To Derivatize or not to Derivatize: That is the Question

I. Review of Gas Chromatography
   A. Basic Chromatography
   B. Gas Chromatography
   C. Introduction to GC/MS

II. Analytical derivatization for Gas Chromatography
   A. What is Analytical Derivatization and Why we do it
   B. General requirements and Chemical Reactions
   C. Derivatization Techniques
      1. Silylation
      2. Acylation
      3. Alkylation
      4. Specialized, including Chiral

III. Summary

Basic Chromatography
Sample
Introduction
Mobil Phase
Stationary Phase
Detection
Interpretation

Basic Chromatography

Basic Chromatography

Gas Chromatography

Basic Chromatography

Gas Chromatography

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The Gas Chromatograph

Flash Vaporization
- Instantaneous Vaporization
- Hotter than Boiling Point of Sample

Gas Chromatograph

Injection Systems . . . All Glass Systems
- Steroids
- Carbohydrates
- Amino Acids
Chromatography is a PARTITIONING Process

MOBILE PHASE  →  STATIONARY PHASE

GAS
LIQUID (GLC)
SOLID (GSC)

LIQUID
LIQUID (LLC)
SOLID (LSC)

Gas-Liquid Chromatography

COLUMN
Granular Support
Stationary (Liquid) Phase

Partitioning

COLUMN
Granular Support
Stationary (Liquid) Phase

High Solubility
Low Vapor Pressure

Low Solubility
High Vapor Pressure
Peak Migration

Injection

Migration

Elution

MINUTES

Illustration of Multipaths

Particle size distribution affects band spreading

Column and Solvent Efficiency

Normal

Increased Column Efficiency
(more theoretical plates)

Increased Solvent Efficiency
(greater ratio of retention times)

Peak Resolution

- Flow Rate
- Temperature
- Liquid Phase
- % Liquid Phase
Resolution as a Function of Temperature

20% Liquid Phase at 30°C

20% Liquid Phase at 40°C

20% Liquid Phase at 50°C

20% Liquid Phase at 60°C

Resolution as a Function of % Liquid Phase

20% Liquid Phase at 50°C

10% Liquid Phase at 50°C

5% Liquid Phase at 50°C

1% Liquid Phase at 50°C

Tailing Factor

\( \frac{a}{b} \times 100 = \text{Tailing Factor} \)

\( c = 0.1h \)

Packed vs. Capillary

COLUMN

Carrier Gas

Liquid Phase

PACKED

1/8" or 1/4" O.D.

OPEN TUBULAR (Capillary)

0.01" or 0.02" I.D.
Open Tubular Column

Tubing Wall

Tubing Wall

Liquid Phase

Open Tubular Columns

- More Liquid
- Larger Samples
- Fast Analysis
- High Resolution

S.C.O.T.

Tube Wall

Celite Support

Liquid Phase

Comparison of Capillary and Packed Columns

<table>
<thead>
<tr>
<th></th>
<th>CAPILLARY</th>
<th>PACKED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inside Diameter, mm</td>
<td>0.25 - 0.5</td>
<td>2</td>
</tr>
<tr>
<td>Maximum Plates/m</td>
<td>3,000</td>
<td>2,400</td>
</tr>
<tr>
<td>Maximum Practical Length, m</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>Maximum Total Plates</td>
<td>300,000</td>
<td>14,400</td>
</tr>
<tr>
<td>Amount Liquid Phase, %</td>
<td>--</td>
<td>5</td>
</tr>
<tr>
<td>Liquid Film Thickness, μ</td>
<td>0.1 - 1</td>
<td>5</td>
</tr>
<tr>
<td>Mesh Range</td>
<td>--</td>
<td>580/100</td>
</tr>
<tr>
<td>Permeability, x10^7 cm²</td>
<td>200 - 800</td>
<td>2</td>
</tr>
<tr>
<td>Average Linear Velocity, cm/sec.</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>Average Flow Rate, ml/min</td>
<td>1 - 3</td>
<td>20</td>
</tr>
<tr>
<td>Maximum Sample Size, μl</td>
<td>0.01 - 0.02</td>
<td>1 - 5</td>
</tr>
</tbody>
</table>

Thermal Conductivity Detector

Filaments

Gas Flow

OUT

IN
Thermal Conductivity Cell

Flame Ionization Detector

Ionization Reaction

Electron Capture Detector

$\text{CH}_4 + \text{O}_2 \rightarrow \text{CHO}^+ + \text{e}^-$
Introduction to GC/MS

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Why Mass Spectrometry?

