

Guide to Derivatization Reagents for GC

A large number of reagents are used to prepare derivatives for gas chromatography, but most of the derivatization reactions fit into one of three categories: acylation, alkylation, or silylation. This bulletin describes each category, and presents information on how to choose the proper reagent based on the functional group(s) of the compound to be derivatized.

Key Words

- acylation ● alkylation ● silylation
- derivatization reagents ● derivatives

Considerations When Derivatizing an Analyte

Gas chromatography is used to separate volatile organic compounds. By modifying the functionality of a molecule to increase – or sometimes decrease – volatility, derivatizing reagents enable chromatographers to analyze compounds that otherwise are not readily monitored by GC. Derivatization also reduces analyte adsorption in the GC system and improves detector response, peak separations, and peak symmetry.

Derivatives are used for the following reasons:

- to improve resolution and reduce tailing of polar compounds (–OH, –COOH, =NH, –NH₂, –SH, and other functional groups)
- to analyze relatively nonvolatile compounds
- to improve analytical efficiency and increase detectability
- to improve stability of compounds

The choice of a derivatizing reagent is based on the functional group requiring derivatization, the presence of other functional groups in the molecule, and the reason for performing the derivatization. The chemical structure and properties of the molecule influence the reagent choice.

In choosing a suitable derivatization reagent, certain criteria must be used as guidelines. A good reagent:

- produces a derivatization reaction that is 95-100% complete
- will not cause any rearrangements or structural alterations during formation of the derivative
- does not contribute to loss of the sample during the reaction
- produces a derivative that will not interact with the analytical (GC or HPLC) column
- produces a derivative that is stable with respect to time

Supelco offers helpful free technical literature for most derivatization reagents (see page 9).

Glassware for Derivatization

Vials with 0.1-10.0mL capacity accommodate sample plus solvent and reagent in quantities typically used in gas chromatography. Vials must be suitable for temperature extremes. Vials supplied with open-center screw caps can be sealed with rubber septum stoppers or Teflon®-lined discs. The heavy walls and excellent sealing properties of Supelco™ micro-reaction vials allow samples to be heated safely to moderately high temperatures. Ground bottoms give these vials added stability on a flat surface, and are convenient for pencil markings. Thermostatically controlled heating units with aluminum blocks drilled to fit the vials precisely are available from several manufacturers, including Supelco.

Note: Although a Teflon lining generally is quite inert, it can be dissolved by some samples and reagents.

Deactivation of Glassware

Because the surface of laboratory glassware is slightly acidic, it can adsorb some analytes — particularly amines. In low level analyses, such losses can be significant. To prevent sample loss through adsorption, glassware used in low level analyses usually is silanized. Silanization masks the polar Si-OH groups on the glass surface by chemically binding a nonadsorptive silicone layer to the surface, in effect “derivatizing” the glass. In the most common silanization procedure, the glassware is treated with a solution of 5-10% dimethyldichlorosilane (DMDCS) in toluene for 30 minutes. The deactivated glassware is rinsed with toluene, then immediately thereafter with methanol.

Adsorption also can be reduced by adding a compound that competes for the adsorptive sites on the glass surface. A small amount (often less than 1%) of an alcohol, such as butanol, added to the solvent significantly reduces adsorption losses.

Contents

Topic	Page
Considerations When Derivatizing an Analyte	1
Acylation	2
Alkylation	3
Silylation	3
Derivatization Reagent Selection Guide	4
Troubleshooting	8
Ordering Information	9

Sample Handling

Most lab personnel transfer samples and reagents with pipettes. For sensitive reagents, we recommend using a microliter syringe, which reduces exposure to atmospheric moisture. Syringes with Teflon-tipped plungers are more convenient than conventional syringes with all-metal plungers, particularly for transferring volatile reagents. The Teflon plunger tip forms a better seal and facilitates withdrawal of the reagent from a sealed vial.

Any syringe will retain some reagent in the barrel. A syringe with an all-metal plunger, if not properly cleaned, is prone to corrosion and seizing. The best cleaning procedure is to remove and wash the plunger, and use a vacuum to pull solvent through the syringe. A seized plunger sometimes can be freed by soaking the syringe in a container filled with methanol.

Injection Ports

When working with silylating reagents, use a silanized glass injection port or make injections directly onto a glass column. Use of a stainless steel injection port frequently yields erratic and irreproducible results. The problem may not become apparent until after several weeks of use, when corrective action may include replacing the injector.

Reaction Time

Reaction time varies greatly among compounds. Many materials can be derivatized by the reagents described here in a matter of seconds or minutes at room temperature, while others require extended periods at elevated temperatures. For a compound with unknown reactivity, the progress of the derivatization can be monitored by periodic chromatographic analysis of aliquots of the reaction mixture. Disappearance of the reagents or appearance of product peaks can be used to determine the reaction's progress.

Heating often increases the yield of derivative and/or shortens the reaction time. Before using heat, consider the thermal stability of the analytes and reagents involved.

Water

Water in the reaction mixture often can hinder the reaction and/or hydrolyze the derivative, reducing the yield of derivative for analysis. Tightly seal opened reagents during storage. If necessary, add sodium sulfate to the reaction mixture to trap water present in the sample.

Chromatography

We offer a wide range of general purpose and specially tested capillary GC columns for evaluating underivatized and derivatized analytes. For descriptions of our capillary columns, please refer to the current Supelco catalog.

