

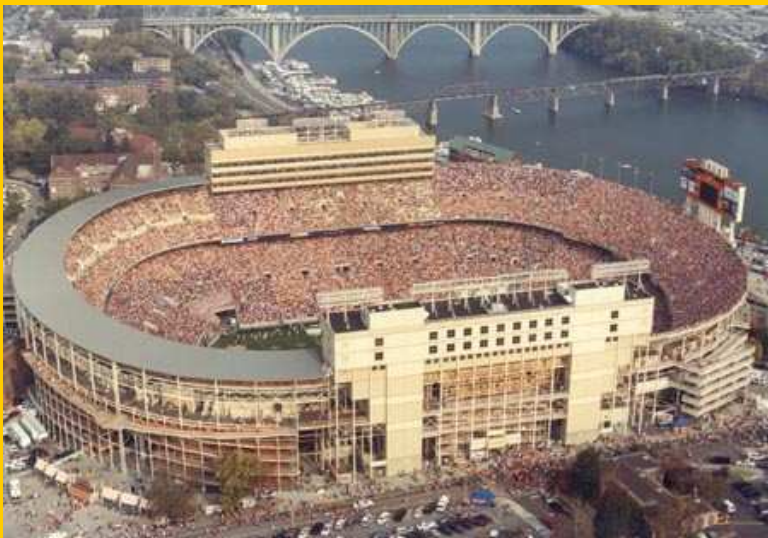
Packed-Column SFC-Based Analysis of Lipid Modification Reactions: Overview and Applications

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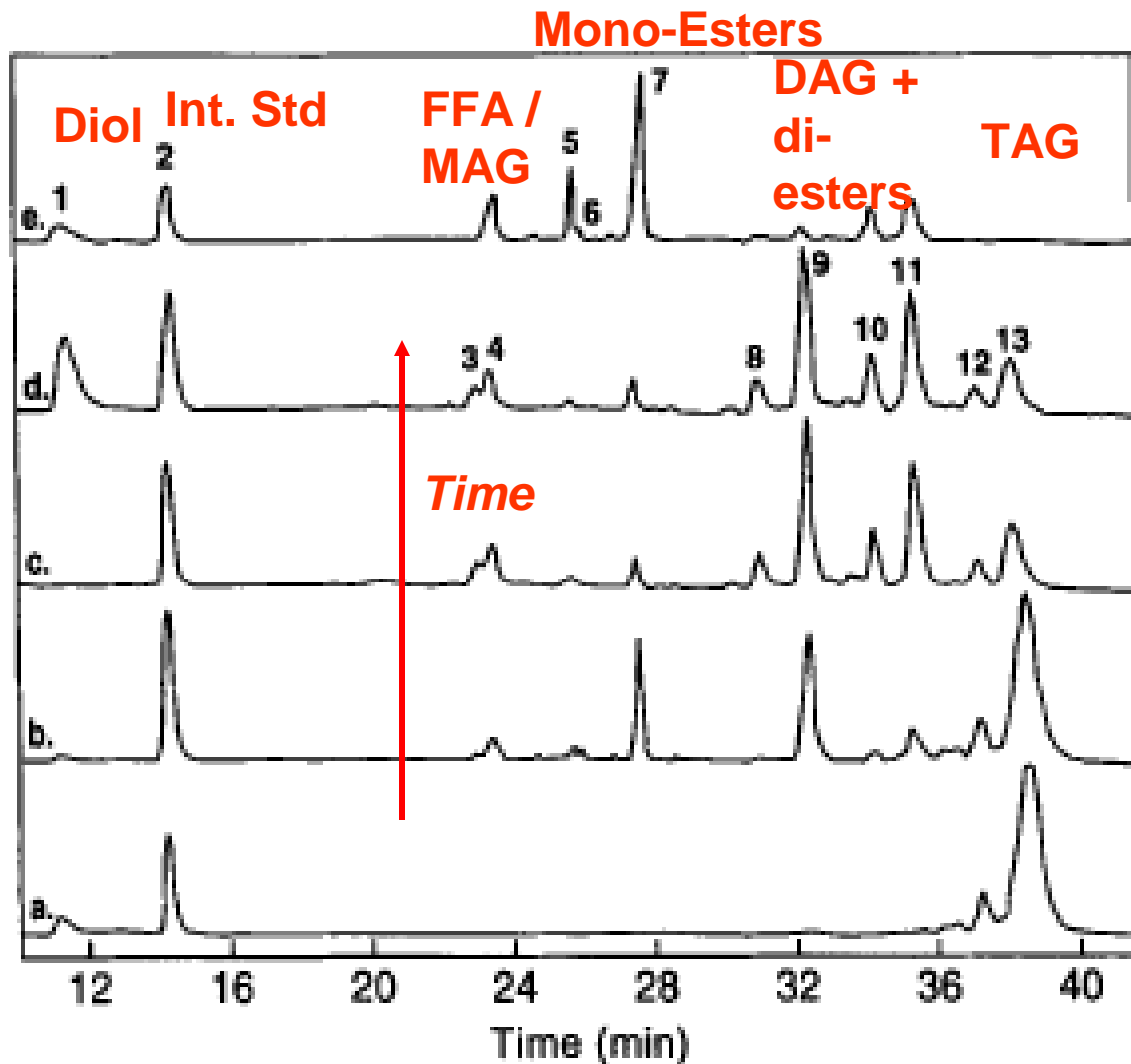
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Why SFC for Lipid Analysis?

