Analysis of Fatty Acids and Fatty Acid Esters in Saw Palmetto by Supercritical Fluid Chromatography (SFC) without Derivatization

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Introduction

- Fatty Acid content of natural products is typically determined by GC or HPLC techniques which both involve chemical modification of the compounds
- GC requires the derivatization of the fatty acids to methyl esters (FAME) leaving doubt as to whether the esters found are naturally occurring or formed through the derivatization process
- SFC is capable of analyzing fatty acids without derivatization as well as the natural occurring methyl esters

Experimental Design

- Develop a method for the separation of fatty acids and triglycerides present in saw palmetto oil using supercritical fluid chromatography as the analysis technique and eliminate the need for derivatization of the sample.

Advantages of Capillary SFC

- High resolution capillary column separations
- Use of universal detectors such as the flame ionization detector (FID)
- Elute compounds without the use of added modification of the mobile phase
- The use of pressure programming to effect phase solubility change
- The use of low temperatures to analyze temperature sensitive compounds

SFC Instrumentation

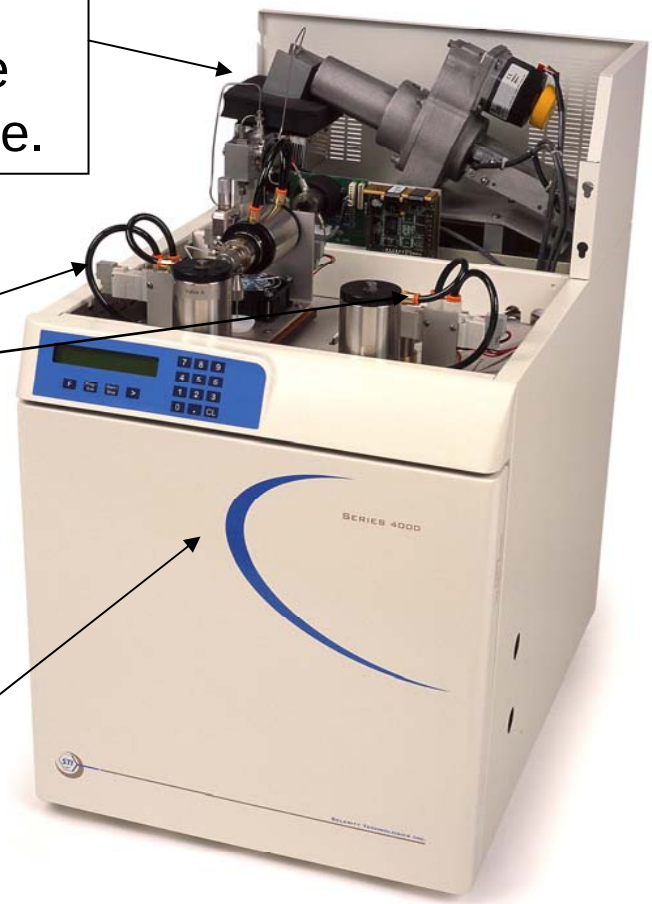
- Selerity Technologies Series 4000 SFC
  - Flame ionization detector for universal detection
  - Syringe pump for pulse-free carbon dioxide delivery
  - Thermally stabilized pressure transducer for accurate pressure (retention time) control
  - EzChrom Elite data acquisition software package

Series 4000 SFC

The high pressure syringe pump is conveniently place on the back of the oven cabinet to save laboratory space.

The SFC system configured for capillary column use is equipped with split/splitless injection and an FID

The high capacity oven moves the air rapidly through the oven cavity for rapid heating and cooling.

