

TheReporter

Reprinted from Volume 15, No. 4, 1996

T296024

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Use of BSTFA Silylating Reagent to Prepare Volatile Derivatives for GC

K. Kiefer, K. Herwehe

We examined the purity of a widely used silyl reagent, bis(trimethylsilyl)trifluoroacetamide, the amount of reagent needed to derivatize a test analyte, and the effects of a catalyst on the reactivity of the reagent. BSTFA purity for four suppliers ranged from 98.8 to 99.8%. A 1:1 molar ratio of derivatizing reagent to active hydrogen in the analyte molecule was needed to drive the reaction to completion; a 2:1 ratio was preferred. With a catalyst, however, the reaction between 500µL of test standard and 50µL of BSTFA went to completion. Based on this study, and others, we have incorporated purity and reactivity specifications into the quality control criteria for our reagents.

Gas chromatography is used to separate volatile organic compounds. By modifying the functionality of a molecule to increase—or sometimes decrease—volatility, derivatizing reagents enable chromatographers to analyze compounds that otherwise are not readily monitored by GC. Derivatization also reduces analyte adsorption in the GC system and improves detector response, peak separations, and peak symmetry.

The chemical properties of the compounds to be analyzed dictate which derivatizing reagent to use, and at what concentration. In silylation, an active hydrogen is replaced by an alkylsilyl group, most often a trimethylsilyl (TMS) group, $\text{Si}(\text{CH}_3)_3$. TMS derivatives are preferred because of the ease of reaction, freedom from side reactions, reproducibility of the derivatization, and the availability of these reagents. Silyl derivatives are more volatile, less polar, and thermally more stable than their parent compounds. Many materials can be silylated in seconds or minutes at room temperature, but others can require extended periods at elevated temperature.

We investigated the reactivity of a common silyl reagent, bis(trimethylsilyl)trifluoroacetamide (BSTFA), using seven lots of reagent from four raw material suppliers. Several variables were monitored to determine their roles in derivatizing an analyte: BSTFA purity, the amount of BSTFA needed to derivatize a reference analyte (an alcohol), and the effect of adding a catalyst to the BSTFA.

Impurities in the reagent could interfere with the speed or completeness of the reaction, or with the chromatography of the derivatized analyte. To examine BSTFA purity, we injected 1µL of the reagent, neat, into the GC. Product purity for the four suppliers was 98.8-99.8%. At present, impurities that could affect the reactivity of the reagent are not detected by capillary GC. We are working to develop reliable analyses for these impurities.

The ideal derivatization reaction will occur at room temperature, in less than 15 minutes, with a derivative yield of 99.9% or more. We examined the silylating properties of BSTFA on a test analyte, using GC peak area as the measure. The test solution consisted of 1-decanol (10mg/mL) and tridecane (5mg/mL) in hexane. 1-Decanol, a primary alcohol, is easily silylated, and the product is relatively stable. Tridecane, an inert hydrocarbon, was the internal standard. It will not react with BSTFA and elutes near the 1-decanol and 1-decanol-TMS ether peaks. We evaluated the reactivity of the reagent by combining 500µL of the 1-decanol/tridecane test solution with 50µL, 100µL, 200µL, 500µL, and 1000µL of BSTFA. The materials were mixed and allowed to stand 5-10 minutes. 1µL of the resulting product was analyzed on a nonpolar capillary GC column. The chromatogram should show two peaks—the derivative and the internal standard. The absence of the alcohol peak indicates that the reaction is complete.

The chromatograms in Figure A are representative results, for one raw material supplier and one lot of BSTFA. 50µL of BSTFA did not completely derivatize the 1-decanol because the silylating reagent was not added at a 2:1 molar ratio relative to active hydrogen, the ratio recommended to drive the reaction to completion (1). In fact, the ratio was 1:10 (50µL BSTFA:500µL analytical standard). This low ratio produced a 22% yield of 1-decanol-TMS ether. In some cases 1-decanol was completely converted to the TMS ether when 100µL or 200µL of reagent was used. This result, however, varied from supplier to supplier and lot to lot. 500µL of reagent from any lot completely derivatized the alcohol.