- **Low solvent background signal: m**
- **Many detector choices available**
- **Quicker analysis times possible**
- **Low temperature: no degradation of oxygenated molecular groups; double bonds**
- **Derivatization of analytes not needed**
- **Low column wear-and-tear; reduced solvent waste**

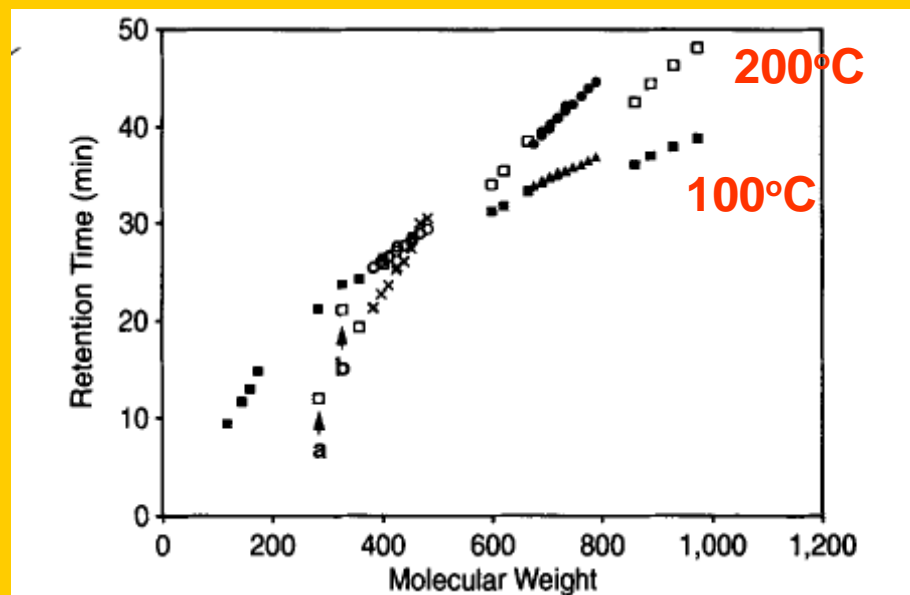
Capillary SFC: Analysis of Enzymatic Alcoholysis of TAG



- Column: SB-Methyl-100 (10 m x 50 μ m i.d)
- Pure CO₂
- 100°C
- FID
- 125 atm (9 min); 5 atm/min to 400 atm
- Hayes and Kleiman, *JAOCS*, 73:1691-1697 (1996)

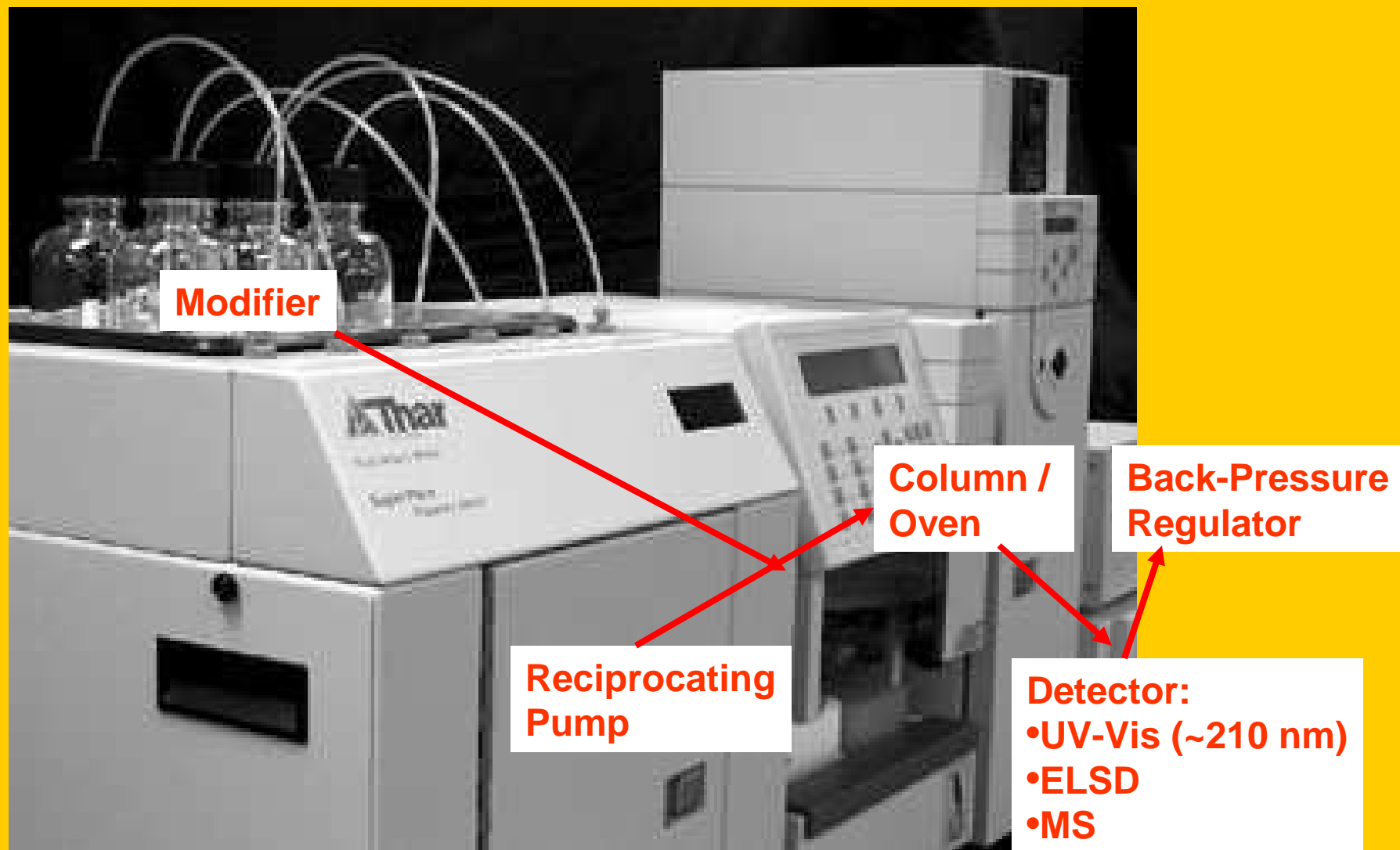
Capillary SFC of Neutral Lipids: Summary

