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, $–NH_2$, –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

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-methytrifluoroacetamide, respectively.

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

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 aliphatic-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.

Diazoalkales

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 □- or □-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, $Si(CH_3)_3$, 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

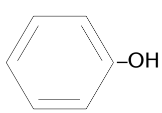
The troubleshooting guide on page 8 can assist you in solving derivatization problems. The guide lists problem symptoms (far left column), possible causes of the problem (second column), and suggested solutions. At the back of this bulletin we have included a blank page for you to record your own observations 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 O ‖	*Acylation*	TFAA	Trifluoroacetamides	Most reactive and volatile of fluorinated anhydrides. Ideal with FID, ECD, TCD. Used in identifying methamphetamine.
R–C–NH$_2$ *Primary*		PFPA	Pentafluoropropionamides	Requires lowest analysis temperature of fluorinated anhydrides. Ideal with FID,ECD,TCD. Used in identifying opiates, benzoylecgonine.[6]
O H		HFBA	Heptafluorobutylamides	Most sensitive fluorinated anhydride for EC detection. Ideal with FID,ECD,TCD.[6]
R–C–N–R *Secondary*	*Alkylation*	TMAH	Methyl amides	A favorite reagent for drugs, especially barbiturates. Flash alkylation.[1] Exception: meprobamate - analyzed by direct GC analysis as the free base.
		DMF-dialkylacetals	N-(N,N-dimethyl)aminomethylenes	Ideal for wet samples where excess reagent forms corresponding alcohol.[2]
Barbiturates	*Silylation*	BSA	Trimethylsilyl amides	Highly reactive, universal reagent. See observations for carbonyls.
Benzodiazepines Imides		BSTFA	Trimethylsilyl amides	Highly reactive, universal reagent, more volatile than BSA. See observations for carbonyls.
Proteins		BSTFA + TMCS	Trimethylsilyl amides	TMCS acts as a catalyst - assists in derivatizing amines.
		MTBSTFA	TBDMCS amides	Strong, yet mild silylating reagent. Derivatives 10,000 times more stable to hydrolysis than TMS derivatives.
		MTBSTFA + TBDMCS	TBDMCS amides	TBDMCS acts as a catalyst - assists in derivatizing amines.
Amines H	*Acylation*	Acetic anhydride	Acetates	Use with primary and secondary amines.
R–C–NH$_2$ H		MBTFA	Trifluoroacetamides	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.
Primary H H R–C–N–R		TFAA	Trifluoroacetamides	Most reactive & volatile of fluorinated anhydrides. Derivatives volatile for FID, ECD, TCD. Good for trace analysis with ECD.
H		TFAI	Trifluoroacetamides	Good for trace analysis with ECD. No acid byproducts – byproduct, imidazole, is inert.
Secondary		PFPA	Pentafluoropropionamides	Requires lowest analysis temperature of fluorinated anhydrides. Ideal with FID, ECD, TCD. Good for trace analysis with ECD. Used to identify catecholamines.
Alkaloids Amino acids		HFBA	Heptafluorobutylamides	Ideal with FID, ECD, TCD. Most sensitive to EC – good for trace analysis with ECD. Used to identify amphetamines, phencyclidine, catecholamines.[6]
Amino sugars	*Alkylation*	PFBBr	Pentafluorobenzyl ethers	Ideal with ECD.
Amphetamines		DMF-dialkylacetals	N-(N,N-dimethyl)aminomethylenes	Rapid reactions, convenient to use. Use with sterically hindered a mines.[2]
Biogenic catecholamines		NBB	Boronates	Converts α-amino acids, hydroxy acids, hydroxy amines, keto acids, diols to more easily chromatographed derivatives.
Carbamates Hydroxyl amines		TMAH	Methyl amides	A favorite reagent for drugs, especially barbiturates. Exception: meprobamate - analyzed by direct GC analysis as the free base.
Nitrosamines Nucleotides	*Silylation*	BSA	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.
Nucleosides Urea		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_4).