A powerful analytical technique used to:

1. Identify unknown compounds
2. Quantify known materials down to trace levels
3. Elucidate the structure of molecules (mol. wt.)

Also, needs only micrograms of samples

Mass Spectra of Carbon Dioxide

A mass spectrum is a graph of ion abundance vs. mass-to-charge ratio

Why Gas Chromatography?

- Capable of resolving complex volatile mixtures (>450 peaks for coffee aroma)
- Uses small sample size (micrograms to picograms)
- Fast analyses — usually a matter of minutes
- Good quantitation — 1-2% RSD normally
Capabilities of GC/MS

- Combines advantages of both techniques:
  1. High resolving power of GC
  2. Positive identification of MS
- Requires only small samples (micrograms to picograms)
- Quantitative trace analysis ~ ppm, ppb
- Mass range of 10-800

Limitations of GC/MS

- Expensive ($40 K to $250 K)
  - Bench-top systems $40 K to $80 K
- Complex to operate (improving)
  - Must know GC, MS, vacuum and interpretation
- Only volatile samples

Combining GC and MS

- GC 760 torr Heated Output
- Requires Interface
- MS High Vacuum Heated Input

Types of interfaces:
- jet separator — packed columns
- direct interface — capillary columns

Interfaces

- Jet Separator
  - Column Flow
  - To MS source (separate Vacuum)
  - To Vacuum

- Capillary Direct Interface
  - GC Oven
  - Heated Transfer Line
  - Fused Silica Column
  - MS Source
  - Separate Vacuum
Ionization Sources

- Electron Impact
- Chemical Ionization
- Negative Chemical Ionization

Electron Impact Source

Entire source is at high vacuum

Chemical Ionization

- Produces ions by gentle process of proton transfer from an ionized reagent gas (e.g., CH$_5^+$)
- Ionization is made by collision of sample molecule with reagent ions
- Leads to simpler and more easily interpretable mass spectra

Comparison of CI and EI Spectra
Mass Analyzers

- Single-Focusing Magnetic Sector
- Double-Focusing Electrostatic/Magnetic
- Quadrupole
- Ion Trap
- Time of Flight

Design of a Double-Focusing Separator

Quadrupole Mass Analyzer
Ion Trap Mass Analyzer

- End Cap
- Filament
- RF Ring Electrode
- Electron Current
- Ions
- End Cap
- Electron Multiplier

Mass Analyzer Time of Flight (T.O.F.)

- Source
- Collector
- Different Masses Give Different Flight Times

Ion Detectors
- Discrete-Dynode Electron Multiplier
- Continuous-Dynode Electron Multiplier
- Ion Sensitive Photoplates
- Channel Electron-Multiplier Array

Discrete-Dynode Electron Multipliers

- Ion Beam*
- Amplifier
- A. Conversion dynode (emits electrons)
- B. Secondary electrons (ions to electrons)
- C. Second dynode — emits electrons (electrons to electrons)

*Dynode is a metal plate (copper-beryllium)
The Mass Spectrometer

1. Inlet System
   - GC or LC or SFC

2. Ion Source
   - Produces Ions
   - E. I.
   - C. I.

3. Mass Analyzer
   - Separates (m/e)
   - Magnetic Sector
   - T.O.F.
   - Quadrupole
   - I.T.D.

4. Detector
   - Discrete Dynode
   - Continuous Dynode

GC Analysis of a Hydrocarbon Mixture

1. 2-Methylbutane
2. Pentane
3. Cyclopentane
4. Nexane
5. Benzene

Fragmentation of Hexane in a Mass Spectrometer

- Molecular ion, \(\text{M}^{++}\) (m/z = 86)
- \(\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3\)
- \(\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3^{+}\)
- \(\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2^{+}\)
- \(\text{CH}_3\text{CH}_2\text{CH}_2^{+}\)
- \(\text{CH}_3\text{CH}_2^{+}\)

