

4.5.05

AOAC Official Method 2003.05
Crude Fat in Feeds, Cereal Grains, and Forages
Randall/Soxtec/Diethylether Extraction-Submersion Method
First Action 2003
Final Action 2006

[Applicable to the analysis of forages, cereal grains, and animal feeds other than baked or expanded products, dried milk or milk products, fishmeal, or oilseeds at concentrations from 0.5 to 100% fat. It is applicable to the same matrixes as **920.39** (see 4.5.01) and **930.09** (see 3.5.07).]

Caution: Store solvents in metal containers in solvent cabinet or solvent room that conforms to applicable safety legislation. Ethers are extremely flammable. Have no open flames in the laboratory where the analysis is being performed. Avoid inhaling vapors. Use solvents in a properly operating hood equipped with explosion-proof lighting, wiring, and fan. Diethylether has the potential to form shock-sensitive, explosive peroxides with age. Check each new container of ether for peroxides when it is opened. Also check partial containers of ether that have not been used for several months before using them again. *Do not* use ether that contains peroxides. Dispose as hazardous material. Stabilized ether may be used. Ground electrical equipment and maintain in proper working order. Follow manufacturer recommendations for installation, operation, and safety of all extraction equipment. Make sure all solvent is evaporated from cups before placing them in the oven to avoid a fire or explosion.

See Table **2003.05** for results of the interlaboratory study supporting acceptance of the method.

A. Principle

The Randall modification of the standard Soxhlet extraction submerges the test portion in boiling solvent, reducing the time needed for extraction. The solvent dissolves fats, oils, pigments, and other soluble substances, collectively termed “crude fat.”

A dried, ground test portion is extracted by a 2-step process: In the first step, the thimble containing the test portion is immersed into the boiling solvent. The intermixing of matrix with hot solvent ensures rapid solubilization of extractables. The thimble is then raised above the solvent and the test portion is further extracted by a continuous flow of condensed solvent. The solvent is evaporated and recovered by condensation. The resulting crude fat residue is determined gravimetrically after drying.

The solubility characteristics of different solvents may result in slight differences in crude fat results. For this reason, the report should reflect the solvent used. Example: % Crude Fat, Ether Extraction.

B. Apparatus

(a) *Solvent extraction system.*—Multiple position extraction unit conducting 2-stage Randall extraction process with solvent recovery cycle, with Viton or Teflon™ seals compatible with ether.

(b) *Thimbles and stand.*—Cellulose thimbles and stand to hold thimbles.

(c) *Extraction cups.*—Aluminum or glass. (Extraction temperature settings may differ; consult manufacturer’s operating instructions.)

Items (a)–(c) are available as Soxtec systems from Foss or other Randall-type extraction systems.

C. Reagents

(a) *Anhydrous diethylether.*—Purified for fat extraction, Fisher E492 labeled “For Fat Extraction” is also stabilized, or E134-4, or equivalent. To prevent ether from absorbing water, purchase it in small containers and keep containers tightly closed. Petroleum ether cannot be substituted for diethylether because it does not dissolve all of the plant lipid material.

Table 2003.05. Interlaboratory results for crude fat in animal feed, cereal grain, and forage, diethylether extraction (submersion) method

Material	Mean	No. of labs ^a	s _r	RSD _r , %	s _R	RSD _R , %
Dehydrated alfalfa	4.44	10	0.21	4.62	0.23	5.1
Corn silage	2.09	9(1)	0.02	1.02	0.06	3.06
Mixed bird seed	7.19	10	0.19	2.64	0.23	3.17
Texturized feed	3.20	9(1)	0.1	3.22	0.22	6.77
Fat supplement	96.38	10	4.82	5.00	4.82	5.00
Medicated goat feed	1.89	10	0.04	2.09	1.08	4.5
Feedlot concentrate pellets	1.53	10	0.14	9.26	0.32	21.0
Cellulose (blank)	0.17	10	0.05	26.1	0.08	48.3
Calf starter medicated	2.96	8(2)	0.05	1.58	0.06	1.9
Calf feed medicated	3.42	9(1)	0.05	1.44	0.12	3.50
Meat meal/hulls mix	4.99	10	0.22	4.33	10.55	11.08
Swine feed	2.77	10	0.07	2.61	0.15	5.5
Broiler starter	7.05	10	0.1	1.35	0.16	2.33
High oil corn	7.69	10	0.1	1.31	0.22	2.93

^a Number of laboratories retained after eliminating the number of laboratories in parentheses.