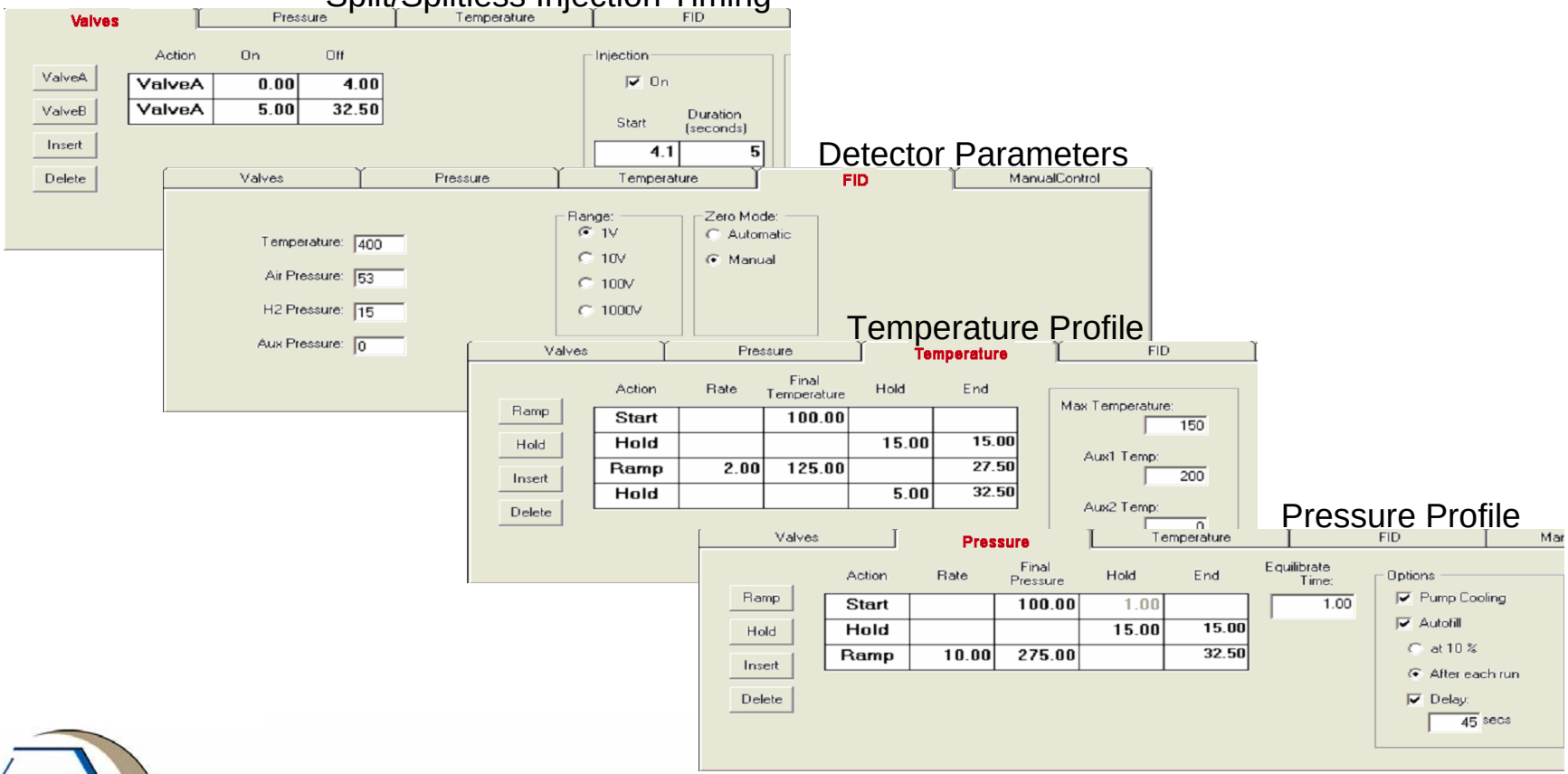


Split/Splitless Injection for Capillary SFC

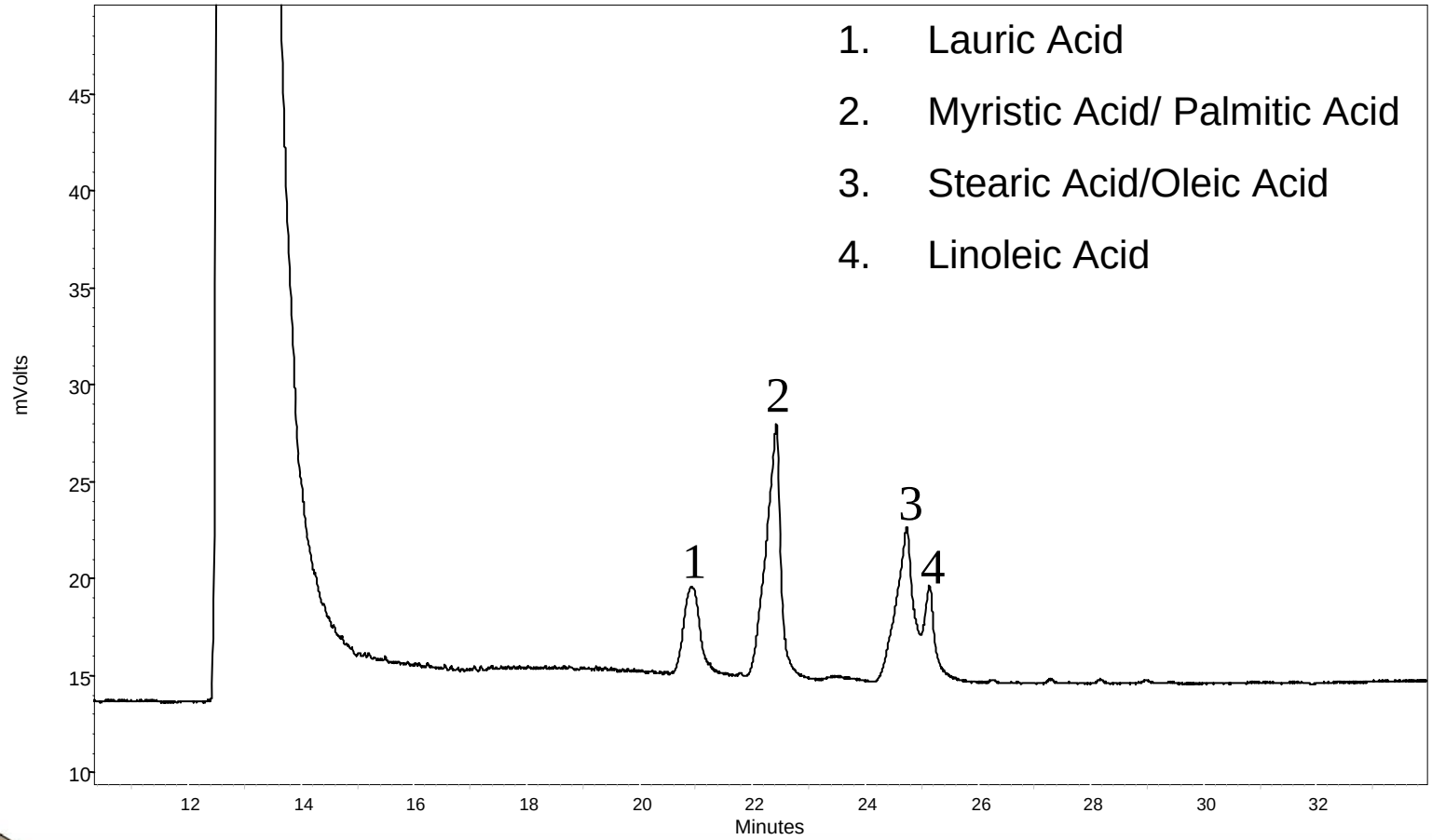
- Direct injection of the sample onto the column while splitting off dead volume
- Increases sample capacity
- Improves peak shape
- Decreases solvent affects of early eluting peaks
- Separates volatiles from solvent

