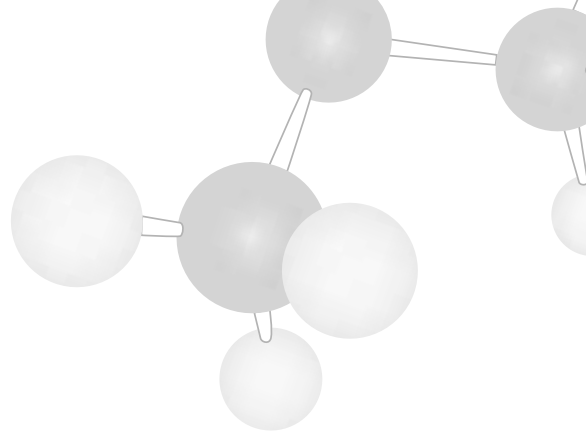


**GC  
DERIVATIZATION  
REAGENTS**



## DERIVATIZATION



### Derivatization

Derivatization is primarily performed to modify an analyte's functionality in order to enable chromatographic separations. For more than 30 years, Regis has been a leader in the manufacture of highly pure derivatization reagents for gas chromatography.

The formation of chemical derivatives to facilitate meaningful analysis has long been a common practice in gas chromatography. For the analytical chemist, judicious use of derivatization can be the key to unlocking and simplifying a great many complex and perplexing separation problems. Derivatization, accomplished through alteration of functional groups, provides:

- Increased sample volatility
- Improved selectivity and chromatographic efficiency
- Enhanced detectability



Additional procedures and references are available on our Web site at: [www.registech.com](http://www.registech.com).



## GAS CHROMATOGRAPHY (GC) DERIVATIZATION

Sample volatility or thermal stability is crucial in GC applications. If a sample does not possess these important characteristics, GC analysis is highly unproductive. Derivatization techniques have been developed to address these issues to insure successful separations. In GC Derivatization, replacement of active hydrogen in functional groups, such as -COOH, -OH, -NH, and -SH, is the primary area of concern and is accomplished through either silylation, acylation or alkylation.

### Silylation

Silylation is the most widely used derivatization procedure for sample analysis by GC. Silylation reagents are popular because they are easy to use and readily form derivatives. In silylation, an active hydrogen is replaced by an alkylsilyl group, such as trimethylsilyl (TMS) or *t*-butyldimethylsilyl (*t*-BDMS). Compared to their parent compounds, silyl derivatives are more volatile, less polar, and more thermally stable. As a result, GC separation is improved and detection is enhanced.

Silylation reagents are generally moisture sensitive, requiring them to be sealed under nitrogen to prevent deactivation. The derivatives of TMS reagents are also moisture sensitive. In response to this difficulty, *t*-BDMS reagents were introduced, which enabled the formation of derivatives 10,000 times more stable to hydrolysis than the TMS ethers.

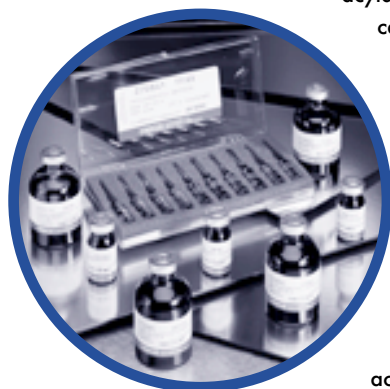
Both TMS and *t*-BDMS reagents are suitable for a wide variety of compounds, offer excellent thermal stability and can be used in a variety of GC conditions and applications.

Analysis by the popular combination of gas chromatography and mass spectrometry (GC/MS) often requires special sample derivatization. Particularly effective in these applications are MTBSTFA and deuterio-MTBSTFA-d.



### Acylation

Acylation reagents offer the same types of advantages available from silylation reagents: creating less polar, more volatile derivatives. However, in comparison to silylating reagents, the acylating reagents more readily target highly polar, multi-functional compounds, such as carbohydrates and amino acids. In addition, acylating reagents provide the distinct advantage of introducing electron-capturing groups, thus enhancing detectability during analysis.