Acylation

Acylation, an alternative to silylation, is the conversion of compounds that contain active hydrogens (-NH, -OH, -SH) into amides, esters, or thioesters through the action of a carboxylic acid or carboxylic derivative. Acylation has many benefits:

- It improves analyte stability by protecting unstable groups.
- It can confer volatility on substances such as carbohydrates or amino acids, which have so many polar groups that they are nonvolatile and normally decompose on heating.
- It assists in chromatographic separations which might not be possible with underivatized compounds.
- Compounds are detectable at very low levels with an electron capture detector.

In halocarbons, the presence of a carbonyl group adjacent to a halogenated carbon enhances the electron capture detector (ECD) response. Acylation also has been used to form fragmentation-directing derivatives for mass spectrometry and chromogenic derivatives for HPLC.

Perfluoro Acid Anhydrides

Acylation of amino, hydroxy, and thiol groups to the perfluoroacyl derivatives reduces polarity. The derivatives also are both stable and highly volatile. Although fluorinated anhydride derivatives are used primarily with electron capture detectors (ECD), they can be used with flame ionization detectors (FID). These reagents react with alcohols, amines, and phenols to produce stable derivatives. Fluorinated anhydrides are used in derivatizing samples for drug of abuse confirmation.

The perfluoro acid anhydrides and acyl halide reagents form acidic byproducts which must be removed prior to the GC analysis, to prevent damage to the chromatography column. Acylations with anhydride reagents normally are performed in pyridine, tetrahydrofuran, or other solvent capable of accepting the acid byproduct. Amine bases also may be used as catalysts/acid acceptors.

Perfluoroacylimidazoles

Perfluoroacylimidazoles offer advantages over perfluoro acid anhydrides for preparing perfluoroacyl derivatives. The reactions are smooth and quantitative, and produce no acid byproducts that must be removed prior to the injection.

The activated amide reagents also yield no acid byproducts, producing only imidazole and N-methyltrifluoroacetamide, respectively.

The perfluoroacylimidazoles react with hydroxyl groups and both primary and secondary amines, and quantitatively acylate indole alkalamines.

General Acylation Reagents

N-Methyl-bis(trifluoroacetamide) (MTBTFA) trifluoroacylates primary and secondary amine, hydroxyl, and thiol groups under mild non-acidic conditions. Reactions with amines generally proceed at room temperature. Hydroxyl derivatizations are slower; heat is recommended. N-methyltrifluoroacetamide, the principal byproduct of the derivatization reaction, is stable and volatile, and does not interfere with the chromatography.

Alkylation

Alkylation involves adding an alkyl group (aliphatic or aromatic) to an active functional (H) group. Replacement of hydrogen with an alkyl group is important because the derivative has lower polarity, relative to the parent substance. Alkylation reagents are used to modify compounds containing acidic hydrogens, such as carboxylic acids and phenols. The resulting products are ethers, esters, thioethers, thioesters, n-alkylamines, and n-alkylamides. Alkylation of weakly acidic groups (e.g., alcohols) requires strongly basic catalysts (sodium or potassium methoxide). More acidic OH groups (phenols, carboxylic acids) require less basic catalysts (hydrogen chloride, boron trifluoride).

DMF-Dialkylacetals

Dimethylformamide dialkyl acetals are used to esterify acids to their methyl esters. Hydroxyl groups are not methylated. Carboxylic acids, phenols, and thiols react quickly, to give the corresponding alkyl derivatives. N,N-dimethylformamide dimethylacetals are moisture sensitive.

Diazoalkanes

In the presence of a small amount of methanol as catalyst, diazomethane (a yellow gas, usually used as an ethereal solution) reacts rapidly with fatty acids, forming methyl esters. Elimination of gaseous nitrogen drives the reaction. The yield is high and side reactions are minimal. However, diazomethane is carcinogenic, highly toxic, and potentially explosive. Diazomethane is not ideal for esterifying phenolic acids because the phenolic hydroxyl groups also are methylated (at a slower rate), which can lead to mixtures of partially methylated products.

Esterification and Transesterification Reagents

Esterification, the reaction of an acid with an alcohol to form an ester, is the most popular alkylation method. Alkyl esters offer excellent stability, and provide quick and quantitative samples for GC analysis. The process involves the condensation of the carboxyl group of the acid and the hydroxyl group of the alcohol, with elimination of water. Results are best in the presence of a catalyst (e.g., hydrogen chloride), which is removed with the water.

Transesterification is the displacement of the alcohol portion of an ester by another alcohol. This reaction has been widely used for making esters of higher alcohols from esters of lower alcohols. In the presence of an acidic or basic catalyst, methanol can be used to transesterify fats or oils.

General Alkylation Reagents

Pentafluorobenzylbromide is convenient for making esters and ethers, and has been used in trace analyses. This strong lachrymator should be used in a hood. Hexacyclooctadecane and pentafluorobenzylbromide are used to prepare pentafluorobenzyl - dilute with 50mL 2-propanol. 1mL of this reagent will derivatize up to 0.3mg phenols.)

Esterate-M is used to prepare methyl and other esters of long chain fatty acids by reaction with dimethylformamide and dimethylacetal. Aldehydes and ketones are conveniently derivatized by forming oximes with o-alkylhydroxylamine HCl reagents. o-Methylhydroxylamine HCl has been used with ketosteroids, prostaglandins, saccharides, aldoacids, and ketoacids. N-butylboronic acid reacts with 1,2- or 1,3-diols or with α - or β -hydroxy acids to form 5- or 6-member ring nonpolar boronate derivatives. The derivatives are prepared simply by adding n-butylboronic acid to a solution of the hydroxy compound in dimethylformamide.

