Note: This paper lists all the types of artifacts noted in our laboratory in the last 20 years and ones we have found in the literature. It is a modified (not refereed) copy of a paper [J.L. Little, J. Chromatogr. A, 844 (1999) 1-22] containing additional examples found since the paper was published.

Abstract

Trimethylsilyl derivatives are routinely employed in gas chromatography to increase the volatility and stability of organic compounds containing active hydrogens. Normally only the desired derivative is formed when organic compounds are derivatized with common silylation reagents. However, some compounds form additional unexpected derivatives or by-products (artifacts). Artifact formation leads to multiple peaks for the same compound or unexpected components in the gas chromatographic analysis of mixtures. This review includes silylation artifacts identified in our laboratory by mass spectrometry during the last 20 years and references to those found in the literature. Also, means of avoiding artifact formation are discussed in detail.

Keywords: Mass spectrometry; Derivatization; Artifacts; Artefacts; Trimethylsilyl derivatives; Silylation; Silyl derivatives; Gas chromatography; GC/MS; GC-MS; Gas chromatography-mass spectrometry; BSA; BSTFA, Accurate mass, Formalin.
1. Introduction

Note: This paper lists all the types of artifacts noted in our laboratory in the last 25 years and ones we have found in the literature. It is a modified (not refereed) copy of a paper [J.L. Little, J. Chromatogr. A, 844 (1999) 1-22] containing additional examples found since the paper was published.

Trimethylsilyl derivatives are routinely employed [1-5,67] in gas chromatography to increase the volatility and stability of organic compounds containing active hydrogens (see Figure 1). Normally only the desired derivative is formed when organic compounds are derivatized with common silylation reagents such as BSA [N,O-bis(trimethylsilyl)acetamide] and BSTFA [N,O-bis(trimethylsilyl)trifluoroacetamide].

![Chemical Structures](image)

However, some functional groups such as aldehydes, amides, carboxylic acids, esters, ketones, and phenols under certain conditions form additional unexpected derivatives from silylation reagents and their by-products (e.g. 1 and 2). We refer to these unexpected derivatives as silylation artifacts. Furthermore, even the derivatization reagent can react with itself, inorganic reagents, other organic reagents, or organic solvents to yield artifacts.

Artifacts are a common problem in analytical chemistry [6] and are noted in a wide variety of chromatography techniques. Artifact is either spelled artifact or artefact and both spellings are acceptable. The former spelling was employed in this article since searches [6] of several common databases showed that scientists prefer to spell the word with an "i".

Artifact formation in silylation reactions leads to multiple peaks for the same compound or unexpected components in the gas chromatographic analysis of mixtures. This leads to confusion about the concentration of a component or the number of components present in the sample. For quantitative
analyses, the responses for the multiple components can be summed (assuming equal responses) or
derivatization conditions changed to avoid artifact formation. This report includes the types of artifacts
noted in our laboratory during the last 20 years and references to those found in the literature. Also, means
of avoiding artifact formation are discussed. The majority of the examples noted in this report are included
in a soon to be published paper [71], but this report (not refereed) includes many examples added since the
paper was accepted for publication.

2. Experimental

2.1. Instrumentation and sample preparation

Gas chromatography/mass spectrometry (GC/MS) data were obtained on Finnigan/MAT 4023,
VG/Micromass 70, VG/Micromass Autospec, Hewlett Packard MSD, and Finnigan/MAT TSQ-700 mass
spectrometers. The source temperature was set at 250 °C on magnetic mass spectrometers and at 150 °C on
quadrupole mass spectrometers.

All reactions were performed in 2-ml disposable glass vials with crimp tops. Septa were sealed by
crimping an aluminum top. Typically 1-5 mg of a sample were dissolved in 0.5 ml of a suitable nonprotic
solvent such as N,N-dimethylformamide (DMF), dimethylsulfoxide (DMSO), tetrahydrofuran (THF),
acetonitrile, toluene, pyridine, etc. The sample was then mixed with 0.5 ml of derivatization reagent
(BSTFA, BSA, etc.). Occasionally the sample was dissolved directly in the derivatization reagent and no
solvent was employed. Samples were heated for 15-30 minutes at 50-80 °C. Solvent vapors are easily
contained in the sealed vials, but caution should be employed when the solvents are heated significantly
above their boiling points. Under these conditions, the septum could rupture. Reinforced vials are
commercially available for performing reactions at temperatures in excess of 100 °C.

Most GC separations were performed on relatively non-polar capillary columns with bonded liquid
phases such as DB-17 or DB-5 (J&W, 30-m length, 0.25-µm film thickness, and 0.32 mm I.D.). The
columns were typically programmed from 50 °C to 300 °C at 12-15 °C/min with a helium backpressure of
12 psi. Under these conditions a non-retained component traveled through the column at approximately 30
cm/second. Normally 1-3 microliters of the solutions were injected in the split mode or 1 microliter in the
splitless mode with an injection port temperature of 275-310 °C.

A wide variety of conditions were employed for sample derivatization depending on the class of
compounds and sample matrix. Several references [1-5,67] discuss factors to consider in optimizing
silylation reactions including reaction mechanisms, choice of solvents, choice of reagents, catalysts,
temperatures, reaction times, etc. In addition, many distributors of silylation reagents offer excellent
technical information.

2.2. Chemical ionization mass spectrometry

Molecular weights were confirmed by ammonia or isobutane chemical ionization (CI) mass spectral
data. Ammonia was normally the preferred gas (pressure set to yield a ratio of m/z 18 to 35 of
approximately 10:1). Several compounds did not yield molecular weight information with either ammonia
or isobutane as the CI gases. These compounds were analyzed with CI gas mixtures of either 3%
methylamine in methane or 3% dimethylamine in methane. The pressures of these gases were optimized
by setting the ratio of the gas phase monomers (m/z 32 and 46, respectively) to the gas phase dimers (m/z
63 and 91) at approximately 10:1.

2.3 Accurate mass electron impact mass spectral data

Accurate mass electron impact mass spectral data was obtained on a Micromass Autospec GC-MS
(magnetic instrument) at about 5000 resolution. Several different ions in 8 (n=1) including the molecular
ion (C₆H₁₂NO₂F₃Si, measured 215.0591, calculated 215.0589, 0.9 ppm error), the molecular ion-methyl
(C₆H₁₃NO₂F₃Si, measured 200.0358, calculated 200.0355, 1.5 ppm error), the molecular ion-methyl-
formaldehyde (C₅H₇NOF₃Si, measured 170.0258, calculated 170.0249, 6 ppm error), and the Si(CH₃)₂F
ion (measured 77.0212, calculated 77.0223, 14 ppm error). Accurate mass data were also obtained for 2,
the silylation by-product from BSTFA. Several ions were measured including the molecular ion
(C₅H₁₀NOF₃Si, 185.0471 measured, 185.0484 calculated, 6.9 ppm error), molecular ion-methyl
(C₄H₇NOF₃Si, 170.0248 measured, 170.0249 calculated, 0.6 ppm error), molecular ion-methyl-CF₂
(C₃H₇NOSiF, 120.0281 measured, 120.0281 calculated, 0 ppm error), and the Si(CH₃)₂F ion (measured
77.0224, calculated 77.0223, 1.3 ppm error). The measured errors are within the capability of our
measurement (control chart data over several years, n=191, one standard deviation 5.4 ppm).