- + Universal separation by MW (nonpolar column)
- + Pure CO₂ → FID detector allowable
- - Injected volume indirectly controlled
- - Frit Restrictor
 - Mobile phase velocity is not externally controlled
 - Variation of retention time (week-to-week)
 - Difficult to change/fix



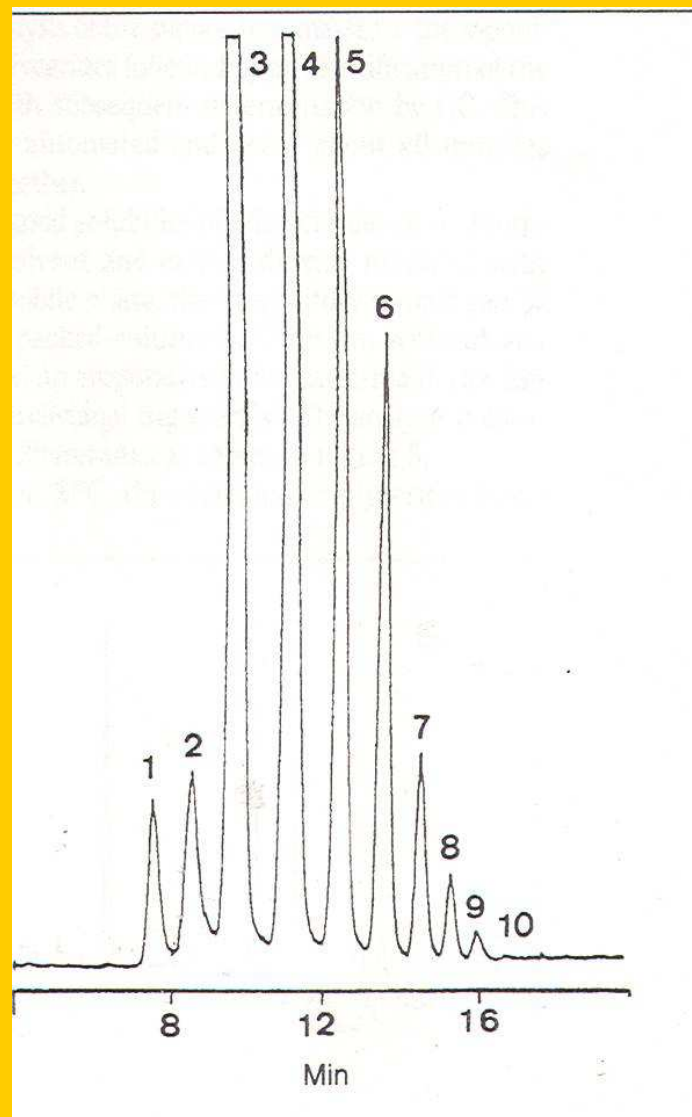
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Packed-Column SFC