- m/z:
  - 71
  - 57
  - 43
  - 29

- Relative abundance (%):
  - 10
  - 100 (base peak)
  - 75
  - 40
Mass Spectrum of Hexane

C₆H₁₄; Mol. Wt. = 86

Mass Spectrometry of Isomers

N-Butane

Iso-Butane

Why High Vacuum Systems?

- Low pressure is essential for production of free electrons and ions
- Permit free passage of the electrons, sample ions and fragments
- Mean free path of ions is increased high vacuum
- To minimize ion-molecule collisions
**Total Ion Chromatogram**
- Universal Response
- Scans a specified mass range (say 40 to 240 AMU)
- Not as sensitive as SIM
- Slower acquisition rates can compromise with quantitation
- Permits retrieving mass spectra
- Best for qualitative analysis

**Selective Ion Monitoring**
- Monitors several discrete m/z values (target compounds)
- Increases S/N ratio
- Permits greater acquisition rate
- Best for quantitative analysis

**TIC vs. SIM**
- SIM: m/z = 18
- Water
- TIC: 10-200 AMU
- Water
- Carbon Dioxide
- Phenol

**For More Information**
Analytical Derivatization for Gas Chromatography

Derivatization

Definition

• The chemical modification of an existing compound to produce a new compound having properties that are suitable for a specific analytical procedure.

Requirements

The Ideal Derivatization Procedure Will...

• Accomplish the desired modification.
• Proceed quantitatively, or at least reproducibly.
• Produce products which are readily distinguishable and separable from the starting materials.
• Proceed rapidly with simple and straightforward laboratory techniques.
• Be relatively selective while being applicable to a number of similar compounds.
• Involve reagents and reactions which present no unusual hazards.

Analytical Derivatization

What are the Reasons for Derivatization?

• Impart Volatility
• Decrease Adsorption
• Improve Resolution
• Increase Stability
• Improve Detectability
• Assist in Structure Determination
The most commonly used derivatization procedures involve the substitution of active hydrogens on the compound to be derivatized with a variety of functional groups. These functional groups impart the desired characteristics to the compound, while eliminating the adverse effects of the polar active hydrogens.

\[ R_1 - AH + R_2 - D \rightarrow R_1 - AD + R_2 - H \]

Where atom "A" = Oxygen, Sulfur, Nitrogen or similar atoms
Where atom "D" = Functional group on the derivatization reagent

Derivatization Techniques

- Silylation
- Acylation
- Alkylation
- Specialized

Silylation

Definition

- The introduction of the silyl group into a molecule, usually by substitution of active hydrogen; occasionally by replacement of the metal component of a salt.

The most frequently used derivatives for gas chromatography analysis are:

- Trimethylsilyl — $\text{Si(CH}_3\text{)}_3$
- $t$-Butyldimethylsilyl — $\text{Si(CH}_3\text{)}_2\text{C(CH}_3\text{)}_3$

Examples: Trimethylsilyl (TMS) = $\text{Si(CH}_3\text{)}_3$

\[ R - \text{OH} \overset{TMS}{\rightarrow} R - \text{O} - \text{TMS} \]
\[ R - \text{NH}_2 \overset{TMS}{\rightarrow} R - \text{N} - \text{TMS} \]
\[ R - \text{C} - \text{OH} \overset{TMS}{\rightarrow} R - \text{C} - \text{O} - \text{TMS} \]
Silylation

Advantages
• Wide range of applications
• Variety of reagents available
• Easily prepared
• Excellent thermal stability
• Excellent chromatographic characteristics

Disadvantages
• Silylation reagents are moisture-sensitive
• TMS and TBDMCS derivatives are easily hydrolyzed
• Derivatives cannot be made in aqueous solutions
• Must use aprotic organic solvents
• Silylating reagents and silyl derivatives react with many column materials
• Silicone residues build up in GC detectors

