

# 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

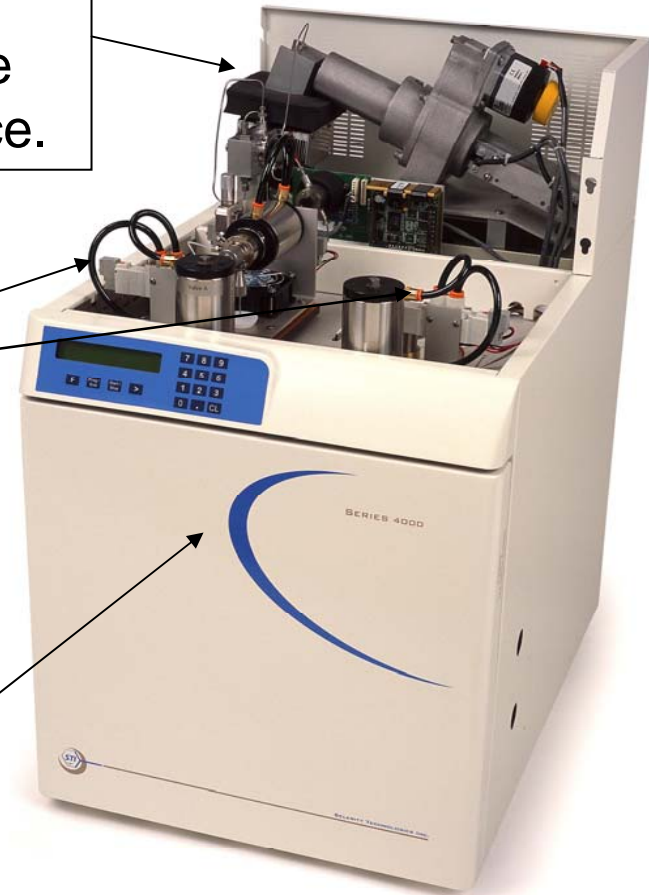


# Series 4000 SFC

The high pressure syringe pump is conveniently placed 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 effects of early eluting peaks
- Separates volatiles from solvent



# Run Conditions

Column: SB-Cyanopropyl-25, 50 $\mu$ m ID X 195  $\mu$ m ID, 0.25 film, 10 m

## Split/Splitless Injection Timing

**Valves**

Action	On	Off
ValveA	0.00	4.00
ValveB	5.00	32.50

Injection:  On

Start: 4.1      Duration (seconds): 5

Buttons: ValveA, ValveB, Insert, Delete

## Detector Parameters

**FID**

Temperature: 400

Air Pressure: 53

H2 Pressure: 15

Aux Pressure: 0

Range:  1V,  10V,  100V,  1000V

Zero Mode:  Automatic,  Manual

Buttons: Ramp, Hold, Insert, Delete

## Temperature Profile

**Temperature**

Action	Rate	Final Temperature	Hold	End
Start		100.00		
Hold			15.00	15.00
Ramp	2.00	125.00		27.50
Hold			5.00	32.50

Max Temperature: 150

Aux1 Temp: 200

Aux2 Temp: 0

Buttons: Ramp, Hold, Insert, Delete

## Pressure Profile

**Pressure**

Action	Rate	Final Pressure	Hold	End
Start		100.00	1.00	
Hold			15.00	15.00
Ramp	10.00	275.00		32.50

