If this SOP is accessed electronically, it is an uncontrolled copy and will not be updated.



STANDARD OPERATING PROCEDURE

# PERCENT LIPIDS IN TISSUE

EXT-LIPID
Revision 2
September 20, 2010

Approved By: _____ Supervisor — 8/30/10 Date

_____ QA Manager — 8/30/10 Date

_____ Laboratory Manager — 8/30/10 Date

**COLUMBIA ANALYTICAL SERVICES, INC.**
1317 South 13th Avenue
Kelso, Washington 98626

© Columbia Analytical Services, Inc. 2010

Annual review of this SOP has been performed and the SOP still reflects current practice.
Initials: _____ Date: _____
Initials: _____ Date: _____
Initials: _____ Date: _____

DOCUMENT CONTROL
NUMBER: _____
Initials: _____ Date: _____

If this SOP is accessed electronically, it is an uncontrolled copy and will not be updated.



# PERCENT LIPIDS IN TISSUE

## 1. SCOPE AND APPLICATION

1.1. This procedure is used to determine the lipid content in biological tissue. The procedures used are based on the procedure described in the Puget Sound Protocols, Bligh & Dyer, and the EPA Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories.

1.2. This procedure is typically applied to marine tissues. In some cases, the procedure can be used to determine lipids in other biological tissue.

## 2. METHOD SUMMARY

2.1. A homogenized portion of the tissue sample is extracted with organic solvent. The extract is evaporated using moderate heat and the lipid weight is determined. Percent lipids is calculated from the weight measured.

2.2. The extraction may either be performed independently of the extraction done for other organics analyses or a portion of the extract from another extraction may be used for the percent lipids determination

## 3. DEFINITION

Lipids - Naturally occurring compounds that are soluble in such organic solvents as dichloromethane, hydrocarbons, chloroform, benzene, and alcohols. This includes a diverse range of compounds, like fatty acids and their derivatives, carotenoids, terpenes, steroids, cholesterols, and bile acids

## 4. INTERFERENCES

4.1. Since the procedure is used to provide an estimation of lipid content based on non-volatile extracted material, interferences from co-extracted components that are not considered lipids may occur.

4.2. Due to the nature of the procedure, interferences attributed to laboratory procedures are not typically encountered.

## 5. SAFETY

If this SOP is accessed electronically, it is an uncontrolled copy and will not be updated.



Personal protective equipment will include safety glasses (with side shields), gloves, and a lab coat. Follow normal precautions as per the CAS Safety Manual.

## 6. SAMPLE HANDLING AND PRESERVATION

Tissue samples should stored at -20 ± 4°C until analysis. When frozen, samples should be analyzed within 1 year of sampling. Project-specific protocols may apply to sample storage and holding time requirements.

## 7. APPARATUS

7.1. Evaporating pans: Aluminum weighing pans.

7.2. VOA vials, 40mL, precleaned.

7.3. Tissuemizer.

7.4. Drying oven, capable of maintaining a temperature of 70°C.

7.5. Hotplate, capable of maintaining a temperature of 70°C.

7.6. Analytical balance, capable of weighing to 0.1mg.

## 8. REAGENTS

8.1. Sodium sulfate (granular, anhydrous), $Na_2SO_4$. Purify by heating at 400ºC for 4 hours in a shallow tray or crucible, or by pre-cleaning the sodium sulfate with dichloromethane.

8.2. Extraction solvents:

8.2.1. Dichloromethane

8.2.2. Chloroform/Methanol (1:1)

## 9. PREVENTIVE MAINTENANCE

9.1. Balance calibration checks are performed daily for each day analyses are performed. The results of these checks are recorded in the designated logbook for balance calibration verification located in the extractions lab.

9.2. Periodic balance servicing is performed by an outside qualified vendor.

## 10. RESPONSIBILITIES

It is the responsibility of the analyst to perform the analysis according to this SOP and to complete all documentation required for data review. Analysis and interpretation of the results are performed by personnel in the laboratory who have demonstrated the ability to generate

If this SOP is accessed electronically, it is an uncontrolled copy and will not be updated.



acceptable results utilizing this SOP. This demonstration is in accordance with the training program of the laboratory. Final review and sign-off of the data is performed by the department supervisor/manager or designee.

## 11. PROCEDURE

Note: If samples are suspected to be high in lipids (i.e. whole fish), the % lipids must be done before samples are run on GPC to prevent overloading of GPC column. The maximum amount of lipid that can be loaded on the GPC column is 2 g. If lipid is over 2g see the extraction supervisor for assistance.

11.1. PSEP Option:

11.1.1. Homogenize the tissue sample using the CAS *SOP Tissue Sample Preparation (MET-TISP)*. If using a portion of extract from an analytical extraction, use 2 ml of extract from the 10 ml intermediate volume before GPC cleanup and skip to section 11.1.6.

