

When you see something like “10 mM ammonium acetate in 90:10 acetonitrile : **buffer**” in a method, you may be wondering how exactly this mobile phase solution is prepared. For example, is it 10 mM with respect to the total solution (*i.e.* with acetonitrile added) or just with the aqueous component? Do you just mix 900 mL acetonitrile and 100 mL **buffer**? These ambiguities can lead to inconsistencies in the way the mobile phase is prepared and hence in the resulting data.

**To avoid these issues, please follow these step-by-step instructions:**

1. **Buffer Stock Solution** (100 mM Ammonium acetate aqueous solution): Weigh 3.854 g ammonium acetate and quantitatively transfer to a 500 mL volumetric flask. Dilute to mark with DI water. This makes a 100 mM solution.

2. **Buffer Solution** (10 mM Ammonium acetate aqueous solution): Pipet 100 mL Stock Solution into a 1000 mL volumetric flask and dilute to mark with DI water. This makes a 10 mM solution.

3. **90/10 Mixture**: Pipet 100 mL **Buffer** Solution into a 1000 mL volumetric flask and dilute to mark with acetonitrile. This makes your mobile phase solution “10 mM ammonium acetate in 90:10 acetonitrile : **buffer**”

*These instructions help to ensure you get reproducible results with respect to the mobile phase.*