

Gas Chromatography Reagents

Derivatization

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 standards in the field of analytical derivatization. The first book, *Handbook of Analytical Derivatization Reactions* by Daniel R. Knapp¹, provides a general collection of analytical derivatization methods for chromatography and mass spectrometry (MS) that involves formation of covalent derivatives prior to analysis. The second book, *Silylation of Organic Compounds* by Alan F. Pierce,² "was a significant factor in the transfer of silylation reactions from the relatively esoteric field of organosilicon chemistry to the status of perhaps the most widely practiced of derivatization methods."³

Compounds or compound mixtures are derivatized before analysis for the following reasons:

1. To make a compound that otherwise could not be analyzed by a particular method suitable for analysis.⁴
2. To improve the analytical efficiency of the compound.^{5,6}
3. To improve the detectability of the compound.⁷

Suitability

Often compounds cannot be analyzed because they are not in a form that is suitable for the particular analytical technique. Examples include nonvolatile compounds for GC analysis,^{8,9,10} insoluble compounds for HPLC analysis and materials that are not stable using the conditions of the technique.¹¹ The derivatization procedure modifies the chemical structure of the compounds, allowing analysis by a desired technique.¹²

Efficiency

Direct analysis can be difficult when compounds interact with each other or with the column. These interactions can lead to poor peak resolution and/or asymmetrical peaks that make proper peak integration difficult or impractical. This interference can be reduced with conversion to derivatized products.^{13,14} Compounds that exhibit co-elution can often be separated by using the appropriate derivatization methods.

Detectability

As demand increases for the analysis of increasingly smaller amounts of materials, it becomes important to extend the detectability range of the materials in question. This increased sensitivity can be accomplished by improved detector design that is directed toward specific atoms or functional groups.

Another popular approach to increase detectability is the use of derivatization. Enhanced detectability can be achieved by increasing the bulk of the compound, or by introducing atoms or functional groups that strongly interact with the detector.^{16,17} This technique is performed in gas chromatographic applications, with the addition of halogen atoms for electron capture detectors,^{18,19} and with the formation of TMS derivatives to produce readily identifiable fragmentation patterns and mass ions.²⁰

Types of Derivatization

Compounds containing functional groups with active hydrogens (-COOH, -OH, -NH and -SH) are usually derivatized prior to analysis by gas chromatography. These functional groups have a tendency to form intermolecular hydrogen bonds that affect the volatility, their tendency to interact deleteriously with column packing materials and their thermal stability. Silylation, acylation and alkylation are derivatization techniques used to alter these functional groups to improve their thermal and chromatographic character.

The ideal derivatization procedure will:

1. Accomplish the desired modification.
2. Proceed quantitatively, or at least reproducibly.
3. Produce products that are readily distinguishable and separable from the starting materials.
4. Proceed rapidly with simple and straightforward laboratory techniques that will be both selective and applicable to a number of similar compounds.
5. Involve reagents and reactions that present no unusual hazards.



Thermo Scientific Silylation Reagents

Silyl derivatives are the most widely used derivatives for gas chromatographic applications. Usually they are formed by the replacement of the active hydrogens from acids, alcohols, thiols, amines, amides and enolizable ketones and aldehydes with the trimethylsilyl group. A variety of reagents is available for the introduction of the trimethylsilyl group. These reagents differ in their reactivity, selectivity and side reactions and the character of the reaction products from the silylation reagent itself. Considerable literature is available to assist you in the selection of the most suitable silylation reagent for your particular compounds or systems.^{1,2}

Silylation reagents and trimethylsilyl derivatives are hydrolytically unstable and must be protected from moisture. However, the rate of hydrolysis for various reagents and derivatives is different, and sometimes it is possible to prepare derivatives in the presence of small amounts of moisture,²¹ or to isolate and purify derivatives by extraction in an organic solvent, followed by washing with aqueous solutions.²² Reagents that introduce a t-butyl dimethylsilyl group instead of the trimethylsilyl group were developed for greater hydrolytic stability.²³ These derivatives provide improved stability against hydrolysis and provide distinctive fragmentation patterns, making them useful in GC/MS applications.²⁴

Most trimethylsilyl and t-butyl dimethylsilyl derivatives offer excellent thermal stability and are suitable for a wide range of injector and column conditions. However, as the 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 (TG-WaxMS) and free fatty acid phases (TG-WaxMS A).

