



Prepare a 16mM Ammonium Acetate Buffer 90:10 in Acetonitrile for HPLC - Tips & Suggestions

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Step-by-Step Instructions to Prepare 16 mM Ammonium Acetate in 90:10 Acetonitrile:Aqueous Phase

This procedure produces a mobile phase containing 16 mM ammonium acetate in a solvent mixture of approximately 90% acetonitrile and 10% aqueous phase (made from buffer stock and DI water).

1. Prepare the Buffer Stock (500 mM Ammonium Acetate)

1. Weigh **3.85 g** of ammonium acetate.
2. Transfer it into a **100 mL volumetric flask**.
3. Add **DI water** up to the 100 mL mark.
4. Mix until the salt is fully dissolved.
5. Filter the solution through a **0.45 µm nylon filter**.
6. Store the buffer stock at **2–8°C**.

Result: This is a **500 mM ammonium acetate stock solution**.

2. Prepare the Final 90:10 Mobile Phase (16 mM Ammonium Acetate)

1. Add **32 mL** of the **500 mM buffer stock** into a **1000 mL (1 L) volumetric flask**.
2. Add **68 mL DI water** to the same flask.
3. Fill the flask to the final 1000 mL mark with **acetonitrile**.
4. Mix thoroughly.
5. (Recommended) Filter the final mixture through a **0.45 µm nylon membrane** before use.

3. Final Composition

- The total buffer added contributes to a final concentration of:
 $(32 \text{ mL} \div 1000 \text{ mL}) \times 500 \text{ mM} = 16 \text{ mM ammonium acetate}$
- The solvent composition is:
~90% acetonitrile + ~10% aqueous phase (buffer + water)

These instructions help to ensure you get reproducible results with respect to the mobile phase used in our Application Notes.

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