11.1.2. Weigh 2 grams of tissue into a VOA vial. Dry the sample with sodium sulfate.

11.1.3. Add 10mL dichloromethane.

11.1.4. Using a tissuemizer, homogenize the tissue/solvent mixture until sample is thoroughly mixed.

11.1.5. Vortex and centrifuge at 100xG for 10 minutes.

11.1.6. Heat sample pans in oven at 70ºC for one hour. Remove from oven and weigh as soon as pans are no longer warm (less than 5 minutes). Weigh the pans to the nearest 0.0001 gram.

11.1.7. Pipet 2mL of the extract into a DCM-rinsed glass syringe with a new filter. Filter the extract into a pan and evaporate to dryness. Using a hot plate with temperature <70ºC. Place pans in oven at 70ºC for one hour.

11.1.8. Remove from oven and weigh as soon as pans are no longer warm (less than 5 minutes). Weigh the pans to the nearest 0.0001 gram.

11.1.9. Calculate percent (%) lipids and GPC loading.

11.2. Bligh & Dyer Option:

11.2.1. Prepare pans: Heat aluminum sample pans in oven at 70° C for a minimum of one hour. Cool pans in a desiccator for a minimum of 30 minutes. Weigh pans to the nearest 0.0001 gram.

If this SOP is accessed electronically, it is an uncontrolled copy and will not be updated.



11.2.2. Homogenize tissue in accordance with CAS *SOP Tissue Sample Preparation (MET-TISP).*

11.2.3. Weigh the appropriate amount of tissue into a scintillation vial for samples ≤ 3 grams, into a 40 mL VOA vial for samples 3 - 6 grams, or into a 250 mL beaker for large sample 6 - 30 grams.

11.2.4. Add the appropriate amount of 1:1 Chloroform/Methanol (v/v):

≤ 2 grams, add 10 mL
> 2 - 5 grams, add 25 mL
5 - 10 grams, add 50 mL
10 - 30 grams, add 100 mL

11.2.5. Using a tissumizer, homogenize the sample/solvent mixture for approximately 1 minute or until sample is thoroughly dessimated. Rinse the tissuemizer with solvent. Collect the rinsate with the sample extract. Centrifuge the sample extract at 1000xG for 10 minutes. Transfer extract to a clean VOA vial, leaving the tissue behind. Rinse the tissue portion by adding 1-2 mL of solvent to the tissue. Vortex, centrifuge, and combine with the extract in the VOA vial. Examine the extract to verify that a single phase is present. High water content can result in phase separation. In these cases, add solvent in 1 mL increments until a single phase is achieved.

11.2.6. **Low Volume Extractions**: Back extract by adding 7 mL of sodium sulfate/DI water (25g sulfate/1000mL H2O) to the extract). Vortex extract and centrifuge at 1000xG for 10 minutes. Lipids will be retained in bottom chloroform layer. Pipette the top water layer off the bottom chloroform solvent layer and discard. If colloidal proteinaceous material is observed, add 0.5 mL of sodium sulfate/DI water, vortex, and centrifuge for another 10 minutes. Discard the top water layer.

11.2.7. **High Volume Extractions**: Transfer the extract with rinses into a 250 mL separatory funnel. Back extract 2 times with 100 mL sodium sulfate/DI water, discarding the top water layer each time (lipids will be retained in the bottom layer). Filter the bottom layer through muffled anhydrous sodium sulfate into a KD. Rinse the funnel with 10 - 20 mL of dichloromethane. Concentrate to approximately 5 mL on the S-evap, keeping the temperature ≤ 80°C. The extract may need to be centrifuged or filtered at this point.

11.2.8. Concentrate the extract to approximately 2mL using nitrogen.

11.2.9. Place pans on a hot plate at a setting of 3. Filter the extracts through 3mL syringes into the pans and evaporate to dryness. DO NOT LET EXTRACTS

If this SOP is accessed electronically, it is an uncontrolled copy and will not be updated.



BOIL. Heat sample pans in oven at 70° C for a minimum of one hour. Cool pans in a desiccator for a minimum of 30 minutes. Weigh pans to the nearest 0.0001 gram and record the weight.

## 12. QA/QC REQUIREMENTS

12.1. QC Samples Required

12.1.1. Analyze one sample in triplicate per 20 samples.

12.1.2. Analyze one blank, an empty aluminum pan, per twenty samples.

12.2. Acceptance Criteria

12.2.1. RPD for duplicates should be <20%.

12.2.2. Method blank weight should be less than the absolute value of 0.5mg.

12.3. Calculations

12.3.1. Calculate percent (%) lipids

PSEP Option:

$$\%\,Lipids = \frac{\left[\left(Wt_{dish+lipid(g)} - Wt_{dish(g)}\right) - Blank\right] \times 5}{Sample\ Wt.(g)} \times 100$$

Bligh & Dyer Option:

$$\%\,Lipids = \frac{\left[\left(Wt_{dish+lipid(g)} - Wt_{dish(g)}\right) - Blank\right]}{Sample\ Wt.(g)} \times 100$$

12.3.2. Calculate number of (g) loaded on GPC (5 ml):

$$\frac{\%\ Lipid}{200} \times Sample\ Wt.(g)$$

12.4. Reporting

12.4.1. Fill out the Excel report for % lipids found at R:\SVG\Forms\lipids.

If this SOP is accessed electronically, it is an uncontrolled copy and will not be updated.



