

Preparative scale chiral chromatography

CHIRALS

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INTRODUCTION

The human body is a chiral world and contains thousands of chiral forms of biologically active molecules, which are responsible for controlling our health throughout our lives. The body is very sensitive to chirality, and two enantiomeric forms of a drug would encounter many different chiral molecules and would not necessarily interact with them in the same, or even a beneficial, way. Therefore, strict industry regulations governing chiral purity for active pharmaceutical ingredients (APIs) have been established. These regulations place high demands on the pharmaceutical industry, which has made – and will continue to make – considerable efforts to manage chiral purity.

For the production of enantiopure drugs (meaning drugs that contain only molecules having the same chiral sense), various methods can be applied. Chromatography, a fast and scalable method to access pure compounds, has become increasingly important in the development phase of new APIs. Because so many potential candidates are eliminated during the first clinical studies, it may not make sense to develop an optimized synthesis toward the enantiopure product at this early stage. Chromatography offers a powerful approach to chiral separations because the process yields both enantiomers, and yields are generally higher than those achieved in the chemical racemate separation by crystallization. In addition, chromatography does not require a functional group (acid or base) in the molecule, allowing salt formation.

CHIRAL SEPARATION TECHNIQUES

There are several different approaches to obtaining enantiopure material: chiral synthesis, chiral crystallization and chiral chromatography. Each approach has

advantages and disadvantages, depending on the amount of material, its physicochemical properties, processing costs and timelines involved.

A chiral synthesis offers the most elegant, yet complex solution. It also requires a chiral starting point; and racemisation has to be prevented. The chiral purity must be monitored throughout the whole synthesis. The advantages are apparent in the long term: the reaction can be scaled up to production size; and the unwanted enantiomer, which would otherwise make up half of the material, will not be wasted. On the other hand, it takes time to develop a chiral synthesis route. Depending on the necessary additional synthesis steps, this can be more expensive than the achiral route.

An achiral synthesis tends to be quicker and cheaper than a chiral synthesis; however, the resolution step can also be time-consuming and difficult. An achiral synthesis can be considered, followed by a chiral crystallization or chiral chromatography. The advantage of chiral crystallisation is that it may be repeated on a large scale fairly easily. On the other hand, this approach is not applicable in all cases. The crystallization process itself can also be lengthy. Depending on the molecule, the mother liquor needs to be enriched by the desired enantiomer (1) or seeded by enantiopure crystals of this target enantiomer. Often the yield is not as high as compared to a chromatographic resolution.

An achiral synthesis followed by chiral chromatography is a good alternative. Chromatography in general is a flexible and mild separation technique, and especially well-suited to sensitive products. Chiral stationary phases (CSPs) offer several advantages; the solutes are unmodified and the separations can be rapid. The development time is substantially shorter than for a chiral synthesis, but CSPs are expensive and the solvent consumption is rather high, depending on the possible sample loading. The separation system (eluent mixture and

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CSP) has to be specifically selected for the particular separation at hand.

Chromatographic Processes for Prep Scale Chiral Separations

For preparative scale separations, the available techniques are the batch elution chromatography by liquid chromatography (LC) and by supercritical fluid chromatography (SFC) as well as the continuous simulated moving bed (SMB) process, which is based on LC.

Batch Elution Chromatography

For the batch elution process (Figure 1), a single column packed with chiral stationary phase (CSP) is usually used. A defined volume of racemic feed solution is injected repeatedly. The two enantiomers separate (due to different affinity to the CSP) as they are washed through the column by the eluent. At the end of the column, the enantiomers are collected in different fractions. Injection and fraction collection can be automated. Chiral separations are classical binary separations, but the batch elution process is not limited to two fractions. This has an advantage in the removal of impurities.

For the batch elution process the solvent consumption is rather high compared to a process like the simulated moving bed. Another possibility using a single column would be the application of a steady-state recycling (SSR). This process is a cyclic, one-column process similar to SMB. However, SMB is continuous, whereas SSR remains a discontinuous, repetitive process.

