



## Gas Chromatographic Analysis of Free Fatty Acids in Saw Palmetto

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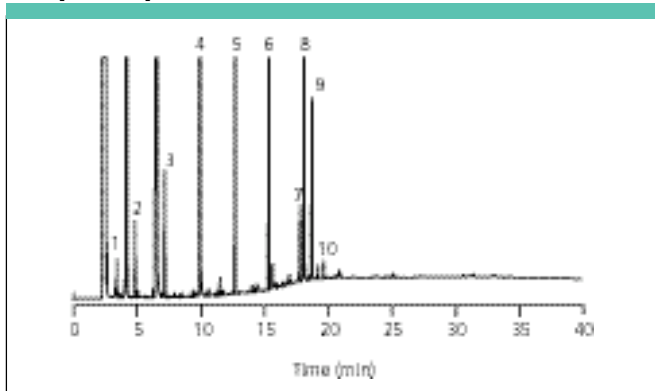
**This application shows the analysis of free fatty acids present in saw palmetto using Meth-Prep™ I derivatizing reagent and an Alltech AT™-FAME capillary column. The compounds of interest produced sharp, narrow peaks that did not exhibit peak tailing.**

The berries from saw palmetto (*Serenoa Repens*) have been used in folk medicine in sex-related therapies. Reportedly, they have been used by men and women to increase sexual vigor. They have been used to tone and strengthen the reproductive systems of men and to treat symptoms associated with enlarged prostate. Saw palmetto has also been used to increase the size of underdeveloped mammary glands and to reduce enlarged ovaries in women.

Saw palmetto berries contain a number of physiologically active compounds, including fatty acids, phytosterols, and monoacylglycerides. The berries are commercially available in many forms, including teas, capsules, tinctures, liquids, and semisolid extracts. Many of these forms use ground saw palmetto berries combined with filler materials that may include vegetable oils such as pumpkin oil.

We chose to use a GC procedure to characterize the free fatty acids because of its resolving power relative to HPLC. The free acids in the saw palmetto sample are first converted to their volatile methyl esters. The sample preparation must be carried out with special care so the triglycerides from the filler materials are not also converted into FAMES.

### Sample Preparation



**Figure 1:** Analysis of saw palmetto free fatty acids as FAMES on an AT™-FAME capillary column. Peaks: 1 = C6:0 methyl ester, 2 = C8:0 methyl ester, 3 = C10:0 methyl ester, 4 = C12:0 methyl ester, 5 = C14:0 methyl ester, 6 = C16:0 methyl ester, 7 = C18:0 methyl ester, 8 = C18:1 methyl ester, 9 = C18:2 methyl ester, 10 = C18:3 methyl ester.

The free fatty acids in the sample are derivatized prior to GC analysis. Combine 30 mg of saw palmetto sample with 1 mL of Meth-Prep™ I reagent (Alltech Part No. 18005) following the procedures supplied with the reagent. Meth-Prep™ I derivatizes fatty acids to form fatty acid methyl esters in the injection port of the GC at temperatures greater than 240 °C.

Meth-Prep™ I is a 0.2 N aqueous solution of (*m*-trifluoromethylphenyl)trimethyl-ammonium hydroxide. When mixed with a sample, free fatty acids are extracted into the aqueous medium. When the aqueous layer is injected into a GC, the free fatty acids and the derivatizing reagent form a salt that thermally decomposes to the FAMES. Triglycerides present in the sample are not affected by this procedure and do not interfere with the determination.

### Instrumentation and Equipment

We used a gas chromatograph equipped with a split/splitless injector and a flame ionization detector and a Heliflex® AT™-FAME capillary (30 m × 0.25 mm, 0.25 μm  $d_p$ , Part Number 12436, Alltech Associates).

### Experimental Conditions

Helium was the carrier gas at a linear velocity of 25 cm/s. The injector temperature was 240 °C. Sample injection volume was 1 μL with a split ratio of 25:1. The FID temperature was 260 °C. The separation was done with the following temperature program: initial temperature of 110 °C, hold for 1 min, ramp temperature at 8 °C/min to a final temperature of 240 °C, hold for 25 min.

### Conclusion

The free fatty acid content of commercially available saw palmetto nutraceuticals can easily be analyzed using Meth-Prep™ I derivatizing reagent and the AT™-FAME capillary column (Figure 1). Meth-Prep™ I reagent is a one-step derivatizing reagent that quickly and cleanly derivatizes fatty acids in the GC injection port in seconds. The AT™-FAME column is a stable bonded polyethylene glycol capillary column especially designed and tested for the reproducible analysis of fatty acid methyl esters.

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