References

- Knapp D.R. (1979). *Handbook of Analytical Derivatization Reactions*, John Wiley & Sons: New York.
- Pierce, A.E. (1968). *Silylation of Organic Compounds*, Pierce Chemical: Rockford, IL.
- Pierce, A.E. (1968). *Silylation of Organic Compounds*, Pierce Chemical: Rockford, IL. p. 2.
- Sweeley, C.C., *et al.* (1963). Gas-liquid chromatography of trimethylsilyl derivatives of sugars and related substances. *J. Am. Chem. Soc.* **85**, 2495-2507.
- Khalifa, S. and Mumma, R.O. (1972). *J. Agric. Food Chem.* **20**, 632.
- Sinsheimer, J.E. and Smith, R.V. (1967). Methods for the qualitative and quantitative analysis of some hydroxystilbenes. *J. Pharm. Sci.* **56**, 1280.
- Poole, C.F. (1976). *Chem. Ind. (London)*, 479.
- Brittain, G.D. and Schewe, L. (1971). In *Recent Advances in Gas Chromatography*, Domsky, I.I. and Perry, J.A., Eds., Marcel Dekker: New York, NY.
- Sakauchi, N. and Horning, E.C. (1971). *Anal. Lett.* **4**, 41-42.
- Sullivan, J.E. and Schewe, L.R. (1977). Preparation and gas chromatography of highly volatile trifluoroacetylated carbohydrates using *N*-Methylbis (trifluoroacetamide). *J. Chromatogr. Sci.* **15**, 196-197.
- Hallgren, B. and Larsson, S. (1962). *J. Lipid Res.* **3**, 31.
- Samuelsson, K. and Samuelsson, B. (1969). Gas-liquid chromatography-mass spectroscopy of cerebrosides as trimethylsilyl ether derivatives. *Biochem. Biophys. Res. Commun.* **37**(1), 15-21.
- Langer, M., *et al.* (1960). *Chem. Ind. (London)*, **378**.
- Neeman, M., *et al.* (1959). *Tetrahedron*, **6**, 36.
- Marfey, P. (1984). Determination of D-amino acids. II. Use of a bifunctional reagent, 1,5-Difluoro-2,4-Dinitrobenzene. *Carlsberg Res. Commun.* **49**, 591-596.
- Ehrsson, H., *et al.* (1971). *Acta. Pharm. Suecica.* **8**, 319.
- Koshy, K.T., *et al.* (1975). *O*-(2,3,4,5,6-Pentafluorobenzyl) hydroxylamine hydrochloride as a sensitive derivatizing agent for the electron capture gas liquid chromatographic analysis of keto steroids. *J. Chromatogr. Sci.* **13**(Feb), 97-103.
- Walle, T. and Ehrsson, H. (1970). *Acta. Pharm. Suecica.* **7**, 389-406.
- Benington, F., *et al.* (1975). Identification and separation of indolealkylamines by gas liquid chromatographic analysis of their heptafluorobutryl derivatives. *J. Chromatogr.* **106**, 435-439.
- Kelly, R.W. and Taylor, P.L. (1976). *tert*-Butyldimethylsilyl ethers as derivatives for qualitative analysis of steroids and prostaglandins by gas phase methods. *Anal. Chem.* **48**(3), 465.
- Lau, H.L. (1966). *J. Gas Chromatogr.* **4**, 136.
- Tallent, W.H. and Kleinman, R. (1968). *J. Lipid Res.* **9**, 146.
- Mawhinney, T.P. and Madson, M.A. (1982). *N*-Methyl-*N*-(*tert*-butyldimethylsilyl) trifluoroacetamide and related *N*-*tert*-butyldimethylsilyl amides as protective silyl donors. *J. Org. Chem.* **47**, 3336-3339.
- Bazan, A.C. and Knapp, D.R. (1982). Improved derivative of 6-keto-prostaglandin F_{1α} for gas chromatographic-mass spectrometric analysis. *J. Chromatogr.* **236**, 201-207.

Thermo Scientific Acylation Reagents

Acylation is the conversion of compounds (through the action of a carboxylic acid or a carboxylic acid derivative) that contain active hydrogens such as -OH, -SH and -NH to esters; thioesters; and amides.¹ In chromatographic applications, the acylation reaction is used primarily for converting the above classes of compounds into derivatives that are better suited for chromatography² or that give a greater response to the chromatographic detection system than the parent compound.³

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.

Acyl derivatives are also useful in MS applications in which they influence the fragmentation patterns of the compounds to be studied.⁴

References

1. Donike, M. (1973). Acylation with bis (acylamides). *N*-Methyl-bis (trifluoroacetamide), two new reagents for trifluoroacetylation. *J. Chromatogr.* **78**, 273-279.
2. Sullivan, J.E. and Schewe, L.R. (1977). Preparation and gas chromatography of highly volatile trifluoroacetylated carbohydrates using *N*-Methyl-bis (trifluoroacetamide). *J. Chromatogr. Sci.* **15**, 196-197.
3. Benington, F., *et al.* (1975). Identification and separation of indolealkylamines by gas liquid chromatographic analysis of their heptafluorobutyryl derivatives. *J. Chromatogr.* **106**, 435-439.
4. Borga, O., *et al.* (1971). Quantitative determination of nortriptyline and desmethylnortriptyline in human plasma by combined gas chromatography-mass spectrometry. *J. Chromatogr.* **4(12)**, 837-849.

Thermo Scientific Alkylation Reagents

When used in derivatization for gas chromatography, alkylation represents the substitution of an active hydrogen by an aliphatic or aliphatic-aromatic¹ (benzyl) group. This technique is used to modify those compounds containing acidic hydrogens, such as carboxylic acids and phenols. The principal chromatographic use of this reaction is the conversion of organic acids into esters, which produce better chromatograms than the free acids.

In addition, alkylation reactions can be used to prepare ethers, thioethers and thioesters; *N*-alkylamines; and amides.² As the acidity of the active hydrogen decreases, the strength of the alkylating reagent must be increased. As the reagents and conditions become harsher, the selectivity and applicability of the methods become more limited.

References

1. Kawahara, F.K. (1968). Microdetermination of derivatives of phenols and mercaptans by means of electron capture gas chromatography. *Anal. Chem.* **40(6)**, 1009.
2. Kananen, G., *et al.* (1972). Barbiturate analysis – a current assessment. *J. Chrom. Sci.* **10**, 283-287.