Run Conditions

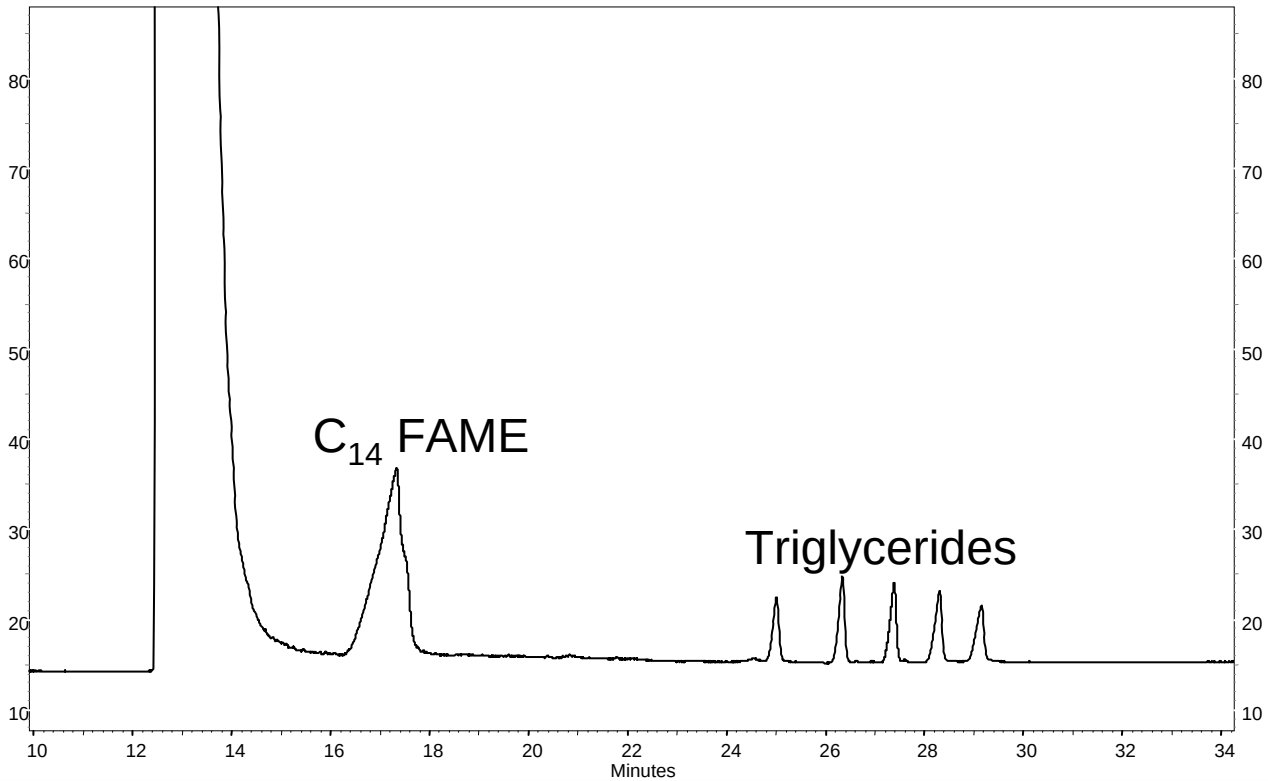
Column: SB-Cyanopropyl-25, 50μm ID X 195 μm ID, 0.25 film, 10 m



