

High-pressure liquid chromatography (HPLC) is one of the most used techniques the pharmaceutical and other chemical industries to separate, qualitate and quantitate the components of a mixture. The mixture is carried through the stationary phase in a column by a solvent, known as the mobile phase, under high pressure.

When it comes to choosing the mobile phase for use in HPLC, the solvent selected will depend on the type of interactions needed between the analyte and the stationary phase. While there are many different modes of chromatography, this post will concentrate on normal-phase and reversed-phase chromatography. Mobile phases are typically made up of two different components: a weak component that promotes retention of the analytes on the stationary phase, and a strong component what promotes elution of the analyte from the column.

### **Mobile phase for normal-phase chromatography**

The weak portion of the mobile phase for normal-phase HPLC must be a non-polar (lipophilic) solvent. This is because separation in normal-phase HPLC relies on the attraction of the analyte to the polar stationary phase. The non-polar will not attract the molecules of the analyte, thus boosting the retention time of the analyte in the stationary phase. When the analytes are needed to elute from the column, a polar organic solvent is then used as the strong portion of the mobile phase.

The most commonly used normal-phase mobile phase solvents used are:

- Hexane
- Heptane
- Chloroform
- Benzene
- Ethyl Acetate Dichloromethane
- Ethanol
- Isopropanol



### **Mobile phase for reversed-phase chromatography**

When it comes to the mobile phase used for reversed-phase HPLC (the most commonly used mode of HPLC), the weak solvent must be polar in nature so it can help repel or push the analyte toward the non-polar, hydrophobic stationary phase as it passes through the column. Again, the result is increased retention time of the analyte.

The most commonly used mobile phase for reversed-phase HPLC is water. The water is mixed with an organic modifier in a ratio of 0% to 100%. The modifier can be acetonitrile, methanol, or another polar organic solvent. Increasing the organic modifier, or strong solvent, will help elute the analytes from the column.

### **Mixing of solvents in the mobile phase**

Since it is rare to use a single solvent in an HPLC mobile phase, it is important to take the following into consideration when mixing the solvents:

- **Miscibility** – Solvents being mixed together should be miscible with each other, meaning one needs to be able to dissolve with the other. Using immiscible solvents together can cause permanent damage to columns.
- **Temperature** – Solvents should be the same temperature before they are mixed. Letting them sit for a while in the lab will ensure this.
- **Purity** – Solvents must be of HPLC grade or higher to ensure analysis will have low background and to minimize long term damage to the system. This includes the water used, which should be put through reverse-osmosis.
- **UV cut-off wavelength** – Is the lowest wavelength that the solvent can be detected at. The solvents must be chosen such that they have a UV cut-off wavelength which is lower than the analytes in the analysis.
- **Refractive index** – The solvents, when mixed, should have a refractive index that is significantly different than that of the sample.
- **Viscosity** – The solvents should have a low viscosity to ensure it can easily flow through the stationary phase for effective mass transfer. Solvents with higher viscosities will cause higher backpressures in the system which could damage components and ruin columns.

- **Compressibility** – The compressibility of the solvent can affect the flow rate of the mobile phase. Flow rates are kept consistent by the pumping system, but the flow rates of the solvents used should be consistent with each other whenever possible.

Reach out to our team if you have questions about commonly used HPLC mobile phase solvents or if you need technical assistance with your work. Phenomenex offers a free, 24/7, online Technical Support service – [Chat Now](#).

Share with friends and coworkers:

- [Click to share on LinkedIn \(Opens in new window\)](#)
- [Click to share on Facebook \(Opens in new window\)](#)
- [Click to share on Twitter \(Opens in new window\)](#)
- [Click to share on WhatsApp \(Opens in new window\)](#)
- [Click to email a link to a friend \(Opens in new window\)](#)