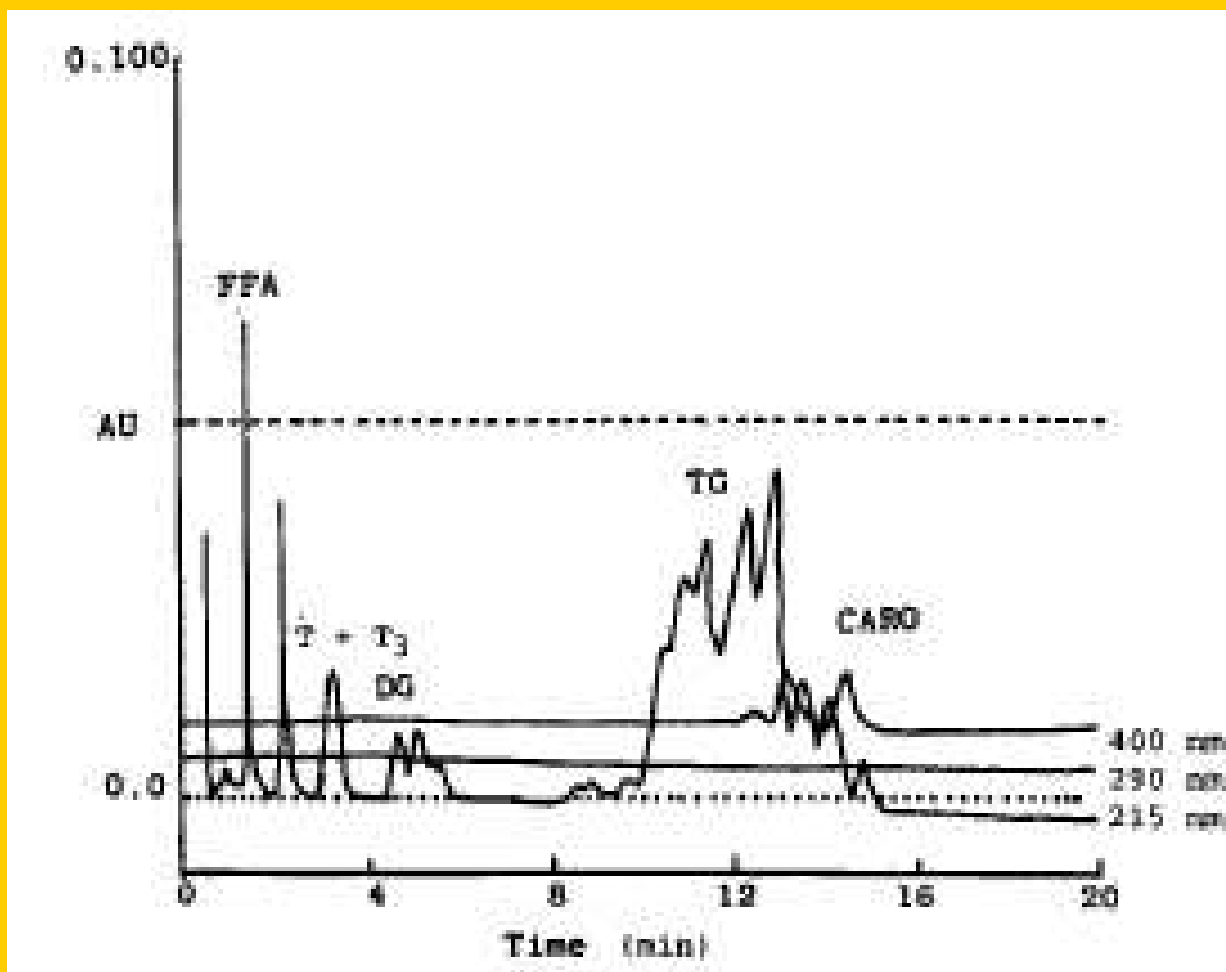
TAG Seps Based on MW / Carbon Number

- 1-10: TAG-32 to TAG-50
- Cyanopropyl column
- MeOH modifier: 2.4 vol %
- 2.6 mL / min
- 150°C
- 190 bar (0-9 min); ↑ 225 bar (14 min)
- ELSD
- Anton et al., *J Chrom Sci* 32: 430 (1994).



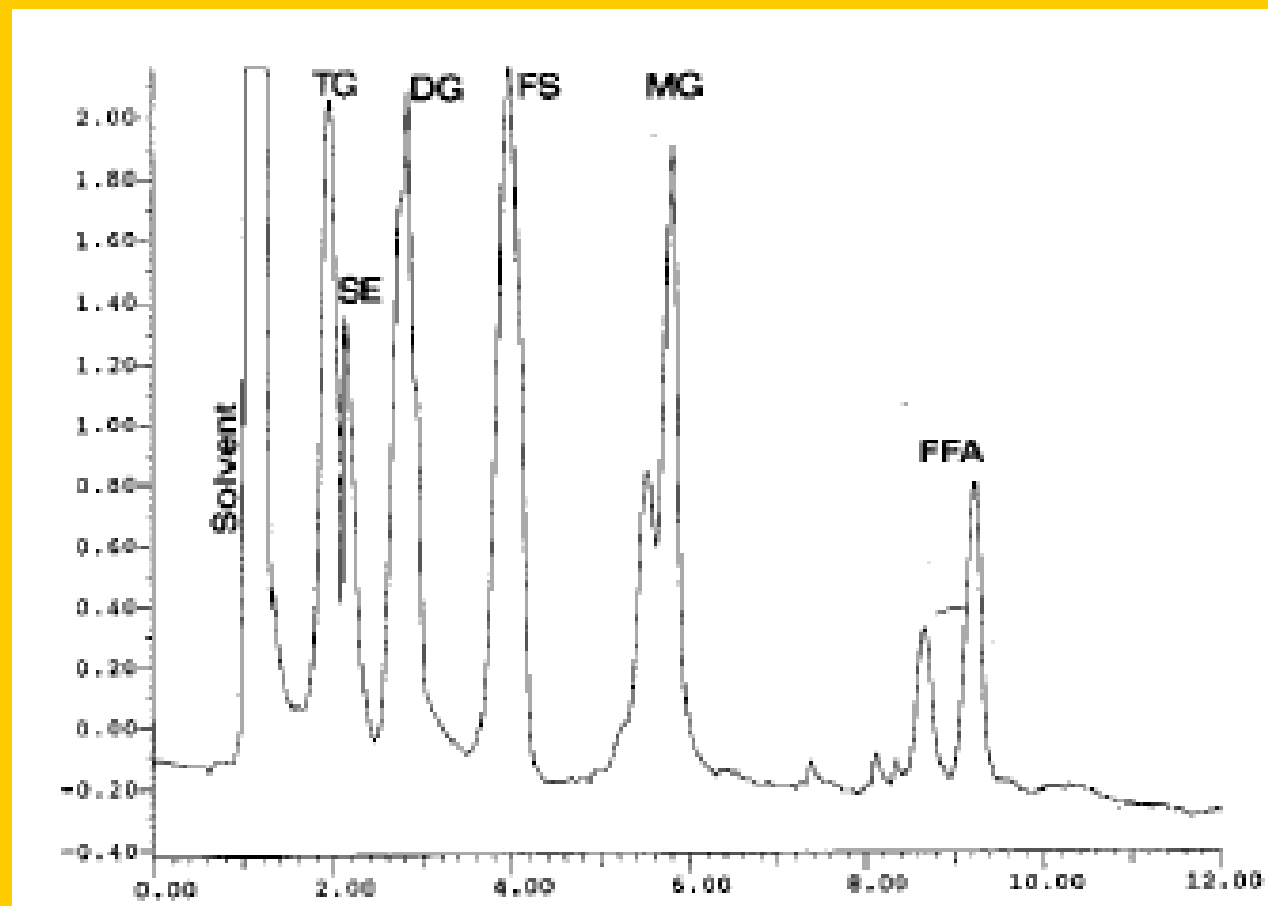
Separation of Glycerides + Lipid Fractions from Crude Palm Oil

- C₁₈ column
- EtOH, 6%
- 3.2 mL/min;
- 180 bar (isobaric)
- 70°C
- Choo et al., *JAOCS* 73: 523 (1996)



