
Preparation of TMS Derivatives for GC/MS

Introduction

Trimethylsilyl (TMS) ethers are a convenient way to derivatize a variety of functional groups prior to GC analysis. The reagent we use is bis(trimethylsilyl)trifluoroacetamide (BSTFA). It will react quickly and quantitatively with alcohols, acids, and amines, and the by-products are volatile and can be injected directly into the GC without extraction. Pyridine is added as a basic catalyst to speed reaction with sterically hindered groups. The primary drawback to this method is that the TMS derivatives are unstable, and will commonly last only a few days before significant hydrolysis becomes a problem.

Materials

- BSTFA
- Anyhdrous pyridine
- Methanol (GC grade)
- DCM (GC grade)
- 50 mL syringe (**note A**)
- heating block (65°C)
- GC vials

Special Hazards and Warnings

- ☒ BSTFA are both toxic, carcinogenic, and smell truly horrible. Keep them in the hood and off your hands and clothes.
- ☒ Capped vials can sometimes burst while being heated. Keep the hood sash lowered while samples are heating, and wear safety glasses.

Procedure

1. Transfer sample to a GC vial in an appropriate aprotic solvent (DCM, ether, hexane, etc.). Remember that protic solvents like methanol cannot be present, as they will react with the reagent (**note B**). Add any internal standard.
2. Add 25 μL BSTFA and 25 μL pyridine (**note C**) to the sample. This amount is sufficient for a sample containing $<100 \mu\text{g}$ of total derivatizable material and dissolved in $\sim 100 \mu\text{L}$ of solvent. If your sample is bigger, you will need to scale up the amount of reagents being added, or take a smaller aliquot of your sample for the reaction.
3. Cap the vial tightly and heat at 65°C for ~ 20 minutes. The heating step is simply to ensure that the reaction goes to completion, so the exact time and temperature are not critical. You should not need to heat samples for any longer than 30 minutes.

4. Let the sample cool to room temperature then inject on the GC/MS. Store derivatized samples in the freezer to help extend their lifespan.
5. Important! BSTFA is very corrosive to metal syringe needles and plungers. Be sure to clean all syringes that come in contact with BSTFA thoroughly, first using methanol then DCM. This includes the autosampler syringe on the GC/MS.

Notes

A. BSTFA will quickly corrode syringe plungers, leading to a stuck syringe. Using a teflon-tipped plunger will help to minimize this, as will careful and immediate cleaning of the syringe with methanol after every use. The glass capillary autopipetor is an even better option. It may be helpful to designate a single syringe which is only to be used with BSTFA.

B. The BSTFA itself can be used as a solvent with good success. In cases where minimal dilution is desired, the sample can be transferred to a GC vial using DCM, the solvent completely removed by evaporation, then the sample redissolved (with sonicating) in BSTFA + pyridine.

C. BSTFA will react very slowly with tertiary or otherwise hindered hydroxyl groups, such as those often found on sterols. The addition of anhydrous pyridine as a catalyst will greatly speed the reaction of these groups. Addition of pyridine for other compounds is not necessary, but for most samples the addition of pyridine can only help matters, thus its addition is included as part of the standard protocol.

D. If samples were previously derivatized, but have been sitting in the freezer too long and have degraded, try adding more BSTFA (with heating) to the existing sample. Unless it has gotten wet, this usually works to rederivatize the analytes.

E. Injecting BSTFA into the GC injector often will temporarily improve peak shapes. This is because the BSTFA will react with bare silica surfaces in the liner and column, and helps to (temporarily) deactivate them. Consider it an added bonus.

Troubleshooting

The most common symptom is that some or all of the peaks in your chromatogram are not derivatized with TMS groups. The most likely cause is that the reagent has gone bad. Once opened, it has a shelf life of only a month or two in the freezer, less if it is opened frequently. Dry the sample back down, break out some new BSTFA, and try again. Second most likely cause is water in the reaction, either because your sample is wet, one of the solvents is wet, or you used wet glassware or syringe. Dry the sample over anhydrous sodium sulfate. Try fresh aliquots of solvents if you suspect them. Finally, make sure there is no methanol (protic solvent!) in your sample.