GC Derivatization

What is GC Derivatization?

Derivatization is the process of chemically modifying a compound to produce a new compound which has properties that are suitable for analysis using a GC.

Why Derivatize

- To permit analysis of compounds not directly amenable to analysis due to, for example, inadequate volatility or stability
- Improve chromatographic behavior or detectability

Many compounds do not produce a useable chromatograph (i.e. multiple peaks, or one big blob), or the sample of interest goes undetected. As a result it may be necessary to derivatize the compound before GC analysis is done.

Derivatization is a useful tool allowing the use of GC and GC/MS to be done on samples that would otherwise not be possible in various areas of chemistry such as medical, forensic, and environmental.

References:

1. *Regis 1998-99 Chromatography Catalog*

2. Knapp, D. R. *Handbook of Analytical Derivatization Reactions*; John Wiley and Sons; New ork, 1979

3. Blau, K., King, G. *Handbook of Derivatives for Chromatography*; Heyden & Sons Ltd.; London, 1979

What Does Derivatization Accomplish?

- Increases volatility (i.e. sugars):
  - Eliminates the presence of polar OH, NH, & SH groups
  - Derivatization targets O,S, N and P functional groups (with hydrogens available
- Increases detectability, I.e. steroids/ cholesterol

- Increases stability

- Enhances sensitivity for ECD (Electron Capture Detection). The introduction of ECD detectable groups, such as halogenated acyl groups, allows detection of previously undetectable compounds

The main reason for derivatizing is to impart volatility to otherwise non-volatile compounds. The low volatility may result from the size of the molecule and the resultant large dispersion forces holding the molecule together. Smaller molecules may have a low volatility due to the strong intermolecular attractions between polar groups. In the latter case, masking the polar groups by derivatization can yield dramatic increases in volatility.

Derivatization can also be used to decrease volatility to allow analysis of very low molecular weight compounds, to minimize losses in manipulation and to help separate sample peaks from solvent peak.

Some compounds, which can be volatilized, undergo partial thermal decomposition in the GC so they need to be made more stable.

Polar samples tend to adsorb on the active surfaces of the column walls and the solid support. Reduction of this adsorption can be accomplished by derivatization.

Derivatization can be used to improve detectability of sample compounds for ECD. In general, the halogenated substituents increase electron affinity in the following order I > Br> Cl > F (Though they show little increase in volatility).

Derivatization serves to accentuate the differences in the sample compounds to facilitate the chromatographic separation.

Main Types of Derivatization

- Silylation
  - readily volitizes the sample. Most prevalent method

- Alkylation
  - Used as the first step to further derivatizations or as a method of protection of certain active hydrogens

- Acylation
  - commonly used to add fluorinated groups (ECD)

Silylation

Silylation produces silyl derivatives which are more volatile, less stable, and more thermally stable.

- Replaces active hydrogens with a TMS (trimethylsilyl group).

References:
1. *Regis 1998-99 Chromatography Catalog*, pages 86-88

- Silylation occurs through nucleophilic attack (SN2). The better the leaving group, the better the siliylation.
- Silylation reagents will react with water and alcohols first. Care must be taken to ensure that both sample and solvents are dry.

- Solvents should be as pure as possible. This will eliminate excessive peaks. Try using as little solvent as possible as this will prevent a large solvent peak.

- Pyridine is the most commonly used solvent. Although pyridine may produce peak tailing, it is an acid scavanger and will drive the reaction forward
- In many cases, the need for a solvent is eliminated with silylating reagents. (If a sample readily dissolves in the reagent, it usually is a sign that the derivatization is complete).

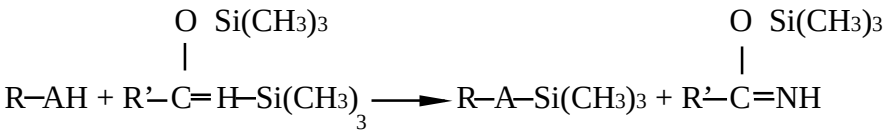
- Ease of reactivity of functional groups towards silylation. Many reagents require heating (not in excess of 60 degrees C for about 10-15 minutes, to prevent breakdown). Hindered products may require long term heating.
- The ease of reactivity of the functional group toward silylation follows the order:

Alcohol > Phenol > Carboxyl > Amine > Amide
hydroxyl hydroxyl

- The order of alcohols being:

Primary > Secondary > Tertiary

General Reaction Mechanism



References:
1. Knapp, D.R. *Handbook of Analytical Derivatization Reactions*; pages 8-10; John Wiley & Sons; New York, 1979

The silylation reaction is driven by a good leaving group, which means a leaving group with a low basicity, ability to stabilize a negative charge in the transitional state, and little or no π backbonding between the leaving group and silicon atom.

