INSTRUCTIONS

BSTFA + TMCS
N,O-bis(Trimethylsilyl)trifluoroacetamide with Trimethylchlorosilane

TS-38831 TS-38833 TS-38840 TS-38832 TS-38834 0322

Product Description

NUMBER	DESCRIPTION
TS-38831	**BSTFA + 1% TMCS,** *10 x 1 mL ampule package*
TS-38832	**BSTFA + 1% TMCS,** *10 g Hypo-Vial™ Sample Storage Vial*
TS-38833	**BSTFA + 1% TMCS,** *25 g Hypo-Vial™ Sample Storage Vial*
TS-38834	**BSTFA + 1% TMCS,** *100 g Hypo-Vial™ Sample Storage Vial*
TS-38840	**BSTFA + 10% TMCS,** *10 x 1 mL ampule package*
	N,O-bis(Trimethylsilyl)trifluoroacetamide with 10% Trimethylchlorosilane
	CAS No. 75-77-4
	BSTFA + 1% TMCS and BSTFA + 10% TMCS are clear, colorless to very light yellow liquids which are very sensitive to moisture. They are packaged under nitrogen in Hypo-Vial™ Sample Storage Vials or 1 mL ampules. Upon contact with water or water vapor, they hydrolyze to form *N*-(trimethylsilyl)trifluoroacetamide, trifluoroacetamide, hydrochloric acid, and hexamethyldisiloxane.

Handling Precautions

Flammable. Harmful by inhalation. Danger of cumulative effects. Irritating to the eyes, respiratory system and skin. Keep away from sources of ignition-No Smoking. In case of contact with the eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. In case of insufficient ventilation, wear suitable respiratory equipment.

Silylation

Effect of Moisture

Water decomposes both TMS reagents and derivatives. Pierce silylation reagents are packaged under nitrogen in Hypo-Vial™ Sample 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 Material should be withdrawn from the vials with a hypodermic syringe.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 with the ampules 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 opened ampule is placed in a larger container filled with dry nitrogen or if the material is carefully transferred to an oven dried container which 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 where the derivatizations are being carried out.

In most cases, the silylation reagent is used in large excess. 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 [$(CH_3)_3SiOSi(CH_3)_3$]. A small amount of the siloxane may be present in the reagent. Hexamethyldisiloxane is quite inert and does not interfere in the reaction nor does it produce by-

products 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 are usually completely derivatized within 5 minutes at room temperature, 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, 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, the progress of the derivatization can be monitored by periodic gas chromatographic 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 TMS derivatives over other derivatives is their thermal stability. They are routinely used at column and injector temperatures of 300°C. 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 gas chromatography is used to determine the purity of the reagents, it is necessary to use injector temperatures of 125-150°C and glass-lined injection ports. Care must be used when temperatures above 75°C are needed for a derivatization procedure as decomposition of these reagents can be significant at these temperatures.

Hydrolytic Stability

Although the TMS derivatives are quite thermally stable, 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 room temperature, but TMS amino acids hydrolyze readily. The rates of hydrolysis 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 due to structural and steric features of the molecule. Unless there are data to support the stability of the particular TMS derivative, storage of these compounds should be approached with caution. 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 generating these groups, such as alcohols, acids, primary and secondary amines, mercaptans, primary amides and enolizable ketones, should be avoided. Often an excess of the silylation reagent itself can act as the solvent, 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 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 more often used as they tend to facilitate the reaction. Pyridine is an excellent solvent for TMS reactions and acts as an HCl acceptor for those reactions using organochlorosilanes[1] Although some regard pyridine as a silylation catalyst, there are many instances where silylation reactions actually are slower in pyridine than other solvents.[2] In addition, pyridine may also have other undesirable effects, such as the promotion of secondary products and other chromatographic anomalies.[3] 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 can often be successfully performed in this solvent if the reactants are thoroughly mixed by stirring or if a co-solvent such as dioxane is used[4] Review of the literature for procedures for similar compounds is recommended if these general guidelines fail to give satisfactory results. Of course, our Technical Assistance Staff is always ready to give you a hand.

Selection of Columns

Both silylating reagents and TMS derivatives react with, and are sensitive to, active hydrogen atoms. For this reason, 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 (such as TR-WAX, etc.) and free fatty acid phase (TR-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 extends the useful range of gas chromatographic techniques. An additional advantage of this class of stationary phases is the availability of a wide range of polarities. Non-polar methyl silicones such as TR-1, and TR-1MS are the most widely used phases for TMS derivatives and should be the starting point for column selection. The phenyl methyl silicones such as TR-50MS are good choices when a more polar phase is needed. CyanopropylPHENUL silicones such as TR-1701 and TR-FAME have been used very successfully when highly polar phases are required.

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 we particularly recommend Silyl-8™ Column Conditioner (Product No. TS-38014) for packed columns. Inject 10-50 µL samples at 2-3 minute intervals until conditioning is complete. Maintain the column at 150-200°C (185°C is ideal). After the final injection, raise the column temperature to or near the maximum recommended temperature for the column for 5-10 minutes. Thereafter, the TMS reagents 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 non-volatile residues which can be silylated.