Commonly Used Silylation Reagents

- BSA
  - Strong silyl donor
    - Similar to BSTFA and MSTFA
    - Reacts with all active hydrogen compounds
    - Alcohol, phenols, carboxylic acids, amines, amides, thiols
    - Usually requires anhydrous condition
    - TMCS 1% - 10% frequently used as catalyst
    - Sometimes reacts quantitatively under mild conditions
    - Reaction products often interfere with volatile derivatives
    - Silicon fouling of detectors is common

- BSTFA
  - Strong silyl donor
    - Similar to BSA and MSTFA
  - Frequently used with TMCS 1 - 10%
  - Alone and with TMCS, most commonly used derivatizing agent
  - Reacts with all active hydrogen compounds
    - Alcohol, phenols, carboxylic acids, amines, amides, thiols
  - Usually requires anhydrous condition
  - Often reacts quantitatively under mild conditions
  - Reaction products more volatile than those from BSA
  - Much less detector fouling than with BSA
MSTFA

- Strong silyl donor
  - Similar to BSA and BSTFA – always monovalent
- Frequently used with TMCS 1 - 10%
- Reacts with all active hydrogen compounds
  - Alcohols, phenols, carboxylic acids, amines, amides, thiols
- Most volatile reagent and reaction product
  - Used for derivatizing small volatile molecules
- Better than BSA in avoiding detector fouling
- Usually requires anhydrous conditions

TMSI

- Strongest silyl donor for hydroxyl groups
- Reacts quickly and smoothly with hydroxyl and carboxyl
- Does not react with amines or amides
- Can derivatize hydroxyl in the presence of amines
- Permits selection or successive derivatization of hydroxyls and amines
- Preferred reagent for sugar
- Can tolerate small amounts of water as in syrups
- Can derivatize even highly hindered hydroxyls

TMSDEA

- Strongly basic silylating agent
- Very volatile reagent
- Excellent for derivatizing low molecular weight carboxylic acids
- Reaction can be driven to completion by removal of diethylamine (bp 55°C)
- Good for preparation of TMS standards
- Relatively weak silyl donor

TMCS and HMDS

- Weak silyl donors
- Among the oldest (first) silylating reagents
- Usually used in combination with each other
- Excellent for derivatization of sugars and simple carbohydrates
- Usually combined with pyridine and other solvents
- TMCS can form derivatives of sodium salts of acids and phenols
**MTBSTFA**

- Produces t-butyldimethylsilyl (TBDMS) derivatives
- Strong silyl donor
  - Slightly less than BSA, BSTFA, MSTFA
- Reacts with all active hydrogen compounds
  - Alcohols, phenols, carboxylic acids, amines, thiols
- Derivative much more hydrolytically stable than TMS
- Produces characteristic fragmentation patterns on GC/MS
- Bulky group, reaction may be difficult due to steric hindrance
- Frequently used with t-butyldimethylchlorosilane (TBDMCS) as a catalyst

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**Ready-to-Use Tri-Sil® Reagents**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Description</th>
<th>Formulation</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tri-Sil®</td>
<td>A reagent-catalyst-solvent mixture for one-step derivatization</td>
<td>HMDS:TMCS: Carbohydrates, phenols, steroids, organic acids, alcohols, and some amines.</td>
<td>Not recommended for 3-keto steroids.</td>
</tr>
<tr>
<td>Concentrate</td>
<td>A concentrated reagent-catalyst system</td>
<td>HMDS:TMCS</td>
<td>Has same applications as Tri-Sil®, but offers greater latitude in applications (e.g. pyridine for sugars, DMF for 3-keto steroids, and DMSO for tertiary alcohols).</td>
</tr>
<tr>
<td>Formula &quot;P&quot;</td>
<td>A reagent-solvent system where BSA is the active silylating agent. A one-step derivatizing system.</td>
<td>BSA:Pyridine Hydroxy and (2.5 mEq/ml) polyhydroxy compounds, amines, acids, amides, phenols, amino acids, carboxylic acids and steroids.</td>
<td>Not recommended for carbohydrates.</td>
</tr>
<tr>
<td>Formula &quot;D&quot;</td>
<td>A reagent-solvent system where BSA is the active silylating agent. A one-step derivatizing system.</td>
<td>BSA:DMF (2.5 mEq/ml)</td>
<td>Same applications as Tri-Sil® BSA Formula &quot;P&quot; above, but where DMF is recommended as a solvent. A preferred formulation for phenols, particularly highly hindered phenols, where DMF is required for a smooth complete reaction.</td>
</tr>
</tbody>
</table>

