Fatty Acids Analysis Using GC - Theory and Practice
Gas Chromatography

- Gas-liquid chromatography
  - Adsorbed inner surface of capillary column
- Gas-solid chromatography
  - Porous polymer beads or zeolites
- Separates \textit{vaporized} sample as a consequence of partition between a stationary phase and a mobile phase
Application Areas for GC

- Composition Analyses
  - Fatty Acid Analysis
  - Cholesterol, Tocopherols, and Phytosterols
  - Amino Acid Analysis
  - Spices

- Quality Analyses
  - Lipid Oxidation: hexanal and hydrocarbons
  - Volatiles - Odor and Flavor Compounds
GC Unit

- GC Oven
- Column
- Detector
- Sample Injector
- Control System
Inlet Conditions

- Split/Splitless Inlet
  - Sample Concentration
- Inlet Temperature
  - High Enough to Volatilize Sample
  - Peak Tailing
Autosampler
Oven Temperature Conditions

- Initial and Final Temperature
  - Column
  - Sample
- Constant Temperature
- Programmed Temperature
  - Uniform Increase
  - Multiple Step Increase
Important Parameters to be Considered in GC Analysis

- Column Selection
- Column Flow Rate
- Oven Temperature Conditions
- Inlet Conditions
- Injector: Auto/Manual
- Detector Selection
- Gas Selection: Depending on Detector
Selection of Column

- Sample
- Stationary Phase Type
- Stationary Phase Film Thickness
- Column ID
- Column Length
Stationary Phase

- Bonded Phase
  - Stationary phases are immobilized/crosslinked within the tubing
- Nonbonded Phase
  - Stationary phases are coated on the wall of tubing
- The stationary phase dictates the min/max temperatures at which column can be used.
Selection of Phase Type

- Differences in the chemical and physical properties of the injected organic compounds and their interactions with the stationary phase are the basis for the separation process.

- Phase Polarity: “Like Likes Like”
  - Nonpolar Molecules: n-Alkanes
  - Polar Molecules: Compounds with one or more atoms of Br, F, Cl, O, N, P, S
  - Polarizable Molecules: with one or more of double or triple carbon-carbon bonds
Stationary Phase Film Thickness

- Increasing film thickness
  - Increases the maximum sample capacity
  - Increases analyte retention time
  - Reduces column efficiency
  - Reduces the upper temperature limit
- Thin films are good for analytes with high boiling points
- Thick films are good for analytes with low boiling points (volatile organics and gases)
Phase Ratio (Beta value)

- Expresses the ratio of the gas volume and the stationary phase volume in a column

\[
\text{Beta value} = \frac{\text{Column radius (um)}}{2 \times \text{phase film thickness (um)}} = \frac{r}{2d_f}
\]
Selection of a Column using a Phase Ratio

<table>
<thead>
<tr>
<th>Beta value</th>
<th>Sample Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 100</td>
<td>Highly volatile, Low MW compounds</td>
</tr>
<tr>
<td>100-400</td>
<td>General purpose analyses, wide range of compounds</td>
</tr>
<tr>
<td>&gt; 400</td>
<td>High MW compounds</td>
</tr>
</tbody>
</table>
Column Length

- Shorter column (< 15-m) is good for screening or simple samples
- A longer column (> 60-m) is good for complex or volatile analyses
  - provides greater resolution than a shorter column
  - increases analyses time
  - increases the pressure to move the sample through the column
Theoretical Plate

- A series of discrete but continuous, narrow, horizontal plate in a column at which equilibrium of solute between the mobile and stationary phase is assumed to take place.