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 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 Materials

Although the inertness of silylated glass columns provides ideal surfaces for columns 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 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 the decomposition of TMS reagents and derivatives at elevated temperatures is catalyzed by metals or metal ions. If difficulties are encountered with these reagents, it is a good idea to check to see that there is no contamination by metals.

Considerations for the use of BSTFA + TMCS

BSTFA is an effective trimethylsilyl donor with donor strength approximately the same as its unfluorinated analog BSA, *N,O*-bis(trimethlysilyl)acetamide. It reacts with a wide range of polar compounds to replace labile hydrogens with a -$Si(CH_3)_3$ group. Therefore, it is used to prepare volatile and thermally stable derivatives for gas chromatography and mass spectrometry.

One of the particular advantages of BSTFA over many of the other silylating reagents is the volatility of its by-products, mono-(trimethylsilyl)trifluoro-acetamide and trifluoroacetamide. For example, in the gas chromatographic analysis of some of the lower boiling TMS-amino acids and TMS-Krebs cycle acids, the retention times of these derivatives cause them to be co-eluted with the by-products from most TMS derivatization reagents.[5] Good chromatographic separations can be obtained with BSTFA, as the by-products from this reagent usually elute with the solvent front.

Amides, many secondary amines and hindered hydroxyls will not be derivatized completely by BSTFA alone; however, when a catalyst such as TMCS is added, many of these compounds can be derivatized satisfactorily. The mechanism for the catalytic effect of TMCS is not well understood; however, there is little doubt that the addition of the relatively weak silyl

donor, TMCS, to BSTFA will enhance the donor strength of the stronger donor, BSTFA.[6] The TMCS may participate through the formation of a reactive intermediate. Clearly, in those cases where amounts of TMCS up to 20% are used,[7] the TMCS is not acting in a purely catalytic role.

The donor strengths of BSA and BSTFA are comparable and the reactivity enhancement from the addition of TMCS appears to be similarly comparable. Therefore, it is generally safe to assume that whenever a procedure calls for BSA + TMCS, BSTFA + TMCS can be substituted. This substitution is particularly appropriate when the peaks of interest have relatively low retention times and tend to be obscured by the derivatization reagent or the primary reaction products from the derivatization reagent. In some cases the combination of BSTFA and TMCS is a more powerful silyl donor than the comparable BSA and TMCS solution. In most cases the addition of 1% TMCS is sufficient to achieve the desired derivatization. If after using this reagent under forcing conditions (150°C for 12 hours) it appears that derivatization is not complete, additional TMCS may be added up to a final concentration of about 30%.

BSTFA + 1% TMCS or BSTFA + 10% TMCS 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 at least a two to one molar ratio of BSTFA + TMCS per active hydrogen is recommended. Best results are obtained when the products of the silylation reaction are soluble in the final reaction mixture.

Protocols for Silylating with BSTFA + TMCS

Note: Because of the reactivity of BSTFA + TMCS solutions, they are extremely sensitive to moisture and should be handled under as dry conditions as practicable. All glassware and syringes should be carefully dried.

In many cases, derivatizations are effectively completed at room temperature and without solvent. When there is no information available for a particular compound, it is recommended that these conditions be tried first. If derivatization is not complete under these conditions, the addition of TMCS as a catalyst, the use of an appropriate solvent, and the use of higher temperatures should be considered.

Concentrations of TMCS between 1% and 10% can conveniently be prepared in a syringe by withdrawing the appropriate amounts of BSTFA or BSTFA + 1% TMCS and BSTFA + 10% TMCS to add to the reaction mixture. For example, to obtain 0.100 mL of a BSTFA solution with 6% TMCS, simply withdraw 0.060 mL of BSTFA + 10% TMCS and 0.040 mL of BSTFA or BSTFA + 1% TMCS. As the exact concentration of TMCS is seldom critical, either BSTFA or BSTFA + 1% TMCS can be used as the lower component in most cases. In critical applications or applications where data are to be published, only BSTFA and BSTFA + 10% TMCS should be used unless the proportions are adjusted for the proper concentrations.

Without Solvent

1. Combine 1-10 mg of sample and 0.1-0.5 mL of BSTFA + TMCS in a clean, dry 3 mL Reacti-Vial™ Small Reaction Vial (Product No. 13222).
2. Cap, mix well, and let stand for 5-10 minutes or until the sample has dissolved.
3. Inject an appropriate size sample for column and detector.

With Heat

1. Combine 1-10 mg of sample and 0.1-0.5 mL of BSTFA + TMCS in a clean, dry 3 mL Reacti-Vial™ Small Reaction Vial.
2. Cap, mix well, and heat at 60°C for 15 minutes.
3. Cool to room temperature and inject an appropriate sample.