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**Acylation**

**Definition**

- The introduction of an acyl moiety into a molecule via substitution of an active hydrogen

The most frequently used derivatives for gas chromatography:

- Trifluoroacetyl
- Pentfluoropropionyl
- Heptfluorobutyryl
**Acylation**

**General Reaction Scheme**

- **Advantages**
  - Derivatives are hydrolytically stable
  - Perfluoro derivatives increase volatility
  - Increased sensitivity by added molecular weight
  - Increased detectability by ECD by added halogen atoms
  - Reacts with alcohols, thiols and amines
  - Can be used to activate carboxylic acids for esterification

- **Disadvantages**
  - Derivatives are frequently difficult to prepare
  - Reaction products often must be removed before analysis
  - Reaction must be done in non-aqueous system
  - Reagents are moisture-sensitive
  - Reagents are hazardous and odorous

**Commonly Used Acylation Reagents**

- **Perfluoroacyl Anhydrides**
  - Trifluoroacetic Acid Anhydride (TFAA)
  - Perfluoroacetic Acid Anhydride (PFAA)
  - Hexafluorobutyric Acid Anhydride (HFAA)

- **Perfluoroacyl Imidazoles**
  - Trifluoroacetyl imidazole (TFAI)
  - Perfluoroacetyl propionylimidazole (PFPI)
  - Hexafluorobutyrylimidazole (HFBI)

- **MBTFA**
  - N-methyl-bis-(trifluoroacetamide)

**Perfluoro Acid Anhydrides**

- Produce perfluoroacyl derivatives of alcohols, thiols and amines
- Derivatives are relatively stable to hydrolysis
- Derivatives are useful for ECD, FID and TCD detection
- Usually used with basic solvent
- Produce characteristic MS fragmentation
- Are widely used for drug analysis
- Produce acid byproducts which must be removed before GC analysis
**Perfluoroacylimidazoles**
- Produce perfluoro derivatives of alcohols, amines and thiols
- Quantitatively acylate indol alkylamines
- Derivatives are relatively stable to hydrolysis
- Derivatize both primary and secondary amines
- Produce no acidic byproducts
- Reagents are very reactive with water
- Substance to be derivatized must be dry
- Cannot use in protonated solvents

**MBTFA N-Methyl-bis(Trifluoroacetamide)**
- Forms trifluoroacetyl derivatives of alcohols, amines and thiols
- Reacts with both primary and secondary amines
- Reactions with amines generally complete in 30 minutes at room temperature
- Reacts more slowly with alcohols than amines
- Byproduct is stable and volatile
- Excellent for mono-, di- and trisaccharides

**Alkylation**
*Definition*
- The introduction of an alkyl moiety into a molecule via substitution of an active hydrogen

The most frequently used derivatives for gas chromatography analysis are:
- Methyl — CH₃
- Perfluorobenzyl — CH₂F₂F₃

**General Reaction Scheme**
\[
R\text{C}OH + R'\text{X} \rightarrow R\text{C}OR' + HX
\]
\[
AR\text{OH} + R'\text{X} \rightarrow AR\text{OR'} + HX
\]
\[
R\text{SH} + R'\text{X} \rightarrow R\text{SR'} + HX
\]
Where \(R'\) — \(X\) = the alkylation reagent
Alkylation

• Advantages
  - Wide range of reagents available
  - Wide range of derivatives can be produced
  - Reaction condition can vary from strongly acidic to strongly basic
  - Some reactions can be done in aqueous systems
  - Derivatives are generally stable

