TMS Derivitization for GC-MS

Taken from: https://www.agilent.com/cs/library/usermanuals/Public/G1676-90001_Fiehn.pdf.

Key points to consider:

- Thoroughly dry metabolite samples before derivation. Reagents are not compatible with water or protic solvents.
- Use enough reagent to completely derivatize all metabolites.
- Derivatized samples have a 24- hour shelf life. Before doing a large study, conduct a test to determine the maximum sample size that is completely derivatized with the recommended protocol.

This protocol is a general protocol for mixed compound derivatization which includes methoximation of keto groups to prevent enolization or chemical loss and trimethylsilation of labile hydrogens on polar compounds.

Internal Standard

An internal standard can be used to monitor changes in retention time or signal amplitude. An aliquot of an internal standard (example: 5 µL of myristic acid d27) stock solution is added to the biological extracts. The sample is then evaporated to dryness.

Methoxyamination

Add 10 µL of a 40 mg/mL solution of methoxyamine hydrochloride (Product # 67546; Sigma- Aldrich; St Louis, MO) in pyridine (Product # TS- 27530; Thermo; Rockford, IL). This mixture is gently shaken at 30 °C for 90 minutes.

Trimethylsilylation

Add 90 µL of N- Methyl- N- trimethylsilyltrifluoroacetamide with 1% Trimethylchlorosilane (MSTFA +1% TMCS) (Product # 48915; Thermo; Rockford, IL) to the methoxyaminated samples. The mixture is incubated at 37 °C for 30 minutes. The derivatized samples are cooled to room temperature before being transferred into GC vials.