Simulated Moving Bed Chromatography - Manufacture of Enantiopure Drugs

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Abstract

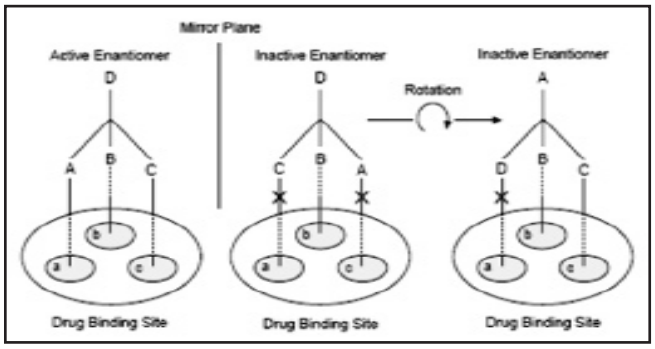
Evidence has been recorded that single enantiomers of chiral drugs are more effective than the racemic mixture in several cases. This has created a huge demand for enantiopure drugs in the market. SMB is a viable technology to produce these on a large scale economically.

1. Introduction

Need for Single Enantiomeric Molecules

The development of rational drug design now takes into consideration the interaction between a drug and the binding site as shown in the fig.1 below.[1]

Figure 1 : The Hypothetical Interaction Between the 2 Enantiomers of a chiral Drug and its Binding Site[2]



Ref: 1

Since these receptors and enzymes are chiral in nature, one stereoisomer often shows greater therapeutic activity than another of its enantiomer. Just one of the above enantiomer binds substantially to the receptor while the other will bind loosely and may or may not show pharmaceutical activity. There are several cases in which one of the enantiomers shows greater activity than the other.

For e.g., levorotary–isomer of all β-blockers is more potent in blocking β-adrenoceptors than their dextrorotary-isomer, such as S (-)-propranolol is 100 times more active than its R (+)-antipode.[2] Many other examples have been compiled in Table 1. below. [2]

Thus, a single pure enantiomer is often more effective therapeutically than the racemate mixture. Without the therapeutically inactive isomer, the drug will be more potent and in some cases will reduce the toxicity which is associated with the lesser active enantiomer.

E.g. Ketamine is an intravenous anesthetic. The (+)-isomer is more potent and less toxic than its (-) - antipode. [2]

With evidence being found that in many cases, a single isomer of a chiral drug shows greater activity; production of pure enantiomers has been one of the prime objectives for pharmaceutical companies. In 2000, exactly 10 years ago, the annual sales of the chiral drug industry touched $ 133 billion mark. About 40% of all dosage-form drug sales in 2000 were of single enantiomers.[3]

In the year 2004, among the 16 newly approved synthetic drugs, 13 were chirals and the rest, only three, were achiral. All the approved chiral synthetic drugs went to market as single enantiomers.[4]

Table 1 : Isomer potency of some representative drugs

Main pharmacological effect of the drug	Isomer potency
β-Adrenoceptor blocking drugs (β-blockers): propranolol, acebutolol, atenolol etc.	l > d (d = inactive) Ex: S(-)-Propranolol > R(+)-Propranolol
Anesthetics: Ketamine, Isoflurane	d > l (l = inactive) Ex: S(+)-Ketamine > R(-)-Ketamine
Analgesic, Anti-inflammatory: (NSAID): ibuprofen, ketoprofen, benoxaprofen, fenoprofen, etc.	d > l Ex: S(+)-ibuprofen > R(-)-ibuprofen
Calcium channel antagonists: verapamil, nicardipine, nimodipine etc.	l > d Ex: S(-)-Verapamil > R(+)-Verapamil

2. Importance of Resolution

Pure enantiomers can be prepared by asymmetric synthesis. Some of the ways in which stereoselectivity is induced in a reaction are
(1) Use of polarized light [5]: It is an impractical method.
(2) Chiral solvent
(3) Chiral additive
(4) Asymmetric catalysts

There are many inconveniences associated with asymmetric synthesis. The synthesis of one enantiomer of high optical purity (e.e) is much more difficult than the racemate. The process can be uneconomical due to the greater number of steps and the costly enantiomeric reagents needed.[6] Racemic purification is usually a simpler route. Among the methods used to achieve resolution, diastereomeric crystallization processes are usually the first choice. But they require an optically pure reactant, enrichment of the desired enantiomer in the mixture and often result in low yields. It involves three steps: making a salt, performing the separation and then recovering the product from the salt. Each step increases the cost of producing the final API. A major problem associated with this method is choosing the appropriate resolving agent, and the nature and composition of the solvent. Ampicillin, a β-lactam antibiotic is still manufactured in an enantiomeric pure form using diastereomeric crystallization using D-camphorsulphonic acid as a resolving agent.[7]
Chiral membranes are in the developmental stage at present and are not used for large scale production.