• Disadvantages
  - Limited to amines and acidic hydroxyls
  - Conditions frequently severe
  - Reagents often toxic
  - Optimization for particular compounds usually necessary

Commonly Used Alkylation Reagents

• BF₃ • Methanol
• Methyl 8® Reagent
• MethElute™ Reagent
• Diazomethane
• Pentafluorobenzyl Bromide

Alkylation Reagents

Boron Trifluoride in Methanol

BF₃•CH₃OH

Used primarily for esterification procedures with fatty acids; however, phenolic hydroxyls may be derivatized

Alkylation Reagents

Dimethylformamide Dialkylacetals

R = Methyl
Ethyl
Propyl
n-Butyl

Used for carboxylic acid esterification. Analytical applications have been expanded to include alcohols, phenols, steroid carbonyls, amino acids, primary and secondary amines, and thiols.
Alkylating Reagents

**Trimethylanilinium Hydroxide TMPAH**

![](image)

MethElute™ Reagent – 0.2M TMPAH in Methanol.

Used for on-column methylation of amines, hydroxyls and carboxyls.

**Alkylation Reagents**

Most versatile reagent for preparation of methyl esters; fast and quantitative with no organic byproducts.

Diazomethane and its precursors are toxic and dangerous.

**Alkylating Reagents**

Diazomethane

![](image)

**Alkylating Reagents**

Pentafluorobenzyl Bromide

![](image)

Used for the preparation of pentafluorobenzyl derivatives of carboxylic acids, phenols, sulfonamides and some mercaptans. Can be used for determination of trace amounts of carboxylic acids, phenols, and mercaptans in water. Most useful for ECD detection due to the introduction of five fluorine atoms.

**Solvents for Derivatization**

- Acetonitrile
  - M.W. 74.10
  - bp 81.9°C
- Dimethylsulfoxide
  - M.W. 78.13
  - bp 199°C
- Tetrahydrofuran
  - M.W. 72.10
  - bp 69°C
- Dimethylformamide
  - M.W. 73.09
  - bp 153°C
- Pyridine
  - M.W. 79.10
  - bp 115.2°C
- Ethyl Acetate
  - M.W. 88.11
  - bp 77°C

High boiling point

Oxidizes to form peroxides

High boiling point

Can have side products

Best solvent for most derivatization
Chiral Separations

Chiral Chemistry
- Isomers: molecules that have the same molecular formula but a different arrangement of atoms.
- Chiral centers or asymmetric carbons: carbon atoms having four different groups or atoms attached.

Types of Optical Isomers

Enantiomers
- Isomers that are mirror images of each other but cannot be superimposed

Diastereomers
- Stereoisomers that are not mirror images of each other

Meso Compounds
- Sets of stereoisomers with a plane of symmetry making them not optically active

If \( n \) is the number of chiral centers, then \( 2^n \) is the number of stereoisomers

Separation of Chiral Compounds

Distereomers
- Diastereomers may have different chemical and physical properties and can usually be separated by classical methods

Enantiomers
- Enantiomers have identical chemical and physical properties except for their ability to rotate the plane of polarized light. Special techniques must be used for separation and identification

Chromatographic separation of enantiomers
Separation on chiral column
Precolumn derivatization with chiral derivatizing reagents, then separation on chromatographic columns

S-(-)-N-(trifluoroacetyl)prolyl chloride (S-(-)-TFAPC)

(-)-\( \alpha \)-methoxy-\( \alpha \)-trifluoromethylphenylacetic acid (MTPA), \((S)-(-)-N\)-(trifluoroacetyl)prolyl chloride (S-TPC); 2,3,4,6-tetra-\( \alpha \)-acetyl-\( \beta \)-D-glucopyranosyl isothiocyanate, \( R \)-(+)-\( \alpha \)-phenylethyl isocyanate, 2,3,4-triacetyl-\( \alpha \)-D-arabinopyranosyl isothiocyanate

Chiral Drugs

Fig. 1. Structures and stereochemical notation for ephedrine, pseudoephedrine, methamphetamine, and methamphetamine.
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