2.4. Electron impact mass spectral data

The major ions and relative intensities of the majority of the components (TMS=trimethylsilyl)
discussed in the text, but whose mass spectra are not displayed or found in commercial databases, are listed
in this section. Full spectra will be sent for consideration as entries in the Wiley Registry of Mass Spectral
Data [7] and the NIST/EPA/NIH Mass Spectral Database [8]. The mass spectra are listed with the relative
intensities and the number of halogens present noted in parentheses.

5: 73(30), 147(100), 189(80), 213(40,1Cl), 236(10,1Cl), 305(10), 326(10,1Cl), 380(20), 415(30,1Cl);
per-TMS derivative of disilicic acid, CAS No. 20638-18-0: 73(100), 147(60), 207(13), 221(50), 281(15),
295(10), 327(13), 341(15), 399(10), 415(15), 529(8), 503(7), 591(45); bis-(TMS) derivative of hydrogen
peroxide: 59(6), 117(5), 119(4), 133(60), 163(100); per-TMS derivative of sulfuric acid: 45(6), 66(13),
73(32), 131(7), 133(4), 147(100), 227(57); per-TMS derivative of phosphorous acid: 45(17), 61(10),
73(75), 131(8), 133(15), 135(14), 147(93), 195(8), 207(100), 221(5), 283(21), 298(94); bis-TMS derivative
derivative of sulfurous acid: 43(30), 45(57), 58(20), 59(32), 66(30), 73(80), 131(5), 147(100), 211 (8); bis-TMS
59(6), 66(13), 73(32), 131(7), 133(4), 147(100), 227(57); per-TMS derivative of phosphorous acid: 45(17), 61(10),
73(75), 131(8), 133(15), 135(14), 147(93), 195(8), 207(100), 221(5), 283(21), 298(94); bis-TMS derivative
derivative of sulfurous acid: 43(30), 45(57), 58(20), 59(32), 66(30), 73(80), 131(5), 147(100), 211 (8); bis-TMS
derivative of phosphorous acid: 45(8), 73(35), 98(7), 133(8), 135(12), 147(22), 195(8), 211(100), 226(6);
20: 44(20), 56(20), 69(41), 71(27), 99(16), 127(100); 23: 42(7), 61(100), 172(8), 185(62), 233(26); 24:
45(5), 61(100), 73(37), 100(17), 105(15), 149(50), 168(8), 216(5), 245(2); BSA artifact with MW 245:
43(20), 45(15), 73(100), 75(13), 115 (18), 116(30), 130(10), 131(9) 147(90), 190(10), 230(15), 245(10); BSA
artifact with MW 275: 56(38), 73(100), 113(18), 114(12), 117(18), 131(8), 147(40), 172(5), 260(15),
275(5).

3. Results and discussion

In many of our examples, significant concentrations of silylation artifacts are only noted in the
derivatization of reaction mixtures or crude samples, and not in the derivatization of pure samples.
Apparently components not present in the pure samples lead to the formation of these artifacts. Many
materials are reported [9] to catalyze the silylations of compounds with BSTFA and BSA. Catalysts
reported include trimethylchlorosilane (TMCS), trifluoroacetic acid, hydrogen chloride, potassium acetate,
piperidine, O-methylhydroxylamine hydrochloride, pyridine, oxalic acid, and trimethylbromosilane. Thus,
it is not surprising that silylation artifacts are noted in reaction mixtures or crude samples since they often
contain salts, bases, and acids as contaminants.

Attempts were made to isolate several of the artifacts by preparative gas chromatography for
characterization by proton nuclear magnetic resonance (NMR) analyses. However, the only artifact
successfully isolated was an artifact of an amide. This is not surprising since many silylated components
tend to decompose when exposed to moisture in the air. Therefore, the majority of the artifacts noted in our
laboratory were identified by interpretation of electron impact mass spectra, by confirmation of molecular
weights with chemical ionization data, and by proposing structures from reasonable reaction mechanisms.

3.0. Incomplete derivatization

Multiple peaks can be noted due to incomplete silylation of compounds. Several excellent references
[1-5,67] discuss factors to consider in optimizing silylation reactions including reaction mechanisms,
solvents, derivatization reagents/reagent mixtures, catalysts, temperatures, reaction times, etc. In addition
many distributors of silylation reagents offer technical information on the selection and use of silylation
reagents.

An interesting article [81] was noted concerning the partial derivatizations of 3-hydroxy-3-
methylglutaric acid (HMG) and 3-hydroxyisovaleric acid (HIVA).
The authors noted that methods (biological matrices) employing hydrochloric acid acidification and sodium chloride saturation resulted in partial silylation of HMG and HIVA. Incomplete silylation was minimized by three different approaches. One approach substituted sulfuric acid for acidification and sodium sulphate for the saturating salt. The improvement was attributed to the fact that the sulfate formed a weaker adduct with the hydroxyl groups of HMG and HIVA. Thus the hydroxyl groups were free for derivatization. Another approach employed pyridine as a solvent in the derivatization when using hydrochloric acid and sodium chloride sample preparation route. The pyridine was proposed to minimize formation of hydroxyl adducts with chloride. Another approach, used in conjunction with the first two, employed a DEAE-Sephadex anion exchange to remove chloride.

3.1. Aldehyde artifacts

Aldehydes form artifacts in a variety of ways with silylation reagents and are often formed at very high concentrations. Aromatic aldehydes were noted to react with MSA \([10-12]\) or MSTFA \([13]\) to yield the following types of artifacts:

![Chemical structures of HMG and HIVA](image)

The MSTFA adducts of aromatic aldehydes \([13]\) show characteristic molecular ion-H (M-1) and the molecular ion-N(CH\(_3\))COCF\(_3\) (M-126) fragments in their electron impact mass spectra.

We have noted similar types of artifacts when aromatic aldehydes such as methyl 4-formylbenzoate or 4-formylbenzoic acid are reacted with either BSA or BSTFA in DMF:
The molecular formulae of the two preceding artifacts were confirmed by accurate mass EI and their molecular weights by ammonia chemical ionization mass spectrometry for the methyl 4-formylbenzoate derivative.

