GC Derivatization

Pierce 2003-2004 Applications Handbook & Catalog

Derivatization

The chemical literature contains an abundance of data on derivatization, most of which is relevant to particular compounds, classes of compounds and derivatization reagents. Two books are recognized as standard reference in the field of analytical derivatization. The first, Handbook of Analytical Derivatization Reactions by Daniel R. Knapp, is a general collection of analytical derivatizations for chromatography and mass spectroscopy (MS) involving the formation of covalent derivatives before analysis. The second, Silylation of Organic Compounds by Alan E. Pierce, was a significant factor in the transfer of silylation reactions from the relatively esoteric field of organo-silicon chemistry to the status of perhaps the most widely practiced of derivatization methods.

Suitability

Often compounds cannot be analyzed by a particular method because they are not in a form that is amenable to the analytical technique. Examples of this problem are nonvolatile compounds for gas chromatographic analysis and insoluble compounds for high performance liquid chromatography (HPLC) analysis. Many materials that are not stable under the conditions of the technique also fall into this category. The derivatization procedure modifies the chemical structure of the compounds so that they can be analyzed by the desired technique.

Efficiency

The direct analysis of many compounds and mixtures of compounds is difficult because of interactions between the compounds themselves or between the compounds and the column. These interactions can lead to poor peak resolution and/or unsymmetrical peaks, which make proper peak identification difficult or impractical. In many cases, conversion to derivatized products will reduce the interaction interfering with analysis. Compounds that co-elute with or that are poorly resolved from other sample components can frequently be resolved if one or more of them is converted to an appropriate derivative.

Detectability

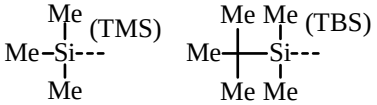
As the need expands for the analysis of increasingly smaller amounts of materials, it becomes important to extend the range of detectability of the materials in question. This increased sensitivity can be accomplished by improved detector design, especially those designs directed toward specific atoms or functional groups. Another widely used approach is increasing the detectability of compound itself by derivatization. This enhanced detectability can be achieved by increasing the bulk of the compound or by introducing atoms or functional groups that interact strongly with the detector. In gas chromatographic applications, examples of this technique are the addition of halogen atoms for electron capture detectors and the formation of TMS derivatives to produce readily identifiable fragmentation patterns and mass ions.

Types of Derivatization

The bulk of analytical derivatization reactions used for gas chromatography (GC) fall into three general reaction types: alkylation, acylation and silylation. **For GC analysis, compounds containing functional groups with active hydrogens (e.g., -COOH, -OH, -NH and -SH) are of primary concern.** The tendency of these functional groups to form intermolecular hydrogen bonds affects the inherent volatility of compounds containing them, their tendency to interact deleteriously with column packing materials and their thermal stability. Alkylation, acylation and silylation all are used to modify these classes of compounds.

Silylation

The term "silylation" usually is used to abbreviate trimethyl-silylation [Si(CH3)3]. It is also used to designate the attachment of other silyl groups such as dimethylsilyl [SiH(CH3)2], t-butyldimethylsilyl [Si(CH3)2C(CH3)3] and chloromethyldimethylsilyl [SiCH2Cl(CH3)2].



Silyl derivatives are probably the most widely used derivatives for GC applications. They are usually

formed by the replacement of the active hydrogens from acids, alcohols, thiols, amines, amides and enolizable ketones and aldehydes with the trimethylsilyl group. A wide variety of reagents are available for the introduction of the trimethylsilyl group. These reagents differ in their reactivity, selectivity, side reactions and the character of the reaction byproducts from the silylation reagent. Considerable literature is available to assist the researcher in the selection of the most suitable in reagent for the particular compounds or systems in question.