Supercritical Fluid Chromatography (SFC)

Supercritical fluid chromatography can be described as an adaptation of liquid chromatography (LC), where the liquid eluent is replaced by a supercritical fluid. For every substance there is a temperature above which it can no longer exist as a liquid, no matter how much pressure is applied. Likewise, there is a pressure above which the substance can no longer exist as a gas no matter how high the temperature is raised. These points are called the supercritical temperature and supercritical pressure respectively and are the defining boundaries on a phase diagram for a pure substance. When the substance has properties intermediate between a liquid and a gas it is called a supercritical fluid.

Part of the theory of separation in SFC is based on the density of the supercritical fluid which corresponds to solvating power. As the pressure in the system is increased, the supercritical fluid density increases, and correspondingly its solvating power increases. Therefore, as the density of the supercritical fluid mobile phase is increased, components retained in the column can be made to elute. This is similar to temperature programming in gas chromatography (GC) or using a solvent

gradient in High Performance Liquid Chromatography (HPLC).

There are a number of possible fluids which may be used in SFC as the mobile phase. Based on its low cost, low interference with chromatographic detectors and good physical properties (non-toxic, non-flammable, low critical values), carbon dioxide is the standard. The main disadvantage of carbon dioxide (CO₂) is its inability to elute very polar or ionic compounds. This can be overcome by adding a small portion of a second fluid called a modifier fluid. This is generally an organic fluid which is completely miscible with carbon dioxide (e.g., alcohols, cyclic ethers), but can be almost any liquid including water. The addition of the modifier fluid improves the solvating ability of the supercritical fluid and sometimes enhances selectivity of the separation. It can also help improve separation efficiency by blocking some of the highly-active sites on the stationary phase. Modifier fluids are commonly used, especially in packed column SFC.

Supercritical fluid chromatography has several main

advantages over other conventional chromatographic techniques (e.g. HPLC). Compared with HPLC, SFC provides rapid separations with limited use of organic solvents. With the desire for environmentally conscious technology, the use of organic chemicals as used in HPLC could be reduced with the use of SFC. Because SFC generally uses carbon dioxide collected as a by-product of other chemical reactions or is collected directly from the atmosphere, it contributes no new chemicals to the environment. In addition, SFC separations can be done faster than HPLC separations because the diffusion of solutes in supercritical fluids is about ten times greater than that in liquids (and about three times less than in gases). This results in a decrease in resistance to mass transfer in the column and allows for fast, high-resolution separations.

Simulated Moving Bed

The simulated moving bed is a continuous chromatographic binary separation process. The technology itself has its origin in the petrochemical industry, where today several million tons of product are produced each year using mainly zeolites as stationary phases. It is also extensively used in the sugar industry for the production of several mono- and oligosaccharides (2). The SMB process has been adopted for fine chemical and pharmaceutical applications only in the past 15 years, when suitable chiral stationary phases became available on the market.

Within CarboGen and AMCIS, two SMB plants (LicoSep 10-50 from NOVASEP) are used for binary, mainly chiral separations on a scale of 100-4000 g racemate/day. Column

sets of eight columns with 4.8 cm I.D and 2.5 cm I.D. are available and are packed to a standard length of 10 cm. The inlet and outlet flow rates can be up to 250 ml/min, whereas the flow rate in the column loop can be up to 500 ml/min. The pressure in the plant depends on the chosen flow rates and the viscosity of the eluent mixture. The optimal operation pressure is below 50 bar.