The silylation of both aldehydes with BSA also yielded additional artifacts, which incorporated the solvent, DMF. Its molecular formula was confirmed by accurate mass data and its molecular weight by ammonia chemical ionization data for the methyl 4-formylbenzoate artifact. The molecular ion of the artifact was shifted 7 m/z units when perdeuterated DMF was substituted for DMF in the derivatization of methyl 4-formylbenzoate. The corresponding artifacts were not noted when BSTFA was employed as the derivatization reagent. Several structures could be proposed for these BSA-DMF artifacts. Two of the possible structures are shown below:

The imine structure above (structure on left) is more consistent with the large ion noted at m/z 99 in the undeuterated artifact spectrum, which is not easily explained by the structure containing the α,β-unsubstituted amide artifact structure. The ion at m/z 99 was noted as the basepeak in the EI mass spectrum of Compound 19, a DMF artifact. However, silylated compounds often undergo complex rearrangements in their EI spectra and the α,β-unsubstituted amide artifact could be formed by further reaction of the BSA-Aldehyde Artifact with DMF.

The silylation of both aldehydes with BSTFA yielded two additional artifacts in each case. The molecular weight was confirmed by ammonia chemical ionization and the molecular formula by accurate mass EI data for the methyl 4-formylbenzoate artifact. The proposed structures are noted below:
The corresponding artifacts were not noted with BSA. Structures could not be proposed for the other set of artifacts formed with BSTFA and aldehydes. The artifacts had molecular weights of 191 and 249, respectively, for the methyl 4-formylbenzoate and for the 4-formylbenzoic acid artifacts. These artifacts were not noted when BSA was employed in the derivatization reactions.

Aldehydes with α-hydrogens can react with MSTFA [13] to yield two different types of artifacts:

- Enol-form
- MSTFA

The major ions noted in the electron impact mass spectra [13] for MSTFA adducts of aliphatic aldehydes such as 3 are shown below:

- The ion at m/z 184 is thought to be formed [13] by the neutral loss of hexanal from the molecular ion to yield an odd electron ion for the O-trimethylsilyl form of MSTFA. This intermediate ion then loses a methyl radical to yield the fragment ion proposed for m/z 184 above.

We have noted the analogous types of artifacts such as 4 with BSA and aromatic aldehydes. Others [13] propose that this type of artifact is formed directly from the silylation product. However, it could just as well be formed from the derivatization by-product, 1, in the presence of excess BSA.
The electron impact mass spectrum (see Figure 2) of the artifact formed from 2-chlorobenzaldehyde is most consistent with 4 above. However, the trimethylsilyl group readily migrates in the electron impact mass spectra of organic compounds [14]. Thus, another likely structure for the artifact could be 5. Indeed two isomers are sometimes noted in our work for related aldehyde artifacts.

We also have noted smaller concentrations of other artifacts, for example 5a, formed from further reactions of 4 with BSA:

The concentrations of these types of artifacts formed from BSA were significantly reduced in this example by substituting BSTFA for BSA. We also plan to evaluate the use of N-trimethylsilylimidazole (TMSI) to determine if it might also reduce the concentrations of these types of artifacts.

We have also noted acetal artifacts such as 6. This type of artifact is likely formed by the reaction of BSA or BSTFA with the gem-diol (hydrate) of the aldehyde to form a bis(trimethylsiloxy)acetal. The electron impact mass spectrum of the acetal formed from BSA and 4-chlorobenzaldehyde is shown in Figure 3.
An analogous artifact was noted by others [69] for the reaction of MSTFA with the hydrate of glyoxylic acid to yield 6a in addition to the MSTFA adduct, 6b.

\[
\text{HCO} - \text{CO} - \text{OH} \xrightarrow{\text{MSTFA}} (\text{CH}_3)_2\text{SiO} - \text{H} - \text{SiO}(\text{CH}_3)_2 + \text{H}_2\text{O}
\]

\[
\text{6a} \quad \text{6b}
\]

We identified several artifacts (see Figure 4) in the silylation of a 36% Formalin with BSTFA when repeating work performed in a literature reference [15]. Formalin solutions are complex mixtures of “poly-acetals” and “poly-hemiacetals” formed by mixing water, methanol, and formaldehyde gas. The artifacts in the silylation of Formalin solutions are likely formed by the reaction of 7 with one or more of the oligomeric compounds present in the Formalin mixture.

\[
(\text{CH}_3)_3\text{Si} - \text{O} - \text{N} - \text{Si}(\text{CH}_3)_3 + \text{H}_2\text{O} \xrightarrow{\text{BSTFA}} \text{CF}_3\text{N} - \text{H}_2\text{O} + (\text{CH}_3)_3\text{SiOSi}(\text{CH}_3)_3 + (\text{CH}_3)\text{SiOH}
\]

\[
\text{BSTFA} \quad 7
\]

\[
(\text{CH}_3)_3\text{Si} - \text{O} - \text{H} - \text{CH}_2\text{OH} \xrightarrow{\text{BSTFA}} \text{CF}_3\text{O} - \text{H} - \text{O} - \text{Si}(\text{CH}_3)_3
\]

\[
7 \quad 8 \quad (n = 0-4)
\]

The authors of the original study calculated the oligomer distribution of the Formalin solution after derivatizing with BSTFA. However, they did not identify or comment on the silylation artifacts present in the analysis. The concentrations and origin of these artifacts should have been considered in the calculation of the oligomer distribution in their original work.

We identified these artifacts by their EI and CI mass spectra. Compound 8 \((n=1)\) shows a very unusual ion at m/z 77 in its electron impact mass spectrum in Figure 5. We have proposed this ion to have a structure of \(+\text{Si}(\text{CH}_3)_2\text{F}\). We suspect it is formed by some type of an intramolecular rearrangement since its relative intensity decreases as the distance between the trimethylsilyl and trifluormethyl groups increases. This decrease is shown in the electron impact mass spectra for 8 \((n=1\) and \(n=3)\) in Figure 5.

Others have studied a very similar rearrangement [16] that leads to the formation of the \(+\text{Si}(\text{CH}_3)_2\text{F}\) ion in the electron impact mass spectrum of trimethylsilyl trifluoroacetate. B/E linked scan and accurate mass data show that the ion is formed in a multi-step mechanism. A methyl group is initially lost from the molecular ion with subsequent losses of difluorocarbene and carbon dioxide to yield m/z 77. Furthermore, m/ 77 is the base peak [17] in the electron impact mass spectrum of 2, the silylation by-product from BSTFA. We confirmed by accurate mass data that 2 likely fragments by an analogous mechanism as that of trimethylsilyl trifluoroacetate (see Experimental Section).
Thus our data and literature references support the following fragmentation mechanism for 8 (n=1):

\[
\begin{align*}
&\text{CF}_3\text{O} + \text{NH}_2\text{Si(CH}_3\text{)}_3 & \rightarrow & \text{CF}_3\text{O} + \text{NH}_2\text{Si(CH}_3\text{)}_3 + \cdot \text{CH}_3 \\
&\text{m/z 77} & \rightarrow & \text{m/z 120}
\end{align*}
\]

Using a larger excess of the derivatization reagent, BSTFA, might reduce the formation of these artifacts. This would decrease the relative concentration of 7, and thus form lower concentrations of the observed artifacts. However, diluting the sample with more derivatization reagent would make it difficult to detect many of the low-boiling components. Another possibility is substituting another silylation reagent for BSTFA.

