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Product Information

Fatty Acid Extraction Kit for Pet Food

Catalog Number **MAK372**
Store at Room Temperature

TECHNICAL BULLETIN

Product Description

Lipids in food serve as important energy substrates for growth and maintenance. Lipids in foods are composed primarily of triacylglycerides. Other minor components such as phospholipids, free fatty acids and cholesteryl esters may be present. The Fatty Acid Extraction Kit for Pet Food enables the extraction of these lipids in one step. The extracted lipids can then be transesterified and quantified using gas chromatography (GC) with flame-ionization detection (FID).

The Folch method has been conventionally used to extract lipids containing fatty acids from biological samples, using chloroform, methanol, and water to separate lipids from aqueous-soluble compounds.[1] In this procedure, lipids are retained in the lower chloroform layer; whereas, aqueous-soluble compounds are retained in the upper methanol-water layer. The sample is then centrifuged to achieve uniform separation and the bottom chloroform layer is transferred with a pipette to another test tube. An aliquot of the transferred chloroform layer is then transesterified with 14% boron trifluoride in methanol or 1% concentrated sulfuric acid in methanol. This transesterification reaction results in fatty acid methyl esters:

$$R\text{-}COOH \rightarrow R\text{-}COOCH_3$$

The methyl esters can be separated from the transesterification medium with water, and heptane or hexane, and injected directly into a GC-FID system for quantitation.

The Fatty Acid Extraction Kit for Pet Food shortens the extraction process by eliminating the need to centrifuge, pipette, and prepare solvents and standards. Once the sample is homogenized and dissolved in the Extraction Solvent containing the internal standard, it is vortexed and poured into the plunger syringe with filter, which preferentially elutes the chloroform layer containing total lipids. The user then squeezes the plunger to ensure that the lipids are eluted from the syringe filter.

A portion of the total lipid extract containing the total fatty acids can then be transesterified for GC-FID analysis as described in the Procedure. Data comparing the standard Folch method to the Fatty Acid Extraction Kit extraction method are presented under the Results Section.

Components

The kit is sufficient for 40 extractions.

Extraction Solvent containing 0.15 mg/mL of Glyceryl tritridecanoate (13:0-TAG) as an internal standard Catalog Number MAK372A	120 mL
Aqueous Buffer Catalog Number MAK372B	40 mL
Plunger Syringe with Filter Catalog Number MAK372C	40 each

Reagents and Equipment Required but Not Provided.

- Homogenizer to homogenize solid samples
- Capped Pyrex® glass tubes to collect the total lipid extract
- Gas chromatography system (GC), preferably with a flame-ionization detector (FID)
- Polar gas chromatography column
- Sulfuric acid (Catalog Number 258105 or equivalent) in methanol (Catalog Number 1.06011 or equivalent) **OR** Boron trifluoride-methanol solution (Catalog Number B1252 or equivalent)
- Hexane (Catalog Number 227064 or equivalent)

Precautions and Disclaimer
For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
This kit is shipped at ambient temperature. Storage at room temperature is recommended.

Procedure
Sample Preparation
1. Weigh the pet food sample. Add 3 mL of Extraction Solvent to each sample. Lipids can be extracted from up to 0.15 g of sample containing <10% lipids.
2. Homogenize in Extraction Solvent.
3. Add 0.5 mL of Aqueous Buffer to the homogenized sample and vortex.
4. Place the syringe containing the filter on top of a collecting tube that can hold at least 2 mL of liquid.
5. Pour the homogenized sample into the syringe, attach plunger, and push the plunger to elute lipids into the collecting tube. The eluted solvent contains the total lipid extract.
   Note: Avoid excessive plunging. Although the filter selectively traps water/methanol, excessive plunging may inadvertently force water though the filter.
6. The total lipid extract can be transesterified and analyzed by GC-FID.

