Preparing an internal standard for rose volatile extractions. To determine relative amounts of chemicals with the coupled Gas Chromatograph / Spectrometer (GC/MS), we need a chemical with a known concentration which is precisely measured and added to all samples. This helps us check the concentration of an extracted chemical to the concentration of our standard and allows us to determine if our extraction has become evaporated. We are using Nonyl Acetate as our standard, because it is not is not present naturally in our samples, and Nonyl Acetate leaves the GC/MS column in near the middle of the other compounds we are interested in, which makes it a useful marker for GC/MS analysis.

Nonyl Acetate and Dichloromethane both evaporate easily and can become contaminated if we constantly are opening and closing their containers. Because of this, we first we prepare a stock solution which we will further dilute to create a working solution. This way, when we run out of working solution, or our working solution becomes contaminated, we can quickly prepare another solution without opening the larger solvent containers. This helps minimize the risk of contamination of the more expensive chemicals. We add 5 µl of working solution to individual volatile extractions immediately after the extraction is finished, and volatile extractions should be stored in the freezer to avoid evaporation.

**Important**: it is crucial to have clean glassware when working with volatile extractions, as the GC/MS will detect contaminants down to the molecular level. We never use any plastic labware; the solvents can dissolve some plastics and will contaminate our samples. In order to ensure that our glassware is clean before use, we wash our glassware multiple ways. First, with rinse out our glassware three times with water, then with Sparkleen and water, and finally with acetone only. We then put our clean glassware into the oven at 50 °C to dry. We only remove the glassware from the oven right before we use it. This procedure should be followed for any glass and containers which will contact our solvents.

**Stock Solution Preparation:**

Take a previously cleaned volumetric flask of 10 ml from the oven. Fill half of the volumetric flask with dichloromethane. Then add **11.56 µL** of **Nonyl Acetate**, and then more solvent up to the 10 ml line with a Pasteur pipette.

**Final Solution Preparation:** Use stock solution to obtain a **0.2 µg /µL** (1:4 ratio)

100 µL of stock solution

400 µL of dichloromethane

**Usage:**

Add **5 µl** of working solution to each volatile extraction before storage.