Aldehydes readily form hemiacetals and acetals. Sugars are probably the most well known example of acetals formed during silylation. These acetals are formed by intramolecular attack of an alcohol group on the aldehyde group found in reducing sugars at equilibrium. Attempts to silylate these hemiacetals by silylation often lead to several peaks for each individual sugar [18].

Aldehyde artifacts could be avoided in many cases by first converting the aldehyde to its methoxime or hydroxylimine derivatives before silylation. The only significant problem with forming imine derivatives is that they exist as syn- and anti-geometric isomers, which are separated by many non-polar GC columns.

### 3.2. Ketone artifacts

Ketones form the same type of artifacts noted for aldehydes. Ketones with α-hydrogens react to form artifacts through their enol-form as noted below:

\[
\begin{align*}
&\text{R} = \text{HO} \leftrightarrow \text{R} = \text{enol-form} & \text{BSA or BSTFA} & \rightarrow & \text{R} = \text{(CH}_3\text{)}_3\text{SiO} \\
&\text{keto-form} & & & \text{R} = \text{enol-form}
\end{align*}
\]

Pure samples of ketones form varying amounts of these types of artifacts depending on variables [19] such as reaction time or the addition of catalysts such as TMCS. Of course the presence of HCl in crude samples would have the same effect since TMCS would be formed in situ.

\[
\begin{align*}
&\text{HCl} \underset{\text{BSA or BSTFA}}{\rightarrow} \text{(CH}_3\text{)}_3\text{SiCl} & \rightarrow & \text{TMCS}
\end{align*}
\]
Several schemes are available for avoiding artifacts from ketones that can form enol-trimethylsilyl ethers. They include avoiding acid catalyst when using BSA and TMSIM as derivatization reagents or converting the ketone to a methoxime derivative [19]. The methoxime derivative will still yield multiple peaks on certain stationary GC phases due to the formation of syn- and anti-geometric isomers. The formation of silylquinoxalinol derivatives from 1,2-diaminobenzene and a silylation reagent avoids the formation of syn- and anti-geometric isomers for α-keto acids [20].

The electron impact mass spectra of the ketone artifacts formed from 9 and 10 are shown in Figure 6.

\[
\begin{align*}
\text{OH} & \quad \text{O} \\
\text{9} & \quad \text{OH} \\
\text{O} & \quad \text{Si} \\

\text{10} & \quad \text{O} \\
\text{CH}_3 & \quad \text{CH}_3 \\
\end{align*}
\]

The *ortho*-isomer, 9, shows a significantly larger ion at m/z 147 formed from an intramolecular rearrangement. This ion is characteristic of two closely spaced trimethylsilyl groups within a molecule [21]. This ion is much smaller in the *para*-isomer, 10. Therefore, in some cases this ion can be employed to distinguish isomers.

\[
\text{(CH}_3\text{)}_3\text{Si}^{+} \rightleftharpoons \text{Si} \rightleftharpoons \text{CH}_3 \\
m/z 147
\]

The enol-TMS compounds have been noted to further react with diethyl disulfide in the derivatization of steroids. MSTFA/ammonium iodide/ethanethiol was used for the silylation. The MSTFA reacts in situ with the ammonium iodide to product trimethylsilyl iodide, TMSI. TMSI is reported to be one of the most powerful trimethylsilyl donors available [84]. Iodine present is converted into hydrogen iodide by the oxidation of ethanethiol to diethyl disulfide. The resulting disulfide is proposed to further react with the desired TMS-enol derivative to form the sulfide-artifact:
We have not noted the reaction of the reaction by-products (1, 2, and 7) with ketones. This is not surprising since ketones are much less likely to form ketal-like compounds while aldehydes readily form acetal-like compounds. However, others have even noted the by-product of BSFTA, 7, reacting with highly electron deficient ketones [22-23].
The analogous BSA adduct was noted (2-5%) when BSA (no TMCS added) was employed. The formation of the bis(trimethylsiloxy) ketal is not surprising since mesoxalic acid is isolated in crystalline form as its hydrate. Possibly eliminating the TMCS or increasing the ratio of the silylation reagent would form less of the nitrogen-containing artifact. The latter approach would lead to a smaller concentration of 7.

The formation of trimethylsilyl enol-ethers can be useful in the analysis of keto-carbonyl compounds such as acetoacetamides, alkyl acetoacetates, α,α-diketo esters, etc. These classes of compounds elute as very broad peaks or two peaks connected by a valley. This poor chromatography is noted on even highly deactivated bonded-phase capillary columns. The broadening of the peak is due to the dynamic equilibrium of the keto- and enol-forms of the diketo compounds. For example, derivatizing of 11 with BSTFA and N,N-dimethylformamide as the solvent normally yields complete trapping of the enol-form as its trimethylsilyl enol-ether.

The resulting chromatogram yields a very sharp peak that can easily be quantified or two peaks in some cases. Two peaks are sometimes noted because the trimethylsilyl enol-ether can exist in E- and Z- confirmations.

 Others have noted the attack of a trimethylsilyl radical on the enol-trimethylsilyl ether double bond for α,β-ketones such as testosterone [24] that subsequently eliminates a trimethylsilyl group. The reactions forming the artifacts were presumed to be free radical in nature and catalyzed by ultraviolet light or dibenzoyl peroxide [25].
3.3. Carboxylic acid and ester artifacts

Carboxylic acids and alkyl esters tend to form silylation artifacts much less readily than either ketones or aldehydes. Alkyl esters do not normally silylate and carboxylic acids form the expected trimethylsilyl derivatives.

However, malonic acid, α-hydroxymalonic acid, α-methylmalonic acid, and alkyl diesters of malonic acid were shown by mass spectrometry data [26] to form artifacts from the silylation of the enol-form of their ester groups. For example, α-hydroxy malonic acid forms the expected tris-trimethylsilyl derivative and the unexpected tetrakis-trimethylsilyl artifact shown below:

The tris-TMS derivatives of methylmalonic and hydroxymalonic acids were noted to form additional artifacts by reaction with oxygen (see Section 3.9 Oxidation reactions).

Alkyl esters of malonic acid such as dimethyl malonate form a mono-trimethylsilyl artifact:
These types of artifacts are observed because the enol-forms of the compounds are stabilized as α,β-unsaturated esters.

We have noted that carboxylic acids with at least one α-hydrogen occasionally form artifacts. Apparently the derivatization by-product from BSA, 1, can attack the initially formed trimethylsilyl ester to yield an artifact. The electron impact mass spectrum of an artifact, 12 or 13, formed from the reaction of BSA and 1 with pentanoic acid is shown in Figure 7. The electron impact mass spectrum is most consistent with the structure for 13. The electron impact mass spectrum of 12 would be expected to be more similar to that of 3 shown in Figure 2. In particular, 12 should show ions at m/z 43 for the presence of an acetyl group and the loss of ketene from either the molecular ion or the molecular ion-methyl group.

