

The mobile phase used for reversed phase LC can be a pure solvent, a combination of a mixed solvent, or a solvent that contains buffers and additives. The mobile phase solvent acts as a carrier for the analyte through the stationary phase without affecting the results. There are literally hundreds of solvents to choose from, which can make it extremely challenging to decide which one is right for your needs.

Mobile Phase Solvent Characteristics

Fortunately, there are a few characteristics of mobile phase solvents that can help you choose the right one for your reversed phase LC analysis. These characteristics include:

- **Solvent Purity** - The solvent selected as a mobile phase in LC should be HPLC or MS grade, should be filtered and sonicated to ensure minimal absorbance in UV spectra and free of air bubbles.
- **Solvent Viscosity** - The reversed phase mobile phase should have an optimal viscosity, to help ensure an optimal linear velocity through the HPLC column stationary phase which in turn provides narrower and sharper peaks improving the overall sensitivity of the analysis.
- **Refractive Index** - This is a key consideration when using a refractive index detector. Ideally when working with RI detector the mobile phase and sample diluent should have similar refractive index to avoid getting negative peaks, in other words, sample should be prepared in mobile phase.
- **Solvent Boiling Point** - It is best to choose a solvent that has a low boiling

Chromatography Spotlight: Select the Right Mobile Phase Solvent for Reversed Phase LC

point for increased recovery and specially in LC-MS, a more volatile solvent will improve the ionization leading to increase in MS sensitivity.

- **Non-Corrosiveness** - The mobile phase solvent must not corrode the HPLC tubing, pump valves, seals and other parts which falls in the flow path.
- **Solvent Toxicity** - Ensure the solvent is not toxic and that you use the stabilized version.
- **Solvent Miscibility** - Choose a solvent which is perfectly miscible with the sample. The analyte also must be soluble in it. Otherwise, you will experience sample precipitation at the column inlet frits creating high backpressure, baseline issue, less sensitive peaks for your analyte and peak area and peak shape issues in the chromatogram.
- **Mobile Phase Transparency** - Select a mobile phase that transmits at the detection wavelength.
- **Elution Strength** - This is the ability of the solvent to elute target analytes from the stationary phase. The higher the elution strength, the more effective the solvent and the better the selectivity. The mobile phase solvent should have optimal and adequate elution strength to achieve selectivity for the target analytes.

With these characteristics in mind, the preferred mobile phase solvent for most chromatographers is acetonitrile because it has optimal viscosity, good UV transparency, and adequate elution strength.

Other common solvents include methanol, which has a higher viscosity than acetonitrile

(particularly when mixed with water), and tetrahydrofuran, which has a high elution strength, but is more toxic and presents safety issues. Methyl tert-butyl ether can act as a substitute for tetrahydrofuran.

Choosing Mobile Phase Buffers and Additives

Along with choosing your mobile phase solvents, you will also need to choose the appropriate buffers and/or other additives. Buffers are used to control the pH of the mobile phase. Acidic additives (pH of 2 to 4) are used for most pharmaceutical applications, whereas basic mobile phases (pH greater than 8) are used to achieve higher retention and improved peak shapes for basic analytes.

Simple Mobile Phases Are Best

It is becoming the norm to choose simpler mobile phases for reversed phase LC. The practicality behind this is evident, with the advancements in column stationary phase and column manufacturing technology and the availability of extremely sensitive, high-resolution instruments, reduces the need for buffers and other additives that were previously necessary to provide good chromatography.

For example, mixing acetonitrile with water at 95% has traditionally been done to improve the efficiency of the mixing by ensuring the two mobile phases are similar in terms of

viscosity and surface tension. However, this is becoming increasingly unnecessary with pump improvements and the introduction of online mixers.

In addition to this, methods are becoming more robust with the use of simpler solvents that have linear gradient profiles, and with the rapid advancement in high-resolution MS technology it further reduces the need for complex mobile phases in LC methods.

If you would like more information about choosing the right mobile phase, connect with one of our technical specialists through our free online portal — **Chat Now**

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