Care needs to be taken not to inject silylating reagents unto columns which have active hydrogens in the stationary phase, because they will be derivatized. Examples of columns not compatible with silylating reagents are CARBOWAX and free fatty acid phases.

Advantages and Disadvantages of Silylation

Advantages

- Ability to silylate a wide variety of compounds
- Large number of silylating reagents available
- Easily prepared

Disadvantages

- Silylation Reagents are moisture sensitive
- Must use aprotic (no protons available) organic solvents

Silylating Reagents

1. **HMDS (Hexamethyldisilzane).**
   - Weak donor, as it has symmetry
   - If used will attack only easily silylated hydroxyl groups
   - Sometimes found in combination with TMCS
2. **TMCS (Trimethylchlorosilane).**
   - Weak donor, again not commonly used
   - Often found as a catalyst to increase TMS donor potential
   - Bad by-product, HCL
3. **TMSI (Trimethylsilylimidazole).**
   - Not a weak donor, but it is selective (will not target N compounds)
   - Reacts readily with hydroxyls but not with amines
   - Since it is selective, it will target the hydroxyls in wet sugars. It will derivatize the acid sites of amino acids, and will leave the amino group free for fluorinated derivatization (ECD)
4. **BSA (Bistrimethylsilylacetamide).**
   - First widely used silylating reagent
   - Strong silylating reagent- acetamide is a good leaving group. Reacts under mild conditions and produces relatively stable by-products
   - Drawbacks: by-product, TMS-acetamide, will sometimes produce peaks that overlap those of other volatile derivatives. BSA mixtures also oxidize to form silicon dioxide, which can foul FID detectors
5. **BSTFA (Bistrimethylsilyltrifluoroacetamide)**
   - Developed by Gerhke in 1968
   - Reacts similiarly to BSA but the leaving group is trifluoroacetamide, so it acts faster and more completely than BSA
   - BSTFA is highly volatile, and produces by-products that are more volatile than BSA by-products, thus there is little interference with early eluting peaks
   - It can act as its own solvent
   - Combustion product silicon trifluoride, does not foul detectors
6. **MSTFA (N-methyl-trimethylsilyltrifluoroacetamide)**
   - The most volatile of the TMS acetamides
   - Most useful for the analysis of volatile trace materials where the derivatives may be near the reagent or by-product peak
7. **TMS-DEA (Trimethylsilyldiethylamine).**
   - Reagent is used for derivatizing amino acids and carboxylic acids
   - Targets hindered compounds
8. **MTBSTFA (N-methyl-N-t-butyldimethylsilyltrifluoroacetamide).**
   - Replaces the active hydrogen with t-BDMS group

References:
1. *Regis 1998-99 Chromatography Catalog*, pages 86-89,

- Reaction is mild and usually complete upon dissolution
- t-BDMS derivatives are more resistant to hydrolysis and can be up to 10,000 times more stable than TMS derivatives
- Suitable for GC/MS
- Will target sulfonic and phosphoric groups

9. Halo-methylsilyl derivatization reagents. (BMDMCS, and CMDMCS)

- Can produce both silylated and halogenated derivatives (ECD)

Acylation

Acylation reduces the polarity of amino, hydroxyl, and thiol groups and adds halogenated functionalities for ECD. In comparison to silylating reagents, the acylating reagents target highly polar, multi-functional compounds, such as carbohydrates and amino acids.

- Acyl derivatives are formed with acyl anhydrides, acyl halides, and activated acyl amide reagents.
- The anhydrides and acyl halides form acid by-products which must be removed before GC analysis.
- Activated amide reagents, such as MBTFA, have the advantage of not yielding acid by-products.
- Fluorinated acyl groups, going from trifluoracetyl to heptafluorobutyryl , can be used to increase retention times.