The products to be separated are fed to the SMB plant and can be collected continuously. They do not have to be injected in discrete portions as is the case for the batch elution chromatography. The product, separated enantiomers, is collected continuously in two fractions called the extract and the raffinate. This process is more efficient than the discontinuous batch HPLC, where the feed-solution is injected in portions. The productivity (kg separated racemate per day and per kg CSP) is several

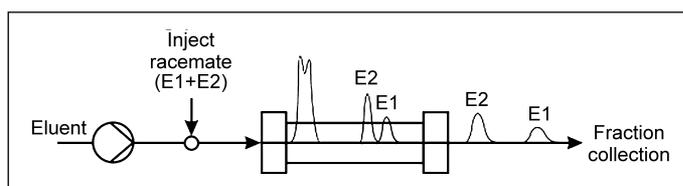


Figure 1 – Batch elution chromatography

times higher and the solvent consumption is substantially lower. This is due to the more efficient use of the CSP volume, since a higher loading of the CSP can be realized.

The principle of the SMB is based on a circular countercurrent flow of a liquid phase (eluent) and a solid phase (CSP) (Figure 2). The racemate to be separated is fed at a certain point to the unit. The two enantiomers have a different affinity to the applied solid phase (CSP). In the countercurrent movement, the less retained enantiomer is carried with the liquid to the raffinate outlet, where it can be collected. The more retained enantiomer is carried with the solid to the extract outlet. The velocities of the solid and the liquid phase are the process parameters that have to be adjusted for each separation task to make the countercurrent principle work.

Technically, it is problematic to physically move the solid without damaging the packing properties of the CSP. Therefore, a real countercurrent process, as described above, seems to be impossible to realize. Nevertheless, a chromatographic set-up that technically approximates the desired countercurrent effect can be implemented. It is called simulated moving bed because the movement of the solid bed (packed HPLC column bed) is not a physical but a simulated movement.

A SMB plant consists of usually six to eight packed HPLC columns (fixed bed), which connect to form a closed loop. The plant has two inlet (feed and fresh eluent) and two outlet ports (extract and raffinate). For example, Figure 2 shows a possible set-up for a SMB plant as it is used within

CarboGen and AMCIS, whereas other set-ups are also common in industry. The flow direction of the circular liquid flow is defined by a pump, which is integrated in the column loop. To simulate a circular solid movement in the opposite direction, all four inlet and outlet ports are periodically and simultaneously shifted by one column position in the direction of the fluid flow. By doing so, each time the ports are shifted one position further, the packing volume of one HPLC column is moved with respect to the inlet and outlet positions in the opposite direction. In this way, a true countercurrent is approximated.



Figure 2 – Simulated Moving Bed (SMB) set-up

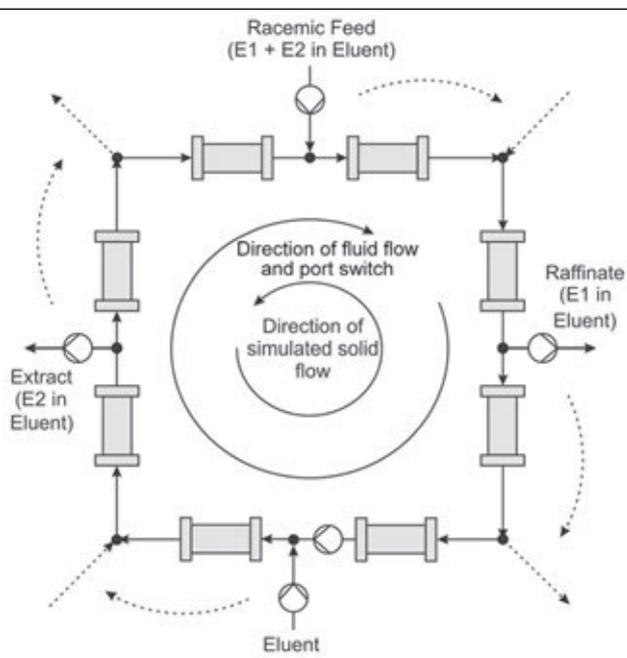
As shown in Figure 2, there are five pumps involved. Between each pair of columns, there is a block of four valves making it possible to connect every inlet and outlet stream to every column position. For an eight column SMB, this makes 32 valves. For a smooth operation, all valves and pumps have to function in a reliable and precise way. Troubleshooting is not an easy task and good knowledge about the plant is necessary.