The formation of this artifact in this particular sample matrix was avoided by employing BSTFA as the derivatization reagent. Furthermore, this artifact was not noted in the derivatization of the pure sample of pentanoic acid with BSA under the same reaction conditions. Apparently some component or mixture of components present in the crude sample catalyzed the formation of the artifact. Possible ways to avoid these acid artifacts are to select a different silylation (BSTFA, TMSIM, etc.) reagent or to select an altogether different type of derivatization reagent [2-5].

3.4. Amine and Amide artifacts

Primary amines can be silylated once or twice depending on the conditions employed and the compound to be derivatized. Secondary amines are also a problem yielding both the underivatized and derivatized forms. The problem of two peaks can be avoided by employing trimethylsilylimidazole, which reacts with hydroxyls but not amines or amides.

Amines have been noted to form two types of artifacts. One is an imine noted when DMF is employed as the solvent (see Section 3.6.a.).

The other is a trifluoroacetamide artifact noted when BSTFA/TMCS is employed as the derivatization reagent [82] with organophosphonofluoridates, ROP(O)(F)CH3. The artifacts’ structures were confirmed by mass spectral and infrared data (see Figure 18). These artifacts are noted when Sarin type nerve agents are decontaminated in aqueous media with monoethanolamine.
The authors were able to reduce the formation of the artifacts by reducing the concentration of BSTFA used in the derivatization reaction [82]. Possibly substituting BSA might also minimize the formation of such artifacts.

Usually the main problem in the silylation of amides is that they can be detected in three different forms after silylation. The amide usually appears in its underivatized form since it tends to be one of the least reactive groups [27]. However, it can form both N-trimethylsilyl and O-trimethylsilyl derivatives.

We have also noted the formation of nitriles in the silylation of aromatic and aliphatic amides. For example, approximately 56% (area % assuming response factors of unity) yield of the nitrile was noted when erucylamide was silylated:

Aromatic amides such as benzamide and terphthalamide only yielded about 0.1% of their respective nitriles when derivatized under similar conditions.
Others [77] have commonly observed nitrile formation from fatty acid amides as a result of injector "autocatalysis." If the glass wool is dirty and/or unsilanized, "autocatalysis" results, even when the sample has been previously silylated.

Aryl and aliphatic carboxamides are known [75] to yield nitriles in high yields under mild conditions using silyl sulfonfonyl polyphosphates. For example, 4-methoxybenzamide was heated at 70-75°C with mesyl trimethylsilyl polyphosphate to yield 90% 4-methoxybenzonitrile. The mesyl trimethylsilyl polyphosphate was prepared by treating MeSO2SiMe3 with P2O5.

Nitriles were noted [76] when the tert-butyldimethylsilyl (t-BDMS) derivatives of arginine and citrulline were formed. The same nitrile forms from both compounds, which helped to confirm the nitrile's structure.

A cyclic amide silylation artifact that we have noted at significant concentrations was formed from the intramolecular condensation of the bis(trimethylsilyl) derivative of 14.

The structure of this artifact, 15, was confirmed by proton NMR analysis and could easily be formed in high yields by the reaction of a pure sample of the starting material, 14, with BSA. The electron impact mass spectrum of the amide artifact is shown in Figure 8. Normally no ions should be noted whose m/z is greater than that of the molecular ion cluster in an electron impact mass spectrum. However, trimethylsilyl derivatives often yield ions [28-29] corresponding to M+1 and M+73 ions due to intermolecular transfer of a proton or the trimethylsilyl group via ion molecule reactions. The relative abundance of these ions will increase as the concentration of the analyte present in the electron impact source increases. Analyzing the compound by chemical ionization allowed the molecular weight of the compound to be determined since this intermolecular transfer of groups is suppressed in the chemical ionization mode.

Decreasing the reaction time and/or temperature of the silylation reaction might significantly reduce this concentration of 15 and the nitrile artifacts. The alcohol groups are likely silylated at room temperature or upon exposure to the hot GC injection port in the presence of excess silylation reagent and should require no additional heating. Another approach would be to decrease the silyl donor strength. The relative silyl donor strengths [30] of common commercial reagents is noted below:

TMSI > BSTFA > BSA > MSTFA > TMSDMA > TMSDEA > MSA > TMCS (with base catalyst) > HMDS

All the silylation reagents are catalyzed by the addition of 1-10% TMCS.

3.5. Reagent artifacts
Trimethylsilyl derivatives of inorganic reagents (see Figure 9) are not truly artifacts since they are expected to undergo derivatization. However, they are often found unexpectedly in crude samples and their presence can catalyze the formation of artifacts [9,19]. The silylation of samples suspected to contain inorganic acids is very desirable since in their underivatized form they can seriously damage GC columns. The per-trimethylsilylated derivatives of silicic acid and disilicic acid are usually noted [31] when samples are isolated from TLC (Thin Layer Chromatography) plates and then derivatized. Phosphorus pentoxide was noted to react with hexamethyldisiloxane [68] to yield a synthetically useful polyphosphoric acid ester mixture. This mixture is composed of cyclic and linear tetramers of phosphoric acid trimethylsilyl esters.

Many of the trimethylsilyl derivatives of the inorganic compounds shown in Figure 9 are already present in large commercial electron impact mass spectral databases such as the NIST/EPA/NIH Mass Spectral Database and the Wiley Registry of Mass Spectral Data. However, there were several spectra not present, which were obtained in our laboratory. In addition, several discrepancies between our spectra and those found in the commercial databases were noted. All of our spectra will be donate to these two commercial databases [7,8].

The discrepancies were noted for the per-trimethylsilylated derivatives of hydrogen peroxide, phosphorous acid, and sulfuric acid. The spectra sent, which were not in either of the commercial databases, were the per-trimethylsilyl derivative of disilicic acid and sulfurous acid and the bis-trimethylsilyl derivative of phosphorus acid.

3.6. Solvent artifacts

Many artifacts are noted from the reaction of either the derivatization reagent, the derivatization by-products, or the analyte with the solvent used in the derivatization reaction. Artifacts noted with several of the more commonly employed silylation reagents are discussed below.

3.6.a. N,N-Dimethylformamide (DMF)

When BSTFA is employed by itself or with other common solvents to derivatize mixtures of hindered phenols such as 16, a mixture of derivatized, 17, and underivatized phenol is obtained. However, when BSTFA and DMF are employed for the reaction, 16 is completely derivatized in 20-30 minutes with heat.

A DMF artifact is always noted at significant concentrations in this derivatization procedure. The artifact was identified by its electron impact mass spectrum (see Figure 10) as 19. The corresponding impurity is not noted using BSA as the derivatization reaction. However, an aldehyde artifact is noted incorporating both BSA and DMF (see section 3.1, BSA-DMF Aldehyde artifact).