References:

1. ***Regis 1998-99 Chromatography Catalog,*** **pages 89,90**

2. Knapp D.R. ***Handbook of Analytical Derivatization Reaction*****; pages 10, 10; Wiley & Sons; New York, 1979**

Acylation converts these compounds with active hydrogens into esters, thioesters, and amides. They are formed with acyl anhydride, acyl halide, and activated acyl amide reagents. The anhydrides and acyl halide reagents form acid by-products, which must be removed before GC analysis. Acylations are normally carried out in pyridine, tetrahydrofuran or another solvent capable of accepting the acid by-product.

The presence of a carbonyl group next to the halogenated carbons enhances the electron capture detector.

Acyl derivatives tend to direct the fragmentation patterns of compounds in MS applications, and so provide helpful information on the structure of these materials.

Acylationg Reagents

1. **Fluorinated Anhydrides:-**

TFAA- Trifluoroacetoic Anhydride
PFPA- Pentafluoropropionic Anhydride
HFBA- Heptafluorobutyric Anhydride

- Most commonly used reagents, as derivatives are suitable for both FID and ECD
- Reacts with alcohols, amines, and phenols to produce stable and highly volatile derivatives
- The acid by-product should be removed, via a stream of nitrogen, before injection unto column. Bases, such as triethylamine, can be added as an acid receptor and promote reactivity
- Ability to adjust retention times for ECD

2. **Fluoracylimidazoles**

TFAI- Trifluoroacetylimidazole
PFPI- Pentafluoropropanylimidazole
HFBI- Heptafluorobutyrylimidazole

- Usually a better choice for making ECD derivatives
- React under mild conditions and their by-products, the imidazole, is not acidic so it will not harm column
- Reagents are extremely sentive to water- will react violently to it.
- Works best with amines and hydroxy compounds

3. **MBTFA-N-Methyl-bis(trifluoroacetamide)**
   - Reacts with primary and secondary amines, slowly with hydroxyl groups and thiols
   - Conditions are mild and the by-products are relatively inert and are non acidic
4. **PFBCI- Pentafluorobenzoyl Chloride**
   - Phenols most receptive site
   - Used for making derivatives of alcohols and secondary amines. Secondary amines will react with this compound.
5. **PFPOH (Pentafluoropropanol)**
   - Used in combination with PFPA
   - Used commonly in applications with polyfunctional bio-organic compounds

References:
1. ***Regis 1998-99 Chromatography Catalog*****, pages 89, 90**

Advantages and Disadvantages of Acylation

Advantages

- Addition of halogenated carbons increased detectability by ECD
- Derivatives are hydrolytically stable
- Increased sensitivity by adding molecular weight
- Acylation can be used as a first step to activate carboxylic acids prior to esterfication (alkylation)

Disadvantages

- Acylation derivatives can be difficult to prepare
- Reaction products (acid by-products) often need to be re moved before analysis
- Acylation reagents are moisture sensitive
- Reagents are hazardous and odorous

References:

1. ***Regis 1998-99 Chromatography Catalog*, pages 91**

2. **Knapp, D. R. *Handbook of Analytical Derivatization Reactions;* pages 6-8; Wiley & Sons; New York, 1979**

Alkylation

Alkylation *reduces molecular polarity by replacing active hydrogens with an alkyl group. These reagents are used to modify compounds with acidic hydrogens, such as carboxylic acids and phenols. These reagents make esters, ethers, alkyl amines and alkyl amides. Reagents containing fluorinated benzoyl groups can be used for ECD.*

- The principal reaction employed for preparation of these derivatives is nucleophilic displacement.

- Alkylation is used to modify compounds with acidic hydrogens, such as carboxylic acids and phenols.

- Alkylation can be used alone to form esters, ethers and amides- or they can be used in conjunction with acylation or silylation.

It is generally used to convert organic acids into esters. As the acidity of the active hydrogen decreases, the strength of the alkylating reagent must be increased. The harsher the reaction conditions or reagents, the more limited the selectivity and applicability of this method.

Alkyl esters have excellent stability and can be isolated and stored for long periods of time.

References:
1. *Regis 1998-99 Chromatography Catalog*, page91

A two step approach is commonly used in derivatization of amino acids, where multiple functional groups on these compounds may necessitate protection during derivatization.

