**STANDARD OPERATION PROCEDURE**

**Faculty of Biosciences, NMBU**

**Method name: Crude fat-Accelerated Solvent Extraction (ASE)**

BIOVIT-no: Arb1045

**1. Introduction**

Accelerated Solvent Extraction (ASE) is an alternative extraction method. The method is compared with the Soxhlet method with HCl – hydrolysis.

The extraction takes place by pumping a solvent into an extraction cell (with the sample inside) which is then given a selected temperature and pressure. The extract is then transferred from the cell to a collection glass. The extract is placed in a water bath under nitrogen to evaporate the solvent and then dried in a vacuum oven. Finally, the sample is weighed.

This is a fast and straightforward method with low solvent consumption.

**2. Reagents**

* Petroleum ether (boiling point 40-60 °C)
* Acetone
* Desiccant (Restek, catalog no. 26033, product name: Diatomaceous Earth)
* Nitrogen gas

**3. Risk assessment**

* Petrol ether:
* Highly flammable
* Avoid skin contact
* Store in a well-ventilated place
* Acetone:
* Highly flammable
* Store in a well-ventilated place
* Desiccant:
* Wear disposable gloves and a dust mask.
* Avoid skin contact
* Emits dust that may be carcinogenic
* Work in a fume hood when disassembling / emptying the extraction cells.

**4. Equipment**

* Weight
* ASE 200, Accelerated Solvent Extractor
* Cells to ASE
* Collection glass for ASE
* Vacuum oven: Heraeus vacutherm
* Metal crucible for weighing
* Equipment for packing cells
* Water bath

**5. Special remarks**

Three different extraction programs are run with different mix of solvents and temperature.

**Program 1:** 100% petroleum ether at 100 ºC

Sample type: Silage, grass, hay, bioprotein and microbes.

**Program 2:** 80% petroleum ether and 20% acetone at 125 °C

Sample type: Fish faeces, intestine cow, concentrate, cat feed, pigs feed, soy, corn, krill, blood meal, beans, sheep manure, liquids and meat

**Program 3:** 70% petroleum ether and 30% acetone at 125 °C

Sample type: Mink faeces, mink feed, fishmeal, pigs feed, krill, yeast, rapeseed, chicken feed.

**6. Sample material**

The sample material must be dry, homogeneous and ground to a size of 1 mm or less.

Liquid/meat samples are mixed well with the desiccant and alternatively dried in the cells at 60 °C overnight.

**7. Work procedure**

Cell packing, dry sample

1. Place 1-2 filters (depending on degree of grinding) in the bottom of the cell and add approx. 1 spatula with desiccant. For finely ground samples 0.5 mm and less use 2 filters.

2. Weigh in approx. 0.5-1.0 g sample in a metal weighing vessel and add approx. 2 spatula spoons desiccant. Mix well!

3. The sample is poured into the cell using a metal funnel.

4. Add 1 spatula with desiccant on top of the cell and screw the lid on tightly.

Cell packing, liquid / meat sample

1. Place 2 filters in the bottom of the cell and add approx. 1 spatula with desiccant.

2. Weigh in approx. 1-2 g of liquid or meat and add 2-3 spatulas of desiccant.

3. The mixture is poured into the cell and 1 spatula with desiccant is added on top the cell.

4. The whole cell with sample and desiccant is dried at 60 °C in an overnight drying cabinet.

5. Remove the samples from the drying cabinet and screw the lid on tightly.

Extraction and evaporation

6. the collection glass is marked, weighed and the lid is screwed on (wear gloves for all handling of the glasses).

7. Cells and glasses are placed on the machine and extraction program is selected (see section 5)

8. When the extraction is complete, remove the collecting tubes (unscrew the cap) and place in a water bath (<60 °C) with nitrogen gas over until the extraction liquid is gone.

9. Place the tubes in a vacuum oven (70 °C) for 30 minutes.

10. The glasses are taken over in a desiccator to cool. (approx. 30 minutes).

11. Weigh the jars and calculate g fat/kg sample.

**8. Calculation**

g fat/kg sample = (Weight tube w/fat – weight tube) \* 1000

 Sample

Where:

Weight glass w/fat = weight of collecting pipe with fat (g)

Weight glass = weight of empty collection pipe (g)

1000 = g/kg

sample = gram weighed sample in the cell (g)

Present as % or g/100g.