We have proposed that 19 is formed from Reaction Intermediate 18 shown below:
Reaction Intermediate 18 is similar to the imidoyl chloride cation formed in the Vilsmeier-Haack synthesis [32]. The total ion chromatogram for the GC/MS analysis of 17 showing the relative retention time and concentration of 19 is shown in Figure 11. It is proposed [33] that the relatively higher dielectric constant of DMF facilitates the solvation of the charge in the transition state of the trialkylyphenols leading to the more efficient silylation of hindered phenols with hexamethyldisilazane.

A mixture of 19 and 20 is noted when a two step derivatization procedure is employed to determine the amount of carboxylic acid present in an acid chloride sample. The acid chlorides are first derivatized with diethylamine to convert acid chlorides to amides. The mixture is then treated with BSTFA in DMF to derivatize any free carboxylic acid present.

The diethylamine employed to derivatize the acid chloride is involved in a “transamidation” reaction with either 19 or Reaction Intermediate 18 to yield 20.

Both 19 and 20 can be avoided by employing another solvent such as pyridine in the derivatization of acid chlorides. Neither the acid chlorides nor free carboxylic acids need DMF to be converted to their respective derivatives.

We have also noted small amounts of the N,N-Dimethylcarbamic acid trimethylsilyl ester, Compound 20a, in samples containing 21. The structure of Compound 20a was confirmed by comparing the retention time and electron impact mass spectrum (see Figure 15) of a purchased reference sample to that of the unknown.
Possibly Compound **20a** is formed by the oxidation of DMF to N,N-dimethylcarbamic acid that is then silylated to yield the observed impurity.

Other artifacts formed from DMF and silylation reagents were also noted in the literature. Amines form silylation artifacts with DMF. For example, octopamine was noted [34-35] to form the following type of artifact:

![Chemical Structure](image1)

This type of DMF artifact was not formed when the glass surfaces of the reaction tubes were silanized [35].

Hexamethyldisilazane (HMDS) was noted to form an artifact with DMF in the derivatization of tertiary alcohols [34,36]. The artifact was not characterized in their work. A reasonable structure for the artifact would be the structure shown below or a similar amidine:

![Chemical Structure](image2)

### 3.6.b. Acetone

The use of acetone as a solvent in the derivatization of compounds with either BSA or BSTFA was noted to yield artifacts. For example, in the analysis of a mixture of hydroquinones, several artifacts such as **21** were observed. The EI mass spectrum of one of these artifacts formed from 2-chlorohydroquinone is shown in Figure 12.
Apparently this artifact is formed by the reaction of acetone with the phenol to give a “hemi-ketal” intermediate, which is then silylated to yield the observed artifact. These artifacts were not noted when a pure sample of the 2-chlorohydroquinone was derivatized in acetone.

Acetone was also noted to dimerize to yield “diacetone alcohol” which can yield three different artifacts, $21a$ - $21c$.

Structure $21b$ is proposed to be the major isomer with MW 260 by its EI mass spectral fragmentation pattern and from literature references [78-80]. There was a minor isomer with MW 260 (concentration ratio of major to minor isomer about 50:1), which is thought to be $21c$. Both spectra are shown in Figure 17. Accurate mass electron impact mass spectrometry data was obtained for both the major and minor isomers and it is consistent with the proposed structures.

3.6.c. Dimethylsulfoxide (DMSO)

Dimethylsulfoxide (DMSO) was noted to give many different artifacts in the preparation of polyester samples for composition analysis [37]. It was proposed that BSTFA reacted with DMSO in a Pummerer Reaction [38] to form Intermediate $22$. 

$21c$
By-products from the silylation reaction, 2 and 5, react with 22 to yield 23 and 24 in reasonably large concentrations. GC/IR, GC/MS, CI GC/MS (accurate mass), and deuterium-labeled reagent experiments were used to identify the artifacts.

\[
\begin{align*}
2 + 7 + 22 & \rightarrow 23 \\
 & \\
24 \\
\end{align*}
\]

Smaller concentrations methylthiomethyl esters, 24a, of acids present in the sample in addition to the desired derivatives such as 24b.

\[
\begin{align*}
\text{CO}_2\text{H} & \quad \text{DMSO} \\
& \quad \text{BSTFA} \\
\text{CO}_2\text{H} & \\
& \\
\text{CO}_2\text{Si(CH}_3\text{)}_3 & \\
\text{CO}_2\text{Si(CH}_3\text{)}_3 & \\
\text{CO}_2\text{CH}_2\text{SCH}_3 & \\
\text{CO}_2\text{Si(CH}_3\text{)}_3 & \\
24\text{a} & \\
24\text{b} & \\
\end{align*}
\]

Similar reactions were noted [39] when N-tert-butyldimethylsilyl derivatives of imidazole were heated with DMSO at very high temperatures.

The DMSO artifacts [37] were minimized in the polyester analysis by substituting BSA for BSTFA. BSA is a weaker silyl donor than BSTFA that retarded the formation of the reaction intermediate. Occasionally one BSA artifact is noted. Its spectrum is compared to the corresponding BSTFA artifact in Figure 19. The molecular formula of the BSA artifact was confirmed by accurate mass data and isotope ratios.

Substitution of BSA for BSTFA does have some restrictions. BSA and its by-products are higher boiling than BSTFA and its by-products and could obscure lower boiling components of interest. Another possible reagent for this application is N-methyl-N-trimethylsilyl trifluoroacetamide (MSTFA), which has not been tested. It and its by-products are more volatile than BSA and its by-products.

3.6.d. Methanol

Methanol artifacts of glycols have been noted when methanol/base is used to hydrolyze polyester samples. The artifacts are also noted when titanium tetraisoproxide methanol is used at high temperatures and pressure to trans-esterify polyesters. These artifacts are probably formed by the oxidation of methanol to yield low levels of formaldehyde. The formaldehyde then forms hemi-acetals, which are detected as their trimethylsilyl derivatives upon derivatization.
EI mass spectra of the trimethylsilyl derivatives of ethylene glycol and glycerin are compared to their artifact spectra, respectively, in Figures 13 and 14. The similarity of the EI spectra of the glycols and their artifact spectra indicate that the compounds undergo a neutral loss of formaldehyde after ionization. The molecular weights of the artifacts were confirmed by ammonia CI data. Possibly these methanol artifacts could be minimized by the addition of antioxidants to the solutions and/or performing the reactions under an inert atmosphere with degassed reagents. Another possibility is to add dilute hydrochloric acid to the samples before derivatization, which could shift the equilibrium towards the glycol.

Surprisingly, none of the cyclic acetals such as 28 and 29 were noted from the intramolecular reaction of formaldehyde with glycerin. Samples enriched in these cyclic acetals were formed by reacting Formalin with glycerin in tetrahydrofuran over Amberlyst 15 resin.