Alkylating Reagents

1. **DMF (dialkylacetals)**
   - These reagents work quickly, derivatizing upon dissolution. Suitable for flash alkylation, where derivatization takes place in the injection port
   - The different alkyl homolgues allow formation of a variety of esters. polarity and volatility of the samples can be adjusted, thereby changing retention time
   - They will react with water to give the corresponding alcohol. Traces of water will not affect the reaction as long as you have an excess of acid.
2. **TBH (tetrabutylammonium hydroxide)**
   - Froms butyl ester, which will allow longer retention times
   - Used most commonly for low molecular weight acids
3. **BF_3 in methanol or butanol**
   - Convenient and inexpensive method for forming esters
4. **PFBBr (Pentafluorobenzyl bromide)**
   Esterifies phenols, thiols, and carboxylic acids

Advantages and Disadvantages of Alkylation

Advantages

- Wide range of alkylation reagents available
- Reaction conditions can vary from strongly acidic to strongly basic
- Some reactions can be done in aqueous solutions
- Alkylation derivatives are generally stable

Disadvantages

- Limited to amines and acidic hydroxyls
- Reaction conditions are frequently severe
- Reagents are often toxic

GC Chiral Derivatization

These reagents target one specific functional group and produce individual diastereomers of each of the enantiomers.

References:
1. *Regis 1998-99 Chromatography Catalog*, page 92

2. Blau, K; King, G *Handbook of Derivatives for Chromatography*; Chapter 13; Heyden & Sons; London, 1979

- GC determination of enantiomeric purity is facilitated by using enantiopure derivatization reagents.

These reagents target one specific functional group and produce individual diastereomers of each of the enantiomers.

There are two ways of separating enantiomers by chromatography:

1. separation on an optically active stationary phase
2. preparation of diastereomeric derivatives that can be separated on a non chiral stationary phase

If an optically pure reagent is used to prepare diastereomeric derivatives, then only two derivatives are formed. The enantiomeric ratio is reflected in the relative peak sizes.

Reagents for optical purity

1. **TPC (N-trifluoroacetyl-L-prolyl chloride)**
   - Used for optically active amines, most notably amphetamines
2. **MCF ((-) menthylchloroformate)**
   - Used for optically active alcohols

GC Derivatization and MS Analysis

GC volatility enhancement is an important consideration in derivatization for MS analysis. Derivatization may be used to reduce the contribution of thermal or catalytic decomposition to the mass spectrum.

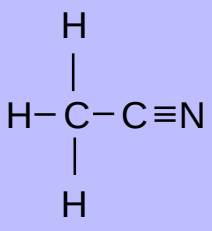
References:
1. Knapp, D. R. *Handbook of Analytical Derivatization Reactions*; page4; Wiley & Sons; New York, 1979

Derivatives are also used to stabilize ions formed in the mass spectrometer to favor structurally informative fragmentation modes.

Solvents for GC Derivatization

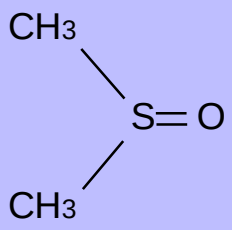
References:
1. Regis 1998-99 Chromatography Catalog; page 92

