INSTRUCTIONS

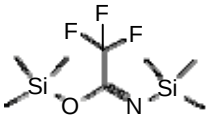
BSTFA

N,O-Bis(trimethylsilyl)trifluoroacetamide

TS-38830 TS-38828 TS-38829

0255.1

Number	Description
TS-38830	**BSTFA,** 10 × 1 ml glass ampules
TS-38828	**BSTFA,** 25 g in Hypo-Vial™ Sample Storage Vial
TS-38829	**BSTFA,** 100 g in Hypo-Vial Sample Storage Vial



BSTFA
$C_8H_{18}F_3NOSi_2$
MW 257.40

Storage: Upon receipt store protected from moisture at 4°C. Product shipped at ambient temperature.

Note: BSTFA [N,O-Bis(trimethylsilyl)trifluoroacetamide] is a clear, colorless to very light yellow liquid which is very sensitive to moisture. In contact with water or water vapor, BSTFA hydrolyzes to form N-(trimethylsilyl)trifluoroacetamide, trifluoroacetamide, and hexamethyldisiloxane.

Caution: BSTFA is flammable, harmful by inhalation, and an irritant. Consult the material safety data sheet for this product to determine proper handling and disposal.

Introduction

BSTFA [N,O-Bis(trimethylsilyl)trifluoroacetamide] is an effective trimethylsilyl (TMS) donor for derivatization of polar compounds to facilitate analysis by gas chromatography. BSTFA reacts with a wide range of polar compounds to replace labile hydrogens with a $-Si(CH_3)_3$ group (TMS). Replacing hydrogen atoms with TMS group produces a volatile and thermally stable derivative of the parent compound for gas chromatography and mass spectrometry.[1,2]

Compared to many other silylation reagents, BSTFA produces more volatile by-products, namely mono(trimethylsilyl) trifluoro-acetamide and trifluoroacetamide. As a result, better chromatographic separations can be obtained with BSTFA because the by-products usually co-elute with the solvent front. With other reagents, the by-products have longer retention times and tend to co-elute with the derivatized products, as in GC analysis of lower boiling-point.[3]

BSTFA can be used at full strength or diluted with a suitable solvent such as pyridine. In most applications it is advisable to use an excess of the silylating reagent and > 2:1 molar ratio of BSTFA to active hydrogen is optimal. Best results are obtained when the products of the silylation reaction are soluble in the final reaction mixture. Amides, many secondary amines and hindered hydroxyls will not be derivatized by BSTFA alone. However, when a catalyst such as trimethyl-chlorosilane (TMCS) is added with the BSTFA, many of these compounds can be derivatized satisfactorily (see instructions for Product No. TS-28832).

Additional Materials Required (depending on protocol used)

- Reaction Vial: Reacti-Vial™ Small Reaction Vials (e.g., Product No. TS-13222; several sizes available)
- Heating/Stirring/Evaporation Apparatus: Reacti-Therm™ Heating Module or Heating/Stirring Module with/without a Reacti-Vap™ Evaporator Apparatus. (Several sizes and models are available; visit the website.)
- Solvent(s): Pyridine (Product No. TS-27530), Acetonitrile (Product No. TS-20062), Tetrahydrofuran (Product No. TS-27860), Dimethylformamide (Product No. TS-20672), Dimethylsulfoxide (Product No. TS-20684), methylene chloride, and/or toluene.
- Dry glassware, syringes and other sample-handling devices

Procedures for BSTFA Silylation without Solvent

BSTFA hydrolyzes and becomes non-reactive when exposed to moisture. Use only dry glassware and syringes. Avoid exposing reagent to moist air when opening vial. In many cases, derivatization is effective at room temperature and without solvent. When no information exists for a particular compound, try this condition first.

Protocol: No Solvent, No Heat

1. Combine 1-10 mg of sample and 0.1-0.5 ml of BSTFA in a clean dry 3 ml Reaction Vial.
2. Cap, mix well, and incubate for 5-10 minutes or until reaction is complete.
3. Inject an appropriate size sample for column and detector.

Protocol: No Solvent with Heat

1. Combine 1-10 mg of sample and 0.1-0.5 ml of BSTFA in a clean, dry 3 ml Reaction Vial.
2. Cap, mix well, and heat at 70°C for 15 minutes.
3. Cool to room temperature and inject an appropriate sample.

