

**Total Fat by Acid Hydrolysis**  
**Filter Bag Technique**  
(For HCl, HClI)

**DEFINITION**

This method determines total fat by releasing complexed lipids using acid hydrolysis. The fat is then capable of being extracted by the fat solvents. The compounds extracted are predominantly triacylglycerides. Small amounts of other lipids having some solubility in the fat solvents are also extracted.

**SCOPE**

This method is applicable food and feed samples with 0-100% fat content.

**APPARATUS**

1. Analytical Balance—capable of weighing 0.1 mg.
2. Dryer—forced air dryer that neutralizes acid fumes and capable of maintaining a temperature between  $60-120 \pm 2^\circ\text{C}$  (ANKOM<sup>RD</sup> Dryer, ANKOM Technology).
3. Hydrolysis instrument—capable of performing the hydrolysis at  $90 \pm 2.0^\circ\text{C}$ . The instrument must also be capable of creating a uniform flow pattern around each sample to ensure uniformity of digestion (ANKOM<sup>HCl</sup> Hydrolysis System, ANKOM Technology).
4. ANKOM Extractor capable of performing extractions under pressure at  $90 \pm 2.0^\circ\text{C}$  (XT10, XT15, XT20, ANKOM Technology).
5. Filter Bags—constructed from chemically inert and heat resistant filter media, capable of being heat sealed closed and able to retain 1 micron particles while permitting solution penetration (XT4, ANKOM Technology).
6. Heat sealer—sufficient for sealing the Filter Bags closed to ensure complete closure (1915, ANKOM Technology).
7. Desiccant Pouch—collapsible sealable pouch with desiccant inside that enables the removal of air from around the Filter Bags (MoistureStop weigh pouch, ANKOM Technology).
8. Celite—diatomaceous earth (Item # XTC, ANKOM Technology).
9. Marking pen—Solvent and acid resistant (F08, ANKOM Technology).

**REAGENTS**

1. Fat solvent used is based on the comparative method targeted. When comparing to ISO 6492 use petroleum ether (B.P.  $35-65^\circ\text{C}$ ). When comparing to Mojonnier method use a mixture of 45% petroleum ether, 45% diethyl ether and 10% ethanol (see Notes, *Caution*).
2. 3N HCl (*Note* – Some strongly bound samples will need 5N HCl)

**PROCEDURE**

1. Prepare sample to a homogenous consistency.
2. Place a labeled Filter Bag on the balance and zero.
3. Weigh 0.5-1g of Celite in the Filter Bag for samples with fat up to 20% and zero balance. For samples with expected fat values  $>20\%$ , *see Numbered Note 1*.
4. Weigh 0.75 - 1.0 g of sample into the bag and record weight ( $W_1$ ). No more than 1.5g of Celite and sample dry matter should be placed in each bag.
5. Using a heat sealer, completely seal the upper edge of the Filter Bag within 4 mm of the top. Use sufficient heat to completely seal the Filter Bag and allow enough cool time (2 sec) before removing the bag from the heat sealer. Shake bag to mix sample and Celite.
6. Place up to 15 bags in the HCl Bag Holder and insert in the chamber of the Hydrolysis Instrument. Include two blank bags (C) with Celite (*see Numbered Note 2*).
7. Add 500 ml of the HCl solution to the chamber.
8. Follow instructions on the instrument screen. Select 60 min. for the hydrolysis time, temperature of  $90^\circ\text{C}$ , and rinse time of 20 min.
9. When the process is complete, remove the bags from the Hydrolysis Instrument and holder. Spread bags out and gently press between paper towels to absorb water from bags. (*see Numbered Note 3*).
10. Place bags back in HCl Bag Holder and dry for 2-3 hrs at  $110^\circ\text{C}$  in the ANKOM<sup>RD</sup> Dryer. (*see Numbered Note 5*).
11. Quickly remove samples from the dryer and place them directly into a Desiccant Pouch containing pH paper. Allow to cool to room temperature and check pH paper. If acid shows on the paper, continue drying. If the pH paper is neutral, record weight ( $W_2$ ) of samples.
12. Extract encapsulated samples in an ANKOM Extractor set at  $90^\circ\text{C}$  for 40–60 min. with the appropriate solvent.
13. Dry extracted samples for 20 min at  $110 \pm 2^\circ\text{C}$
14. Cool in Desiccant Pouch and weigh ( $W_3$ ).

## CALCULATION:

$$\% \text{ Total Fat} = \frac{100 \times (W_2 - (W_3 + (C_1 - C_2)))}{W_1}$$

Where:  $W_1$  = Original weight of sample  
 $W_2$  = Weight of dried sample, Filter Bag and Celite after hydrolysis  
 $W_3$  = Weight of dry extracted sample, Celite and Filter Bag.  
 $C_1$  = Blank Bag dry weight after hydrolysis  
 $C_2$  = Blank Bag weight after extraction.

**NOTES** *Caution* — Most fat solvents are extremely flammable. Use fume hood when handling exposed solvent and use appropriate procedures to avoid static electricity discharge.

## NUMBERED NOTES:

1. Samples with expected fat percentages higher than 20% require higher ratios of Celite to sample. A ratio of 5:1 will be sufficient for most high fat samples. In a few cases ratios as high as 7:1 were found to be beneficial. No more than 1.5g of Celite and sample dry matter should be placed in each bag. The increased volume inside the bags will limit the number of bags processed in the hydrolysis chamber to ten. If fat continues to migrate from the bag then a pre-extraction with solvent may be necessary. Fat migration can be detected by placing a blank bag next to suspect samples (See Numbered Notes, 2).
2. Blank Bags serve as correction factors for slight losses of weight that may occur during extraction. Blank bags with Celite also act as indicators of fat loss during hydrolysis. When the blank bag is placed next to a sample that loses fat, it will absorb some of these compounds. The result is a blank loss during extraction that exceeds the normal expected blank loss of 0.0020g by 0.0005g or more. This is an indication of fat loss during hydrolysis, absorption by the Celite in the blank bag and the loss of that fat during extraction.
3. For uniform treatment of the bags during the blotting procedure, ANKOM has developed a weighted pressure plate for this process (Product ID-H35 HCl Blotter).
4. Even with extensive rinsing some samples may contain residual HCl. This HCl will evaporate during the drying step with no adverse effect on the fat. HCl vapors are highly corrosive so use an appropriate oven or the ANKOM<sup>RD</sup> Dryer with an acid neutralizing filter.
5. More rapid drying can be accomplished in a microwave oven. ANKOM has developed a Microwave Drying System that removes the moisture and the acid fumes (Product ID- MDA Microwave Drying Assembly).