White Paper

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# Strategy for Preparative LC Purification


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## Introduction

Preparative liquid chromatography (LC) is a powerful technique for the isolation or purification of one or more target compounds from a mixture. It has become the main mode of purification in drug discovery and development labs for reaction cleanup, purification of natural products, and isolation of impurities, among other uses. In most cases, preparative LC begins with an analytical separation, confirming the presence of the target compound in the sample. Once a suitable separation has been developed, the method is optimized for purity, yield, and throughput before being scaled to preparative levels.

In this White Paper, Agilent presents an overview of the key steps involved in scaling from analytical to preparative LC. Applying the fundamental chromatographic and mathematical principles outlined in this paper can help ensure predictable and seamless transition to semiprep purification.

## Choosing the right separation mode and stationary phase

The first task facing the chromatographer is selecting the appropriate chromatographic, or separation mode. This is dependent on the chemical and physical nature of the of the solute of interest. Descriptions of some commonly used chromatographic modes can be found in Table 1.

## Optimizing the separation: mobile phase

The choice of the mobile phase can be determined by:

- Optimized selectivity for the separation
- Solvent purity (low levels of nonvolatile contaminants can increase baseline noise)
- Detection type (UV transparency, MS compatibility)
- Volatility (for easy removal from isolated fractions)
- Viscosity (high viscosities cause high backpressures)
- Dissolving power for the components (solubility of sample in mobile phase)
- Cost

**Table 1.** Common chromatographic modes.

| Reversed phase | Normal phase | Ion exchange | Size exclusion | Chirality |
|---|---|---|---|---|
| Most organic substances | Lipophilics, such as oils, fats, lipids | Ions, such as acids, bases, peptides, proteins, nucleic acids | Polymers, including proteins, nucleic acids | Enantiomers |
| Aqueous mixtures with methanol or acetonitrile and additives | Organic solvents | Aqueous buffers, ionic solutions | Aqueous buffers, organic solvents | Aqueous or organic solvents |

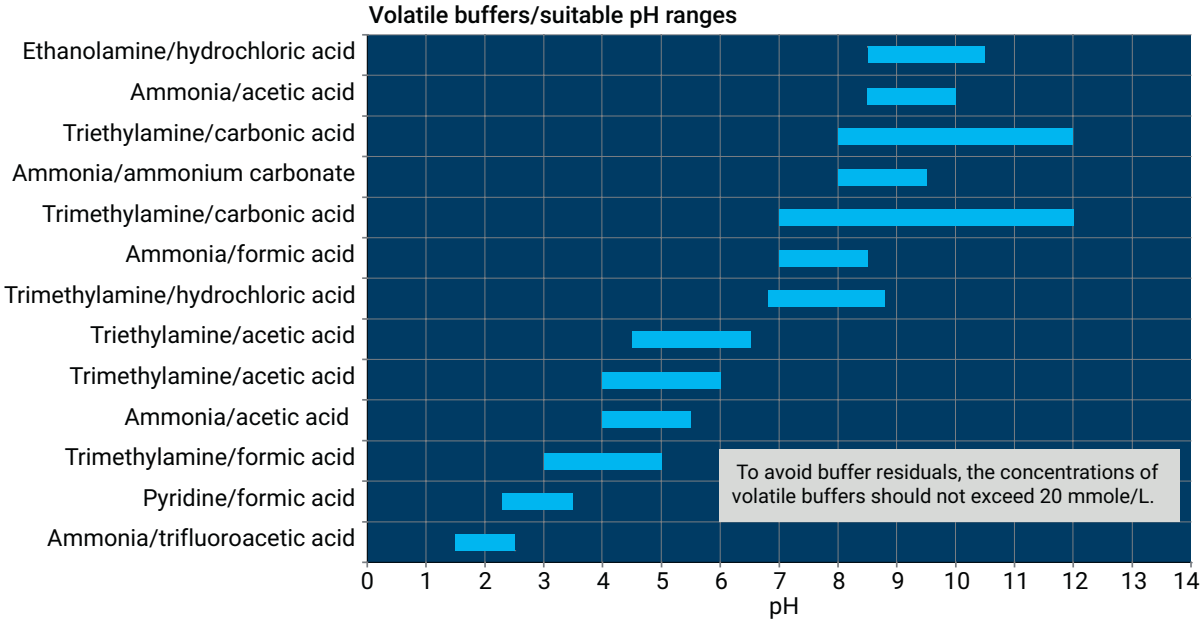


**Figure 1.** Common volatile buffers used in HPLC.

## Optimizing the throughput: sample amount and overloading type

In preparative LC, it is a common practice to overload the column to increase throughput. Overloading will cause the peaks to shift from a Gaussian to a more triangular shape. However, indiscriminate overloading can cause coelution with impurities or the analyte to precipitate out of solution. Loading studies can be performed to determine suitable sample amounts. Performing these studies on an analytical column can conserve valuable sample while minimizing solvent consumption and waste.

Column overloading can be done in two ways:

- **Concentration (mass) overloading:** To determine the maximum concentration that can be injected, several sample solutions of increasing concentration are injected onto the column, keeping the injection volume the same. The highest concentration that does not cause split peaks (a sign of sample precipitation) or coelution should be used.
- **Volume overloading:** If the sample has poor solubility, volume overloading is recommended. The same sample is injected at increasing volumes. The largest volume that does not cause coelution should be used.