Both silylation reagents and trimethylsilyl derivatives are hydrolytically unstable and must be protected from moisture. Although the previous statement is generally true, the hydrolysis rates of various reagents and derivatives are different. It often is possible to prepare derivatives in the presence of small amounts of moisture or to isolate and purify derivatives by extraction in an organic solvent followed by washing with aqueous solutions. Reagents that introduce a t-butyl-dimethylsilyl group in place of the trimethylsilyl group were developed to impart greater hydrolytic stability to the derivatives. These t-butyldimethylsilyl derivatives not only have improved stability against hydrolysis, but they also have the added advantage of distinctive fragmentation patterns, which makes them useful in a variety of GC/MS applications. Most trimethylsilyl and t-butyldimethylsilyl derivatives have excellent thermal stability and are amenable to a wide range of injection and column conditions.

As silylation reagents will derivatize nearly all active hydrogens, it is important that they are not injected onto any column in which the stationary phase contains these functional groups. Examples of packings that are not compatible with silylating reagents are polyethylene glycols (such as CARBOWAX° Polyethylene Glycols) and free fatty acid phases (FFAP).

Effect of Moisture

Water decomposes both TMS reagents and derivatives. Pierce silylation reagents are packaged under nitrogen in Hypo-Vial' Storage Vials or 1 ml ampules. Material should be withdrawn from the vials with a hypodermic syringe. Compensation for pressure changes due to the withdrawal of liquid from the vial may be accomplished by injecting a similar volume of dry nitrogen into the vial. Optimum results are obtained when a fresh ampule is used for each derivatization or group of derivatizations done at the same time. However, multiple uses can be accomplished if the open ampule is placed in a larger container filled with dry nitrogen or if the material is carefully transferred to an oven-dried container that has been cooled to room temperature in a desiccator. If dry needles, syringes and equipment are used, dry box techniques should not be necessary unless the materials to be derivatized require such treatment, or unless there is high humidity in the room in which the derivatizations are being carried out.
In most cases the silylation reagent is used in large amounts. Therefore, very small amounts of moisture in the sample can be tolerated as the water will react with the reagent and be removed chemically from the system. Hydrolysis of TMS derivatives and reagents produces hexamethyldisiloxane [(CH3)3SiOSi(CH3)]. A small amount of the siloxane may be present in the reagent. Hexamethyldisiloxane is quite inert and does not interfere in the reaction or produce byproducts with the sample. Because of its high volatility, it is eluted with the solvent or reagent and usually does not interfere with the chromatogram.

Reaction Times

Reaction times vary greatly from compound to compound. Although many materials can be silylated in a matter of seconds or minutes at room temperature, others may require extended periods at elevated temperatures. Unhindered primary alcohols usually are completely derivatized within five minutes at room temperature (RT), while some compounds may require extended heating at temperatures as high as 150°C in the presence of a catalyst. In general, the ease of silylation in descending order is alcohols (primary, secondary, and tertiary), phenols, carboxylic acids, amines (primary, then secondary) and amides. Tertiary amines will not react with these reagents. When working with a compound with unknown reactivity, the progress of derivatization can be monitored by periodic GC analysis of aliquots of the reaction mixture. Either the disappearance of the starting material or the appearance of product peaks can be used to determine the progress of the reaction.

Thermal Stability

One of the advantages of using TMS derivatives is their thermal stability. They are routinely used at

column and injector temperatures of 300°C, but temperatures of 350°C and above have been used successfully. The TMS reagents themselves are also quite thermally stable; however, the more reactive silyl donors such as BSTFA and BSA will decompose at elevated temperatures, especially in the presence of metals. When GC is used to determine reagent purity, it is necessary to use injector temperatures of 125°C-150°C and glass-lined injection ports. Care must be used when temperatures above 75°C are needed for a derivatization procedure using these reagents because decomposition of the reagents can be significant at these temperatures.

Hydrolytic Stability

Although the TMS derivatives are quite stable thermally, their hydrolytic stabilities are highly variable and these derivatives should be considered to be easily hydrolyzed. TMS derivatives of sugars are quite stable to water at RT, but TMS amino acids hydrolyze readily. The hydrolysis rates for other compounds generally lie between these two extremes. The general order of hydrolytic stability is alcohols, phenols, carboxylic acids, amines and amides; however, wide variations occur among these groups as a result of structural and steric features of the molecule. Unless there is data to support the stability of the particular TMS derivative, approach storage of these compounds with caution. Many (most) silylated samples are best stored with excess silylating reagent present.

