

The mobile phase used in HPLC has a profound effect on the separation of the sample by directly affecting the retention and differential migration of the components in the sample. For this reason, it is critical that mobile phase preparation be done correctly and as consistently as possible. What follows are some tips and tricks for mobile phase preparation that will ensure the highest possible accuracy of the separation and analysis.

Use a Simple Mobile Phase

Using a simple mobile phase will lead to a method that is more robust and easier to transfer. These methods benefit organizations since troubleshooting and downtime are minimized when robust methods are implemented.

Use appropriate grade solvents and reagents

It is critical that the components that make up the mobile phase be of the highest purity. When it comes to the solvent, the correct grade should be used based on application. When it comes to the reagents, they must have high purity and not be past their expiry dates.

Base Mobile Phase Preparation on Weight

While it is possible to base mobile phase preparation on volume ratios, volume can change

with variations in temperature, which can affect the accuracy and reproducibility of the separation. For this reason, it is better to base the mobile phase preparation on weight ratios, which will remain consistent even when environmental conditions change.

Mix Mobile Phase Components Separately

If basing mobile phase preparation on volume, it is important to measure the components of the mobile phase separately, then combine them. If the water is measured first, and the organic solvent added to the water, contraction of the solvent mixture will increase its concentration. If the water is added to the solvent mixture, the contraction of the solvent mixture will decrease its concentration.

Use a Pre-Mixed Mobile Phase

The alternative to mixing the mobile phase is to use a premixed one. This is particularly useful in reversed-phase chromatography because it will help avoid temperature changes during mixing, improve the mixing efficiency, and allow the mixture to more effectively degas.

Correcting the Temperature of the Solvent

When relying on volume, the solvent must be brought to room temperature to ensure a consistent volume and the reproducibility of the analysis. This should be done by putting the solvent in either a hot-water or cold-water bath, whichever is needed to appropriately adjust the solvent temperature.

Choose the Correct Filter

When it comes to filtering the mobile phase during preparation, it is important to choose the right type of filter. A membrane-style filter with pore size of 0.45 μm or smaller is recommended for HPLC systems. For UPLC systems, the pore size should be 0.22 μm or lower. This provides the most effective removal of particles from the mobile phase, preventing the system from becoming clogged.

Ultimately, you want the mobile phase preparation to be not only correct, but also consistent. This will ensure the reproducibility of the analyses and protect the integrity of the separations you run.

For more information about mobile phase preparation or if you need technical assistance with your work, Phenomenex offers a free, 24/7, online Technical Support service – **Chat Now**.

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