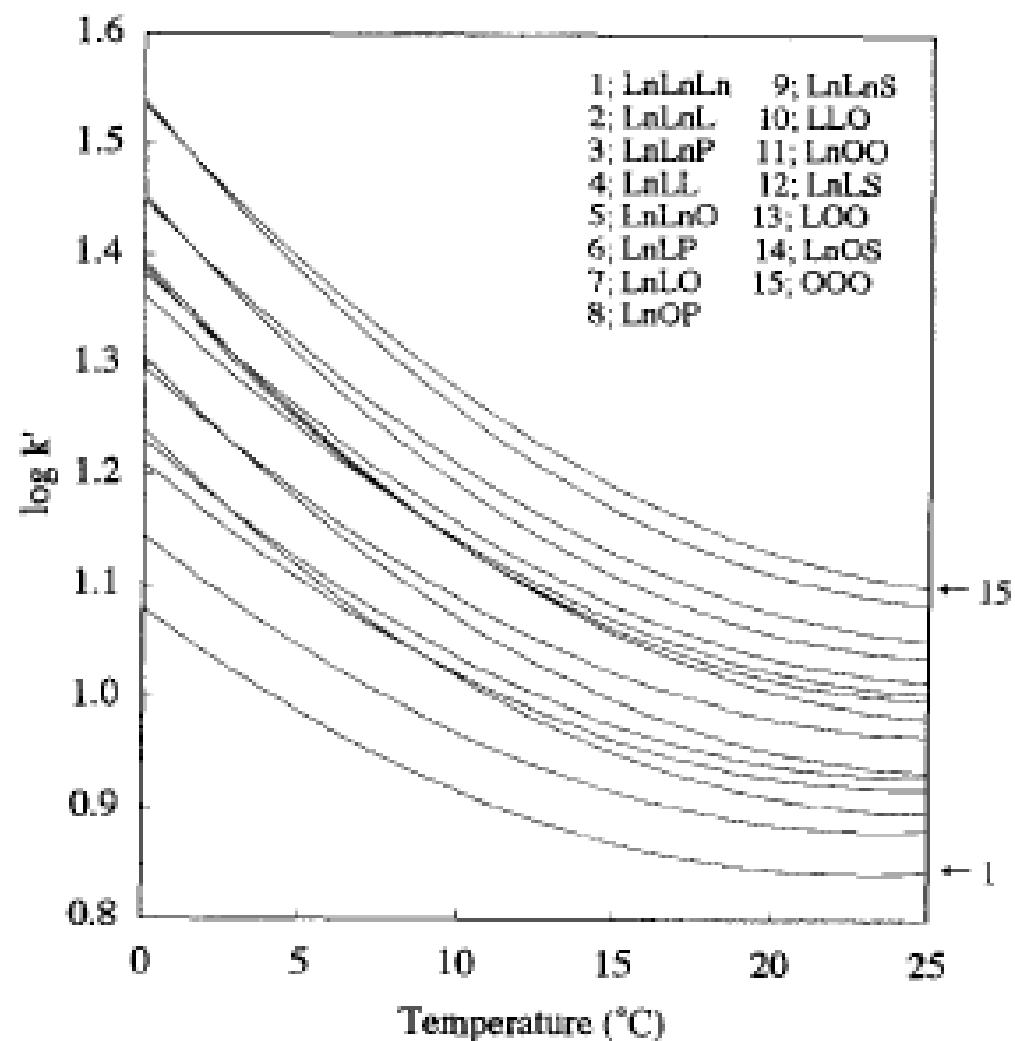
Separation of Glycerides + Sterols

- Aminopropyl silyl column
- MeOH, 8%
- 2.0 mL/min
- 150 bar (isobaric)
- 70°C
- Medvedovici et al, *Chromatographia* 44:37 (1997)



