Product Information

69479 *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide

for GC derivatization, LiChropur®

Storage temperature: 2-8°C

N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) has become one of the most important silylating agents for analytical purposes[1].
The silylation potential of MSTFA is similar to that of Bis(trimethylsilyl)acetamide (BSA) and N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) as trimethylsilyl (TMS) donor and can be used for the silylation of all protic functional groups[2]. It can be increased by addition of a catalyst – mainly Trimethylchlorosilane (TMCS)[1,3,4]. Other catalysts are Trimethylsilylimidazole (TMSI; for the silylation of indolyl-NH)[5,6], potassium acetate[4,7], TMBS and TMIS[7] (for the quantitative derivatization of ketosteroids as their silyl enol ethers).

Features/Benefits

- Very versatile. Reacts with a range of polar organic compounds, replacing active hydrogens with a TMS group.
- Reacts rapidly and more completely than BSA.
- MSTFA and its by-product N-Methyltrifluoro-acetamide is more volatile than many other silylating reagents such as BSA and BSTFA, causing less chromatographic interference.
- MSTFA has good solvent properties and can function as a silylation reagent without adding additional solvents. MSTFA is very soluble in most commonly used silylation solvents such as acetonitrile and pyridine.

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. MSTFA is extremely sensitive to moist-ure and should be handled under dry conditions. Prepare a reagent blank (all components, solvents, etc., *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 using a gentle stream of dry nitrogen, then use neat or add solvent.
2. Add excess silylating reagent. The reagent can be used at full strength or with a solvent.* In most applications it is advisable to use an excess of the silylating reagent.
3. Warm the mixture to 60–90°C for 15–90 min. 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.
4. Dissolve in dry dichloromethane and inject the sample into the GC.

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.

* 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.

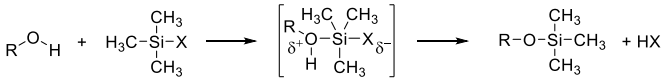
Under extreme conditions compounds may require heating for up to 16 h to drive the reaction to completion. Amino acids may require reaction in a sealed tube or vial. Heat samples cautiously, near the boiling point of the mixture, until a clear solution is obtained. If derivatization is not complete, evaluate the addition of a catalyst, use of an appropriate solvent, higher temperature, longer time and/or higher reagent concentration.

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 MSTFA 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 SLB®-1ms and SLB-5ms. Normal hydrocarbons (carbon-hydrogen analytes with single bonds) are separated by these phases. More polar phases, Equity-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[8,9]

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, –NH2 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 MSTFA, X = CF_3-C=O-N-CH_3) 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 derivatization 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.

Application Examples

MSTFA is the preferred reagent for the silylation of various polar compounds[1,3,10,11]. MSTFA is widely used for the silylation of steroids. Trimethylsilylether derivatives of anabolic steroids in bovine urine, which contain only hydroxyl functional groups (e.g. stilbenes estradiol-like compounds) can be synthesized for GC-MS analysis[4,7,12-16,17]. The derivatization of acetals from lipid fractions of liver after reductive work-up and chromatographical separation, was done with MSTFA as silylating agent[18]. Synthesis and use of reference substances to detect the use of anabolic steroids in man have been described[19,20].
Also, the trimethylsilylation of metabolites of anabolic agents in greyhound racing is carried out only with MSTFA[21]. Carboxylic acids such as fatty acids (capric acid C_{10}, myristic acid C_{14}, stearic acid C_{18}, behemic acid C_{22}, hexacosanic acid C_{26}, mellisic acid C_{30}), can likewise be silylated in a mixture of pyridine/hexane = 1:1[22] or in hexane[23]. The N-nitroso compounds of sarcosine, proline and 2-hydroxyproline were synthesized and silylated[24].
The silylation of β-ketoesters to the 3-trimethyl-siloxy-2-alkene acid alkylester has been described for four acidic esters (3-oxobutanoic acid trimethylsilylester, 3-oxooctanoic acid trimethylsilylester, 3-oxotetradecanoic acid trimethylsilylester and 3-oxodecanedioic acid bis(trimethylsilyl)ester[25]. Ureas and anilines, e.g. 4-chloroaniline, 3.4-dichloroaniline and 4-chloro-3-trifluoromethylaniline as degradation standards of antimicrobial agents have been studied via silylation with MSTFA[26].
Nucleic acids and their constituents[27], hindered phenols (with a mixture of MSTFA and 1% TMCS in pyridine as solvent)[28], 2- and 4-TMS-hydroxyacetophenones[29] and metabolites of piperidine in urine[30] have been silylated. Amino-alkylphenols can be derivatized to N-trifluoro-acetyl-O-TMS-aminoalkylphenols simultaneously by MSTFA and N-methyl-bis-(trifluoroacet-amide)[31,32].

The silylating potential can be reduced by addition of trifluoroacetic acid[32,33] or other protic substances[32].

Two different mixtures have been shown to be of particular use for the determination of anabolic steroids: MSTFA/TMCS/TMSI (100:5:2)[12-14] for the silylation of hydroxyl groups only, and MSTFA/Trimethyliodosilane (TMIS) (100:2 or 500:1, containing a small amount of 1,4-dithio-erythritol to reduce formed iodine)[12,16,34], which yielded TMS ethers as well as TMS enol ethers quantitatively (TMIS has been shown to be the best catalyst for this purpose[7]). The derivatization of heptafluorobutyrates to study the metabolism of 17β,19-nortestosterone in urine of calves after administration with a MSTFA/TMIS mixture (1000:2) has been described[35]. Analysis of buprenorphine in horse urine derivatized at the phenolic hydroxyl group was accomplished using GC-MS[36].

Storage/Stability

Recommended storage conditions for the unopened product are stated on the label. Store in an amber bottle or ampule at room temperature in a dry, well ventilated area. Use only in a well ventilated area. Keep away from ignition sources. Properly stored, this reagent 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

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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.