Procedures for BSTFA Silylation in Organic Solvent

Protocol: Organic Solvent, No Heat

1. Dissolve a 1-10 mg sample in 1.0 ml of a suitable solvent (see Appendix) in a clean, dry 3 ml Reaction Vial.
2. Add 0.1-0.4 ml of BSTFA.
3. Cap, mix well, and let stand for 5-10 minutes.
4. Inject an appropriate sample.

Protocol: Organic Solvent with Heat

1. Dissolve a 1-10 mg sample in 1.0 ml of a suitable solvent (see Appendix) in a clean, dry 3 ml Reaction Vial.
2. Add 0.1-0.5 ml of BSTFA.
3. Cap tightly, mix well, and heat at 70°C for 15 minutes.
4. Cool to room temperature and inject an appropriate sample.

Procedures for BSTFA Silylation of Aqueous Samples

For effective BSTFA derivatization, it is usually necessary to remove all water from aqueous samples before the addition of the BSTFA. Water can be removed by evaporation (Procedure A) or by extracting the compound from the water phase with organic solvent (Procedure B).

Protocol A: Evaporation

1. Evaporate the water from an aqueous sample containing the desired amount of the material to be derivatized by directing a gentle stream of dry nitrogen over the sample maintained at 40-70°C. Sample evaporation is easily accomplished in a 3 ml Reacti-Vial Small Reaction Vial using a Reacti-Therm Heating Module equipped with a Reacti-Vap Evaporator.
2. Many samples will be dry enough at this point to proceed direction with the derivatization procedure (Step 4). If the derivatization is not effective or the sample is viscous, an additional drying step might be required (Step 3).
3. Remove remaining traces of moisture by adding 0.1-0.5 ml of an organic solvent, such as toluene or methylene chloride, which forms an azeotrope with water and then removing the solvent by evaporation as in Step 1. Repeat this step as many times as necessary to achieve a completely dry sample.
4. Derivatize sample according to the protocols previously listed for non-aqueous samples.

Procedure B: Organic Phase Extraction

1. Add an amount of an organic solvent which is immiscible with water, such as methylene chloride or toluene, to the sample and mix well.
2. Transfer the organic layer containing the sample to a clean container.
3. Repeat step 1 using fresh solvent, and combine this organic extract with the first organic layer. In most cases, two extractions are sufficient.
4. Wash the organic extracts containing the sample with two small (one-fifth the volume of the solvent) portions of a saturated sodium chloride solution to remove any water-soluble impurities and most of the water from the solvent layer.
5. Dry the organic extracts over a small amount of anhydrous sodium sulfate, and transfer the organic solvent containing the sample to a clean, dry container.
6. The sample may now be handled as described in the protocols on page 6 for samples with solvents. The solvent system may be changed to a different silylation solvent by evaporation and reconstitution with the desired solvent.

Procedure for BSTFA Silylation of Amino Acids

1. Evaporate an aqueous sample containing from 0.5-6.0 mg of amino acids to dryness at 70°C in a stream of dry nitrogen. Sample evaporation is easily accomplished in a 3 ml Reacti-Vial Small Reaction Vial using a Reacti-Therm Heating Module equipped with a Reacti-Vap Evaporator.
2. Add 0.5 ml of methylene chloride and again evaporate to dryness as above to ensure azeotropic removal of water.
3. Add an internal standard if desired.
4. Add 0.25 ml of BSTFA for each milligram of amino acid and 1 ml of acetonitrile.
5. Seal tightly, mix well, and heat for 2.5 hours at 150°C.
6. Cool to room temperature and inject an appropriate sample.

Appendix: General Information About TMS Silylation

A. Effects of Moisture

Water decomposes both TMS reagents and derivatives. Equilibrate reagent bottles to room temperature before opening to avoid moisture from the air condensing onto the reagent. Avoid or minimize exposing reagent to humid air. For best results, purge the vial head-space with nitrogen gas before reclosing reagent storage vial.

Because the silylation reagent is typically used in large molar excess, very small amounts of moisture in the sample can be tolerated for the reaction. Hydrolysis of TMS derivatives and reagents produces hexamethyldisiloxane [$(CH_3)_3SiOSi(CH_3)_3$]. A small amount of the siloxane may be present in the reagent. Hexamethyldisiloxane is inert, does not interfere in the reaction, does not produce by-products with the sample, and elutes with the solvent.