Acetonitrile



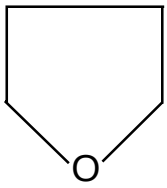
M.W. 41.05
bpt. 81.6 C

Dimethylsulfoxide



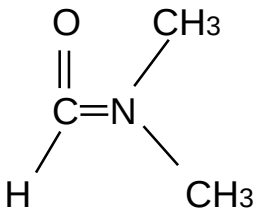
M.W. 78.13
bpt. 189 C

Tetrahydrofuran



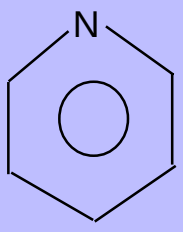
M.W, 72.10
bpt. 66 C

Dimethylformamide



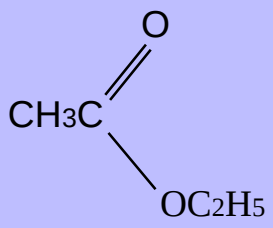
M.W. 73.09
bpt. 153 C

Pyridine



M.W. 79.10
bpt. 115.2 C

Ethyl Acetate



M.W. 88.11
bpt. 77 C

Guide to GC Derivation Methods/ Functional Groups

Functional Group	Method	Reagent	Derivative Note
Alchohols & Phenols	Silylation	BSA	TMS
		BSTFA	TMS
		MTBSTFA	TMS
		Deriva-Sil	TMS
	Acylation	HFBI	Heptafluorobutrates
		HFBA (HFAA)	Heptafluorobutrates
		PFPA (PFAA)	Pentafluoroacetates
		TFAA	Trifluoroacetates
		MBTFA	Trifluoroacetates
	Alkylation	DMF	
		PFB-Br/TBA-H-SO4	
		TBH	
Active Hydrogens	Silyation	BSA	TMS
		BSTFA	TMS
		BSTFA/ TMCS	TMS
		Deriva-Sil	TMS
		Hydrox-Sil	TMS
		MSTFA	TMS
		MTBSTFA	TMS
		TMSI	TMS
	Acylation	PFPOH/PFPA	
	Alkylation	DMF	
		TBH	
Amides	Silylation	BSA	TMS
		BSTFA	TMS
		BSTFA/TMCS	TMS
		Deriva-Sil Conc.	TMS
	Acylation	HFBI	

	Alkylation	DMF Dialkylacetals	
Amines	Silylation	BSTFA	TMS
		MTBSTFA/t-BDMCS	TMS
	Acylation	HFBA (HFAA)	
		PFPA (PFAA)	
		TFAA	
		HFBI	
		PFBCl	
	Alkylation	DMF Diacetals	
AminoAcids	Silylation	BSTFA	
		TMSI	
	Acylation	HFBI (+ silylation)	
	Alkylation	DMF Dialkylacetals	
		TBH	
Carbohydrates & Sugars	Silylation	HMDS	
		Hydroxy-Sil AQ	
		TMSI	
	Acylation	MBTFA	
Carboxylic Acids	Silylation	BSA	
		BSTFA	
		BSTFA/TMCS	
		Deriva-Sil	
		Hydrox-Sil	
		MSTFA	
		MTBSTFA	
		TMSI	
	Acylation	PFPOH/PFPA	
	Alkylation	DMF Diacelkylacetals	
		TBH	
Catecholamines	Silylation	TMSI	

	Acylation	HFBA (HBAA)
		PFPA
		TFAA
		HFBI
Inorganic Anions	Silylation	BSTFA
		MTBSTFA
Nitrosamines	Acylation	HFBA
Sulfonamides	Silylation	BSTFA
	Acylation	HFBA (HFAA)
		PFPA
		TFAA
	Alkylation	DMF Dialkylacetals PFB-Br/ TBA-H-SO4

Butanolic HCl

Over the last year our 3N Butanolic HCl has been a major seller for us both domestically and internationally. It is used to test infants before they are released from hospital to make sure that there are no problems with their metabolism. While this item is not mentioned in our catalog we do sell it in the following sizes:

Product#	Product	List Price
201009	3 N Butanolic HCl 100 ml	$22
201010	3 N Butanolic HCl 500 ml	$100

The Butanolic HCl is used for derivatizing amino acids and acylcarnites. Blood concentrations of one or several of these compounds are either elevated or decreased in a series of metabolic disorders. Over the last 35 years newborns have been screened for these metabolic disorders. Recently they have started using a tandem GC/MS which can measure all these compounds from a single spot of blood. However, these compounds are poorly ionized in the GC/MS. They are therefore butylated (esterfied) not only to increase their ionization level, but also because it somewhat changes their pattern of fragmentation in the GC/MS. The system is then able to trace the particular fragment corresponding to the loss of the butylated fragment and this is used as a reference.

Why Regis

With over 30 years experiencing manufacturing these reagents, we are able to consistently offer pure, high quality reagents. We maintain an excellent stock on our reagents, which enables us to ship out your order immediately.

In the Regis Chromatography Catalog you will find recommendations on which reagent to use to derivatize your compound, along with a procedure we recommend and references. In addition we offer excellent technical support.

Regis will offer better pricing for large scale, bulk or blanket orders.

Who uses Derivatization Reagents

- Large Drug Testing Laboratories
- Forensic Laboratories
- Hospitals
- Small Testing Laboratories
- Environmental Laboratories
- Pharmaceutical Laboratories

Top Selling Reagents

- BSTFA/ Regisil
- MSTFA
- MTBSTFA
- MBTFA
- PFPA
- TPC
- 3N Butanolic HCl