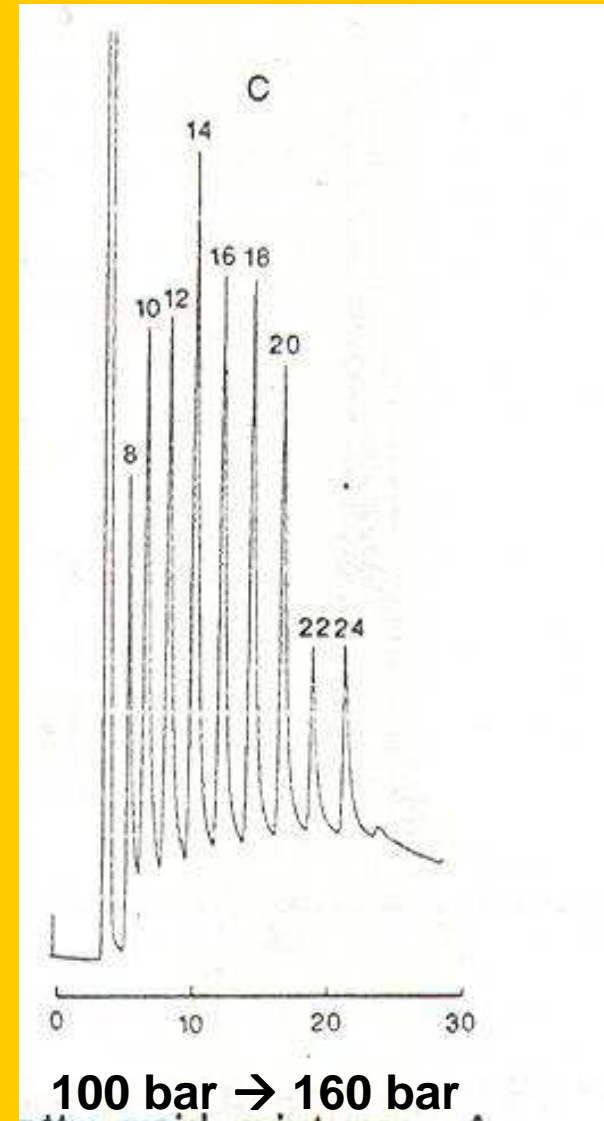
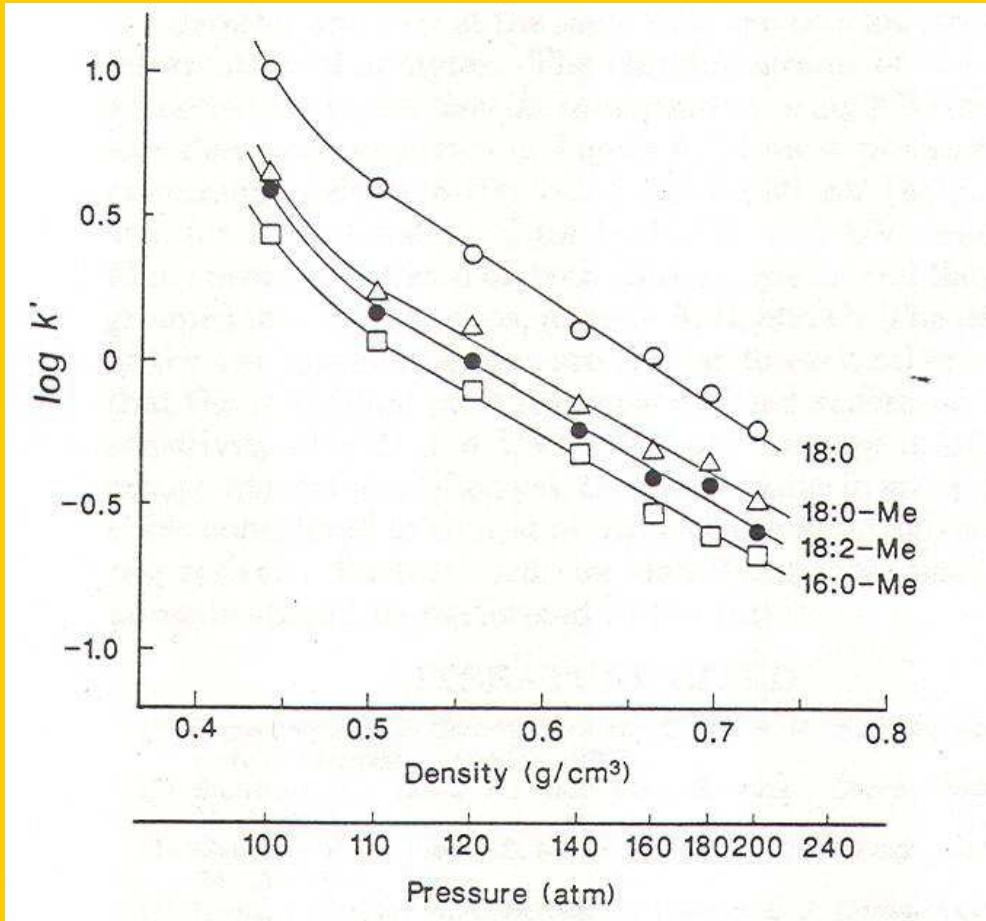
TAG Separations via Subcrit FC

- C_{18} Column
- CO_2 , 3 mL/min
- 150 atm (isobaric)
- UV (210 nm) detector
- Funada and Hirata, *J Chrom A* 764: 301 (1997).



FFA / FAME separation on C₁₈ Column

- 45°C, no modifier, UV-vis (190 nm) detector
- Nomura et al, *Anal Chem* 61: 2076 (1991)



Separation of C₁₈ FFA by Degree of Saturation

- Aminopropylsilyl
- CO₂
- 150 bar (isobaric)
- UV (200 nm)
- 40°C
- Sakaki, *J Chrom Sci* 648: 451 (1993).