B. 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 incubation at elevated temperatures. Unhindered primary alcohols are usually completely derivatized within 5 minutes at room temperature; other compounds require extended heating at 150°C in the presence of a catalyst. In general, the ease of silylation in descending order is alcohols (primary, secondary, tertiary), phenols, carboxylic acids, amines (primary, then secondary), and, finally, amides. Tertiary amines will not react with these reagents. When working with a compound with unknown reactivity, progress of the derivatization can be monitored by periodic gas chromatographic analysis of aliquots of the reaction mixture. Either the disappearance of the reagent or the appearance of product peaks can be used to determine the progress of the reaction.

C. Thermal Stability

One advantage of TMS derivatives over other derivatives is their thermal stability. TMS derivatives are routinely analyzed in 300-350°C columns and injectors. Many TMS reagents themselves are also quite thermally stable, but the more reactive silyl donors such as BSTFA and BSA decompose at high temperatures, especially in the presence of metals. Perform long-incubation derivatization reactions at < 75°C to avoid reagent decomposition. When using the gas chromatograph to determine reagent purity, use injectors at 125-150°C.

D. Hydrolytic Stability

Although the TMS derivatives have good thermal stability, they vary widely in hydrolytic stability. For example, TMS derivatives of sugars are relatively stable to water at room temperature, but TMS amino acids hydrolyze readily. The general order of hydrolytic stability is alcohols, phenols, carboxylic acids, amines, and amides; however, wide variations occur among these groups because of structural differences. Unless empirical testing has established the stability of a prepared derivative, assume that derivatives are not very stable and analyze samples immediately after derivatization.

E. Solvent Suggestions

Because silylation reagents react with active hydrogen atoms, avoid solvents containing or capable of generating these groups (e.g., alcohols, acids, primary and secondary amines, mercaptans, primary amides and enolizable ketones). Excess silylation reagent itself can act as the solvent for derivatization and injection, thereby eliminating the need for additional components in the analytical scheme. In some cases, the silylated product is quite soluble in the reagent even though the parent compound is not. Non-polar organic solvents such as hexane, ether, benzene, and toluene are excellent solvents for the reagents and many reaction products, but they do not accelerate the rate of reaction. Polar solvents such as pyridine, dimethylformamide (DMF), dimethylsulfoxide (DMSO), tetrahydrofuran (THF), and acetonitrile are tend to facilitate TMS reactions. Pyridine is an excellent solvent for TMS reactions and acts as an HCl acceptor for those reactions involving organochlorosilanes.[4] Although some regard pyridine as a silylation catalyst, there are many instances where silylation reactions actually are slower in pyridine than other solvents.[5] In addition, pyridine may also have other undesirable effects, such as the promotion of secondary products and other chromatographic anomalies.[6] 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 are often successfully performed in this solvent if the reactants are thoroughly mixed by stirring or if a co-solvent such as dioxane is used.[7] Review the literature for procedures for similar compounds if these guidelines fail to give satisfactory results.

F. Column Selection

Both silylating reagents and TMS derivatives react with, and are sensitive to, active hydrogen atoms. Therefore, avoid using stationary phase containing these functional groups. Do not use polyethylene glycol or free fatty acid phase (FFAP) columns. TMS derivatives are best processed in silicone-based columns, especially those based on non-polar methyl silicones. If necessary, polar phenyl methyl or cyanopropyl methyl silicone columns can be used.

G. Column Conditioning

For best results, condition columns before analyzing a TMS derivative. Inject a TMS reagent such as HMDS (Product No. 84770; inject 10-50 µl at 2-3 minute intervals) until a stable baseline is attained. Maintain the column at 150-200°C (185°C is ideal). After the final injection, raise the column to near the maximum recommended temperature for 5-10 minutes. Thereafter, injecting samples derivatized with TMS reagents will keep the column conditioned. If the column is inactive or used for non-TMS samples, recondition the column to remove non-volatile residues that can be silylated.

H. Glass Injector Ports

Use glass injector ports or direct on-column injection into glass columns when working with silylating reagents. Erratic and irreproducible results frequently occur when stainless steel injection ports are used for TMS reagents and derivatives. These problems may not begin to become apparent until several weeks of use; however, once they do appear, correction may require replacement of the injector.

References

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