3.7. Silylation reagent artifacts

Three structures are proposed for artifacts frequently noted at lower concentrations in samples derivatized with BSA. They are proposed to be formed by the reaction of BSA and its byproduct, 1. Analogous by-products are not noted with BSTFA since it and its by-product, 2, do not contain any active hydrogens and its by-product does not add as readily across active carbonyls.
3.8. Oxidation Reactions

We have noted that THF (tetrahydrofuran) containing peroxides can lead to artifact formation. Others have also noted oxidations during silylation reactions. Trimethylsilylation of 7-methylpurine nucleosides using BSTFA and TMCS yielded 7-methyl-8-oxo compounds as artifacts [49]. The compounds were shown by $^{18}$O$_2$ labeling experiments to be formed by an oxidation reaction (dissolved oxygen) during the derivatization reaction. The resulting artifacts were found to be very useful for analyzing 7-methylpurine nucleosides because they were amenable to gas chromatography and exhibited structurally diagnostic mass spectra. Components in hydrolyzed DNA were noted to oxidize [50, 74] during silylation for GC/MS analyses. To prevent the artifactual formation of oxidized bases during the silylation, a preparative HPLC method was developed to remove the interferences. Molecular oxygen [51] was involved in the dehydrogenation of nucleosides during vigorous trimethylsilylation to yield artifacts. The reaction was accelerated by heat and certain free radical initiators and was inhibited by diethylthiocarbamate and galvinoxyl free radical. This reaction was found useful as a synthetic approach in nucleoside synthesis. Persilylation of norethynodrel and 5(10)estrene-17β-ol-3-one was noted [52] to aromatize the A-rings of these compounds.

A systematic study [66] of oxidations showed that levels of 8-hydroxyguanine, 8-hydroxyadenine, 5-hydroxycytosine and 5-(hydroxymethyluracil) in commercial calf thymus DNA detected by GC-MS are elevated by increasing the temperature at which derivatization is performed. Their work suggests that artifactual oxidation during derivatization is decreased by reducing the temperature of the derivatization reaction to 23°C and excluding as much air possible.

Tris-trimethylsilyl artifacts of malonates (see Section 3.3 Carboxylic acid and ester artifacts) form varying amounts of oxidation artifacts. For example [72, 73]:

![Diagram of Oxidation Reactions](image-url)
α,β-Ketones such as testosterone [24] are noted to form oxidation artifacts (see Section 3.2, Ketone Artifacts). The reactions forming the artifacts were presumed to be free radical in nature and catalyzed by ultraviolet light or dibenzoyl peroxide [25].

3.9. Other miscellaneous artifacts

Several other references were found in the literature for miscellaneous silylation artifacts. Also, various miscellaneous classes of compounds were noted to form artifacts in our laboratory.

3.9.a. Epoxides

Epoxides [40] were reported to react with TMCS as follows:

\[
\begin{align*}
\text{Epoxide} + (\text{CH}_3)_3\text{SiCl} & \rightarrow \\
\text{TMCS} & \rightarrow \\
\text{OSi(CH}_3)\text{}_3
\end{align*}
\]

Epoxides could react with by-products such as 1, 2, and 7 in a similar manner to yield silyl derivatives; however, no literature references were found for these types of artifacts.

3.9.b. Carbamazepines

The derivatization of a carbamazepine metabolite [70] was shown by mass spectrometry data to yield the expected trimethylsilyl derivative, 24c, and the rearrangement product 9-acridinecarboxaldehyde, 24d.
3.9.c. Carbon dioxide, carbon disulfide, and sulfur trioxide

Silylation artifacts can be formed from carbon dioxide dissolved in samples. Amines such as glycine, serine, alanine, and ethanolamine [41] were noted to form carboxylates. For example:

\[
\text{CO}_2 + \text{H}_2\text{N}\text{CH}_2\text{OH} \xrightarrow{\text{BSTFA}} \text{(CH}_3\text{)}_3\text{SiO}\text{N}-(\text{CH}_2\text{CH}_3)_2
\]

These types of artifacts were avoided by adding dilute hydrochloric acid during the final stages of drying a sample for derivatization. Silylamines add to carbon dioxide to give the following carbamate ester [42-44].

\[
\text{(CH}_3\text{)}_3\text{Si-N-(CH}_2\text{CH}_3)_2 + \text{CO}_2 \rightarrow \text{(CH}_3\text{)}_3\text{Si-O}N(\text{CH}_2\text{CH}_3)_2
\]

Carbon disulfide [42,45] and sulfur trioxide [42,46] also react with silylamines to give similar products.

3.9.d. Prostaglandins

Prostaglandins [47] were noted to form three different chromatographic peaks when silylated with trimethylsilyl imidazole (TMSI) containing piperidine (PIP). For example, a prostaglandin having an unstable β-hydroxy ketone ring structure was found to form the following derivatives:
When a mixture of BSTFA and PIP were used for the derivatization, only one peak was noted by gas chromatography for 25. The piperidine-containing artifact might also have been avoided by employing TMSI with a tertiary amine as a catalyst instead of piperidine.

3.9.e. Glycosides

An artifact was noted to form in the silylation [48] of glycosides with hexamethyldisilazane (HMDS) and TMCS. This artifact was desirable since it formed volatile derivatives of anthocyanin-2-arylbenzopyrilium salts. The compounds are converted into quinoline-like structures such as 26 by reaction of the salts with mono-trimethylsilylamine.

\[
(CH_3)_3SiNHSi(CH_3)_3 + \text{HMDS} \rightarrow \text{26}
\]

3.9.f. Salts of inorganic and organic acids

Salts of inorganic or organic acids are normally not derivatized when reacted with BSA or BSTFA alone. However, under certain circumstances they will be detected as their trimethylsilyl ester derivatives. Ammonium salts of many different inorganic acids [53] were readily derivatized. K or Na salts were first converted to ammonium salts by cation exchange. Several Na and K salts of mono-, di-, and tri-basic organic acids and sodium salts of fatty acids were directly silylated [54] with a mixture of hexamethyldisilazane and trimethylchlorosilane. Na salts of organic acids are converted to trimethylsilyl esters [55] employing mixtures of hydroxylamine hydrochloride/BSA or trimethylchlorosilane/BSA. The conversion of volatile organic acids to sodium salts with subsequent derivatization [55] can be a very useful means of avoiding their loss when samples are concentrated by lyophilization.

\[
\text{O} + \text{TMCS} \rightarrow \text{OSi(CH}_3)^3 + \text{Na}^+ \text{Cl}^-
\]

3.9.g. Triflic acid with amides and esters
The presence of triflic acid in samples leads to silylation artifacts from C-silylation at the α-position of amides and esters. For example, the trimethylsilyl ester of triflic acid was shown [56] to react with N-methylacetamide after aqueous work-up to yield 27.

3.9.h. Flavanones and chalcones

Flavanone aglycons were noted to ring open to their corresponding chalcones [57]. This ring opening was confirmed by GC/MS, silylation of standards, and UV spectrophotometric data.