With Solvent

1. Dissolve a 1-10 mg sample in 1.0 mL of a suitable solvent (*see* Solvent Suggestions, page 4) in a clean, dry 3 mL Reacti-Vial™ Small Reaction Vial.
2. Add 0.1-0.5 mL of BSTFA + TMCS.
3. Cap, mix well, and let stand for 5-10 minutes.
4. Inject an appropriate sample.

With Heat and Solvent

1. Dissolve a 1-10 mg sample in 1.0 mL of a suitable solvent (see Solvent Suggestions, page 4) in a clean, dry 3 mL Reacti-Vial™ Small Reaction Vial.
2. Add 0.1-0.5 mL of BSTFA + TMCS.
3. Cap tightly, mix well, and heat at 60°C for 15 minutes.
4. Cool to room temperature and inject an appropriate sample.

For 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.
2. Add 0.5 mL of methylene chloride and again evaporate to dryness as above to ensure azeotropic removal of water.
3. Add a nonaqueous internal standard if desired.
4. Add 0.25 mL of BSTFA + TMCS for each mg 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.

For Aqueous Samples

For effective BSTFA + TMCS derivatization, it is usually necessary to remove all water from aqueous samples before the addition of the BSTFA + TMCS. This removal of water can either be accomplished by removing the water from the sample (Procedure A) or removing the sample from the aqueous phase by solvent extraction, followed by drying (Procedure B).

Procedure A

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. This evaporation is conveniently carried out in a 3 mL Reacti-Vial™ Small Reaction Vial using a Reacti-Therm™ Heating Stirring Module (Product No. TS-18823) equippedwith a Reacti-Vap™ Evaporator (Product No. 18826).
2. Many samples will be dry enough to go directly to the derivatization step following this drying procedure. If derivatization does not proceed as expected or if the sample is viscous, additional drying may be required. If this is the case, go to step A-3.
3. Last traces of moisture can be removed 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 under the same conditions as the original evaporation. This drying step may be repeated if there is any doubt about the dryness of the sample.
4. The sample may now be handled according to the protocols listed above for non-aqueous samples.

Procedure B

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 the preceding pages for samples with solvents. The solvent system may be changed to a different silylation solvent by evaporation and reconstitution with the desired solvent.

References

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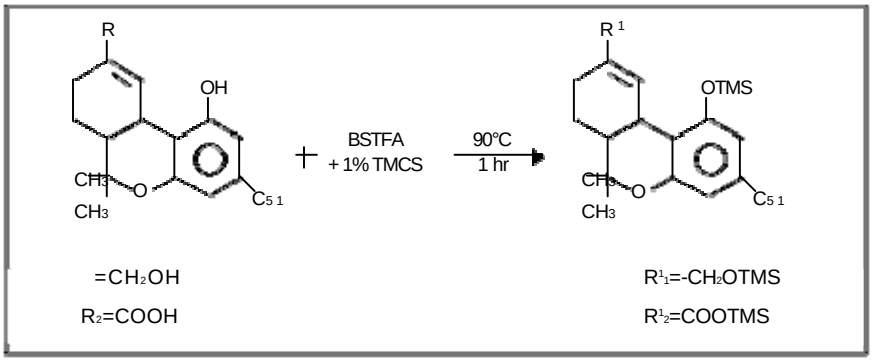
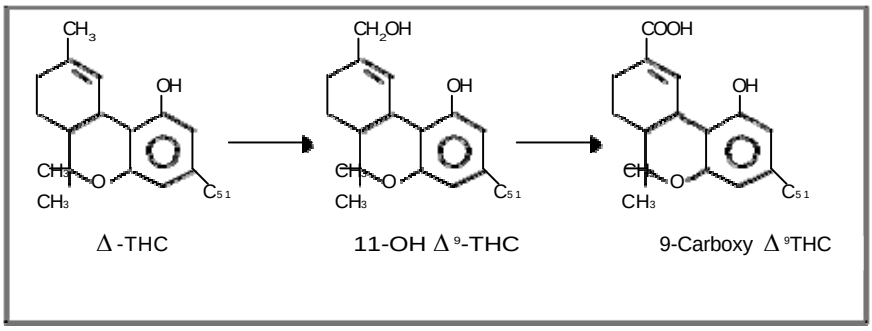
Current versions of product instructions are available at *www.thermo.com/columns*.



TMS Derivatives of Tetrahydrocannabinol (THC) Metabolites[8]



A Major Metabolic Pathway for Δ 9 - THC in Humans

1. Extract metabolites from the physiological fluid with hexane:ethyl acetate (7:1 V/V).
2. Wash organic extract with 0.1 M H_2SO_4.
3. Evaporate to dryness at 60°C in a 1 mL Reacti-Vial™ Small Reaction Vial in a stream of dry nitrogen.
4. Add 100 µL of BSTFA + 1% TMCS, cap and mix well.
5. Heat at 90°C for 1 hour.
6. Cool to room temperature and inject directly into the gas chromatograph.