How to prepare 10mM ammonium acetate buffer 90:10 in acetonitrile for HPLC and LCMS - Tips & Suggestions

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.

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