Dyer Method

1) 5-10g wet sample will be weighed in a pre-weighed 100 mL- conical flask
2) 20 mL methanol (MeOH) and 10 mL chloroform ($CHCl_3$) will be added
3) homogenize the sample for 2 min with an UltraTurrax mixer.
4) ten millilitres $CHCl_3$ will be added a second time.
5) shake the mixture vigorously for 1 min.
6) 18 mL of distilled water will be added (including the water already in the sample).
7) vortex the mixture again for 1 min.
8) separate the two layers by centrifugation for 10 min at 450 g in a thermostatic centrifuge at 20°C.
9) the lower layer will be transferred to a pear-shaped flask with a Pasteur pipette.
10) a second extraction will be done with 20 mL 10% (v/v) MeOH in $CHCl_3$ by vortexing for 2 min.
11) after centrifugation, the lower $CHCl_3$ phase will be added to the first extract.
12) evaporate the sample to dryness (e.g. with a rotavapor)
13) the residue will be further dried at 104°C for 1 h.
14) record the extracted weight and calculate the lipid content

Volume of solvents should be adjusted to the sample size. Wet fish samples should be homogenized with chloroform and methanol in a proportion not higher than:

1g tissue : 1 mL chloroform : 2 mL methanol

References

Bligh EG, Dyer WJ: **A rapid method of total lipid extraction and purification**. *Can J Biochem Physiol* 1959, **37:** 911-917.

Smedes Method (based on de Boer et al. 1999)

1) the 5-10g wet sample will be weighed in a pre-weighed 100 mL-centrifugation glass beaker;
2) 16 mL of propan-2-ol, and then 20 mL of cyclohexane will be added to each centrifugation glass beaker using 20 mL pipettes;
3) the sample will be homogenized with the solvents for 2 minutes using e.g. UltraTurrax;
4) **x** mL of deionized water will be added to obtain a total of 22 g water including the water already in the sample,
   x can be calculated according to the following equation:

$$\mathbf{x\ [mL]} = 22 - \frac{\text{sample wet weight [g]} * \text{water content [\%]}}{100}$$

5) the sample will again be homogenized for 1 minute using e.g. UltraTurrax;
6) when an emulsion occurs, this will be dissolved by adding of HCl (37%) dropwise from a pasteur pipette while gently stirring the mixture with a glass rod;
7) the phases will be separated by centrifuging the mixture for 5 ± 1 minutes at 440 - 460 g;
8) the upper cyclohexane phase will be separated and volumetrically quantified, and transferred to a 10 mL glass centrifugation beaker;
9) after addition of 20 mL of a mixture of cyclohexane/propan-2-ol (87:13, w/w) to the remaining lower watery phase, the phases will again be homogenized for 1 minute and afterwards separated by centrifuging the mixture for 5 ± 1 minutes at 440 - 460 g;
10) again, the upper cyclohexane phase will be separated, volumetrically quantified, and transferred to the same 40 mL glass centrifugation beaker holding the first extract of the corresponding sample;
11) the combined cyclohexane extracts will be concentrated by gentle nitrogen stream to approx. 3 mL; the concentrated extract will be quantitatively transferred into a pre-weighed 10 mL widemouth glass flask;
12) the glass centrifugation beaker used for concentrating the extract will be rinsed with 3 mL of the cyclohexane/propan-2-ol mixture using ultrasonication, and the solvent will be added to the corresponding widemouth flask;
13) the solvents in the flask will be evaporated to dryness (e.g. with a rotavapor)
14) the flask will be further dried for 1 hour at 105;
14) after cooling to room temperature in a desiccator, the dried flask will be weighed, and the lipid content calculated according to:

$$\frac{(\text{weight of dry flask [g]} - \text{weight of empty flask [g]}) * 100}{\text{wet weight of sample [g]}} = \textbf{lipid content in \% of wet weight}$$

Volume of solvents should be adjusted to the sample size. Wet fish samples should be homogenized with propan-2-ol and cyclohexane in a proportion not higher than:

1g tissue:1.6 mL propan-2-ol:2 mL cyclohexane

References

Smedes, F: **Determination of total lipid using non-chlorinated solvent**s. *Analyst* 1999, **124**:1711–1718.

De Boer J, Smedes F, Wells D, Allan A: **Report on the QUASH interlaboratory study on the determination of total-lipid in fish and shellfish.** Round 1 SBT-2. Exercise 1000, 1999, EU, Standards, Measurement and Testing Programme.