Functional Group/ Compound Type	Procedure	Reagent	Derivative	Observations
Carbohydrates	*Acylation*	Acetic anhydride	Acetates	Generally used with pyridine – 1:1 mixture with pyridine will derivatize alditols.
$(CH_2OH)_n$ Starches		MBTFA	Trifluoroacetamides	Reagent of choice for derivatizing sugars. Forms volatile derivatives of mono-, di-, and trisaccharides.
Sugars		TFAI	Trifluoroacetamides	Forms volatile derivatives of mono-, di-, and trisaccharides.
	Silylation	BSA + TMCS	Trimethylsilyl ethers	BSA not recommended for carbohydrates – anomerization will occur. Can be used with some syrups.
		BSTFA + TMCS	Trimethylsilyl ethers	Use with sugar acids, glucuronides.
		HMDS	Trimethylsilyl ethers	Most popular choice for silylating sugar acids and related substances. TMCS will increase silylation potential.
		HMDS + TMCS	Trimethylsilyl ethers	Use with aldoses.[4]
		HMDS + TMCS + pyridine	Trimethylsilyl ethers	Use with oligosaccharides.[4]
		TFA	Trimethylsilyl ethers	
		TMSI	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.
		TMSI + pyridine	Trimethylsilyl ethers	Reagent of choice for silylating aldoses, sugar phosphates, disaccharides containing small amounts of water. Will not derivatize amino groups.
Carbonyls				
>C=O	*Alkylation*	BCl_3-2-chloroethanol	Chloro esters	Use to prepare phenoxy-type acids for ECD.
Acid halides Acid anhydrides		o-Methyloxyamine HCl	Oximes	Use with aldehydes, ketones, ketosteroids. Prevents keto groups from forming enol ethers.[3]
Aldehydes		TFAA	Trifluoroacetates	Most reactive and volatile of anhydrides. No acid byproduct. Good with ECD.
Enols Esters Ketones Hydrazones	*Silylation*	BSA	Trimethylsilyl ethers	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.
Oximes Phenoxy acids Steroids (hydroxy/ keto hormones)		BSTFA	Trimethylsilyl ethers	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.
		BSTFA + TMCS	Trimethylsilyl ethers	TMCS acts as a catalyst, increasing reactivity of BSTFA.
		TMSI + pyridine	Trimethylsilyl ethers	Use with hindered and unhindered steroids.