Equilibrate Time: 1.00

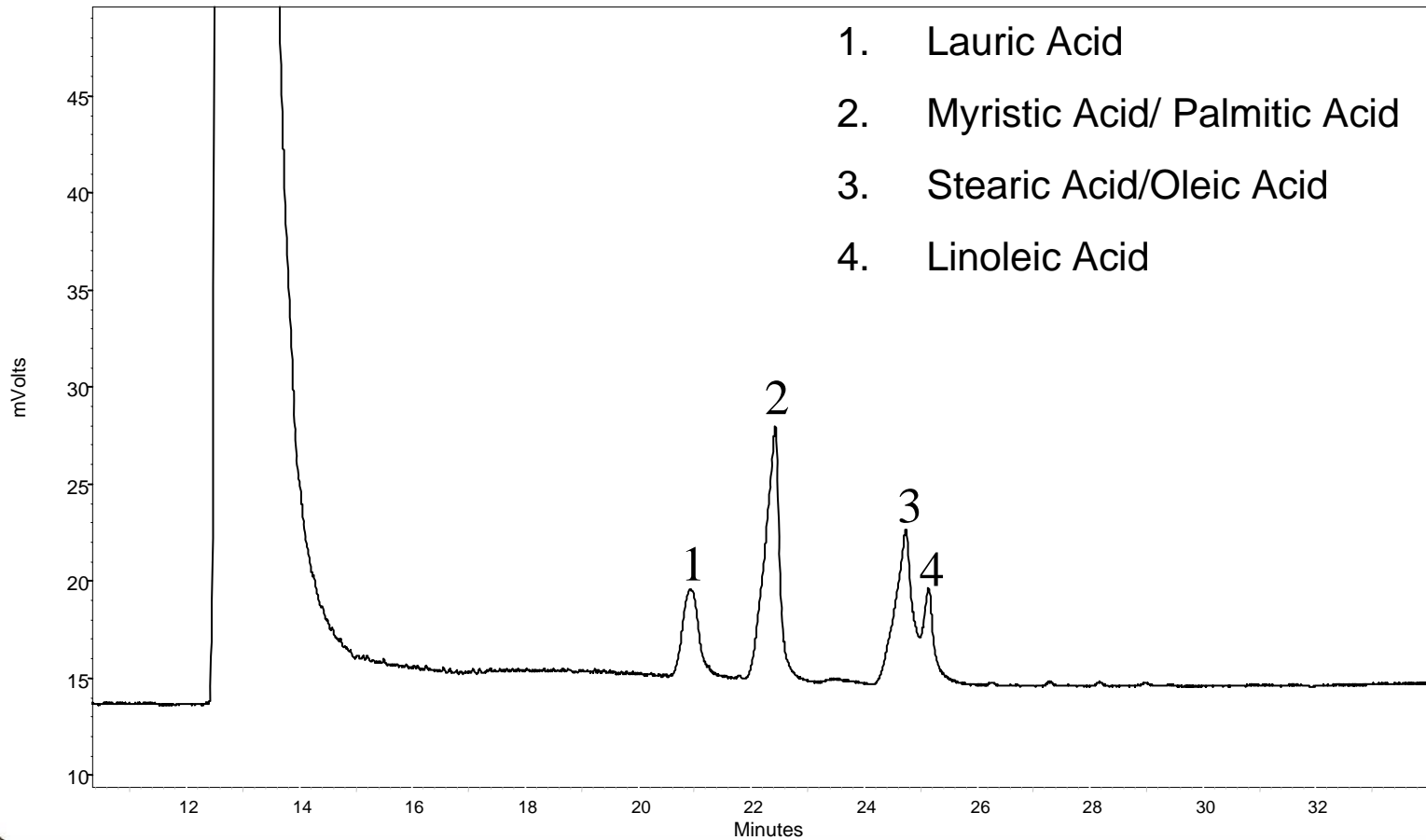
Options:  Pump Cooling,  Autofill,  at 10%,  After each run,  Delay: 45 secs