12.4.2. Samples are weighed to the nearest 0.1mg and report to the nearest 0.1%.

12.4.3. Report results using no more than 3 significant figures.

12.5. Data review

12.5.1. It is the analyst's responsibility to review analytical data to ensure that all quality control requirements have been met for each analytical run. Results for QC analyses are calculated and recorded as specified. All data will be initialed, dated and attached to required data quality worksheet.

12.5.2. The data packet for the sequence is submitted for review by supervisor or designee. The results are transferred to the appropriate report form located in the CAS network directory R:\SVG\WIP.

12.5.3. Refer to the SOP for *Laboratory Data Review Process* for general instructions for data review.

## 13. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

Corrective action measures applicable to specific analysis steps are discussed in the applicable section of this (and other applicable) SOP(s). Also, refer to the SOP for *Corrective Action* for correct procedures for identifying and documenting such data. Procedures for applying data qualifiers are described in the SOP for *Report Generation* or in project-specific requirements.

## 14. METHOD PERFORMANCE

This method was validated through single laboratory studies of accuracy and precision. Refer to the reference method for additional method performance data available.

If this SOP is accessed electronically, it is an uncontrolled copy and will not be updated.



## 15. POLLUTION PREVENTION

It is the laboratory's practice to minimize the amount of solvents and reagents used to perform this method wherever technically sound, feasibly possible, and within method requirements. Standards are prepared in volumes consistent with laboratory use in order to minimize the volume of expired standards to be disposed of. The threat to the environment from solvents and/or reagents used in this method may be minimized when recycled or disposed of properly.

## 16. WASTE MANAGEMENT

The laboratory will comply with all Federal, State, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions as specified in the laboratory Safety Manual, Sections 6 and 7.

## 17. TRAINING

17.1. Training outline

17.1.1. Review literature (see references section). Read and understand this SOP. Also review the applicable MSDS for all solvents used. Following these reviews, observe the procedure as performed by an experienced analyst at least three times.

17.1.2. The next training step is to assist in the procedure under the guidance of an experienced analyst. During this period, the analyst is expected to transition from a role of assisting, to performing the procedure with minimal oversight from an experienced analyst.

17.1.3. Training is documented following the SOP *for Documentation of Training*.

17.1.4. NOTE: When the analyst training is documented by the supervisor on internal training documentation forms, the supervisor is acknowledging that the analyst has read and understands this SOP and that adequate training has been given to the analyst to competently perform the analysis independently.

If this SOP is accessed electronically, it is an uncontrolled copy and will not be updated.



## 18. REFERENCES

18.1. USEPA Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1 - Fish Sampling and Analysis, Third Edition, November, 2000.

18.2. Recommended Protocols for Measuring Selected Environmental Variables in Puget Sound, January 1996 and subsequent chapter revisions.

18.3. Bligh & Dyer, Canadian J. Biochemistry and Physiology, Aug. 1959

18.4. NOAA (National Oceanic and Atmospheric Administration). 1993a. *Sampling and Analytical Methods of the National Status and Trends Program, National Benthic Surveillance and Mussel Watch Projects 1984-1992 Volume 11.Comprehensive Descriptions of Complementary Measurements.* NOAA Technical Memorandum NOS ORCA 71. Coastal Monitoring and Bioeffects Assessment Division, Office of Ocean Resources Conservation and Assessment, National Ocean Service, Silver Spring, MD. July.

18.5. NOAA (National Oceanic and Atmospheric Administration). 1993c. *Sampling and Analytical Methods of the National Status and Trends Program, National Benthic surveillance and Mussel Watch Projects 1984-1992. Volume IV. Comprehensive Descriptions of Trance Organic Analytical Methods.* NOAA Technical Memorandum NOS ORCA 71. Coastal Monitoring and Bioeffects Assessment Division, Office of Ocean Resources Conservation and Assessment, National Ocean Service, Silver Spring, MD. July.

## 19. CHANGES SINCE THE LAST REVISION

19.1. Changed SOP No from SOC-LIPID to EXT-LIPID
19.2. Updated references to Corporate SOPs through-out the document.
19.3. Changed methylene chloride to dichloromethane.
19.4. Sec 12.2.1 Re-worded to clarify
19.5. Added new NOAA references (18)
19.6. Added standard training section 17 to the SOP.
19.7. Removed reference to MDL study (14)