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

- 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
A General Reaction Scheme

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.

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

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

The Ideal Derivatization Procedure Will...

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 — Si(CH₃)₃
- t-Butyldimethylsilyl — Si(CH₃)₂·C(CH₃)₃

Silylation General Reaction Scheme

\[ \begin{align*}
R - OH & \xrightarrow{TMS} R - O - TMS \\
R - NH₂ & \xrightarrow{TMS} R - NH - TMS \quad \text{or} \quad R - N - (TMS)₂
\end{align*} \]

Where A = O, S, N, or similar atoms.

Examples: Trimethylsilyl (TMS) = Si(CH₃)₃
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**: Frequently used with TMCS Catalyst
  - 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**

**Reagent** | **Description** | **Formulation** | **Applications**
---|---|---|---
Tri-Sil® Concentrate 49005 | A concentrated reagent-catalyst system | HMDS:TMCS (3:1) | 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).
Tri-Sil® BSA Formula “P” 49011 | A reagent-solvent system where BSA is the active silylating agent. A one-step derivatizing system. | BSA:Pyridine Hydroxy and (2.5 mEq/ml) polyhydroxy compounds, amines, acids, amides, phenols, amino acids, carboxylic acids and steroids. Not recommended for carbohydrates.
Tri-Sil® BSA Formula “D” 49010 | A reagent-solvent system where BSA is the active silylating agent. A one-step derivatizing system. | BSA:DMF (2.5 mEq/ml) | Same applications as Tri-Sil® BSA Formula “P” 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.

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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: \( \text{CF}_3 \text{C} = \text{O} \)
- Pentfluoropropionyl: \( \text{CF}_3\text{CF}_2\text{C} = \text{O} \)
- Heptafluorobutyryl: \( \text{CF}_3\text{CF}_2\text{CF}_2\text{C} = \text{O} \)
Acylation

General Reaction Scheme

\[ R - OH + R' - C - X \rightarrow R - O - C - R' + HX \]
\[ R - NH_2 + R' - C - X \rightarrow R - NH - C - R' + HX \]
\[ R' - NH + R' - C - X \rightarrow R' - N - C - R' + HX \]
\[ R_2 - NH + R' - C - X \rightarrow N - C - R' + HX \]

where \( R' - C - X \) is the acylating agent

**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 odoruous

Commonly Used Acylation Reagents

**Perfluoroacyl Anhydrides**
- TFAA (Trifluoroacetic Acid Anhydride)
- PFAA (Perfluoropropionic Acid Anhydride)
- HFAA (Heptfluorobutyric Acid Anhydride)

**Perfluoroacyl Imidazoles**
- TFAI (Trifluoroacetylimidazole)
- PFPI (Perfluoropropyrimidazole)
- HFBI (Heptfluorobutyrimidazole)
- 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

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**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** — \( \text{CH}_2\text{F}\)
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

Alkylating Reagents

Boron Trifluoride in Methanol

BF₃ • CH₃OH

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

Dimethylformamide Dialkylacetals

\[
\begin{align*}
\text{R} = & \text{Methyl} \\
& \text{Ethyl} \\
& \text{Propyl} \\
& \text{n-Butyl}
\end{align*}
\]

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

Trimethylanilinium Hydroxide (TMPAH)

MethElute™ Reagent – 0.2M TMPAH in Methanol.

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

Alkylation Reagents

Trimethylanilinium Hydroxide (TMPAH)

Diazomethane

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

Diazomethane and its precursors are toxic and dangerous.

Alkylation Reagents

Pentafluorobenzyl Bromide

PFBBBr

M.W. 250.9
bp 174-175°C
d^2 1.86

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 81.6°C
  - High boiling point

- Dimethylsulfoxide
  - M.W. 78.13
  - bp 189°C
  - Oxidizes to form peroxides

- Dimethylformamide
  - M.W. 73.09
  - bp 153°C
  - High boiling point

- Pyridine
  - M.W. 79.10
  - bp 115.2°C
  - Best solvent for most derivatization

- Tetrahydrofuran
  - M.W. 72.09
  - bp 69°C
  - Can have side products

- Ethyl Acetate
  - M.W. 88.11
  - bp 77°C
  - Can have side products
**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**

**Diestereomers**

- 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
Pre-column derivatization with chiral derivatizing reagents, then separation on chromatographic columns

\[ S(-)-(\text{N-(trifluoroacetyl)prolyl chloride}) \]

\((-\alpha\text{-methoxy-\alpha-trifluoromethylphenylacetic acid (MTPA), (S)-(\text{N-(trifluoroacetyl)prolyl chloride}})\]

\((-\alpha\text{-trifluoroacetyl})\text{prolyl chloride})\]

\(2,3,4,6\text{-tetra-\text{O-acetyl-\beta\text{-d-glucopyranosyl isothiocyanate, R}(+)\text{-phenylethyl isocyanate, 2,3,4-triacetyl-\alpha\text{-d-arabinopyranosyl isothiocyanate}}\]

**Chiral Drugs**

![Chiral Drugs](image)
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

Quality and Experience …
They are what separate Pierce GC products from the competition

When Dr. Alan Pierce founded Pierce Chemical Company in 1950, Pierce was the first commercial producer
of ninhydrin, a chemical used for amino acid analysis. He soon expanded the product line to include fluorine
compounds and organic silicone compounds used for chromatography.

Dr. Pierce, who authored the famous textbook Silylation of Organic Compounds, was recognized throughout
the industry not only as an expert in GC, but also as a teacher and a scientist who cared about his customers.
Although Pierce product lines have grown and diversified during the past 52 years, the scientists of Pierce
Biotechnology (formerly Pierce Chemical Company) are proud to continue Dr. Pierce’s tradition of providing
the GC reagents and solvents – and other products – that scientists around the world trust for performance and
reliability.

Silylation, Alkylation and Acylation Reagents • Purified Silylation Grade Solvents
GC Derivization Systems • Labware

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