SIELC

General Instructions

General Instructions for Mobile Phase Preparation

Summary

When preparing mobile phases with a specified solvent ratio and buffer concentration, follow these steps to ensure accurate and reproducible results.

What Does This Mean?

- **Mobile Phase:** MeCN/H_2O – 60/40%

Means the mobile phase contains **60% acetonitrile** and **40% water** by volume.

- **Buffer: AmFm pH 3 - 20 mM**

Means the **final concentration** of the buffer in the entire mobile phase is 20 mM (w/v or v/v depending on buffer type).

Important: The buffer concentration refers to the whole mobile phase volume, not just the aqueous portion.

How to Prepare

When your method says: Mobile Phase: MeCN/H_2O – 60/40%
Buffer: 20 mM

This means the final mobile phase should contain 60% acetonitrile and 40% aqueous phase, with 20 mM buffer in the total solution.

1. **Prepare a concentrated stock buffer solution** in water.

For example, if the final desired buffer concentration is 20 mM, prepare a higher concentration stock (e.g., 200 mM).

2. **Calculate the volume of stock solution needed** to achieve the desired final buffer concentration in the total volume.

$$C_1 \times V_1 = C_2 \times V_2$$

Example: To get 20 mM in 1 L from 200 mM stock → use 100 mL stock.

3. **Prepare the aqueous phase by mixing the calculated volume of buffer stock with pure water.**

If the aqueous portion is 40% (400 mL total):
→ Mix 100 mL stock + 300 mL water.

4. **Combine the aqueous phase with the organic solvent (e.g., acetonitrile) to achieve the target solvent ratio** (e.g., 60% MeCN, 40% aqueous buffer).

Example: Add 600 mL MeCN to 400 mL aqueous phase for 60/40 MeCN/H_2O.

5. **Mix thoroughly**, then filter and degas before use.

NOTE:

This preparation provides a small deviation of actual reported volume of water because the buffer itself takes some volume (or weight) of the aqueous part of the MP. In above example 20 mM buffer takes about 2 mL (or g) of the aqueous part of the mobile phase. So the actual water/MeCN % ratio is actually 39.8/60.0. However when concentration of the buffer is small (100 mM or less) this deviation can be ignored. And if the preparation is done the same way consistently the result of separation is reproducible.