[1]Flash alkylation: analyte is derivatized in the GC injection port.
[2]DMF-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).
[3]In 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.
[4]With HMDS + TMCS a fine precipitate of NH_4Cl is produced during derivatization. The precipitate does not affect chromatography.
[6]Perfluoro 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				
O	*Alkylation*	PFBBr	Pentafluorobenzyl esters	Used with ECD, UV, MS detection. Use with cannabinoids, carboxylic and fatty acids.
R–C–OH		BCl_3-methanol	Chloro esters	Used to prepare short chain (C1-C10) fatty acids for ECD.
Amino acids		BF_3-butanol	Butyl esters	Used to prepare n-butyl esters of short chain (C1-C10) mono- and dicarboxylic acids.
Cannabinols		BF_3-propanol	Propyl esters	Used to prepare n-propyl esters.
Carboxylic acids		BF_3-methanol	Methyl esters	Use with large samples of C8-C24 fatty acids.
Glycerides		Trimethylsilyldiazomethane	Methyl esters	Used with carboxylic acids. Excess reagent in the presence of methanol reacts
Hydroxy acids				instantly and quantitatively. Reaction easily monitored by disappearance of yellow color of reagent.[5]
Lipids/phospholipids		DMF-dialkylacetals	Methyl esters	Alkylates carboxyl groups; use with sterically hindered carboxylic acids. Also reacts
Prostaglandins				with amines, amino acids, phenols.[2]
Steroids (bile,		Methanolic base	Methyl esters	Use with mono-, di-, triglycerides, glycolipids, sphingolipids.
hydroxy/keto		Methanolic HCl	Methyl esters	Use with fatty acids C9 and longer. Useful for esterifying difficult carboxylic acids (bile acids).
hormones)		Methanolic H_2SO_4	Methyl esters	Use with carboxylic acids and esters (transesterification).
		NBB	Cyclic boronates	Use with carbohydrates, catecholamines, ceramides, sphingosines, corticosteroids,
				hop resin acids, □- and □-hydroxy acids, monoglycerides, monoglyceryl 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.
		TMAH	N-Methyl esters	Use with reactive amino, carboxyl, or hydroxyl groups. Flash alkylation.[1]
	Silylation	BSA	Trimethylsilyl ethers	Derivatives easily formed but generally not stable – analyze quickly.
		BSTFA	Trimethylsilyl ethers	Reacts faster and more completely than BSA. See observations for carbonyls.
		BSTFA + TMCS	Trimethylsilyl ethers	TMCS acts as a catalyst, increasing reactivity of BSTFA.
		TMSI	Trimethylsilyl ethers	Use with fatty acids, cannabinols, steroids. Will derivatize most hindered and
				unhindered steroid hydroxyls. Can be used with some salts.
Ethers				
□C–O–C□	*Silylation*	HMDS + TMCS + pyridine	Trimethylsilyl ethers	Use with epoxides that do not react rapidly with TMCS.[4]
Epoxides		TMCS	Trimethylsilyl ethers	Use with chlorohydrins.
Hydroxyls	*Acylation*	Acetic anhydride	Acetates	Use with alcohols, phenols.
ROH		MBTFA	Trifluoroacetates	Good for trace analysis with ECD.
Alcohols		TFAA	Trifluoroacetates	Good for trace analysis with ECD.
Alkaloids		TFAI	Trifluoroacetates	Good for trace analysis with ECD.
Cannabinoids		PFPA	Pentafluoropropionates	Use with alcohols, phenols. Derivatives volatile for FID, ECD. Good for trace analysis with ECD.[6]
Glycols		HFBA	Heptafluorobutyrates	Use with alcohols, phenols. Derivatives volatile for FID, ECD. Good for trace analysis with ECD.[6]
	Alkylation	PFBBr	Pentafluorobenzyl ethers	Use with alkoxides only. Use with ECD.
		Trimethylsilyldiazomethane[5]	Methyl esters	Not ideal for esterifying phenolic acids – phenolic hydroxyl groups also are
				methylated (at a slower rate) – can lead to mixtures of partially methylated products.
		TMAH	N-Methyl esters	Flash alkylation of phenolic alkaloids.[1]
Phenols		DMF-dialkylacetals	Methyl esters	Use with sterically hindered phenols.[2]
		Hexaoxacyclooctane	Pentafluorobenzyl phenols	Use with phenols for US EPA Method 604 (see General Alkylation Reagents – page 3).
	Silylation	BSA	Trimethylsilyl ethers	Most often used. Good choice for silylating phenols when used in DMF.
		BSTFA	Trimethylsilyl ethers	Good thermal stability.
		BSTFA + TMCS	Trimethylsilyl ethers	Poor hydrolytic stability.
		HMDS	Trimethylsilyl ethers	Use with unhindered alcohols and phenols. Weak donor, usually used with TMCS.
				Appropriate solvent (pyridine, DMF, DMSO) may increase reaction rate.
		MTBSTFA	Trimethylsilyl ethers	
		TBDMSIM	Trimethylsilyl ethers	Use with alcohols. Derivatives 10,000 times more stable to hydrolysis than TMS ethers.
		TMCS	Trimethylsilyl ethers	Weak donor, usually used with HMDS. Can be used with salts. Excellent catalyst for
				forming TMS ethers.
		TMSI	Trimethylsilyl ethers	Strongest silylation reagent for hydroxyls. No reaction with amines or amides.
				Derivatizes sugars in presence of water. Can be used with syrups.

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	Trimethylsilyl ethers	Reaction occurs under mild, non-acidic conditions.
		PFBBr	Trimethylsilyl ethers	Use with ECD.[6]
R–SH	*Alkylation*	Trimethylsilyldiazomethane[5]	Methyl esters	
Mercaptans		DMF-dialkylacetals[2]	Methyl esters	
	Silylation	TMSI	Trimethylsilyl ethers	
Sulfides	*Silylation*	TMSI	Trimethylsilyl ethers	
R–S				
Sulfonic Acids	*Alkylation*	TMAH	N-Methyl esters	
$R-SO_2OH$		PFBBr[6]	Trimethylsilyl ethers	
	Silylation	TMSI	Trimethylsilyl ethers	Reaction is with hydroxyl group.
Sulfonamides	*Acylation*	TFAA	Trifluoroacetates	Stable derivatives.
		PFBBr	Trimethylsilyl ethers	Stable derivatives. Enhances ECD.[6]
		HFBA	Trimethylsilyl ethers	Stable derivatives. Enhances ECD.[6]
$R-SO_2NH_2$	*Alkylation*	DMF-dialkylacetals[2]	Methyl esters	
	Silylation	BSTFA	Trimethylsilyl ethers	

[1]Flash alkylation: analyte is derivatized in the GC injection port.
[2]DMF-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).
[5]Safer substitute for diazomethane.
[6]Perfluoro 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

Books

Handbook of Analytical Derivatization Reactions
D.R. Knapp **23561**

Handbook of Derivatives for Chromatography
K. Blau and J. Halket **26566-U**

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.