3. Resolution Techniques: Chiral Hplc

The difficulties inherent to asymmetric synthesis are conveniently eliminated by using methods of resolution of a racemic mixture into pure isomers. Racemates require cheaper reaction conditions for synthesis than pure enantiomers.

The separation of enantiomers by chromatography has been recognized as being a useful alternative to the more conventional approaches such as enantioselective synthesis and enzymatically catalyzed transformations. Chiral chromatography techniques employ chiral stationary phases (CSP). One enantiomer will have more affinity to the CSP than the other and they thus have different elution times. This makes it possible to separate them relatively easily. The successful application of chromatographic technique to chiral resolution would not have been possible without the design and preparation of efficient CSPs. Many CSPs have been developed, namely Pirkle-type CSP s, Hydrogen-bonding CSPs, cyclodextrin CSPs, protein-bound CSPs, cellulose-derived CSPs. The suitable CSP and a matching mobile phase are determined by trial and error.

Scale up of a chromatographic process is straightforward, making it attractive. The theoretical yield for these methods is 50% because both enantiomers are present. However, if racemization is possible the yield can be increased, thereby increasing the net yield.

HPLC can only be operated in a batch mode, which is a limitation in industry.

A large amount of mobile phase is utilized in eluting both the isomers from the column.

4. SMB: Simulated Moving Bed Chromatography

This technology was first developed by Sorbex ™. Although SMB units are relatively complicated, engineering issues at the scale of pharmaceutical production of enantiopure drugs are well understood.

The first commercially available plant (Licosep) SMB system was offered by Separex in 1991 and was exhibited for the first time in June 1991 during the Achema Exhibition.[8]

5. Principle of SMB

SMB technique uses a countercurrent contact between the mobile phase and the CSP. Countercurrent flow enhances the potential for the separation and, hence, makes the process more efficient. It also allows continuous mode of feed entry, which improves the throughput of the equipment compared to traditional batch chromatography. The principle is best understood by considering the concept of True Moving Bed (TMB) chromatography. TMB is not practical and is purely a theoretical development. (fig. 2) [9]

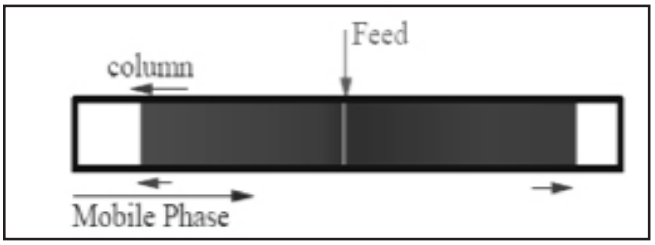


Figure 2 : Continuous separation of two enantiomers in a TMB
Ref : 9

6. TMB

A racemic mixture is introduced in the middle of a long chromatographic column containing a suitable CSP. The column is made to move in a direction opposite to the flow of the mobile phase, at a speed intermediate to the speed of the elution of the two components. The two components will then move in the opposite direction; the isomer with greater affinity moving along with the column whiles the other isomer moving with the mobile phase.

Feed added in a continuous manner will lead to continuous separation and pure components can be collected at the end of the columns.

Since the actual motion of the column or of the solid chiral phase is difficult in practice, it is simulated by breaking the column in discrete units. "Column switching" is performed by simultaneously switching all fluid streams one column forward in a synchronized manner at defined intervals, which has the effect of "moving" the solid phase in the opposite direction of the fluid flow. This sounds difficult, but is easily achieved using valves functioning on the basis of a logic circuit. The switching-times can be calculated based on a number of parameters. When steady state is reached, the system can be operated continuously. If all flow rates and the shift time are determined correctly, raffinate and extract fractions can be withdrawn in high purity, the feed is always added to regions where the two bands overlap, while the products are always withdrawn from regions where the two bands are separated. As such, a high purity (>99%) and high yield (>99%) can be achieved. [10]

The inlets and outlets are always placed in between successive columns. Based on their position, 4 zones can be defined among the columns as shown in fig.3 [8]

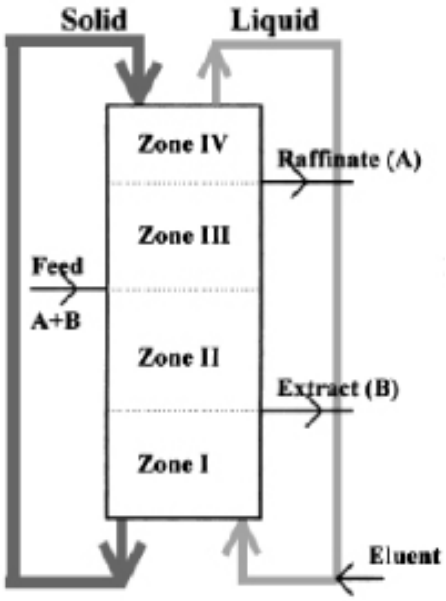


Figure 3 : Zones in SMB column
Ref : 8

Zone 1: It is between Eluent make-up and the extract outlet. Extract is the component which has more affinity for the solid phase.
Zone 2: This zone is between extract outlet and feed inlet.
Zone 3: It is between the feed inlet and raffinate outlet. Raffinate is the component which has lesser affinity for the solid phase.
Zone 4: This lies between the raffinate outlet and the eluent make-up inlet.