Silylation

Silylation is the introduction of a silyl group into a molecule, usually in substitution for active hydrogen. Replacement of active hydrogen by a silyl group reduces the polarity of the compound and reduces hydrogen bonding. The silylated derivative thus is more volatile, and more stable. Detection is enhanced. Many hydroxy and amino compounds regarded as nonvolatile or unstable at 200-300°C have been successfully chromatographed after silylation. Silyl reagents are compatible with most detection systems but, if used in excess, can cause difficulties with flame ionization detectors.

The trimethylsilyl (TMS) group, $\text{Si}(\text{CH}_3)_3$, is the most popular and versatile silyl group for GC analysis. TMS derivatization enables better GC separations and application of special detection techniques. TMS silylating reagents and derivatives react with active hydrogen atoms. Consequently, TMS derivatives should not be analyzed on polyethylene glycol phases or other stationary phases that have these functional groups. Nonpolar silicone phases, such as SPB™-1 and SPB-5, combine inertness and stability with excellent separating characteristics for these derivatives.

Silyl reagents are influenced by both the solvent system and the addition of a catalyst. A catalyst (e.g., trimethylchlorosilane or pyridine) increases the reactivity of the reagent. Silyl reagents generally are moisture sensitive, and should be stored in tightly sealed containers.

Derivatizing Reagent Selection Guide

The table on pages 4-7 of this bulletin summarizes derivatization reagent selection, based on sample type. To choose a suitable reagent, first determine the functional group or compound type that is of interest to you (far left column in the table). There may be several options available (second column from left) – consider what chromatographic tools are available to you, and remember to consider the criteria for choosing a good reagent (page 1). The **Observations** column includes general hints which may assist you in making your selection.

Troubleshooting Guide

The troubleshooting guide on page 8 can assist you in solving derivatization problems. The guide lists problem symptoms (far left column), possible causes of the problem (second column), and suggested solutions. At the back of this bulletin we have included a blank page for you to record your own observations when troubleshooting a derivatization. If you have any helpful hints you would like to share with others, simply fax them to our Technical Service group. If you are unable to solve a derivatization problem, please call our Technical Service group for assistance.

Derivatization Reagent Selection Guide

Functional Group/ Compound Type	Procedure	Reagent	Derivative	Observations
Amides $\begin{array}{c} \text{O} \\ \parallel \\ \text{R}-\text{C}-\text{NH}_2 \\ \text{Primary} \\ \text{O} \quad \text{H} \\ \parallel \\ \text{R}-\text{C}-\text{N}-\text{R} \\ \text{Secondary} \end{array}$	Acylation	TFAA	Trifluoroacetamides	Most reactive and volatile of fluorinated anhydrides. Ideal with FID, ECD, TCD. Used in identifying methamphetamine. Requires lowest analysis temperature of fluorinated anhydrides. Ideal with FID, ECD, TCD. Used in identifying opiates, benzoylgonine. ⁶
		PFPA	Pentafluoropropionamides	Most sensitive fluorinated anhydride for EC detection. Ideal with FID, ECD, TCD. ⁶
		HFBA	Heptafluorobutyramides	A favorite reagent for drugs, especially barbiturates. Flash alkylation. ¹
		TMAH	Methyl amides	Exception: meprobamate - analyzed by direct GC analysis as the free base. Ideal for wet samples where excess reagent forms corresponding alcohol. ²
		DMF-dialkylacetals	N-(N,N-dimethyl)aminomethylenes	Highly reactive, universal reagent. See observations for carbonyls.
		BSA	Trimethylsilyl amides	Highly reactive, universal reagent, more volatile than BSA. See observations for carbonyls.
		BSTFA	Trimethylsilyl amides	TMCS acts as a catalyst - assists in derivatizing amines.
		BSTFA + TMCS	Trimethylsilyl amides	Strong, yet mild silylating reagent. Derivatives 10,000 times more stable to hydrolysis than TMS derivatives.
		MTBSTFA	TBDMCS amides	TBDMCS acts as a catalyst - assists in derivatizing amines.
		MTBSTFA + TBDMCS	TBDMCS amides	
Amines $\begin{array}{c} \text{H} \\ \\ \text{R}-\text{C}-\text{NH}_2 \\ \\ \text{H} \end{array}$ $\begin{array}{c} \text{H} \quad \text{H} \\ \quad \\ \text{R}-\text{C}-\text{N}-\text{R} \\ \\ \text{H} \end{array}$ Primary Secondary	Acylation	Acetic anhydride MBTFA	Acetates Trifluoroacetamides	Use with primary and secondary amines. Use with primary and secondary amines. Principal byproduct, N-methyltrifluoroacetamide, is stable, volatile, does not present problems with GC. Ideal with FID, ECD, TCD. Good for trace analysis with ECD.
		TFAA	Trifluoroacetamides	Most reactive & volatile of fluorinated anhydrides. Derivatives volatile for FID, ECD, TCD. Good for trace analysis with ECD.
		TFAI	Trifluoroacetamides	Good for trace analysis with ECD. No acid byproducts - byproduct, imidazole, is inert.
		PFPA	Pentafluoropropionamides	Requires lowest analysis temperature of fluorinated anhydrides. Used to identify catecholamines.
		HFBA	Heptafluorobutyramides	Ideal with FID, ECD, TCD. Most sensitive to EC - good for trace analysis with ECD.
		PFBBr	Pentafluorobenzyl ethers	Used to identify amphetamines, phencyclidine, catecholamines. ⁶
		DMF-dialkylacetals	N-(N,N-dimethyl)aminomethylenes	Ideal with ECD.
		NBB	Boronates	Rapid reactions, convenient to use. Use with sterically hindered amines. ²
		TMAH	Methyl amides	Converts α -amino acids, hydroxy acids, hydroxy amines, keto acids, diols to more easily chromatographed derivatives.
		BSA	Trimethylsilyl ethers	A favorite reagent for drugs, especially barbiturates. Exception: meprobamate - analyzed by direct GC analysis as the free base.
		BSTFA	Trimethylsilyl ethers	Reagent of choice for simultaneous silylation of amino and hydroxyl groups. Effective without solvent, but also used with solvents such as pyridine or DMF.
		BSTFA	Trimethylsilyl ethers	Reagent and byproducts are volatile. Can act as its own solvent. Can cause detector fouling and noise. If DMF is used as a solvent for silylating secondary amines, n(aminomethylene)-2,2,2-trifluoroacetamides can be formed instead of the TMS derivatives.
		BSTFA + TMCS	Trimethylsilyl ethers	TMCS acts as a catalyst - enhances reactivity of BSTFA.
		HMDS	Trimethylsilyl ethers	Used with TMCS to extend practical range of GC. Gaseous byproduct (NH ₃).

