INSTRUCTIONS

MSTFA + 1% TMCS

N-Methyl-*N*-trimethylsilyltrifluoroacetamide

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48915

0519

HANDLING PRECAUTIONS

Flammable. Harmful by inhalation. Danger of cumulative effects. Irritating to the eyes, respiratory system and skin. Keep away from sources of ignition. 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.

Product Description

NUMBER	DESCRIPTION
48915	**MSTFA + 1% TMCS,** *10 x 1 ml ampules* MSTFA + 1% TMCS is a clear, colorless to very light yellow liquid that is very sensitive to moisture. It is packaged under nitrogen in 1 mL ampules. Upon contact with water or water vapor, it hydrolyzes to form *N*- methyltrifluoroacetamide and hexamethyldisiloxane. With prolonged contact or elevated temperatures, methylamine and trifluoroacetic acid are formed. CAS # 24589-78-4

Storage

Although MSTFA + 1% TMCS is quite stable at room temperature and above, we recommend storage at 4°C or lower. To avoid possible contamination with moisture that may collect from condensation on the cold container, allow the bottle to warm to room temperature in a desiccator to be sure the bottle and septum are dry. Similarly, ampules should be opened at room temperature after drying.

Considerations for the use of MSTFA + 1% TMCS

MSTFA is an effective trimethylsilyl donor with donor strength approximately the same as BSA and BSTFA.[1] It reacts to replace labile hydrogens on a wide range of polar compounds 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 MSTFA over other silylating reagents is the volatility of its byproduct, *N*-methyltrifluoroacetamide. MSTFA is the most volatile TMS-amide available and *N*-methyltrifluoroacetamide has an even lower retention time than MSTFA. TMS derivatives of small molecules can often be analyzed when made from MSTFA, because the by-products and the reagent usually elute with the solvent front. Silylating reagents containing the trifluoroacetyl moiety, such as MSTFA, act as cleaning agents for flame ionization detectors. When a large number of TMS derivatives is to be analyzed using FID, silicone deposits from the excess derivatizing reagent tend to form on the detector and reduce its sensitivity. This buildup is minimized when derivatizing with reagents based on trifluoroacetic acid because the silicone is volatilized as SiF_4. Therefore, BSTFA and MSTFA are recommended over BSA for these applications.

MSTFA + 1% 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 MSTFA per active hydrogen is recommended. 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 MSTFA alone; however, when a catalyst such as TMCS is added, many of these compounds can be derivatized satisfactorily.

Protocols for Silylating with MSTFA + 1% TMCS

NOTE: MSTFA + 1% TMCS is extremely sensitive to moisture and should be handled under as dry of conditions as practical. 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, either higher temperatures or solvent can be employed.

Without Solvent

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

With Solvent

1. Dissolve a 1-10 mg sample in 1.0 mL of a suitable solvent (pyridine, DMSO, DMF, THF, or acetonitrile) in a clean, dry 3 mL Reacti-Vial™ Reaction Vial.
2. Add 0.1-0.5 mL of MSTFA + 1% TMCS.
3. Cap, mix well, and let stand for 5-10 minutes.
4. Inject an appropriate size sample.

With Heat and Solvent

1. Dissolve a 1-10 mg sample in 1.0 mL of a suitable solvent (DMSO, pyridine, DMF, THF, or acetonitrile) in a clean, dry 3 mL Reacti-Vial™ Reaction Vial.
2. Add 0.1-0.5 mL of MSTFA + 1% TMCS.
3. Cap tightly, mix well, and heat at 60°C for 15 minutes.
4. Cool to room temperature and inject an appropriate size 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 a removal of water.
3. Add a non-aqueous internal standard if desired.
4. Add 0.25 mL MSTFA + 1% 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 MSTFA + 1% TMCS derivatization, it is usually necessary to remove all water from aqueous samples before the addition of the MSTFA + 1% 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™ Reaction Vial using a Reacti-Therm™ Heating/Stirring Module (Prod. No's. 18935 and 18970) equipped with a Reacti-Vap™ Evaporator (Prod. No's. 18780 and 18785).
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.

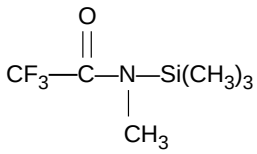
Procedure B

1. Add an amount of an organic solvent that 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 pages 3-4 for samples with solvents. The solvent system may be changed to a different silylation solvent by evaporation and reconstitution with the desired solvent.

References

1. Donike, M. (1969). *J. Chromatogr.* **42,** *103-104.*
2. Foltz, R.L. (1984). Analysis of cannabinoids in physiological specimens by gas chromatography/mass spectrometry. *Adv. Anal. Tox.* **1,** *125-157.*

MSTFA



N-Methyl-*N*-trimethylsilyltrifluoroacetamide
MW 199.1
bp. 70°C/75 mm d_4^{20} 1.11

TMCS

$(CH_3)_3SiCl$

Trimethylchlorosilane
MW 108.64
bp. 57°C d_4^{20} 0.858 mp -40°C