

Isolation of Total Fat by ANKOM^{FLEX} (Method: Total Fat)

Definition

This method is used to isolate Total Fat within a given sample.

Scope

This method is applicable to oil, food, feed and liquid samples.

A. Apparatus

1. ANKOM^{FLEX} Analyte Extractor
2. Digestion Vessels (FLEX54, FLEX55)
3. Magnetic cross stir-bars (9415) – *for use in digestion vessels*
4. Round Bottom Flasks (9364) – *for recovery on the FLEX instrument*
5. Prepacked SPE Columns (FLEX-SPE-02)
6. Fat Filters (FLEX-FF)
7. Analytical Balance – capable of weighing 1mg
8. Weigh tins for sample transfer
9. Disposable pipettes for oil and liquid samples

B. Reagents

1. Use distilled water (DI) throughout
2. n-Hexane (reagent grade or higher)
3. Ethanol (absolute)
4. 3N Hydrochloric Acid (HCl): 280 ml concentrated HCl (36-38%) and 720 ml DI water. Confirm density with Hydrometer (1.055-1.060 g/ml) or titration.

C. Sample Preparation

Table 1 shows maximum recommended sample sizes, to be run on the ANKOM^{FLEX}. Each sample type must be appropriately homogenized or ground, before the analysis.

Table 1. Maximum Recommended Sample Sizes for Analysis on the ANKOM^{FLEX}

Sample Type	Size (g)
Foods & Feeds	1.0
Oils e.g., butter, canola	0.25-0.40
Liquids e.g., cream sauce	1.0

D. Procedure (see the Operator's Manual for more detail)

1. Assemble digestion vessels (digestion vessel + vessel bottom assembly + fat filter) and **add a magnetic cross stir-bar** into each digestion vessel before adding sample.
2. **Foods & Feeds:** Weigh sample into a weigh tin and then quantitatively transfer to the digestion vessels.
 - a. Tare the weigh tin on the analytical balance.
 - b. Weigh sample into tared tin.
 - c. Quantitatively transfer the sample from the tin into the assembled digestion vessel using static free brush. If sample is wet, such as mayonnaise, and residue remains in the tin after transfer, reweigh and subtract from the initial weight of the [tin + sample] to calculate the actual sample weight.
 - d. Note: It is important to not exceed the specified sample size. Exceeding the sample size could result in digestion filters plugging.
 - e. Install digestion vessels on the ANKOM^{FLEX} and follow the instructions in the operating manual on how to: Start an Assay.

3. Oils: Install digestion vessels on the ANKOM^{FLEX} **before** weighing samples.
 - a. Pipette 5ml of distilled water onto the filter through the port on the digestion vessel. This process is done before oil is placed on the filter.
 - b. Using a disposable pipette, draw the oil sample into the pipette.
 - c. Weigh the filled disposable pipette on the analytical balance.
 - d. Insert the disposable pipette into the digestion vessel through the port and empty the contents.
 - e. Re-weigh the pipette and subtract the initial weight from the final weight to calculate actual sample weight.
4. Liquids: Install digestion vessels on the ANKOM^{FLEX} **before** weighing samples. Liquid samples will be quantitatively transferred into digestion vessels.
 - a. Using a disposable pipette, draw the liquid sample into the pipette.
 - b. Weigh the filled disposable pipette on the analytical balance.
 - c. Insert the disposable pipette into the digestion vessel through the port and empty the contents.
 - d. Re-weigh the pipette and subtract the initial weight from the final weight to calculate actual sample weight.
5. Select Method: [Total Fat](#)
6. After the ANKOM^{FLEX} method has ended, the round bottom flasks in the recovery oven will contain the isolated fat. Remove the round bottom flasks for reconstitution or gravimetric transfer.