A combination of the two methods is also possible. Concentration loading is usually preferred over volume loading from a practical point of view, since less solvent has to be removed from collected fractions, either by evaporation or lyophilization.

The results obtained from these analytical studies can be used to determine potential loading on a preparative scale.

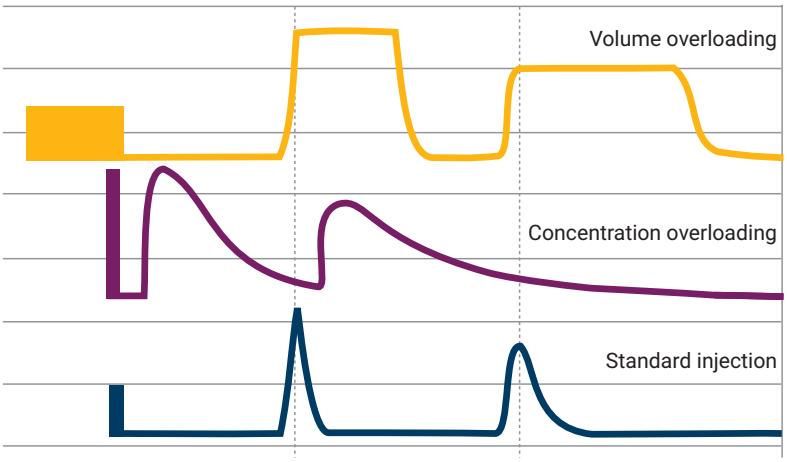


**Figure 2.** Peak shapes of volume and concentration overloading.

## Method scale-up

Three important parameters used to judge the results of a preparative run are purity, yield, and throughput. Since these parameters are interdependent, a preparative method cannot be simultaneously optimized with respect to all three parameters. The most important optimization parameter depends on the application.

In Figure 3, chromatogram 1 shows a preparative HPLC run capable of very high throughput, but the separation of the two compounds is poor. It might be possible to obtain some fractions with high purity for each compound but the recovery, that is, the yield is rather low. In chromatogram 2, the peaks are well separated; therefore, it is possible to get both compounds in high purity and yield, but the throughput is very low. Chromatogram 3 would be an optimized preparative HPLC run with a compromise to all three parameters.

Quick and easy linear scale-up from analytical to preparative scale depends on having both analytical and preparative columns packed with the same stationary phase and particle size. This could be done in three steps:

1. **Refine analytical conditions:** Determine the correct analytical scouting conditions, such as pH, mobile phase, and stationary phase, and optimize the separation for resolution.
2. **Maximize sample loading:** Determine the sample loading on the analytical column by increasing the amount injected until the limit of resolution is reached.

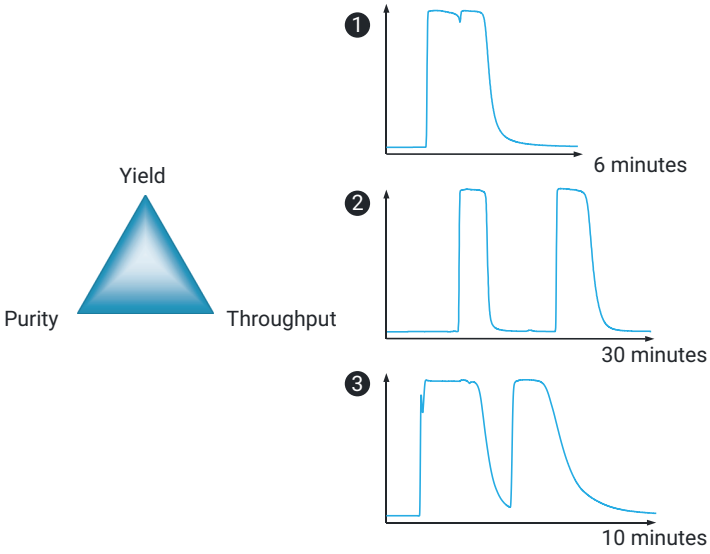


**Figure 3.** Objectives of preparative HPLC.

3. **Calculate and apply linear scale-up formulas:** Two parameters that must be scaled up when going from a smaller id column (analytical) to a larger id column (prep) are flow rate and injection volume. All other parameters such as particle size, stationary phase, and mobile phase must be held constant.

Linear scale-up factors for flow rate and injection volume can be determined using Equations 1 and 2.

Making your injection based on the correct application of these scale-up factors will help ensure a predictable, seamless transition to semiprep scale purification.

$$f_{p,P} = f_{a,A}\left(\frac{d_P}{d_A}\right)^2$$

Where:

$f_{a,A}$ = Flow rate of analytical column

$d_P$ = Internal diameter of preparative column

$d_A$ = Internal diameter of analytical column

**Equation 1.** Scaling to preparative flow rate.

$$V_{inj,P} = V_{inj,A}\left(\frac{d_P}{d_A}\right)^2$$

Where:

$V_{inj,A}$ = Injection volume on analytical column

$d_P$ = Internal diameter of preparative column

$d_A$ = Internal diameter of analytical column

**Equation 2.** Scaling to preparative injection volume.

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