Functional Group/ Compound Type	Procedure	Reagent	Derivative	Observations
Carbohydrates (CH ₂ OH) _n Starches Sugars	Acylation	Acetic anhydride MBTFA	Acetates Trifluoroacetamides	Generally used with pyridine – 1:1 mixture with pyridine will derivatize alditols. Reagent of choice for derivatizing sugars. Forms volatile derivatives of mono-, di-, and trisaccharides.
	Silylation	TFAI BSA + TMCS BSTFA + TMCS HMDS	Trifluoroacetamides Trimethylsilyl ethers Trimethylsilyl ethers Trimethylsilyl ethers	Forms volatile derivatives of mono-, di-, and trisaccharides. BSA not recommended for carbohydrates – anomerization will occur. Can be used with some syrups. Use with sugar acids, glucuronides. Most popular choice for silylating sugar acids and related substances. TMCS will increase silylation potential. Use with aldoses. ⁴ Use with oligosaccharides. ⁴
		HMDS + TMCS HMDS + TMCS + pyridine TFA TMSI	Trimethylsilyl ethers Trimethylsilyl ethers Trimethylsilyl ethers Trimethylsilyl ethers	
		TMSI + pyridine	Trimethylsilyl ethers	Reagent of choice for silylating sugar phosphates in presence of small amounts of water. Can be used with some syrups. Use neat or with solvent.
Carbonyls >C=O Acid halides Acid anhydrides Aldehydes Enols Esters Ketones Hydrazones Oximes Phenoxy acids Steroids (hydroxy/ keto hormones)	Alkylation	BCl ₃ -2-chloroethanol o-Methoxyamine HCl	Chloro esters Oximes	Reagent of choice for silylating aldoses, sugar phosphates, disaccharides containing small amounts of water. Will not derivatize amino groups.
	Silylation	TFAA BSA BSTFA	Trifluoroacetates Trimethylsilyl ethers Trimethylsilyl ethers	Use to prepare phenoxy-type acids for ECD. Use with aldehydes, ketones, ketosteroids. Prevents keto groups from forming enol ethers. ³ Most reactive and volatile of anhydrides. No acid byproduct. Good with ECD. Under mild reaction conditions forms highly stable products with most organic functional groups. Very volatile. Byproduct, TMS-acetamide, may interfere with early eluting peaks. BSA mixtures oxidize to form SiO ₂ , which fouls FIDs. Reacts faster and more completely than BSA. BSTFA and its byproducts are highly volatile and will not interfere with early eluting peaks. Can act as its own solvent. Combustion product, HF, reacts with SiO ₂ , forming volatile products that can cause detector fouling and noise. TMCS acts as a catalyst, increasing reactivity of BSTFA. Use with hindered and unhindered steroids.
		BSTFA + TMCS TMSI + pyridine	Trimethylsilyl ethers Trimethylsilyl ethers	

¹Flash alkylation: analyte is derivatized in the GC injection port.

²DMF-dialkylacetals are recommended for sterically hindered aldehydes, amines, carboxylic acids, and phenols. Shorter chain reagents produce more volatile derivatives than longer chain reagents.

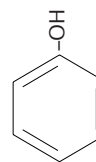
These include DMF-DBA, DMF-DEA, DMF-DMA, DMF-DPA, and Esterate-M (DMF-DMA, 2meq/mL in pyridine).

³In some cases methyl oximes are not resolved from other components of a complex mixture. o-Benzylhydroxylamine HCl forms less volatile derivatives which may be separated. If analysis requires ECD sensitivity, use o-(pentafluorobenzyl)hydroxylamine HCl.

⁴With HMDS + TMCS a fine precipitate of NH₄Cl is produced during derivatization. The precipitate does not affect chromatography.

⁶Perfluoro acid anhydrides produce acidic byproducts which must be removed from the reaction mixture before the derivatives are injected onto the GC column. With perfluoroacylimidazole there are no acid byproducts to remove.