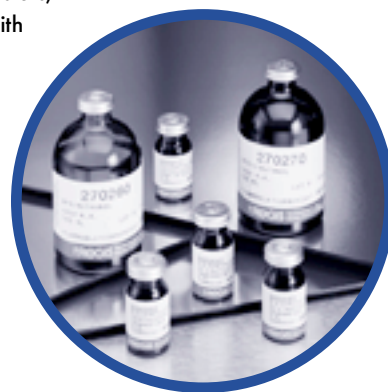
Generally, these reagents are available as acid anhydrides, acyl derivatives, or acyl halides. The acyl halides and acyl derivatives are highly reactive and are suitable for use where steric hindrance may be a factor. Acid anhydrides are supplied in a number of fluorinated configurations, which improve detection. These fluorinated anhydride derivatives are used primarily for Electron Capture Detection (ECD), but can also be used for Flame Ionization Detection (FID). Fluorinated anhydrides are often used in derivatizing samples to confirm drugs of abuse. Despite the special utility of these reagents, their acidic nature requires that any excess or byproducts be removed prior to analysis to prevent deterioration of the column.

## GAS CHROMATOGRAPHY (GC) DERIVATIZATION

### Alkylation

As with other derivatization reagents, alkylation reagents reduce molecular polarity by replacing active hydrogens with an alkyl group. These reagents are used to modify compounds having acidic hydrogens, such as carboxylic acids and phenols. Alkylation reagents can be used alone to form esters, ethers, and amides—or they can be used in conjunction with acylation or silylation reagents. A two-step approach is commonly used in the derivatization of amino acids, where multiple functional groups on these compounds may necessitate protection during derivatization.

Due to the availability of reagents and their ease of use, esterification (the reaction of an acid with an alcohol in the presence of a catalyst to form an ester) is the most popular method of alkylation. Alkylation reagents are available in several configurations that enable the formation of a variety of esters. Alkyl esters are stable, and can be formed quickly and quantitatively. By altering the length of the substituted alkyl group, retention of the derivative can be varied. In addition to the formation of simple esters, alkylation reagents can be used in extractive procedures where biological matrices may be present.



### GC Chiral Derivatization

GC analysis of enantiomeric compounds on nonracemic or achiral stationary phases requires the use of enantiopure derivatization reagents. These reagents generally target one specific functional group to produce diastereomers of each of the enantiomeric analytes. From the resulting chromatograms, calculations are conducted to determine the enantiomeric concentration of the analyte.



## GUIDE TO GC DERIVATIZATION METHODS/FUNCTIONAL GROUPS

Functional Group	GC Derivatization Method		
	Silylation	Acylation	Alkylation
Active Hydrogens	BSA, BSTFA, BSTFA/TMCS, Deriva-Sil, Hydrox-Sil, MSTFA, MTBSTFA, TMSI	PFPOH/PFPA	DMF Dialkylacetals, TBH
Carboxylic Acids	BSTFA, Hydrox-Sil Conc., MTBSTFA, TMSI	PFPOH/PFPA	BF <sub>3</sub> /Methanol, BF <sub>3</sub> / <i>n</i> -Butanol, DMF Dialkylacetals, TBH
Alcohols and Phenols: unhindered and moderately hindered	BSA, BSTFA/TMCS, HMDS, MTBSTFA/ <i>t</i> -BDMCS	HFBI, Fluorinated anhydrides (HFBA, PFPA, TFAA), MBTFA, MCF*	DMF Dialkylacetals, PFB-Br/TBA-H-SO <sub>4</sub> , TBH
Alcohols and Phenols: highly hindered	BSTFA/TMCS, Deriva-Sil, Deriva-Sil Conc.	Fluorinated anhydrides (HFBA, PFPA, TFAA), HFBI, PFBCI	DMF Dialkylacetals, PFB-Br/TBA-H-SO <sub>4</sub> , TBH
Amines: primary and secondary	BSTFA, MTBSTFA/ <i>t</i> -BDMCS	Fluorinated anhydrides (HFBA, PFPA, TFAA), HFBI, MBTFA, PFBCI, TPC*	DMF Dialkylacetals, TBH
Amides	BSA, BSTFA, BSTFA/TMCS, Deriva-Sil Conc.	HFBI	DMF Dialkylacetals, TBH
Amino Acids	BSTFA, TMSI	HFBI (+ Silylation)	DMF Dialkylacetals, TBH
Catecholamines	TMSI	Fluorinated anhydrides (HFBA, PFPA, TFAA), HFBI	
Carbohydrates and Sugars	HMDS, Hydrox-Sil AQ, TMSI	MBTFA	
Inorganic Anions	BSTFA, MTBSTFA		
Nitrosamines		HFBA	
Sulfonamides	BSTFA	Fluorinated anhydrides (HFBA, PFPA, TFAA)	DMF Dialkylacetals, PFB-Br/TBA-H-SO <sub>4</sub>

Derivatization reagents are listed in alphabetical order, not in order of preference.

\* For Chiral Analysis

Source: Knapp, D.R. *Handbook of Analytical Derivatization Reactions*; John Wiley and Sons: New York, 1979.