(b) *Cotton*.—Defatted. Soak medical grade cotton in diethylether or hexanes for 24 h, agitating several times during this period. Remove and air dry.

(c) *Sand*.—Ashed (for ignition boats). EM SX0075-3, or equivalent (CAS 14808-60-7).

(d) *Celite 545*.

D. Preparation of Analytical Sample

Reduce particle size of samples to fineness of 0.75–1 mm.

E. Determination

Weigh 1–5 g test portions containing ca 100–200 mg fat directly into tared cellulose thimbles, according to following scheme:

Crude fat, %	Test portion weight, g
<2	5
5	2–4
10	1–2
>20	1

Record weight to nearest 0.1 mg (S) and thimble number. *Note:* If test sample contains large amounts of urea salts (>5%) or soluble carbohydrates (>15%), glycerol, lactic acid, amino acid salts (>10%) or other water-soluble components, remove by water extraction. Weigh test portion onto filter paper, extract with five 20 mL portions of water, allowing each portion to drain. Place filter paper containing washed test portion into thimble and dry at 102 °C for 2 h. To facilitate filtration, add 1–2 g ashed, acid-washed sand, or Celite to bottom of filter or mix in with test portion before water extraction.

Dry thimbles containing test portions at 102 °C for 2 h. If dried test portions will not be extracted immediately, store in desiccator. Both solvent and test materials must be free of moisture to avoid extraction of water-soluble components such as carbohydrates, urea, lactic acid, and glycerol, which will result in false high values.

An absorbent, such as diatomaceous earth (Celite or Super-Cel), can be added to the test portion when high fat materials, which melt through the thimble during the predry step, are present. Alternatively, defatted cotton can be added before the predry step to absorb the melted fat. If the material melts at 102 °C, place a pretared extraction cup under the thimble during the drying step to catch any melted fat that was unabsorbed and escaped the thimble.

Place defatted (with same solvent to be used for extraction) cotton plug on top of test portion to keep material immersed during the boiling step and prevent any loss of test portion from top of thimble. Prepare cotton plug large enough to hold materials in place, yet as small as possible to minimize absorption of solvent. Adding the cotton plug before the 102 °C, 2 h drying step is acceptable.

Place three or four 5 mm glass boiling beads into each cup, and dry cups for at least 30 min at 102 °C. Transfer to desiccator and cool to room temperature. Weigh extraction cups and record weight to nearest 0.1 mg (T).

Extract, following manufacturer's instructions for operation of extractor. Preheat extractor and turn on condenser cooling water. Attach thimbles containing dried test portions to extraction columns. Put sufficient amount of solvent into each extraction cup to cover test portion when thimbles are in boiling position. Place cups under extraction columns and secure in place. Make sure that cups are matched to their corresponding thimble. Lower thimbles into solvent and boil for 20 min. Verify proper reflux rate which is critical to the complete extraction of fat. This rate depends upon the equipment and should be supplied by the manufacturer. A reflux rate of ca 3–5 drops/s applies to many extraction systems.

Raise thimbles out of solvent and extract in this position for 40 min. Then evaporate as much solvent as possible from cups to reclaim solvent and attain apparent dryness.

Remove extraction cups from extractor and place in operating fume hood to finish evaporating solvent at low temperature. (*Note:* Take care not to pick up any debris on bottom of extraction cup while in hood. Let cups remain in hood until all traces of solvent are gone.)

Dry extraction cups in a 102 °C oven for 30 min to remove moisture. Excessive drying may oxidize fat and give high results. Cool in desiccator to room temperature and weigh to nearest 0.1 mg (F).

F. Calculations

$$\% \text{ Crude fat, diethylether extract} = \frac{F - T}{S} \times 100$$

where F = weight of cup + fat residue, g; T = weight of empty cup, g; S = test portion weight, g.

References: *J. AOAC Int.* **86**, 888(2003); 899(2003).