Buttons: Ramp, Hold, Insert, Delete

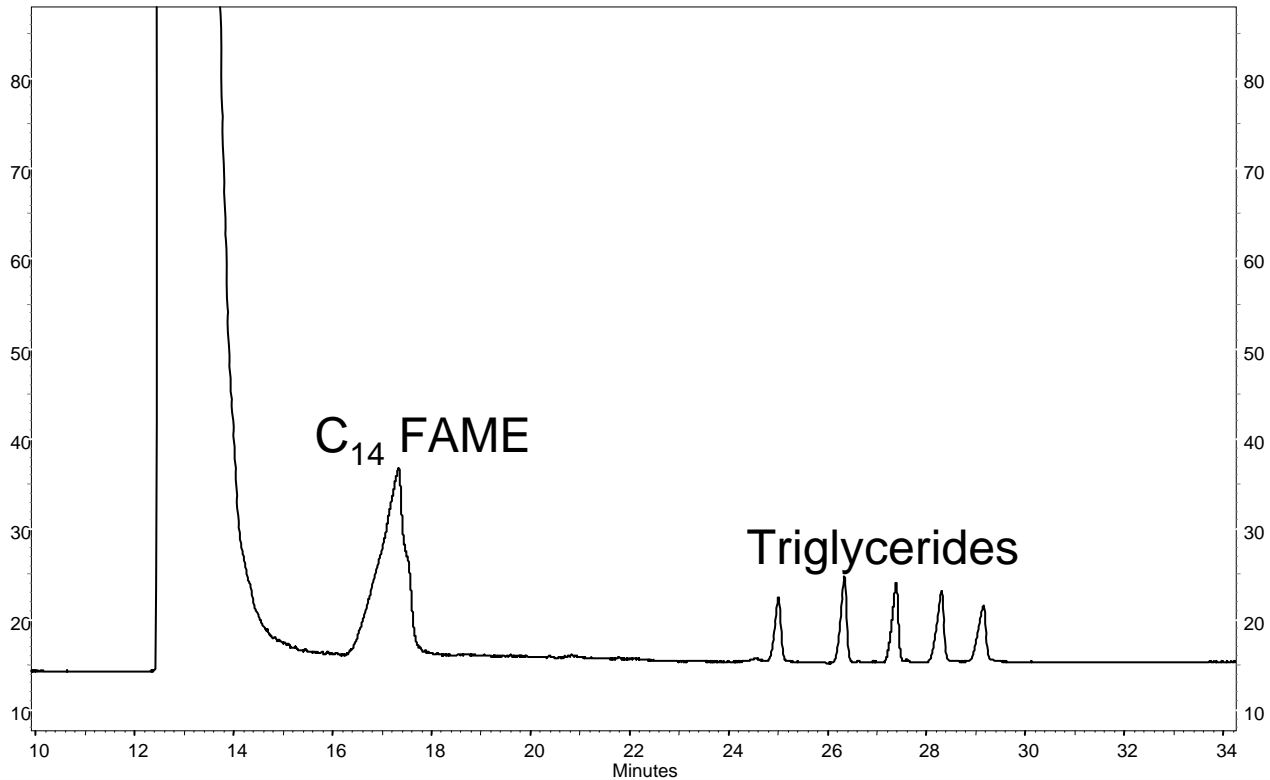




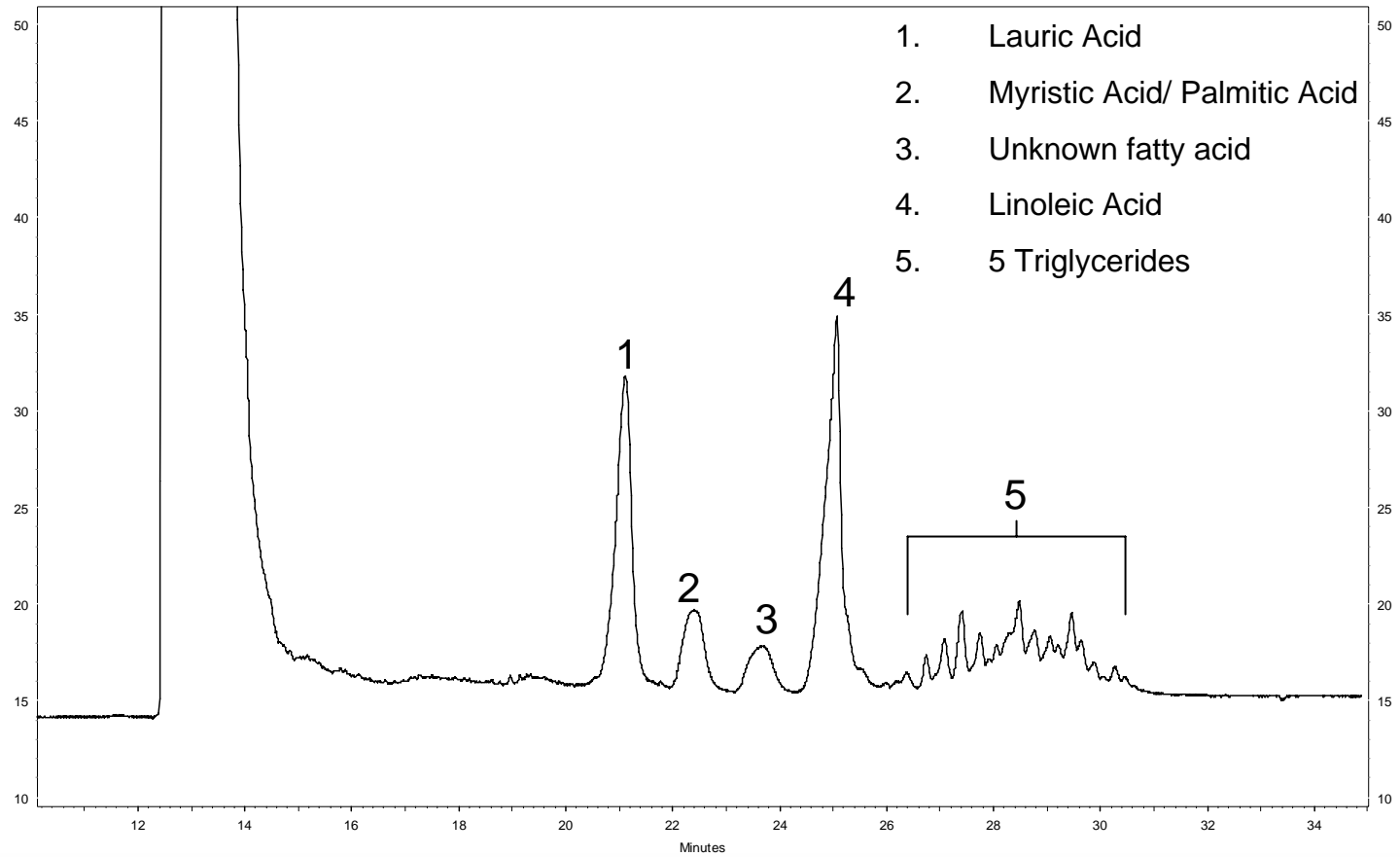
# Fatty Acid Standard Solution



# C<sub>14</sub> 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

