

# Column Chromatography in Pharmaceutical Analysis

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## Commentary

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## DESCRIPTION

In chemistry, a single chemical compound can be isolated from a mixture using a technique called column chromatography. Due to the fact that different chemicals bind to the adsorbent differently and pass through the column at various speeds, chromatography can divide substances into fractions. This method can be employed with a wide variety of adsorbents (normal phase, reverse phase) and solvents, making it widely adaptable. The method is applicable at scales ranging from micrograms to kg. The fundamental benefit of column chromatography is the stationary phase, which is inexpensive and easily disposed of after usage. The latter stops recycling-induced stationary phase deterioration and cross-contamination.

### Stationary phase

Silica gel is the most used in stationary phase for column chromatography, followed by alumina. In the past, cellulose powder has frequently been employed. To perform ion exchange chromatography, Reversed-Phase chromatography (RP), affinity chromatography, or expanded bed adsorption, a variety of stationary phases are available (EBA). Although in EBA a fluidized bed is used, the stationary phases are often finely ground powders or gels and/or are micro porous for an enhanced surface. The dry weight of the analyte combination that can be put to the column and the stationary phase weight have an important ratio. The range of this ratio for silica column chromatography is 20:1 to 100:1.

### Mobile phase

A solvent or combination of solvents called the mobile phase or eluent is utilized to transport the chemicals across the column. To cut down on the time and eluent required to conduct the chromatography, it is chosen so that the retention factor value of the target compound is about between 0.2 and 0.3. Additionally, the eluent has been

selected to enable efficient separation of the various components. In small-scale preliminary experiments, the eluent is frequently adjusted using Thin-Layer Chromatography (TLC) with the same stationary phase.

### **Preparation of column**

A column is created by putting a solid adsorbent inside of a glass or plastic tube with a cylindrical shape. The amount of the substance that is being isolated will determine the size. To hold the solid phase in place, the base of the tube has a filter, a cotton or glass wool stopper, or glass frit. The top of the column may have a solvent reservoir attached to it. The dry method and the wet approach are typically employed to prepare columns. For the dry method, dry stationary phase powder is added to the column first. Next, mobile phase is added, which is then flushed through the column until it is entirely wet and is never allowed to run dry after this point. While moving through the column with the eluent at various speeds, the distinct components are held by the stationary phase differently and apart from one another. They elute one at a time at the column's end. The eluent is collected throughout the entire chromatography procedure in a number of fractions. By using fraction collectors, fractions can be automatically gathered. Running multiple columns at once will boost chromatography's productivity. Multi stream collectors are employed in this situation. Each fraction is examined for dissolved compounds using analytical chromatography, UV absorption spectra, or fluorescence, for example, in order to monitor the eluent flow's composition.

Column chromatography is a very labor-intensive step that takes a lot of time. Many manufacturers, including Biotage, Buchi, Interchim, and Teledyne Isco, have created automated flash chromatography systems that minimize human involvement in the purification process. These systems are commonly referred to as LPLC, or Low Pressure Liquid Chromatography, and operate at pressures between 350 and 525 kPa (50.8 and 76.1 psi). Automated systems will have parts such a gradient pump, sample injection ports, a UV detector, and a fraction collector to collect the eluent that are often found on more expensive High Performance Liquid Chromatography (HPLC) systems. These automated systems often have the ability to separate samples from a few milligrams to a large industrial scale of many kilograms, and they provide a far more affordable and expedient alternative to doing repeated injections on prep-HPLC.