- \[ N = \frac{L}{H} \]
  
  (\( N \) = number of theoretical plate, \( L \) = column length, \( H \) = the height equivalent to a theoretical plate)

- Column efficiency = \( N^{1/2} \)
## Effect of Column ID on the Characteristics of GC Column

<table>
<thead>
<tr>
<th>Column ID</th>
<th>Sample Capacity (ng)</th>
<th>Efficiency TPN/m</th>
<th>Optimum flow rate (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 mm</td>
<td>5-30</td>
<td>5000</td>
<td>0.4</td>
</tr>
<tr>
<td>0.25 mm</td>
<td>50-100</td>
<td>4170</td>
<td>0.6</td>
</tr>
<tr>
<td>0.32 mm</td>
<td>400-500</td>
<td>3330</td>
<td>1.0</td>
</tr>
<tr>
<td>0.53 mm</td>
<td>1,000-2,000</td>
<td>1670</td>
<td>2.8</td>
</tr>
<tr>
<td>0.75 mm</td>
<td>10,000-15,000</td>
<td>1170</td>
<td>5.6</td>
</tr>
<tr>
<td>2 mm</td>
<td>20,000</td>
<td>2000</td>
<td>20</td>
</tr>
</tbody>
</table>

*packed column*

60-m capillary column, 2-m packed column. 0.25 um film for 0.25 and 0.32 mm columns and 1.0 um for megabore columns
Column Flow Rate

- Column Diameter
- Column Length
- Oven Temperature
Detectors

- Flame Ionization Detector
  - The number of ions produced is proportional to the number of reduced carbon atoms in the flame

- Thermal Conductivity Detector
  - The thermal conductivity of hydrogen and helium are about 6-10 times greater than those of most organic compounds.

- Electron Capture Detector
  - Sensitive to halogens, peroxides, quinones, and nitro groups. Important tool for chlorinated insecticides
Flame ionization Detector
Chromatogram from Flame Ionization Detector

Figure 2. Typical chromatogram of a blank water sample fortified at 0.300 µg L⁻¹. Compound identification is shown in Table 1.
Retention Times of Compounds

Retention Time, minutes

Abundance, arb. units

80 °C/min

6 °C/min
Mass Selective Detector

- Electron Impact: Bombarding the sample with high energy electrons, which produces a family of positive particles whose mass distribution is characteristic of the parent species. The complex mass patterns that result are used for identification.

- Chemical Ionization: Use reagent gas. More gentle than EI and less fragmentation. Methane is the most common reagent. Isobutane and ammonia are also used.
Mass Selective Detector

Ion Source
El Fragmentation

3-Phenyl-2-propenal (C₉H₈O), MW = 132.16

3-Pentanol (C₅H₁₂O), MW = 88.15
Compounds Identification
Sample Preparation and Injection

- Solvent Extraction
  - Folch Solution
  - Saponification
- Derivatization
- Sample Injection
  - Auto-injection
  - Manual injection
  - On-column injection
Derivatization

- Improves thermal stability of compounds
- Increases volatility of compounds
- Increases sensitivity
- Improves separation
Derivatizing Reagents

- **Acylation**
  - Converts compounds that contain active hydrogens (-OH, -SH, and -NH) into esters, thioesters, and amide. Fluorination for ECD.

- **Alkylation**
  - Addition of alkyl group (aliphatic or aliphatic-aromatic) to an active functional (H) group. (e.g., esterification of fatty acids)

- **Silylation**
  - Replacement of active H group by a silyl group.
Fatty Acid Methyl Esters (FAME)

- Methanolic hydrogen chloride
  - 5% anhydrous hydrogen chloride in methanol
  - 30 minutes at 50°C

- Methanolic sulfuric acid
  - 1 to 2% concentrated sulfuric acid in methanol.
  - Destruction of PUFA
  - Not recommended for polyunsaturated fatty acids

- Boron trifluoride-methanol
  - 12 to 14% boron trifluoride in methanol
  - 45 minutes at 100°C
Advantages and Limitations of GC

- **Advantages**
  - Very powerful tool for quantification and qualification of various compounds
  - Sample preparation is easy
  - Easy to operate

- **Limitations**
  - Non-volatiles cannot be analyzed
  - For solvent-soluble compounds only
  - Limited for small compounds