

KORY'S GC TECK-TIPS

How should I analyze fatty acids?

We often get questions on how to analyze fatty acids. Most are wondering what columns are best for specific types of samples and if derivatization would be required. The answers depend on the matrix and the information that is required. There are four different types of columns that we recommend for the analysis of fatty acids and advantages of each outlined below:

- ZB-FFAP – This column is used for the analysis of free fatty acids that have not been derivatized. Most of the lower molecular weight compounds like C2-C8 usually aren't derivatized and may be analyzed by headspace. (see app 8186) Higher molecular weight samples are dissolved and then filtered before injecting. (See app 14856) The end result may not be too clean though so lower lifetimes should be expected.
- ZB-WAX – Good resolution of methylated fatty acids. The WAX column offers better selectivity for unsaturated samples than the lower polarity columns. (See app 16320) However, the samples should be methylated (derivatized) before running on the WAX column. Methylation gives improved peak shape and performance on non-FFAP column, it also lowers the boiling point so they will elute at lower temperatures.
- ZB-1HT, ZB-5HT, or other low polarity columns are recommended when methylated acids are present in higher molecular weight and higher boiling point matrices. Some of the matrix may remain in the column as a residue. A lower polarity column is stable to higher temperature allowing for improved elution of contaminants and longer column lifetimes. Lower polarity columns will not provide the same separation of FAMES as higher polarity columns, but are used for the classification of the general character (C14 %, C16%, etc) of a sample. See app 14863. However, some of the higher polarity columns like the MultiResidue columns do offer better resolution with the advantage of the higher temperature limits. See app 16322.
- The BPX-70 is designed for the analysis of cis- and trans- isomers of fatty acid methyl esters. This analysis has become more popular lately with some countries requiring the percentage of trans fat to be printed on the labels. This column excels at resolving these isomers

Two common methylation (derivatization) procedures used for converting Fatty Acids to Fatty Acid Methyl Esters are listed below:

1. Boron Trifluoride procedure:
 - a. About 10mg of dried Fatty Acids are added to a vial with 1mL of BF₃/Methanol solution [Boron trifluoride (~10% (~1.3 M) in Methanol)]
 - b. The tube is flushed with nitrogen and heated to 100C for 5 minutes.
 - c. After cooling, add 1mL of water and 2 mL of pentane.
 - d. Vortex for 1 min and collect the upper hexane phase.
 - e. Blow down to dryness and reconstitute in 50-200uL of non-polar solvent.
2. Methanolic methylation:
 - a. Up to 10 mg of lipids are dissolved in 2 ml hexane followed by the addition of 0.2 ml of 2 M methanolic KOH in a capped plastic tube.
 - b. The tube is vortexed for 2 min at room temperature.
 - c. After a light centrifugation, an aliquot of the hexane layer is collected for GC analysis.

For further support, please contact Phenomenex at the below e-mail address.

Key words: Fatty acids, Isomer, FAMES

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