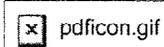


B

ATTACHMENT B



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41.1.28 - Oils and Fats

AOAC Official Method 969.33 Fatty Acids in Oils and Fats

Preparation of Methyl Esters Boron Trifluoride Method First Action 1969

AOAC-IUPAC Method *Codex-Adopted-AOAC Method**

A. Principle

Glycerides and phospholipids are saponified, and fatty acids are liberated and esterified in presence of BF_3 catalyst for further analysis by IR, **965.34E** (see 41.1.36), or GC, **963.22F** (see 41.1.29).

Method is applicable to common animal and vegetable oils and fats, and fatty acids. Unsaponifiables are not removed, and if present in large amounts, may interfere with subsequent analyses.

Method is not suitable for preparation of methyl esters of fatty acids containing major amounts of epoxy, hydroperoxy, aldehyde, ketone, cyclopropyl, and cyclopropenyl groups, and conjugated polyunsaturated and acetylenic compounds because of partial or complete destruction of these groups.

B. Apparatus

(a) *Reaction flasks*.—50 and 125 mL flasks with outer standard taper joints.

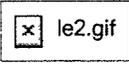
(b) *Condenser*.—Water-cooled, reflux, with 20–30 cm jacket and standard taper inner joint.

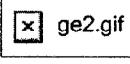
C. Reagents

(a) *Boron trifluoride reagent*.—125 g BF_3 /L methyl alcohol. Available commercially or prepare as follows: Weigh 2 L flask containing 1 L methyl alcohol. Cool in ice bath and with flask still in bath, bubble BF_3 from cylinder through glass tube into methyl alcohol until 125 g is absorbed. Work in hood.

BF_3 must be flowing through glass tube before it is placed in and until it is removed from methyl alcohol to prevent liquid from being drawn into cylinder valve system. Gas should not flow so fast that white fumes emerge from flask. Reagent is stable 2 years. (*Caution*: Remove BF_3 vapors with effective

fume removal device. Avoid contact with skin, eyes, and respiratory tract.)

(b) *Methanolic sodium hydroxide solution*.—0.5M. Dissolve 2 g NaOH in 100 mL methyl alcohol containing  0.5% H₂O. White precipitate of Na₂CO₃ forming on long standing may be ignored.

(c) *Heptane*.—Pure, as determined by GC. If fatty acids containing  20 C atoms are absent in fat or oil, hexane may be substituted.

(d) *Methyl red solution*.—0.1% in 60% alcohol.

(e) *Nitrogen*.—Containing <5 mg O₂/kg.

Check new batches of reagents, particularly BF₃, by preparing and chromatographing methyl esters of pure oleic acid. If extraneous peaks appear (in C₂₀-C₂₂ region with BF₃), reject reagent.

D. Preparation

(Caution: See Appendix B, safety notes on distillation and petroleum ether.)

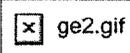
(Work in hood. Wash all glassware immediately after use. If fatty acids containing >2 double bonds are present, remove air from methyl alcohol and flask by passing in stream of N₂ few minutes. Methyl esters should be analyzed as soon as possible. If necessary, heptane solution may be kept under N₂ in refrigerator. For prolonged storage, seal in ampule and store in freezer or add equivalent of 0.005% 2,6-di-*tert*-butyl-4-methylphenol (BHT). For IR analysis, solvent removal must be as complete as possible; for GC, 5–10% solution is suitable.)

Precise weighing is not required. Test sample size need be known only to determine size of flask and amounts of reagents, according to Table 969.33.

Sample ca 350 mg is preferred for GC.

(a) *For fats and oils*.—Add sample to flask and then add methanolic NaOH solution and boiling chip. Attach condenser, and reflux until fat globules disappear (usually 5–10 min). Add BF₃ solution from bulb or automatic pipet through condenser and continue boiling 2 min. Add 2–5 mL heptane through condenser and boil 1 min longer. Remove heat, then condenser, and add ca 15 mL saturated NaCl solution. Stopper flask and shake vigorously 15 s while solution is still tepid. Add additional saturated NaCl solution to float heptane solution into neck of flask. Transfer ca 1 mL upper heptane solution into glass-stoppered test tube and add small amount anhydrous Na₂SO₄ to remove H₂O. If necessary, dilute solution to concentration of 5–10% for GC.

To recover dry esters, transfer aqueous and heptane phases to 250 mL separator. Extract with two 50 mL portions petroleum ether (bp 30–60°C) or hexane. Wash combined extracts with 20 mL portions H₂O until acid-free to methyl red indicator. Dry over anhydrous Na₂SO₄, filter, and evaporate solvent under stream of N₂ on steam bath. If sample is <500 mg, reduce volumes of solvent and H₂O.

More volatile esters may be lost if evaporation is prolonged or if stream of N₂ is too vigorous. For IR spectroscopy, terminate evaporation as soon as solvent is removed. For GC, method is applicable to fatty acids with  8 C atoms, if solvent is not completely removed.

(b) *For fatty acids.*—Add fatty acid to flask, then add BF₃ solution, and continue as in (a) with 2 min boiling under reflux.

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Revised: March 1997

* Adopted as a Codex Reference Method (Type II) for acid hydrolysis, boron trifluoride of linoleate (in the form of glycerides) in special foods.

Table 969.33: Determination of flask size and amount of reagent from approximate test sample size

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