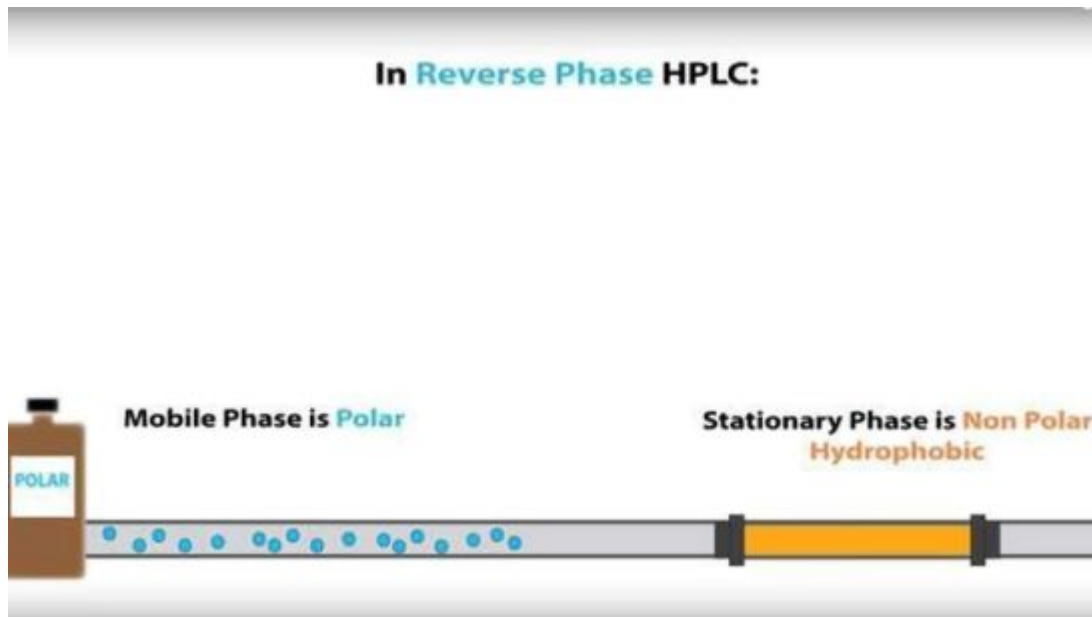


Normal phase [HPLC](#) and [reverse phase HPLC](#) are both types of high-performance liquid chromatography that are used to separate, quantify, identify, and analyze the components of a chemical or biological sample. However, these methods are quite different from each other, and reverse phase HPLC is the better choice in most circumstances.

Reverse Phase vs. Normal Phase HPLC

In normal phase HPLC, the separation of a sample is accomplished by using hydrophilic (easily dissolves in or is absorbed by water) groups in the stationary phase, leaving the polar components to be separated out last. In this case, the stationary phase is polar, and the mobile phase is nonpolar.

In reverse phase HPLC, this process is reversed so that the use of lipophilic (can dissolve in lipids, fats, oils, and non-polar solvents) groups in the stationary phase results in the removal of the polar components first. In this case, the stationary phase is nonpolar, and the mobile phase is polar.



Advantages of Reverse Phase HPLC

The way the reverse phase HPLC process works, water or a water-based solvent can be used in the mobile phase. This comes with several advantages over normal phase HPLC, which include the following:

- Lower costs when compared with other HPLC methods
- Lower toxicity of the solvents, resulting in less harm to the environment
- The need for a smaller sample size while obtaining accurate results

- Reduced solvent evaporation
- The ability to selectively adjust pH to improve sample separation
- The retention of the majority of organic molecules by the stationary phase
- The elimination of skewed analyte retention times
- Increased accuracy of gradient separations

The above benefits speak for themselves, but there is another significant advantage to using reverse phase HPLC. This is the ability to analyze samples containing polar (hydrophilic), non-polar (hydrophobic), ionic, and ionizable compounds, due to the use of a hydrophobic stationary phase.

These are compounds that are similar in many respects, which can make them difficult to separate using other methods. The ability to accurately analyze these substances significantly increases the flexibility of the reverse phase HPLC method when it comes to the analysis of a wide range of sample types.

However, nothing comes without its disadvantages, and reverse phase HPLC is no different. Disadvantages include:

- The need for increased skill and technical expertise
- Difficulty analyzing amines and water-insoluble compounds
- The need to create pressure in the system
- The dissolution of silica at a pH greater than 7.5
- The inability to recover the eluted sample from the column
- The need to use other methods to identify the analytes

Despite these disadvantages, reverse phase HPLC is one of the most commonly used separation techniques. It is used more often than normal phase HPLC because it has such a broad applicability when analyzing samples and the results are easily reproduced.

Reach out to our team if you have questions about reverse phase HPLC 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 \(Opens in new window\)](#)