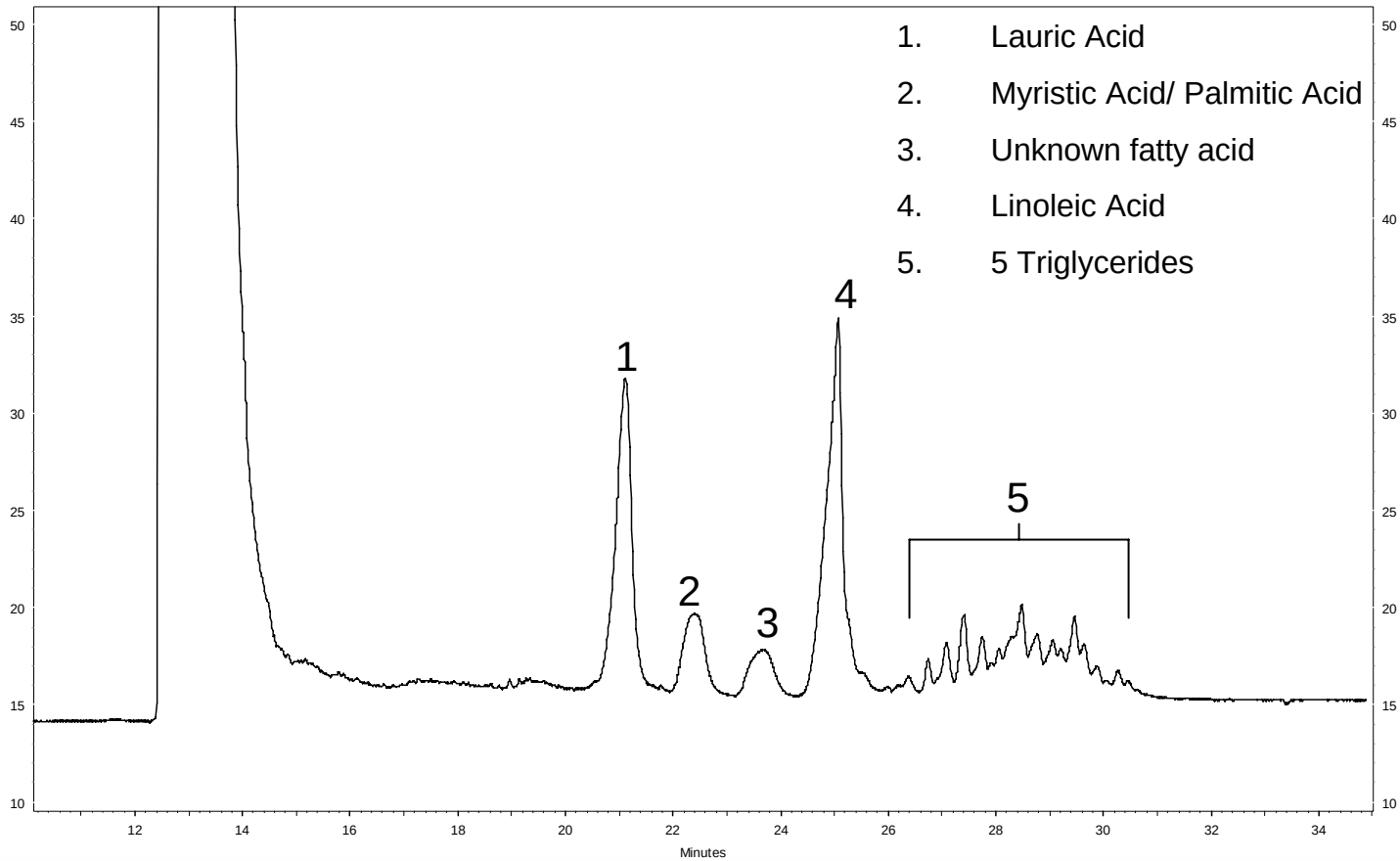
Fatty Acid Standard Solution



C_{14} FAME and Triglyceride Standard Solution



Saw Palmetto Oil



Conclusion

- SFC is an effective tool for the separation of natural.
- Fatty acids can be analyzed by SFC without the added step of derivatization
- FID provides detection of fatty acids and triglycerides where UV would not under HPLC conditions
- Further work using different selection of column stationary phases is needed to further enhance the separations