Solvent Suggestions

Because silylation reagents react with active hydrogen atoms, all solvents containing or capable of ting these groups (e.g., alcohols, acids, primary and secondary amines, mercaptans, primary and enolizable ketones) should be avoided. Often an excess of the silylation reagent itself can act as the solvent, eliminating the need for additional components in the analytical scheme. In some cases, the silylated product is quite soluble in the reagent, although the parent compound is not Nonpolar organic solvents such as hexane, ether, benzene and toluene are excellent solvents for the reagents and the reaction products, but they do not accelerate the rate of reaction. More polar solvents such as pyridine, dimethylformamide (DMF), dimethylsulfoxide (DMSO), tetrahydrofuran (THF) and acetonitrile are used more often because they tend to facilitate the reaction. Pyridine is an excellent solvent for TMS reactions and acts as an HCI acceptor for those reactions using organochlorosilanes. Although some regard pyridine as a silylation catalyst, there are many instances in which silylation reactions actually are slower in pyridine than other solvents. In addition, pyridine also may have other undesirable effects such as the promotion of secondary products and other chromatographic anomalies. DMF is used extensively, especially for steroids and other large molecules. DMSO is useful in the preparation of TMS derivatives of tertiary alcohols and other molecules with limited solubility in other silylation solvents. Although many silylating reagents are not soluble in DMSO, reactions often can be successfully performed in this solvent if the reactants are thoroughly mixed by stirring or if a co-solvent such as dioxane is used. Review of the literature for procedures for similar compounds is recommended if these general guidelines fail to give satisfactory results.

Selection of Columns

Both silylating reagents and TMS derivatives react with and are sensitive to active hydrogen atoms; therefore, all stationary phases containing these functional groups should be avoided. Not only will the packings give unacceptable chromatographic results, but also the use of these reagents will seriously damage or alter the performance of the column. Examples of packings unsuitable for use with these materials are polyethylene glycols (e.g., CARBOWAX° Polyethylene Glycol) and free fatty acid phase (FFAP).

The silicones are the most useful stationary phases for TMS derivatives. They combine inertness and stability with excellent separating characteristics for these types of derivatives. Their inherent Thermal stability, coupled with the thermal stability of the TMS derivatives, greatly extend the useful range of GC techniques. An additional advantage of using this class of stationary phases is the availability of a wide range of polarities. Nonpolar methyl silicones such as SEA-30, OVA-1 and OVA-101 stationary column phases are the most widely used phases for TMS derivatives and should be the starting point for column selection. The phenyl methyl silicones such as OV°-17 and OV°-25 stationary phases are good choices when a more polar phase is needed. Cyanopropylmethyl silicones such as Silar° 9CP and cyanopropylphenylmethyl silicones such as OVA-225

have been used very successfully when highly polar phases are required.

Glass Injector Ports
Use glass injector ports or direct on-column injection into glass columns when working with silylating reagents. Frequently, erratic and unreproducible results begin to occur when stainless steel injection ports are used for TMS reagents and derivatives. These problems may not become apparent until several weeks of use; however, once they do appear, correction may require replacement of the injector. In some cases, insertion of a glass liner will correct the problem, but prevention by use of glass liners prior to the development of problems is the more prudent course.

Column Conditioning
Columns used for TMS derivatives should be thoroughly conditioned before use. Inject a TMS reagent and repeat until a stable baseline is attained. HMDS may be used for this purpose, but Silyl-8`" Column Conditioner (Product # 38015) is recommended for packed columns. Inject 10-50 pl samples at two- to three-minute intervals until conditioning is complete. Maintain the column at 150°C-200°C (185°C is ideal). After the final injection, raise the column for five to 10 minutes. Thereafter, the TMS reagent will keep the column conditioned. If the column is inactive or used for non-TMS samples, it may be necessary to repeat this treatment to remove nonvolatile residues that can be silylated.