Functional Group/ Compound Type	Procedure	Reagent	Derivative	Observations
Carboxyls R-C(=O)-OH	Alkylation	PFBBr BCl ₃ -methanol BF ₃ -butanol BF ₃ -propanol BF ₃ -methanol Trimethylsilyldiazomethane	Pentafluorobenzyl esters Chloro esters Butyl esters Propyl esters Methyl esters Methyl esters	Used with ECD, UV, MS detection. Use with cannabinoids, carboxylic and fatty acids. Used to prepare short chain (C1-C10) fatty acids for ECD. Used to prepare n-butyl esters of short chain (C1-C10) mono- and dicarboxylic acids. Used to prepare n-propyl esters. Use with large samples of C8-C24 fatty acids. Used with carboxylic acids. Excess reagent in the presence of methanol reacts instantly and quantitatively. Reaction easily monitored by disappearance of yellow color of reagent. ⁵ Alkylates carboxyl groups; use with sterically hindered carboxylic acids. Also reacts with amines, amino acids, phenols. ² Use with mono-, di-, triglycerides, glycolipids, sphingolipids. Use with fatty acids C9 and longer. Useful for esterifying difficult carboxylic acids (bile acids). Use with carboxylic acids and esters (transesterification). Use with carbohydrates, catecholamines, ceramides, sphingosines, corticosteroids, hop resin acids, α- and β-hydroxy acids, monoglycerides, monoglycerol ethers, prostaglandins. Reaction achieved by mixing equimolar amounts of sample and reagent in appropriate solvent (several minutes, room temp.). Polar groups on analyte must be on adjacent carbons, or separated by only 1 carbon. Use with reactive amino, carboxyl, or hydroxyl groups. Flash alkylation. ¹ Derivatives easily formed but generally not stable – analyze quickly. Reacts faster and more completely than BSA. See observations for carbonyls. TMCS acts as a catalyst, increasing reactivity of BSTFA. Use with fatty acids, cannabinoids, steroids. Will derivatize most hindered and unhindered steroid hydroxyls. Can be used with some salts.
Lipids/phospholipids Prostaglandins Steroids (bile, hydroxy/keto hormones)		DMF-dialkylacetals Methanolic base Methanolic HCl Methanolic H ₂ SO ₄ NBB	Methyl esters Methyl esters Methyl esters Cyclic boronates	
	Silylation	TMAH BSA BSTFA BSTFA + TMCS TMSI	N-Methyl esters Trimethylsilyl ethers Trimethylsilyl ethers Trimethylsilyl ethers Trimethylsilyl ethers	
Ethers ≡C-O-C≡ Epoxides	Silylation	HMDS + TMCS + pyridine TMCS	Trimethylsilyl ethers Trimethylsilyl ethers	Use with epoxides that do not react rapidly with TMCS. ⁴ Use with chlorohydrins.
Hydroxyls ROH Alcohols Alkaloids Cannabinoids Glycols	Acylation Alkylation	Acetic anhydride MBTFA TFAA TFAI PFPA HFBA PFBBr Trimethylsilyldiazomethane ⁵	Acetates Trifluoroacetates Trifluoroacetates Trifluoroacetates Pentafluoropropionates Heptafluorobutyrate Pentafluorobenzyl ethers Methyl esters	Use with alcohols, phenols. Good for trace analysis with ECD. Good for trace analysis with ECD. Good for trace analysis with ECD. Use with alcohols, phenols. Derivatives volatile for FID, ECD. Good for trace analysis with ECD. ⁶ Use with alcohols, phenols. Derivatives volatile for FID, ECD. Good for trace analysis with ECD. ⁶ Use with alcohols only. Use with ECD. Not ideal for esterifying phenolic acids – phenolic hydroxyl groups also are methylated (at a slower rate) – can lead to mixtures of partially methylated products. Flash alkylation of phenolic alkaloids. ¹ Use with sterically hindered phenols. ² Use with phenols for US EPA Method 604 (see General Alkylation Reagents – page 3). Most often used. Good choice for silylating phenols when used in DMF. Good thermal stability. Poor hydrolytic stability. Use with unhindered alcohols and phenols. Weak donor, usually used with TMCS. Appropriate solvent (pyridine, DMF, DMSO) may increase reaction rate.
Phenols	Silylation	TMAH DMF-dialkylacetals Hexaoxacyclooctane BSA BSTFA + TMCS HMDS	N-Methyl esters Methyl esters Pentafluorobenzyl phenols Trimethylsilyl ethers Trimethylsilyl ethers Trimethylsilyl ethers Trimethylsilyl ethers	
		MTBSTFA TBDMSIM TMCS	Trimethylsilyl ethers Trimethylsilyl ethers Trimethylsilyl ethers	Use with alcohols. Derivatives 10,000 times more stable to hydrolysis than TMS ethers. Weak donor, usually used with HMDS. Can be used with salts. Excellent catalyst for forming TMS ethers. Strongest silylation reagent for hydroxyls. No reaction with amines or amides. Derivatizes sugars in presence of water. Can be used with syrups.
		TMSI	Trimethylsilyl ethers	



Functional Group/ Compound Type	Procedure	Reagent	Derivative	Observations
Nitriles R-C≡N	Undergo many of the same reactions as carboxylic acids – see Carbonyls .			
Thiols	Acylation	MBTFA PFBBr	Trimethylsilyl ethers Trimethylsilyl ethers	Reaction occurs under mild, non-acidic conditions. Use with ECD. ⁶
R-SH Mercaptans	Alkylation Silylation	Trimethylsilyldiazomethane ⁵ DMF-dialkylacetals ² TMSI	Methyl esters Methyl esters Trimethylsilyl ethers	
Sulfides R-S	Silylation	TMSI	Trimethylsilyl ethers	
Sulfonic Acids R-SO ₂ OH	Alkylation Silylation	TMAH PFBBr ⁶ TMSI	N-Methyl esters Trimethylsilyl ethers Trimethylsilyl ethers	Reaction is with hydroxyl group.
Sulfonamides	Acylation	TFAA PFBBr HFBA	Trifluoroacetates Trimethylsilyl ethers Trimethylsilyl ethers	Stable derivatives. Stable derivatives. Enhances ECD. ⁶ Stable derivatives. Enhances ECD. ⁶
R-SO ₂ NH ₂	Alkylation Silylation	DMF-dialkylacetals ² BSTFA	Methyl esters Trimethylsilyl ethers	

¹Flash alkylation: analyte is derivatized in the GC injection port.

