BSA

Product Specification

BSA (N,O-bis(trimethylsilyl)acetamide) is one of the most commonly used silylating reagents. Its reactivity is similar to that of BSTFA, readily silylating a wide range of acidic functional groups such as non-sterically hindered alcohols, amides, amines, amino acids, carboxylic acids, and enols. It is not recommended for use with simple sugars. BSA's silylating potential can be increased by using an appropriate polar solvent, or by adding a catalyst (e.g., 1-10% TMCS).

Features/Benefits

Under mild reaction conditions forms highly stable products with most organic functional groups. TMS derivatives are thermally stable but more susceptible to hydrolysis than their parent compounds.

Reactions are generally fast and quantitative.

Will silylate unhindered hydroxyl groups.

BSA and its byproducts are more volatile than many other silylating reagents, causing less chromatographic interference.

BSA has good solvent properties and usually can function as an efficient silylating reagent without additional solvents. (DMF is the solvent most frequently used to improve efficiency.)

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 – consult MSDS for specific handling information. BSA is extremely sensitive to moisture 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.

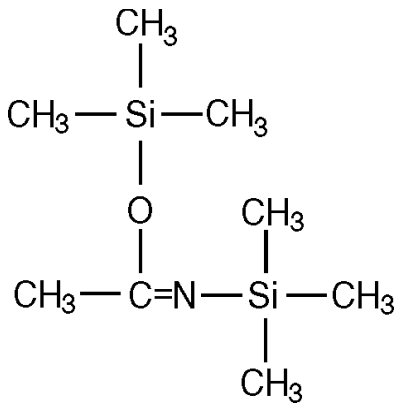
1. Weigh 1-10mg of sample into a 5mL reaction vessel. If appropriate, dissolve sample in solvent (see below). If sample is in aqueous solution, evaporate to dryness, then use neat or add solvent.
2. Add excess silylating reagent. BSA can be used at full strength or with a solvent.* In most applications it is advisable to use an excess of the silylating reagent – at least a 2:1 molar ratio of BSA to active hydrogen. Not all samples are derivatized by BSA alone. For moderately hindered or slowly reacting compounds, use BSA with 1% or 10% TMCS catalyst. BSA may be mixed with other catalysts (trifluoroacetic acid, hydrogen chloride, potassium acetate, piperidine, O-methylhydroxylamine hydrochloride, pyridine).
3. 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.

   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 minutes. Under extreme conditions compounds may require heating for up to 16 hours 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, the addition of a catalyst, use of an appropriate solvent, higher temperature, longer time and/or higher reagent concentration should be evaluated. For hydroxyl groups in sterically unhindered positions in steroids combine 1-10mg sample with 200-500µL BSA. If material is not soluble in BSA, add 100-200µL pyridine. Cap vessel and shake well. Warming (60°C) may accelerate dissolution. For compounds that silylate with difficulty, shake for 30 sec, then heat at 70°C for 15 min.

Properties

Structure:



CAS Number:
10416-59-8

Molecular Formula:
$CH_3C[=NSi(CH_3)_3]OSi(CH_3)_3$

Formula Weight: 203.43

bp: 71-73°/35mm

Flash Point: 53°F (11°C)

d: 0.823

n_D: 1.4170 at 20°C

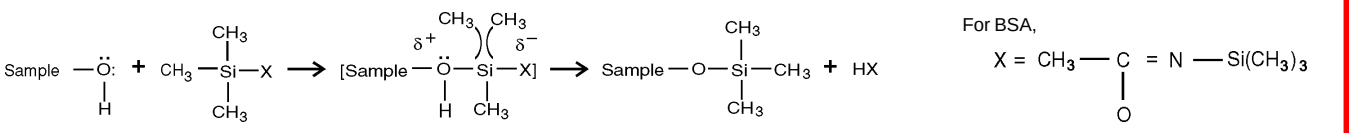
Appearance:
clear, colorless liquid
moisture sensitive

796-0258

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 and are sensitive to active hydrogen atoms. Do not analyze BSA derivatives on stationary phases with these functional groups (e.g., polyethylene glycol phases). Silicones are the most useful phases for TMS derivatives – they combine inertness and stability with excellent separating characteristics for these derivatives. Nonpolar silicone phases include SPB™-1 and SPB-5. Normal hydrocarbons (carbon-hydrogen analytes with single bonds) are separated by these phases. More polar phases, SPB-1701 and SP™-2250, separate carbon-hydrogen analytes that also contain Br, Cl, F, N, O, P, or S

* Nonpolar organic solvents such as hexane, ether, benzene, 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, dimethylformamide (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. Acetonitrile/BSA, 3:1 is recommended for amino sugars.



Adapted from Knapp (2).

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atoms or groups. A highly polar cyanopropylphenylsiloxane phase, SP-2330, is useful for separating fatty acid methyl esters or aromatics.

Mechanism (1,2)

Silylation is the most widely used derivatization procedure for GC analysis. In silylation, an active hydrogen is replaced by an alkylsilyl group, most often trimethylsilyl (TMS). 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, $–NH_2$, 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 silicon atom of the silyl donor, producing a bimolecular transition state. The silyl compound leaving group (X) 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 compound 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: primary > secondary > tertiary, and for amines: primary > secondary.

Toxicity – Hazards – Storage – Stability

BSA is a flammable, moisture-sensitive liquid. It may irritate eyes, skin, and/or the respiratory system. Store in a brown bottle or amber ampul at room temperature, in a dry, well ventilated area away from ignition sources. Use only in a well ventilated area and keep away from ignition sources.

Properly stored, this reagent is stable indefinitely. Recommended storage conditions for the unopened product are stated on the label. Moisture will decompose both TMS reagents and derivatives. To exclude moisture, Supelco packages this product under nitrogen. 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

1. K. Blau and J. Halket *Handbook of Derivatives for Chromatography* (2nd ed.) John Wiley & Sons, New York, 1993.
2. D.R. Knapp *Handbook of Analytical Derivatization Reactions* John Wiley & Sons, New York, 1979.

Additional Reading

L.W. Woo and S.D. Lee, *Capillary Gas-Chromatographic Determination of Proteins and Biological Amino-Acids* J.Chromatogr., B, Biomed. Appl., 665 (1), 15-25 (1995).

C.F. Poole in *Handbook of Derivatives for Chromatography* K. Blau and G.S. King, Eds., Heyden & Son, Ltd. p 152 (1977).

J.D. Nicholson, Analyst, 103: 193 (1978).

J. Drozd, *Chemical Derivatisations in Gas Chromatography* Elsevier (1981).

C.E. Kientz and A. Verweij, J. Chromatogr., 355: 229 (1986).

P. Englmaier, Fresenius' Z. Anal. Chem., 324: 338 (1986).

L.A. Cole, et al., Analyst, 116: 1287 (1991).

N. Hirota, *et al.,* J. Chromatogr., 425: 237 (1988).

Ordering Information

Description	Cat. No.
BSA	
144 ampuls x 0.1mL	**33035-U**
20 ampuls x 1mL	**33036**
25mL	**33037**

For information about BSA + TMCS reagent, refer to Product Specification 496018.

Microreaction Vessels with Hole Caps and Septa	
1mL, pk. of 12	**33293**
3mL, pk. of 12	**33297**
5mL, pk. of 12	**33299**
Books	
Handbook of Derivatives for Chromatography K. Blau and J. Halket	**Z24,6220**
Handbook of Analytical Derivatization Reactions D.R. Knapp	**23561**

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