Column Materials
Although the inertness of silylated glass columns provides ideal surfaces for use with TMS reagents and derivatives, it is not necessary to use glass. Stainless steel columns are widely used with very satisfactory results. The problems mentioned earlier seem to arise in the transition from liquid to gaseous phase in contact with corroded stainless steel in injectors. Once in the gaseous phase, TMS reagents and derivatives appear to be quite stable in the presence of stainless steel columns.

Experience has shown that metals or metal ions catalyze the decomposition of TMS reagents and derivatives at elevated temperatures. If difficulties are encountered with these reagents, it is a good idea to check for contamination by metals.

Alkylation
Used in derivatization for GC, alkylation represents the replacement of an active hydrogen by an aliphatic or aliphatic-aromatic (e.g., benzyl) group. This technique is used to modify those compounds containing acidic hydrogen's such as carboxylic acids and phenols. The principal chromatographic use of this reaction is the conversion of organic acids into esters that produce better chromatograms than the free acids. Alkylation reactions can also be used to prepare ethers, thioethers and thioesters, N-alkylamines, amides and sulphonamides. As the acidity of the active hydrogen decreases, the strength of the alkyl ting reagent must be increased. As the reagents and conditions become harsher, the selectivity and applicability of the method become more limited.
In general, the products of alkylation are less polar than the starting materials because an active hydrogen has been replaced by an alkyl group. Probably the largest application of alkylation for analytical derivatization is the conversion of organic acids into esters, especially methyl esters. Although TMS derivatives of carboxylic acids are easily formed, these compounds suffer from limited stability. The alkyl esters, on the other hand, afford excellent stability and can be isolated and stored for extended periods if necessary.

Diazomethane is the quickest and cleanest method available for the preparation of analytical quantities of methyl esters. The reaction of diazomethane with a carboxylic acid is quantitative and essentially instantaneous in ether solutions. Methyl esters are prepared by titrating an ether solution of the acid with diazomethane until the yellow color of the diazomethane persists. Diazomethane, however, is toxic and there are reports of explosions during the distillation of the material. Small amounts of diazomethane for analytical derivatization can be made safely and conveniently.

BF3-Methanol is the method of choice for the preparation of methyl esters of fatty acids when plenty of sample is available. Sample sizes of 100 u1-500 ul are easily derivatized, and the isolation of the methyl esters is simple and quantitative when dealing with acids having chain lengths from C8 to C24. Special techniques are needed for the successful isolation of the more volatile fatty acid methyl esters below C8. When derivatizing fatty acids containing multiple double bonds, the presence of the strong Lewis acid

BF3 can cause changes in the nature of the unsaturation resulting in ghost peaks. The reagent should be used with caution in such cases. Methyl-8" Reagent is also a general purpose derivatizing reagent for the preparation of methyl derivatives. This reagent has wider applicability for the derivatization of a number of functional groups containing reactive hydrogens. Because the principal reaction product is DMF, isolation of the derivative is not required and the reaction mixture can be injected directly into the gas chromatograph. This reagent is an excellent first choice for a derivatization of a compound for which there is no published method available.

Acylation is the conversion of compounds that contain active hydrogens (e.g., -OH, -SH and -NH) into esters, thioesters and amides, respectively, through the action of a carboxylic acid or a carboxylic acid derivative. The principal use of acylation in chromatographic applications is the conversion of compounds containing active hydrogens into derivatives that are more easily chromatographed, or that give a greater response to the chromatographic detection system than the parent compound. An important example of this application is the insertion of perfluoroacyl groups into a molecule to enhance the detectability of the substance by electron capture. The presence of a carbonyl group adjacent to the halogenated carbons enhances the electron capture detector (ECD) response. In addition, acyl derivatives tend to direct the fragmentation patterns of compounds in MS applications, and provide useful information on the structure of these materials.