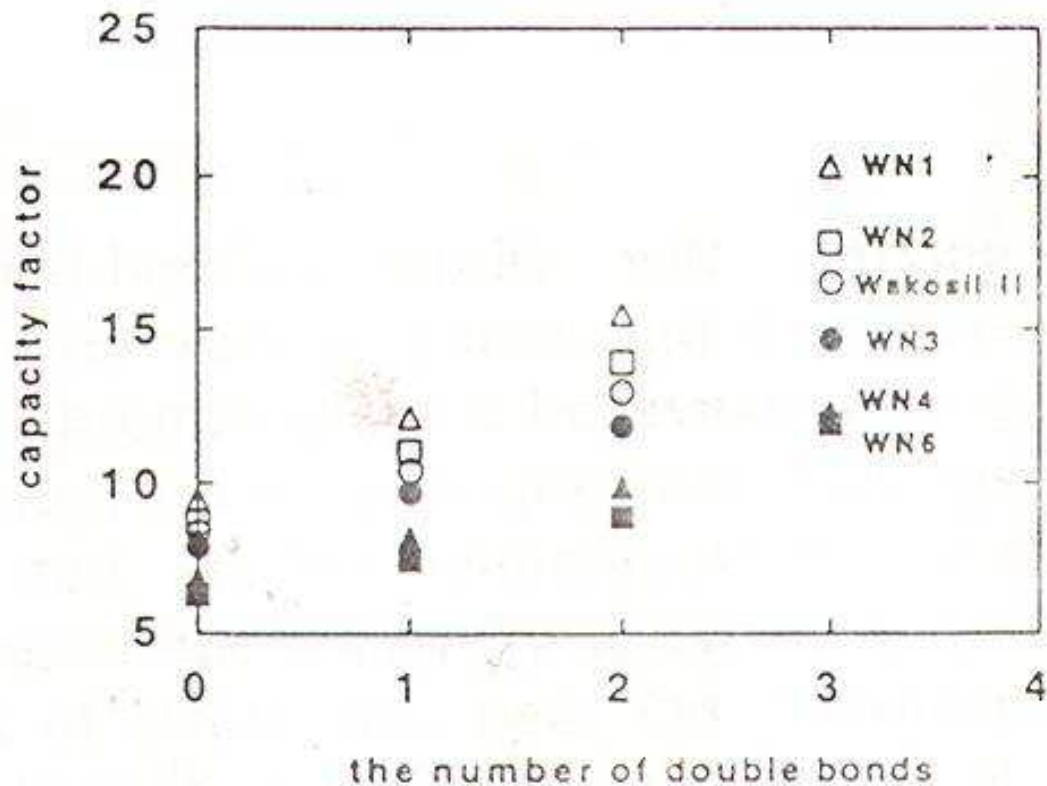
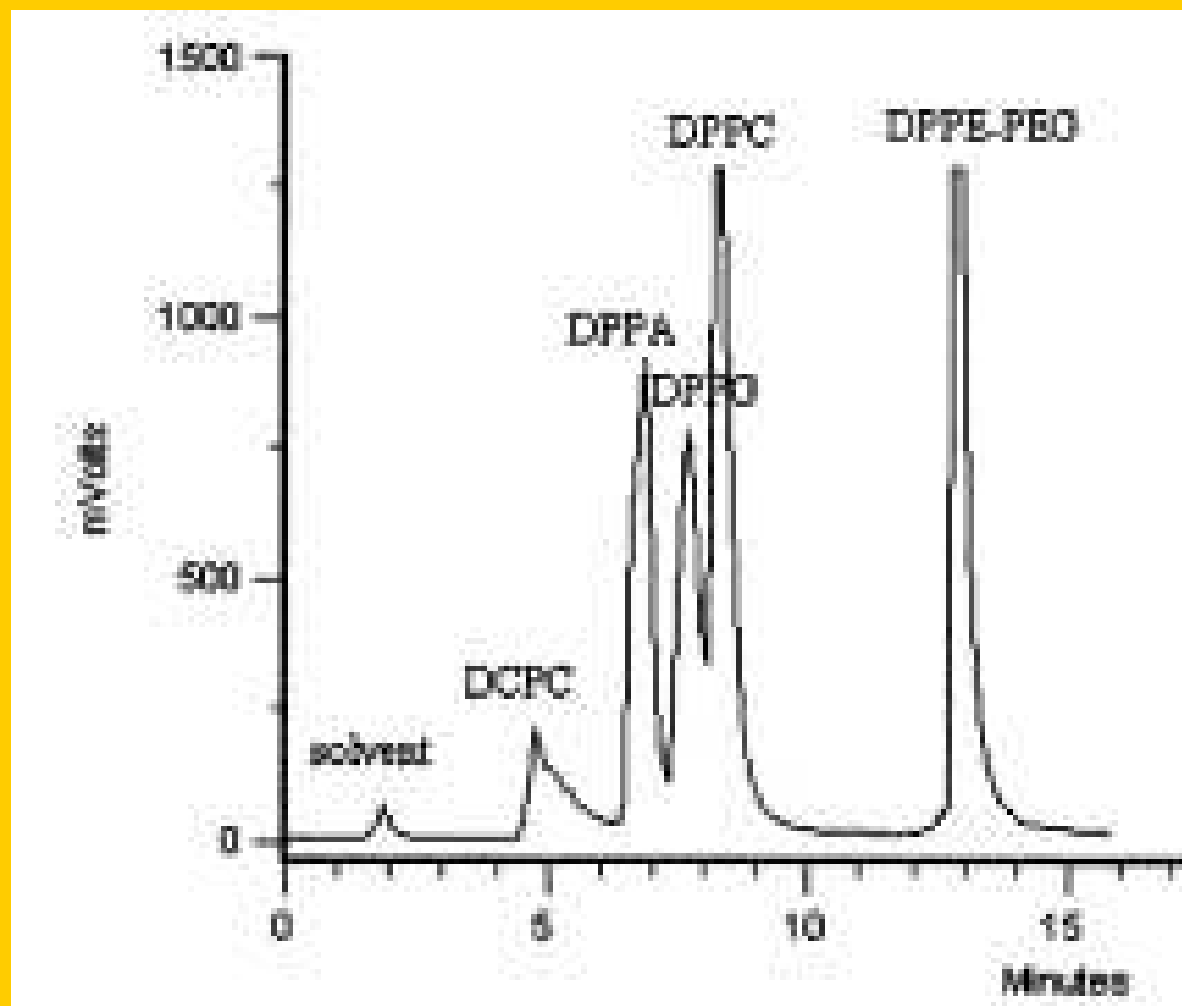


Fig. 6. Plots of capacity factors of fatty acid methyl esters

Phospholipid Separations

- C₈ column
- EtOH / MeOH / CF₃COOH;
gradient
- 2 mL / min
- 125 bar
- 70°C
- ELSD
- Eckard et al,
J Chrom A
826: 241
(1998)