²DMF-dialkylacetals are recommended for sterically hindered aldehydes, amines, carboxylic acids, and phenols. Shorter chain reagents produce more volatile derivatives than longer chain reagents. These include DMF-DBA, DMF-DEA, DMF-DMA, DMF-DPA, and Esterate-M (DMF-DMA, 2meq/mL in pyridine).

⁵Safer substitute for diazomethane.

⁶Perfluoro acid anhydrides produce acidic byproducts which must be removed from the reaction mixture before the derivatives are injected onto the GC column. With perfluoroacylimidazole there are no acid byproducts to remove.

Useful Literature

Handbook of Analytical Derivatization Reactions

D.R. Knapp **23561**

For additional information and prices refer to the current Supelco catalog.

Supelco Technical Literature

Product Specification sheets containing detailed information about reagent physical properties, typical derivatization procedures, reaction mechanisms, storage information, etc. are available, free, for most of the acylation, alkylation, and silylation reagents described in this bulletin. To request this free literature see the table on page 9.

Supelco also produces a wide variety of technical literature, all of which is free. You may request this literature by using the business reply card located at the back of our catalog or by calling our Ordering and Customer Service Department (800-247-6628 or 814-359-3441).

Troubleshooting the Derivatization Reaction and Analysis

With few exceptions, possible causes and remedies listed here specifically address the derivatization process. It is assumed that an appropriate column and analytical conditions, and other general considerations, are used.

Symptom	Possible Cause	Remedy
Missing peaks or solvent peak only	<ol style="list-style-type: none"> 1. Impurities in solvent, starting material, catalysts, or extract may interfere with derivatization (e.g., plasticizers from vial, inorganics used in sample synthesis, preservatives or antioxidants in solvents). 2. Reagent deteriorated. 3. Reagent:sample ratio too low. 4. Rate of reaction too slow. 5. Water in reaction mix. 6. Wrong reagent. 7. Sample adsorbed to glassware. 	<ol style="list-style-type: none"> 1. Use only highest purity materials at all steps in sample preparation process. 2. Store reagent properly to prevent oxygen/water contamination, temperature damage (see product specification sheet). 3. Use more reagent for same amount of sample. 4. Reevaluate reagent concentration, time, temperature. Consider heating the reaction mix (consider thermal stability of the analytes and reagents). A catalyst will increase the reactivity of some reagents. 5. Remove water by adding sodium sulfate to sample. Store reagent properly to prevent oxygen/water contamination. 6. Reevaluate reagent selection. 7. Deactivate glassware, inlet sleeve, and column by silanization.
Extra peak(s)	<ol style="list-style-type: none"> 1. Reagent interacting with column. 2. Impurities from sample, solvent, reagents, sample vial, other labware. 3. Derivative undergoing hydrolysis. 4. Derivative reacting with solvent. 	<ol style="list-style-type: none"> 1. Verify that reagent is compatible with analytical column. 2. Inject solvent and reagent blanks, solvent rinse from unused vial, etc. to isolate source of impurities. 3. Remove water by adding sodium sulfate to sample. Store reagent properly to prevent oxygen/water contamination. 4. Use a solvent that does not have an active hydrogen, alcohol, or enolizable ketone group (e.g., hexane, toluene, etc.).
Detector response low	<ol style="list-style-type: none"> 1. Low yield of derivative – reaction did not go to completion. 2. Detector (FID) dirty. 3. Sample components absorbed by inlet liner or column. 	<ol style="list-style-type: none"> 1. Add more reagent, increase temperature or heating time, or add catalyst. Water may be present; add sodium sulfate to sample. 2. Clean FID per instrument manual. 3. Inject standard on column known to be performing well. If results are good, remove inlet liner and check cleanliness. Use new, deactivated liner or replace glass wool and packing. Rinse bonded phase column or remove 1-2 coils from inlet end of nonbonded column. If performance is not restored, replace column.
No sample separation after adding reagent and heating	<ol style="list-style-type: none"> 1. Septum in reaction vial not sealed. 	<ol style="list-style-type: none"> 1. Prepare a new sample and derivatize. Be sure vial is sealed.
Low Yield	<ol style="list-style-type: none"> 1. Improper handling technique: extra steps allow more room for error (e.g., low boiling components could be lost during sample concentration); sample too dilute; wrong solvent. 2. Impurities in solvent, starting material, catalysts, or extract interfering with derivatization (e.g., plasticizers from vial, inorganics used in sample synthesis, preservatives or antioxidants in solvents). 3. Reagent deteriorated. 4. Reagent:sample ratio too low. 5. Rate of reaction too slow. 6. Water in reaction mix. 7. Wrong reagent. 8. Sample adsorbed to glassware. 9. Carrier, air, detector (FID) hydrogen, or make-up gas flow set incorrectly. 	<ol style="list-style-type: none"> 1. Reevaluate technique, if possible eliminate steps in which analyte could be adsorbed or otherwise lost (unnecessary transfers, etc.). 2. Use only highest purity materials at all steps in the sample preparation process. 3. Store reagent properly to prevent oxygen/water contamination, temperature damage (see product specification sheet). 4. Use more reagent for same amount of sample. 5. Reevaluate reagent concentration, time, temperature. Consider heating the reaction mix (consider thermal stability of the analytes and reagents). A catalyst will increase the reactivity of some reagents. 6. Remove water by adding sodium sulfate to sample. Store reagent properly to prevent oxygen/water contamination. 7. Reevaluate reagent selection. 8. Deactivate glassware, inlet sleeve, and column by silanizing. 9. Measure flows and set according to instrument manufacturer's recommendations.