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	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.	1. Use only highest purity materials at all steps in sample prepartion 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)	1. Reagent interacting with column. 2. Impurities from sample, solvent, reagents, sample vial, other labware. 3. Derivative undergoing hydrolysis. 4. Derivative reacting with solvent.	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	1. Low yield of derivative – reaction did not go to completion. 2. Detector (FID) dirty. 3. Sample components absorbed by inlet liner or column.	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	1. Septum in reaction vial not sealed.	1. Prepare a new sample and derivatize. Be sure vial is sealed.
Low Yield	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.	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
Acetic Anhydride	497121
BSA (N,O-bis(trimethylsilyl)acetamide)	496017
BSA + TMCS	496018
BSA + TMCS + TMSI	496019
BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide)	496020
BSTFA + TMCS	496021
DMDCS (dimethyldichlorosilane)	496022
DMDCS in toluene	496023
HMDS (hexamethyldisilazane)	496024
HMDS + TMCS	496025
HMDS + TMCS + pyridine	496026
Methanolic Base, 0.5N	497007
Methanolic HCl, 0.5N, 3N	497099
Methanolic H_2SO_4	497018
Perfluoro Acid Anhydrides	497104
Pentafluorobenzyl Bromide, Hexaoxacyclooctadecane	497103
Rejuv-8™	496066
TBDMSIM (N-t-butyldimethylsilylimidazole)	496065
TMAH	496180
TMCS (trimethylchlorosilane)	496028
TMSI (N-trimethylsilylimidazole)	496029
TMSI + pyridine	496030
Trifluoroacetic acid	496027
BCl_3-2-chloroethanol	496122
BCl_3-methanol	496123
BF_3-butanol	496124
BF_3-methanol	496125

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-*p*-toluenesulfonamide [60858-95-9]
Diazald-N-methyl-^{13}C-N-methyl-d_3	N-Methyl-^{13}C-d_3-N-nitroso-*p*-toluenesulfonamide [102832-11-1]
DMDCS	Dimethyldichlorosilane [75-78-5]
DMF-DBA	N,N-Dimethylformamide / Di-*tert*-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-(*tert*-Butyldimethylsilyl)-N-methyl-trifluoroacetamide [77377-52-7]
NBB	n-Butylboronic acid [4426-47-5]
PFBBr	Pentafluorobenzylbromide [1765-40-8]
PFPA	Pentafluoropropionic anhydride [356-42-3]
TBDMCS	t-Butyldimethylchlorosilane [18162-48-6]
TBDMSIM	N-(*tert*-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**
PFPA	
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.

Alkylation Reagents (contd.)

Description	Cat. No.
Esterification Reagents	
BCl_3-2-Chloroethanol (11% w/w)	
10 x 1mL	**33056-U**
10mL	**33055-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**
30mL	**33348**
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, 10 x 1mL	**33081**
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_2SO_4 (10% H_2SO_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**
NBB (n-Butylboronate), 10 x 2mL	**33090-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**
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**
REACTA-SIL T/P (TMSI in pyridine)	
10 x 1mL	**39,4645-10X1ML**
25mL	**39,4645-25ML**
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.
Acylation Sampler Kit	**505862**
3 x 1mL of each of the following (except as noted):	
Acetic anhydride (3 x 2mL)	
Heptafluorobutyric anhydride	
Pentafluoropropionic anhydride	
Trifluoroacetic anhydride	
Silylation Sampler Kit	**505846**
3 x 1mL of each of the following:	
BSA	
BSTFA	
BSTFA + TMCS, 99:1 (Sylon BFT)	
HMDS + TMCS, 3:1 (Sylon HT)	
TMSI	

Description	Cat. No.
FID Alkylation Sampler Kit	**505854**
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	
ECD Alkylation Sampler Kit	**505870**
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	

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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