Tocopherol Separations

L. Jiang et al. / *J. Chromatogr. A* 1005 (2003) 153–164

157

UV-Vis @
205 nm

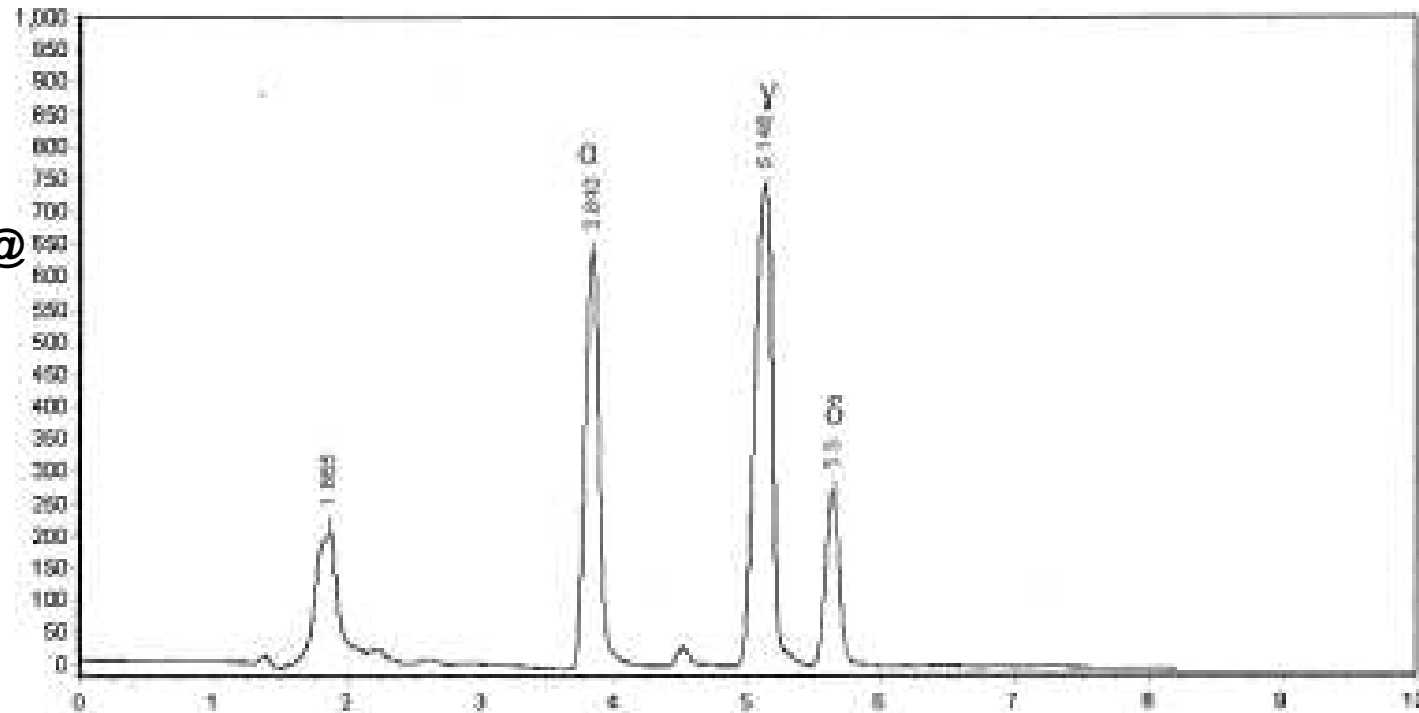
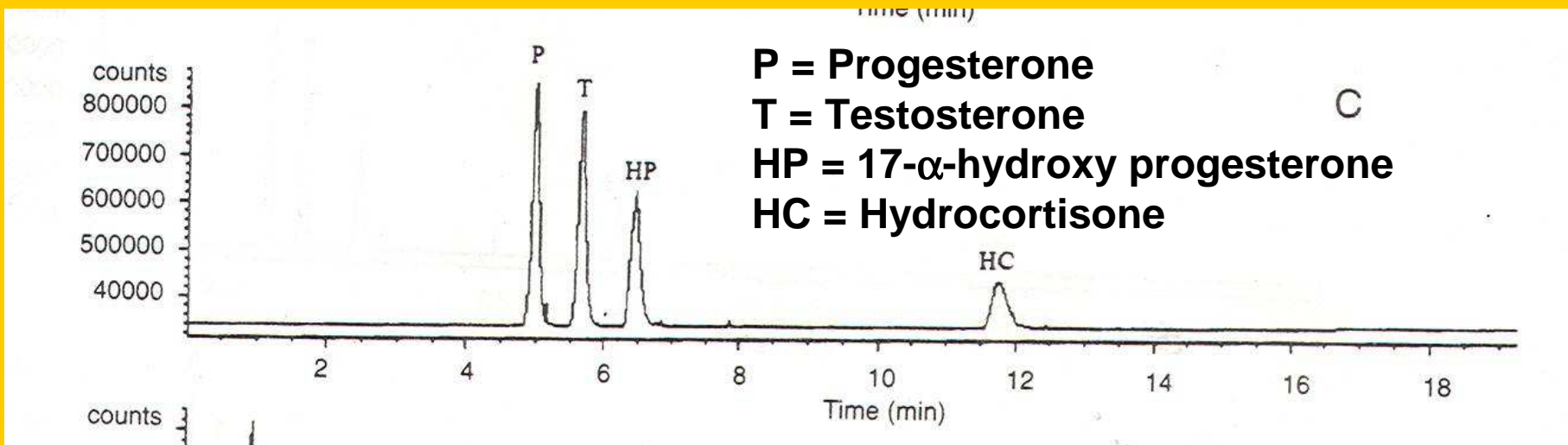


Fig. 2. Chromatogram of tocopherols. Conditions: 3.98% ethanol, 18 MPa, 40 °C, Waters Spherisorb 5 μ m silica column (250 \times 3.9 mm I.D.).

Jiang et al, *J Chrom A*, 1005: 155 (2003)

Separation of Steroids



- **Cyanopropyl Column**
- **5% MeOH modifier**
- **2.0 mL / min**
- **60°C**
- **100 bar (1.5 min); \uparrow 350 bar (15 bar / min)**
- **ELSD**
- **Thompson et al, *J Chrom Sci* 34: 361 (1996).**

Conclusions

- **SFC / Packed Columns allow for effective universal separations of neutral and saponifiable lipids**
- **Separation of lipid classes / carbon number-based separations: use of C₁₈ (or cyanopropyl) column, ~180 bar, ~70°C**
- **Subcritical FC / C₁₈ column is effective for separating TAG by carbon number and degree of saturation**

Conclusions (cont'd)

- **Separation of saponifiable lipids requires polar columns; more effort to optimizing pressure and gradient programming conditions**
- **SFC can provide at worst equal separations as obtained by HPLC and/or GC; sometimes can provide shortened run times compared to HPLC**
- **Advantages of packed-column SFC vs HPLC: more detector choices; reduced solvent use**