The SMB process is a cyclic process. The inlet and outlet ports are switched from column to column. When all valve settings have reached their initial position again, a cycle has been completed. When the plant is switched on, the columns are filled with just the eluent mixture. Then, the loading starts at the feed inlet point. After that, an internal concentration profile starts to build up and a few port switches later; the first separated enantiomers can be collected at the outlets.

It takes about ten cycles for the plant to reach the steady state, where the quality of the collected product remains stable. The typical port switch time is between 0.5 to

2.0 minutes. A cycle consists of six to eight switches (depending on the number of columns). Ten cycles would last between 30 minutes and 2.7 hours. This is the start-up time of the particular SMB separation. It is possible that the operating parameters (pump settings) will have to be adjusted a few times, based on the measured purities at the outlets. Every time the setting is changed, the plant has to be started up and brought to steady state again, before a new measurement can be taken. This implies that it takes some time to start the unit and to reach the desired purity. Therefore, it doesn't make sense to use this technology when the amount of material to be separated is too small.

To develop an SMB separation, a screening of different CSPs and eluent mixtures has to be performed. Next, a combination providing a good separation at low retention times is chosen. For a normal SMB process, the material for the feed solution has to be dissolved in the eluent used for the separation. It is crucial that the material is reasonably soluble in the chosen eluent mixture. SMBs are run under extremely overloaded conditions, one reason for their efficiency. To design a separation, a series of overloaded injections has to be done to



HOW TO SELECT THE SEPARATION PROCESS

For each individual separation task, the most efficient separation technique should be chosen. This largely depends on the scale of the separation (Figure 3).

The advantage of the SMB is the separation speed, the high productivity, low need of CSP and low solvent consumption compared to preparative HPLC systems. The SMB process can be scaled up easily to big production size units. The disadvantages are the necessary start-up procedure, which make the process unsuitable for small scale separations. The standard SMB is limited to binary separations, so possible impurities might accumulate in one or the other enantiomer fraction.

Advantages of batch elution separations are that they are not limited to two fractions and present impurities can be cut out easily. The starting procedure is straightforward and fast compared to SMB plants. The disadvantages of prep HPLC is the low loading and high solvent consumption. Scale-up possibilities are limited.

Batch elution SFC technology is on the rise and the available plants on the market are getting better and bigger. It also has the advantage of not being limited to two fractions. The main eluent, CO₂, is sustainable and cheap and the product can be easily removed from the solvent. The productivity is high and the SFC technology is increasingly competitive with SMB.

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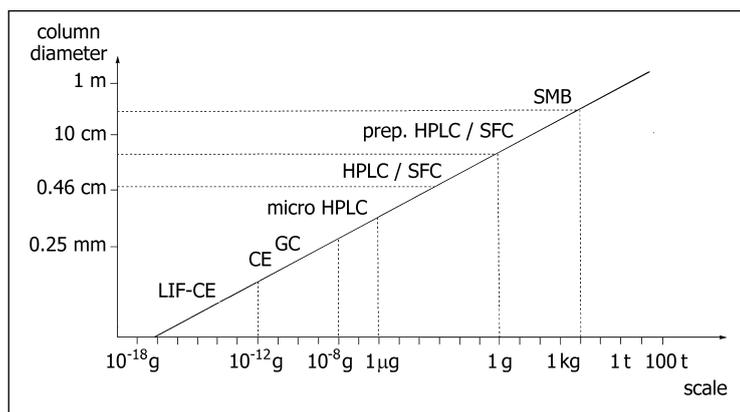


Figure 3 – Separation techniques and scales

investigate the retention behavior under these conditions. Separation design software is used to calculate the optimal plant parameters, which are the recommended feed concentration, switch time and pump settings.

A hand is shown holding a molecular model. The model consists of several spheres (black, white, and grey) connected by thin white rods, representing a chemical structure. The hand is positioned at the top right, with fingers gently grasping one of the spheres. The background is dark, making the hand and the model stand out.

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