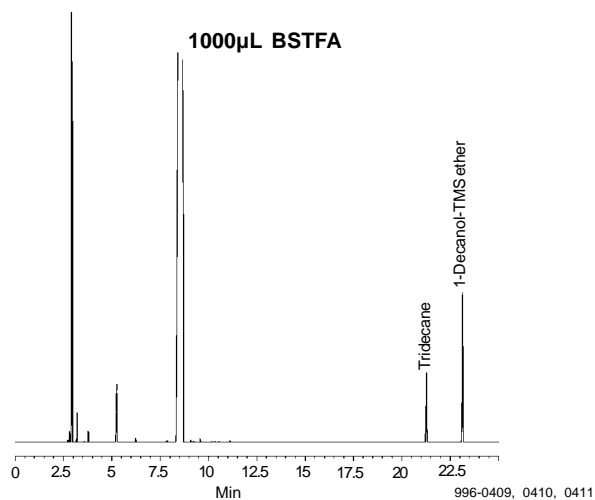
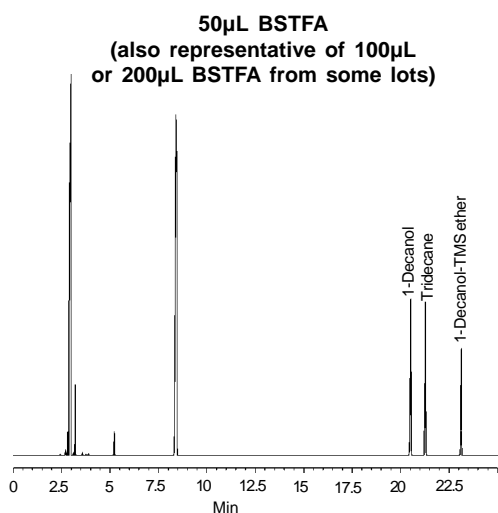
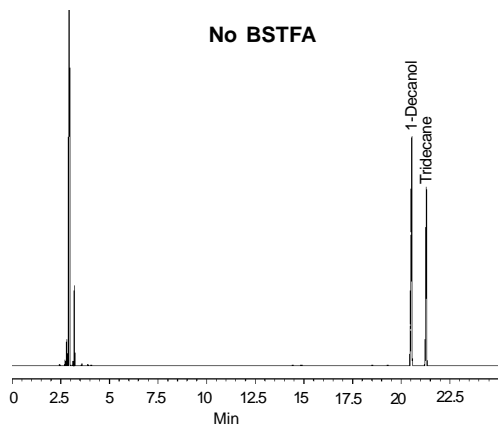
Because unhindered primary alcohols are derivatized easily, only 500µL of BSTFA was required to completely convert 1-decanol to the TMS ether. We recommend using excess reagent, however (e.g., 1000µL). Excess reagent can compensate for water or impurities in the sample, and helps to preserve the sample from hydrolysis if the reaction medium is to be stored prior to the GC analysis. The reaction of 1000µL BSTFA with 500µL test solution meets the criteria for ideal silylation: reaction occurs at room temperature, in less than 15 minutes, and with a reaction yield of 99.9%.

The silyl donor strength of a reagent also is influenced by the addition of a catalyst. With the addition of 10µL of trimethylchlorosilane (TMCS), the reaction between 500µL of test solution and 50µL of BSTFA went to completion. The solvent used also can affect the yield of derivative. Hexane, a nonpolar material, is a suitable solvent for this reaction, but will not accelerate the rate of reaction.

Reprinted from Volume 15, No. 4, 1996

Figure A. Evaluating the Silylation of 1-Decanol with BSTFA

Column: PTE™-5, 30m x 0.25mm ID, 0.25µm film
 Cat. No.: 24135-U
 Oven: 50°C (4 min) to 280°C at 6°C/min
 Carrier: helium, 20cm/sec
 Det.: FID, 250°C
 Inj.: 1µL of (500µL of 10mg/mL 1-decanol + 5mg/mL tridecane in hexane, + 0, 50, or 1000µL BSTFA), split (100:1), 250°C



Based on this study, and others, we have incorporated purity and reactivity specifications into the quality control criteria for our silyl reagents. We also are attempting to identify impurities that might affect the reactivity of the reagent. Studies such as this also indicate that it is important to know the structure of the compound to be derivatized and the number of active hydrogens present. This will indicate the amount of reagent needed to drive the reaction, and how strong the reagent should be. In general, we recommend using at least 10-20% excess reagent, to account for any water or other impurities that might be in the sample. If derivatization is incomplete, or if you are working with an unknown compound, evaluate the effects of adding a catalyst, changing the solvent, and/or modifying the reaction temperature, reaction time, and reagent concentration.

The benefits of using BSTFA as a silyl reagent are speed, simplicity, efficiency, freedom from side reactions, and reproducibility. It also is reassuring to know that your supplier carefully monitors the quality of the reagent you are using.

Ordering Information:

Description	Cat. No.
BSTFA Reagent	
144 ampuls x 0.1mL	33084
20 ampuls x 1mL	33024
25mL	33027
BSTFA+TMCS, 99:1	
144 ampuls x 0.1mL	33154-U
20 ampuls x 1mL	33148
25mL	33155-U
50mL	33149-U
PTE-5 Capillary GC Column	
30m x 0.25mm, 0.25µm film	24135-U

For more information about BSTFA and BSTFA + TMCS, request publications 496020 and 496021.

Reference

1. K. Blau and J.M. Halket, eds. *Handbook of Derivatives for Chromatography* (2nd ed.) John Wiley & Sons (1993).

Reference not available from Supelco.

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Fused silica columns manufactured under HP US Pat. No. 4,293,415.