Transesterification
1. Aliquot 100 µL of the total lipid extract from Sample Preparation, step 5 and dry under nitrogen for transesterification.
   Note: Preliminary testing may be required to establish the appropriate volume to utilize. Less than 5 mg total lipids per sample is sufficient to ensure efficient transesterification.
2. Two suggested reactions for transesterification:
   a. After drying, add 1 mL of 1% concentrated H_2SO4 in methanol and 0.5 mL of hexane. Cap and heat at 70 °C for 3 hours. Add 1 mL of hexane and 1 mL of distilled water.
      OR
   b. Add 1 mL of 14% Boron trifluoride-methanol solution (Catalog Number B1252) and 0.3 mL of hexane. Cap and heat at 95 °C for 1 hour. Add 1 mL of hexane and 1 mL of distilled water.
3. After completing Step 2a or 2b, vortex and centrifuge at 500 × *g* for 5 minutes.
4. Transfer the top hexane layer and dry under nitrogen. Reconstitute the transesterified lipids with 65–100 µL of hexane and add to a GC vial. Inject into a GC-FID system with appropriate column. GC/MS can also be used for quantitation, after establishing response factors for each fatty acid. Volume adjustments may be necessary depending on instrument sensitivity.

Results

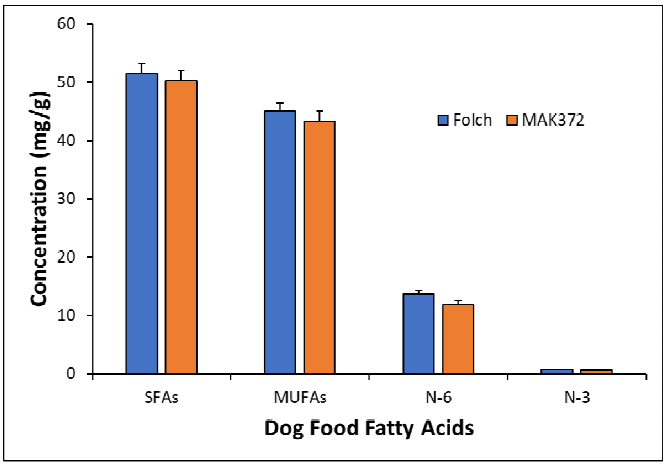
Calculation of GC-FID Results Concentration (mg of fatty acid / g sample) equals:

$$\frac{\text{Amount of internal standard (mg)} \times \text{Area of sample Lipid peak}}{\text{Area of internal standard} \times \text{Weight of tissue (g)}}$$

Amount of internal standard = 0.45 mg when using 3 mL of Extraction Solvent (per sample), which contains Glyceryl tritridecanoate as an internal standard.

Data comparing Folch standard method to MAK372 kit method:

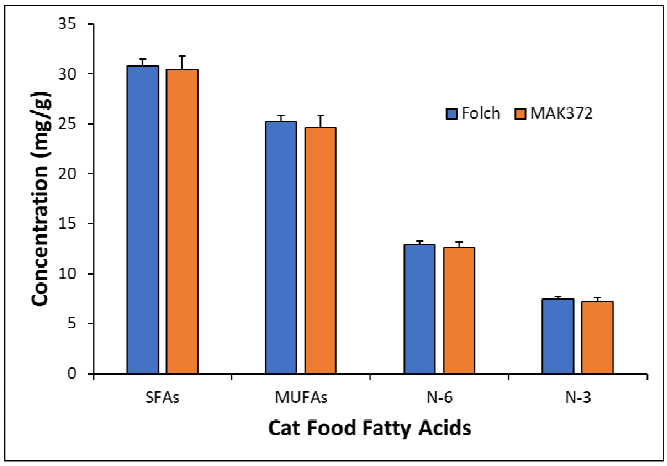
Figure 1.
Dog food fatty acid concentrations (mg/g)



Lipids were extracted from dog food with the Folch or MAK372 kit method, transesterified, and quantified with GC-FID.

SFA = saturated fatty acids
MUFA = monounsaturated fatty acids
n-6 PUFA = omega-6 polyunsaturated fatty acids
n-3 PUFA = omega-3 polyunsaturated fatty acids

Figure 2.
Cat food fatty acid concentrations (mg/g)



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References

1. Folch, J. et al., A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226**, 497-509 (1957).

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