Why We Derivatize:

1. To reduce the polarity and enhance the volatility of high molecular weight polar drugs making them more suitable for analysis via GC-MS (Figure 1).
2. To increase the molecular weight of very volatile drugs, thereby resulting in a more complex mass spectrum that improves the selectivity for that particular drug. An important consideration in derivatizing drugs for GC/MS analysis is that the spectrum of the resulting compounds should contain at least three ions that are unique to that analyte and not a result of the matrix.

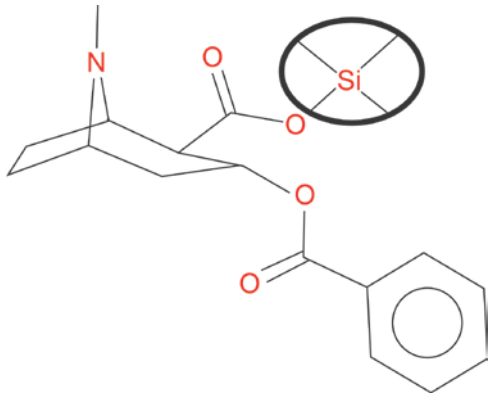

Figure 1. Trimethylsilyl derivative of benzoylecgonine. The underivatized compound has a carboxyl group and is too polar to pass through a GC column.

How to choose a derivatizing agent

Common derivatizing agents for drugs are trifluoroacetic anhydride (TFAA), pentafluoroacetic anhydride (PFAA), and N,O-bis(trimethylsilyl)- trifluoroacetamide (BSTFA, MTBSTFA). TFAA and PFAA react with alcohols, phenols and amines to form floroacyl esters and amides. The main disadvantage of the acid anhydrides is that they are extremely corrosive and can damage the capillary column of the instrument. Excess derivatization reagent must be removed by evaporation prior to injection and the derivatized analytes must be dissolved in a volatile solvent.

For most drugs, conversion to the trimethylsilyl (TMS) ethers, esters and amides, using BSTFA (or MTBSTFA) is found to be very simple and effective. When preparing these derivatives, it is necessary to evaporate the drug extracts to complete dryness prior to derivatization. There is no need to remove any excess BSTFA (MTBSTFA) prior to injection like what is required with agents such as HFAA and PFAA because it does not pose a risk to instrumentation. Another advantage of BSTFA (or MTBSTFA) is that it boils at 145°C at atmospheric pressure; therefore the solvent is not likely to evaporate when stored, increasing its shelf-life.

Derivatizing agents are usually stored at room temperature or in a dessicator. Refrigeration should be avoided due to humid conditions shortening the life and effectiveness of the product.

Common compounds found in a forensic/clinical setting along with their targeted functional groups and derivatizatizing agent of choice for are listed in Table1.

Drug	Derivatized Functional Group	Derivative (BSTFA unless other specified)
amphetamine	-N_2H	Spectrum of BSTFA derivative not definitive; therefore prepare the 4-carbethoxyhexafluorobutyryl amide (4-CB)
methamphetamine	-NH_2	Spectrum of BSTFA derivative not definitive; therefore prepare the 4-carbethoxyhexafluorobutyryl amide (4-CB)
phentermine	-NH_2	Spectrum of BSTFA derivative not definitive; therefore prepare the 4-carbethoxyhexafluorobutyryl amide (4-CB)
cocaine	none	
benzoylecgonine	-CO_2H	TMS ester
morphine	-OH (two)	di-TMS ether
codeine	-OH	mono-TMS ether
6-monoacetylmorphine	-OH	mono-TMS ether
dihydrocodeine	-OH	mono-TMS ether
hydrocodone	enol –OH formed from =O	mono-TMS ether di-TMS ether
oxycodone	-OH enol –OH formed from =O	TMS ether di-TMS ether
norcodeine	-OH -NH_2	One peak: TMS ether and TMS amide
hydromorphone	-OH enol –OH formed from =O	mono-TMS ether di-TMS ether
oxymorphone	-OH (two) enol –OH formed from =O	mono-TMS ether di-TMS ether tri-TMS ether
phencylidine (PCP)	none	
9-carboxy-11-nor-Δ^9-tetrahydrocannabinol	-OH -CO_2H	One peak: mono-TMS ether and mono-TMS ester

Classification of Derivatizing Agents

Silylation

Silylation is the most popular derivatization procedure for GC sample analysis. Silylation reagents are easy to use and readily form derivatives. In silylation, an active hydrogen such as that found in acids, alcohols, thiols, amines, amides, enolizable ketones and aldehydes is replaced by trimethylsilyl (TMS) or t-butyldimethylsilyl (t-BDMS). Compared to their parent compounds, silyl derivatives are more volatile, less polar, and more thermally stable. As a result, GC separation is improved and detection is enhanced.

Acylation

Acylation reagents are typically available as acid anhydrides, acyl derivatives, or acyl halides. Acylation reagents offer similar advantages to silylation reagents. They create less polar, more volatile derivatives. As opposed to silylating reagents, acylating reagents target highly polar, multi-functional compounds, such as carbohydrates and amino acids. Acylating reagents also introduce electron-capturing groups to the derivatized sample; enhancing analytical detection. Acyl halides and acyl derivatives are highly reactive. Typically they are used where steric hindrance may be an issue. Due to the acidic nature of these reagents any excess material or byproducts must be removed prior to sample analysis to prevent GC inlet or column degradation.

Alkylation

Alkylation reagents replace active hydrogens with an alkyl group. These reagents are used to modify compounds having acidic hydrogens, such as carboxylic acids and phenols. Alkylation reagents can be used alone to form esters, ethers, and amides or they can be used in combination with acylation or silylation reagents. Esterification is the most popular method of alkylation. Alkyl esters are stable and form quickly and quantitatively. Alteration of the length of the substituted alkyl group can be used to alter the GC retention times of derivatives.