Product specification sheets for most Supelco reagents are available free of charge. These publications contain information about the reagent: physical properties, use, benefits, mechanism of action and typical derivatization procedures, toxicity, hazards, and storage. To obtain free copies, contact our Order Processing department.

Refer to These Publications for Descriptions of Reagents and Step-by-Step Procedures for Derivatization

Reagent	Publication No.
Acetic Anhydride	T497121
BSA (N,O-bis(trimethylsilyl)acetamide)	T496017
BSA + TMCS	T496018
BSA + TMCS + TMSI	T496019
BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide)	T496020
BSTFA + TMCS	T496021
DMDCS (dimethyldichlorosilane)	T496022
DMDCS in toluene	T496023
HMDS (hexamethyldisilazane)	T496024
HMDS + TMCS	T496025
HMDS + TMCS + pyridine	T496026
Methanolic Base, 0.5N	T497007
Methanolic HCl, 0.5N, 3N	T497099
Methanolic H ₂ SO ₄	T497018
Perfluoro Acid Anhydrides	T497104
Pentafluorobenzyl Bromide, Hexaoxacyclooctadecane Rejuv-8™	T497103
TBDMSIM (N-t-butyltrimethylsilylimidazole)	T496065
TMAH	T496180
TMCS (trimethylchlorosilane)	T496028
TMSI (N-trimethylsilylimidazole)	T496029
TMSI + pyridine	T496030
Trifluoroacetic acid	T496027
BCl ₃ -2-chloroethanol	T496122
BCl ₃ -methanol	T496123
BF ₃ -butanol	T496124
BF ₃ -methanol	T496125

Acronyms

Acronym	Chemical Name [CAS No.]
BSA	N,O-Bis(trimethylsilyl)acetamide [10416-59-8]
BSTFA	Bis(trimethylsilyl)trifluoroacetamide [25561-30-2]
Diazald-N-methyl- ¹³ C	N-Methyl- ¹³ C-N-nitroso- <i>p</i> -toluenesulfonamide [60858-95-9]
Diazald-N-methyl- ¹³ C-N-methyl-d ₃	N-Methyl- ¹³ C-d ₃ -N-nitroso- <i>p</i> -toluenesulfonamide [102832-11-1]
DMDCS	Dimethyldichlorosilane [75-78-5]
DMF-DBA	N,N-Dimethylformamide / Di- <i>tert</i> -butyl acetal [36805-97-7]
DMF-DEA	N,N-Dimethylformamide / Diethyl acetal [1188-33-6]
DMF-DMA	N,N-Dimethylformamide / Dimethyl acetal [4637-24-5]
DMF-DPA	N,N-Dimethylformamide / Dipropyl acetal [6006-65-1]
DMP	2,2 Dimethoxypropane [77-76-9]
HFBA	Heptafluorobutyric anhydride [336-59-4]
HMDS	1,1,1,3,3,3-Hexamethyldisilazane [999-97-3]
MBTFA	N-Methylbis(trifluoroacetamide) [685-27-8]
MNNG	1-Methyl-3-nitro-1-nitrosoguanidine [70-25-7]
MTBSTFA	N-(<i>tert</i> -Butyldimethylsilyl)-N-methyl-trifluoroacetamide [77377-52-7]
NBB	<i>n</i> -Butylboronic acid [4426-47-5]
PFBBr	Pentafluorobenzylbromide [1765-40-8]
PFFA	Pentafluoropropionic anhydride [356-42-3]
TBDMCS	<i>t</i> -Butyldimethylchlorosilane [18162-48-6]
TBDMSIM	N-(<i>tert</i> -Butyldimethylsilyl)imidazole
TFAI	1-(Trifluoroacetyl)imidazole [1546-79-8]
TMCS	Trimethylchlorosilane [75-77-4]
TMSDEA	Trimethylsilyldiethylamine
	(N,N-Diethyl-1,1,1-trimethylsilylamine) [996-50-9]
TMSI	Trimethylsilylimidazole [18156-74-6]

Ordering Information:

Acylation Reagents

Description	Cat. No.
Acetic Anhydride	
10 x 2mL	33085
HFBA	
10 x 1mL	33170-U
MBTFA	
10 x 1mL	39,4939-10X1ML
5mL	39,4939-5ML
PFFA	
10 x 1mL	33167
25mL	33168
TFAA	
10 x 1mL	33165-U
25mL	33164
TFAI	
10 x 1mL	39,4920-10X1ML
5mL	39,4920-5ML

Alkylation Reagents

Description	Cat. No.
DMF-Dialkylacetals	
DMF-DBA	
10 x 1mL	39,5005-10X1ML
5mL	39,5005-5ML
25mL	39,5005-25ML
DMF-DEA (1,1-Diethoxytrimethylamine)	
10 x 1mL	39,4971-10X1ML
5mL	39,4971-5ML
25mL	39,4971-25ML
DMF-DMA	
10 x 1mL	39,4963-10X1ML
5mL	39,4963-5ML
25mL	39,4963-25ML
DMF-DPA	
10 x 1mL	39,4998-10X1ML
5mL	39,4998-5ML
25mL	39,4998-25ML
Diazoalkales[■]	
Diazald	
25g	D28000-25G
100g	D28000-100G
500g	D28000-500G
1kg	D28000-1KG
Diazald-N-methyl- ¹³ C (99 atom % ¹³ C)	
250mg	27,7614-250MG
1g	27,7614-1G
Diazald-N-methyl- ¹³ C-N-methyl-d ₃ (99 atom % ¹³ C, 99 atom % d ₃)	
250mg	29,5981-250MG
1g	29,5981-1G
MNNG	
10g	12,9941-10G
25g	12,9941-25G
(Trimethylsilyl)diazomethane (2.0M solution in hexanes)	
5mL	36,2832-5ML
25mL	36,2832-25ML

■ For more information, request Aldrich publication AL-180.

