Product Information

85431 Silylating mixture I according to Sweeley
(HMDS+TMCS+Pyridine 2:1:10) for GC derivatization, LiChropur®

Storage temperature: 2-8°C

Silylating mixture I according to Sweeley[1] is composed of hexamethyldisilazane (HMDS), trimethylchlorosilane (TMCS) and pyridine in the ration of 2:1:10 (v/v). HMDS, a weak trimethysilyl donor, was the first reagent used to prepare TMS derivatives. It is most often used in silylating mixtures. HMDS has the desirable property of reacting more selectively, in some instances, than other reagents. It is a popular choice for silylating acids, alcohols, amines, and phenols. Reactions proceed faster when HMDS is used in combination with TMCS and pyridine.

Features/Benefits

- Silylating mixture I has greater silylating potential than HMDS alone – it will derivatize alcohols, bile acids, phenols, steroids (except 3-ketosteroids), sterols and sugars which will not be completely derivatized by HMDS. The reagent is versatile, fast and easy to use. It can be used without solvent.

Typical Procedure

This procedure is intended to be a guideline and may be adapted as necessary to meet the needs of a specific application. Always take proper safety precautions when using a silylating reagent. Silylating mixture I is extremely sensitive to moisture and should be handled under dry conditions.

Prepare a reagent blank (all components, solvents *except sample*), following the same procedure as used for the sample.

1. Weigh 1-10 mg of sample into a 5 mL reaction vessel. If appropriate, dissolve sample in solvent. If sample is in aqueous solution, evaporate to dryness, then use neat or add solvent.
2. Add excess silylating mixture. It can be used at full strength or with a solvent.* In most applications it is advisable to use an excess of the silylating reagent – at least a 2:1 molar ratio of the mixture to active hydrogen.**
3. Allow the mixture to stand until silylation is complete. To determine when derivatization is complete, analyze aliquots of the sample at selected time intervals until no further increase in product peak(s) is observed.

   Derivatization times vary widely, depending upon the specific compound(s) being derivatized. Many compounds are completely derivatized as soon as they dissolve in the reagent. Compounds with poor solubility may require warming. A few compounds will require heating at 70°C for 20-30 min. Under extreme conditions compounds may require heating for up to 16 h to drive the reaction to completion.

   If derivatization is not complete, evaluate the addition of a catalyst, use of an appropriate solvent, higher temperature, longer time and/or higher reagent concentration.

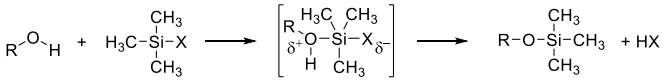
* Nonpolar organic solvents such as hexane, ether, and toluene are excellent solvents for the reagent and the reaction products; they do not accelerate the rate of reaction. Polar solvents such as pyridine, DMF, dimethylsulfoxide (DMSO), tetrahydrofuran (THF), and acetonitrile are more often used because they can facilitate the reaction. Pyridine is an especially useful solvent because it can act as an HCl acceptor in silylation reactions involving organochlorosilanes.
**The combination of HMDS and TMCS can produce a precipitate, ammonium chloride. This salt usually does not affect chromatography of the derivative, but Tallent, et al.,[2] found that ammonium chloride can cause extraneous peaks with products containing epoxide rings. Some analysts separate the salt by allowing it to settle, or by centrifuging the material and removing the supernate. Tallent, et al.,[2] dissolve the silyl compound in hexane and wash it with water. Formation of ammonium chloride can be avoided by using trifluoroacetic acid as the catalyst for HMDS, or using BSA as the silylating reagent.

Use a glass injection port liner or direct on-column injection when working with silylating reagents. Erratic and irreproducible results are more common when stainless steel injection ports are used.

TMS derivatives and silylating reagents react with active hydrogen atoms. Do not analyze HMDS+TMCS+pyridine derivatives on stationary phases with these functional groups (*e.g.* polyethylene glycol phases). Silicones are the most useful phases for TMS derivatives combining inertness and stability with excellent separating characteristics. Nonpolar silicone phases include SPB™-1 and SPB-5. Normal hydrocarbons (carbon-hydrogen analytes with single bonds) are separated by these phases. More polar phases, SPB-1701 and SP™-2250, separate carbon-hydrogen analytes that also contain Br, Cl, F, N, O, P, or S atoms or groups. A highly polar cyanopropylphenylsiloxane phase, SP-2330, is useful for separating fatty acid methyl esters or aromatics.

Mechanism[3-4]

Silylation is the most widely used derivatization procedure for GC analysis. In silylation, an active hydrogen is replaced by an alkylsilyl group. Compared to their parent compounds, silyl derivatives generally are more volatile, less polar, and more thermally stable.
Silyl derivatives are formed by the displacement of the active proton in –OH, –COOH, =NH, $-NH_2$ and –SH groups.



The general reaction for the formation of trialkylsilyl derivatives is shown above.

The reaction is viewed as a nucleophilic attack upon the Si atom of the silyl donor,producing a bimolecular transition state. The leaving group X for HMDS, X = $NHSi(CH_3)_3$), for TMCS, X = Cl, for pyridine, X = C_5H_5N) must possess low basicity, the ability to stabilize a negative charge in the transition state, and little or no tendency for π (p-d) back bonding between itself and the silicon atom.
The ideal silyl leaving group X must be such that it is readily lost from the transition state during reaction, but possesses sufficient chemical stability in combination with the alkyl silyl group to allow long term storage of the derivatizing agent for use as required. As the formation of the transition state is reversible, the derivati-zation will only proceed to completion if the basicity of the leaving group X exceeds that of the group it replaces. The ease of derivatization of various functional groups for a given silyating agent follows this order: alcohol > phenol > carboxylic acid > amine > amide. Within this sequence reactivity towards a particular silylating reagent will also be influenced by steric hindrance, hence the ease of reactivity for alcohols follows the order: prim. > sec. > tert., and for amines: prim. > sec.

Storage/Stability

Recommended storage conditions for the unopened product are stated on the label. Store in an amber bottle or ampule at 2-8°C in a dry, well ventilated area. Use only in a well ventilated area. Keep away from ignition sources.
Properly stored, this mixture is stable indefinitely. Moisture will decompose both TMS reagents and derivatives. To exclude moisture, this reagent is packaged under inert gas. If you store an opened container or transfer the contents to another container for later reuse, add desiccant. **Before reuse, validate that your storage conditions adequately protected the reagent.**

References

1. C. C. Sweeley *et al*., *J. Am. Chem. Soc.* **1963**, *85*, 2497.
2. W. H. Tallent, R. Kleiman, *J. Lipid. Res.* **1968**, *9*, 146.
3. K. Blau and J. Halket, *Handbook of Derivatives for Chromatography* (2nd ed.), John Wiley & Sons, New York, 1993.
4. D.R. Knapp, *Handbook of Analytical Derivatization Reactions,* John Wiley & Sons, New York, 1979.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses.
Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.