3.9.i. α,β-Unsaturated lactones

Compounds containing α,β-unsaturated lactone rings are derivatized to form enol-TMS ethers. The conversion is quantitative when TMCS and a powerful silyl donor are employed [58].

3.9.j. Secondary silylamines with isocyanates

Secondary silylamines react with isocyanates to give N-silylureas [42,59-61].

The primary silylamines add to the isocyanate to give two different products, which were shown by NMR data to be in equilibrium with each other via silyl-proton exchange [42,61]. Isothiocyanates and silylamines form analogous products [42,61].

3.9k. Alkylammonium salts
We noted that bis(hydroxyethyl) methyl tallow ammonium chlorides salts (some referred to commercially as Ethoquads) such as \(28\) yields a mixture of tertiary amines and alkyl chlorides when derivatized with BSTFA. Apparently the intermediate bis(trimethylsilyl) derivative, \(29\), thermally decomposes during the derivatization reaction or upon contact with the GC injection port. These types of artifacts are useful for determining the types of groups attached to ammonia. Field desorption and MALDI data yielded molecular cations and chloride cluster ions for these types of materials with no fragmentation.

The GC-MS results for the silylated sample of \(28\) are shown in Figure 16.

### 3.9.1. Azides

We noted that azides with adjacent methylene groups decompose with time in BSTFA solutions to form imines. Apparently the azide first decomposes to a nitrene, which rearranges to an imine. The imine is silylated by excess BSFA present in the sample to yield the azide artifact.

### 4. Summary of ways to avoid or minimize artifact formation

Normally silylation reactions yield the desired derivative with minimal optimization of reaction conditions. However, many different artifacts can be formed under certain circumstances. In addition, multiple peaks can be noted due to incomplete silylation of compounds. Several excellent references [1-5,67,81] discuss factors to consider in optimizing silylation reactions including reaction mechanisms, solvents, derivatization reagents/reagent mixtures, catalysts, temperatures, reaction times, etc. In addition many distributors of silylation reagents offer technical information on the selection and use of silylation reagents.
Several ways of to avoid or to minimize artifact formation were noted in our studies and literature references. Ones that we have found generally useful are summarized below:

- The first step is to characterize all components in the mixture by electron impact GC/MS. In some cases, additional analyses were required by chemical ionization GC/MS, accurate mass, and isotope labeling to identify unknowns. Silylation conditions can only be efficiently optimized if components are identified and reactions leading to their formation understood. Several references [14,28-29,62-64] discuss the general interpretation of the electron impact mass spectra of silylated compounds. Other references discuss the fragmentation of MSTFA adducts [13] and the trimethylsilyl derivatives of nucleotides [65].

- Reaction times and derivatization reagent concentrations should be optimized for the components/functional groups of interest. For many compounds, derivatization reactions are complete upon mixing or injection onto the hot GC injection port when excess derivatization reagent is employed.

- Select a different silylation reagent. Selecting a weaker silyl donor [30] will often minimize artifacts formed from over-silylation.

- By-products from silylation reactions form artifacts by adding to analytes or by reacting with themselves or with solvents. Thus selecting another derivatization reagent can avoid or minimize many of these types of artifacts.

- Select another solvent for the derivatization. Polar solvents increase the rate of silylation, but we have often noted artifacts from their reactions with analytes and the silylation reagents.

- Perform silylations under nitrogen or argon to avoid artifacts formed by atmospheric oxygen.

- Derivatization methods developed for pure standards often yield different products than those noted for crude samples containing additional solvents, inorganic acids, inorganic salts, etc. Therefore it is best to develop derivatization procedures for compounds with matrices identical or similar to the targeted process samples.

- There is a multitude [2-5] of other derivatization reactions for organic compounds. Consider a totally different class of derivatization reagent.

- Use one class of reagent to derivatize one type of functional group in a sample and a different class of reagents to derivatize another type of functional group. For example, a combination of oxime reagents and silyl reagents is used to derivatize keto-acids.

- Switch to a GC column which doesn't require derivatization of the sample or analyze the sample by HPLC without derivatization.

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Fig. 1. Functional groups normally derivatized by silylation reagents.
Fig. 2. Electron impact mass spectrum of 4, an aldehyde artifact formed with BSA.
Fig. 3. Electron impact mass spectrum of 6 formed from silylation reagent and gem-diol form of aldehyde.
Fig. 4. GC/MS total ion chromatogram of trimethylsilyl derivatized Formalin solution showing retention times of BSTFA artifacts.
Fig. 5. Electron impact mass spectra of two artifacts, 8 (n=1,3), in Formalin solution derivative.
Fig. 6. Electron impact mass spectra of isomeric silylation artifacts 9 (top) and 10 (bottom) showing difference in intensity of m/z 147.
Fig. 7. Electron impact mass spectrum of acid silylation artifact, 13.
Fig. 8. Electron impact mass spectrum of 15 showing ions formed from intermolecular transfer of proton and trimethylsilyl groups.
Fig. 9. Trimethylsilyl derivatives of inorganic compounds.
Figure 10: Electron impact mass spectrum of artifact, 19, commonly noted in reaction mixtures of DMF and BSTFA.
Fig. 11. GC/MS total ion chromatogram of silyl derivative of hindered phenol showing retention time and relative concentration of BSTFA-DMF artifact, 19.
Fig. 12. Electron impact mass spectrum of silylation artifact, 21, formed from acetone as solvent.
Figure 13: Electron Impact Mass Spectrum of Ethylene Glycol (Top) and Ethylene Glycol-Formaldehyde Artifact (Bottom).
Figure 14: Electron Impact Mass Spectrum of Glycerin (Top) and Glycerin-Formaldehyde Artifact (Bottom).
Figure 15: Electron impact mass spectrum of carbamate artifact noted in silylation of sample in DMF.
Figure 16: GC-MS Results for a Tetra-Alkylammonium Salt Derivatized with BSTFA
Figure 17: Electron Impact Mass Spectra of Acetone or Acetone Alcohol Artifacts, 21b and 21c
Figure 18: Artifacts from Saran Type Nerve Agents

**NAME:** O-isopropyl-O’-(2-amino)ethyl methylphosphonate  
**CAS:** 14646-03-8  
**ABBR:** GB-MEA  
**MW:** 181.09  
**MOLECULAR FORMULA:** C6H16NO3P  
**CONFIRMATION STATUS:** Y

**NAME:** O-Isopropyl-O’-2-(trifluoroacetamido)ethyl methylphosphonate  
**CAS:** TBD  
**ABBR:** GB-TFA  
**MW:** 277  
**MOLECULAR FORMULA:** C8H15F3NO4P  
**CONFIRMATION STATUS:** N  
**REMARKS:** Derivatization artifact
Figure 19: Comparison of Electron Impact Mass Spectra of Artifacts formed from BSA and Dimethyl Sulfoxide (Top) and BSTFA and Dimethyl Sulfoxide (bottom).