Trademarks

Omegawax, REACTA-SIL, Rejuv-8, SP, SPB, Supelco, Sylon — Sigma-Aldrich Co. Teflon — E.I. du Pont de Nemours & Co., Inc.

Alkylation Reagents (contd.)

Description	Cat. No.
Esterification Reagents	
BCl ₃ -2-Chloroethanol (11% w/w) 10 x 1mL	33056-U
BCl ₃ -Methanol (12% w/w) 20 x 1mL	33353
20 x 2mL	33089-U
400mL	33033
BF ₃ -Butanol (10% w/w) 10 x 5mL	33126-U
100mL	33125-U
BF ₃ -Methanol (10% w/w) 20 x 1mL	33356
19 x 2mL	33020-U
10 x 5mL	33040-U
5mL	26,4121-5ML
250mL	26,4121-250ML
400mL	33021
BF ₃ -Propanol (14% w/w) 5g	15,6825-5G
100g	15,6825-100G
500g	15,6825-500G
Methanolic Base (0.5N) 2N, 30mL	33352
2N, 100mL	33080
Methanolic HCl 0.5N, 20 x 1mL	33354
0.5N, 10 x 5mL	33095
3N, 20 x 1mL	33355
3N, 10 x 3mL	33051
3N, 400mL	33050-U
Methanolic H ₂ SO ₄ (10% H ₂ SO ₄ v/v in methanol) 6 x 5mL	506516
TMAH, 0.2M in methanol 10 x 1mL	33358-U
10mL	33097-U
General Alkylation Reagents	
DMP (2,2-Dimethoxypropane), 25g	33053
Esterate M, 25mL	33140
Hexaoxacyclooctadecane (18 crown 6), 25g	33003-U
O-Methoxyamine HCl, 5g	33045-U
Pentafluorobenzyl bromide, 5g	33001

Silyl Reagents

Description	Cat. No.
BSA, derivatization grade 144 x 0.1mL	33035-U
20 x 1mL	33036
25mL	33037-U
BSA + TMCS, 5:1 (Sylon™ BT) 20 x 1mL	33018
25mL	33019-U
BSA + TMCS + TMSI, 3:2:3 (Sylon BTZ) 144 x 0.1mL	33151
20 x 1mL	33030
25mL	33031-U

Silyl Reagents (contd.)

Description	Cat. No.
BSTFA, derivatization grade 144 x 0.1mL	33084
20 x 1mL	33024
25mL	33027
BSTFA + TMCS, 99:1 (Sylon BFT) 144 x 0.1mL	33154-U
20 x 1mL	33148
25mL	33155-U
50mL	33149-U
HMDS 30mL	33350-U
100mL	33011
HMDS + TMCS, 3:1 (Sylon HT) 20 x 1mL	33046
REACTA-SIL® Concentrate (HMDS:TMCS, 2:1) 25mL	39,4610-25ML
HMDS + TMCS + Pyridine, 3:1:9 (Sylon HTP) 20 x 1mL	33038
25mL	33039
N-Methyl-N-(trimethylsilyl)trifluoroacetamide 10 x 1mL	39,4866-10X1ML
5mL	39,4866-5ML
25mL	39,4866-25ML
MTBSTFA, derivatization grade 10 x 1mL	39,4882-10X1ML
5mL	39,4882-5ML
25mL	39,4882-25ML
MTBSTFA + TBDMCS, 99:1 10 x 1mL	37,5934-10X1ML
5mL	37,5934-5ML
25mL	37,5934-25ML
TFA 10 x 1mL	33077
25mL	33075
100mL	33076
TMCS, derivatization grade 100mL	33014
TMSI, derivatization grade 25mL	33068-U
TMSI + Pyridine, 1:4 (Sylon TP) 20 x 1mL	33159-U
25mL	33156-U
t-Butyldimethylsilylimidazole-dimethylformamide 10 x 1mL	33092-U

Silyl Reagents for Deactivation of Glassware and Chromatographic Supports

Note: All Supelco™ glass GC columns are silane treated.

Qty.	Cat. No.
DMDCS 100mL	33009
5% DMDCS in Toluene (Sylon CT) 400mL	33065-U
Rejuv-8 Silylating Agent 25mL	33059-U

Description	Cat. No.	Description	Cat. No.
Acylation Sampler Kit 3 x 1mL of each of the following (except as noted): Acetic anhydride (3 x 2mL) Heptafluorobutyric anhydride Pentafluoropropionic anhydride Trifluoroacetic anhydride	505862	FID Alkylation Sampler Kit 3 x 1mL of each of the following: BF ₃ -Methanol Methanolic Base Methanolic HCl (0.5N) Methanolic HCl (3N) TMAH, 0.2M in methanol	505854
Silylation Sampler Kit 3 x 1mL of each of the following: BSA BSTFA BSTFA + TMCS, 99:1 (Sylon BFT) HMDS + TMCS, 3:1 (Sylon HT) TMSI	505846	ECD Alkylation Sampler Kit 3 x 1mL of each of the following (except as noted): BCl ₃ -2-Chloroethanol, 11% w/w BCl ₃ -Methanol, 12% w/w Hexaoxacyclooctadecane, 18 crown 6 (1 gram) Pentafluorobenzylbromide	505870

Notes on Derivatization Procedures

We recommend using this space to record helpful tips and troublesome problems you encounter in working with derivatization reagents. You may want to photocopy this form and send us tips we can share with others, or let us know your problems and we will try to help. Please fax this information to 800-359-3044 or 814-359-5468.

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