As the inlets and outlets are switched, the zones will also shift correspondingly. (fig. 4) [9]

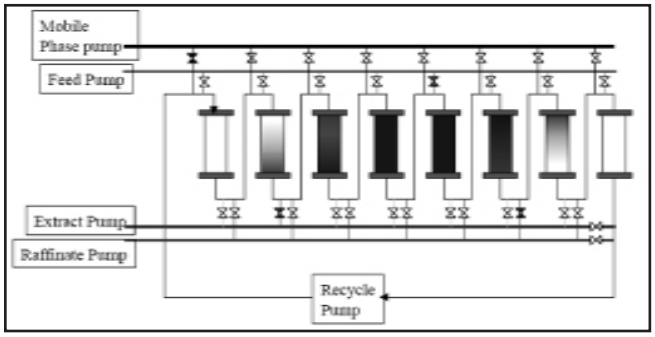


Figure 4 : Schematic diagram of SMB Chromatographic Separation of a binary mixture of stereoisomers. Ref : 8

7. Parameters Affecting Performance

One of the key issues while developing an SMB process is the determination of the zone flow rates and switching time. This determines the optimum position and yield of the desired products. Many mathematical models of SMB have been developed; based on

(1) The actual behavior of compounds in the countercurrent flow

(2) Comparison with TMB and with suitable modifications.

The steps while designing a SMB which would allow one to process a given amount of feed per unit time, based on TMB model are as follows :

Step A: Acquisition of Relevant Physico-Chemical Parameters:
Viz. Equilibrium adsorption isotherms, Column efficiency (number of theoretical plates), estimation of the pressure-drop, porosity, etc.

The adsorption isotherm must be carefully selected. For low concentration ranges, the concentration of a component on the stationary phase may be assumed to be varying linearly with that in the liquid phase. However, a non-linear competitive Langmuir type of adsorption isotherm is frequently used. [8] The column efficiency is calculated by the Van Deemter or Knox equations. The pressure drop per unit length of the column is calculated by Kozeny-Carman equation for laminar flow. [8]

In several cases, a simple column chromatography elution using the chosen phases is carried out to determine various constants.

Step B: Calculation of TMB
The feed composition, feed flowrate and both the phases are known. With the above data, flowrates are calculated for desired yield, assuming optimum conditions.

Step C: Calculation of SMB
Based on above flowrates, the actual SMB behaviour is simulated. The remaining parameters are determined viz. shift period, number of columns and column characters, viz. diameter and length.

The above calculations are simplified by softwares employing numerical methods and visual simulations. The "Triangle Theory" proposed by Morbidelli and his group is particularly useful to determine operating conditions for desired separation. [11]

Studies have shown that the differences between the TMB model and the SMB modification can be decreased by increasing the number of columns per zone.[8] Pharmaceutical industries typically use 6 to 16 columns in the SMB.[8]

The chromatographic resolution of bi-naphthol enantiomers was considered for simulation purposes by Pais L. S., Loureiro J. M., Rodrigues A. E. The chiral stationary phase was 3, 5-dinitrobenzoyl phenylglycine bonded to silica gel and a mixture of 72:28 (v/v) heptanes / isopropanol was used as eluent. The feed concentration is 2.9 g L^{-1} for each enantiomer. The adsorption equilibrium isotherms were determined by the Separex group and were found to be

$$q_A^* = \frac{2.69c_A}{1 + 0.0336c_A + .0.0466c_B} + \frac{0.10c_A}{1 + c_A + 3c_B}$$

$$q_B^* = \frac{3.73c_B}{1 + 0.0336c_A + .0.0466c_B} + \frac{0.30c_B}{1 + c_A + 3c_B}$$

q_i^* = concentration on each solid phase particle
c_i = concentration in mobile phase

The operating conditions used in a SMB configuration 2-2-2-2 at 25 °C recycling

flow rate 35.38 mL min^{-1}, feed 3.64 mL min^{-1},
eluent 21.45 mL min^{-1}, extract 17.98 mL min^{-1}
and raffinate 7.11 mL min^{-1}.

The internal concentration profiles were measured using the 6-port valve of the Licosep SMB pilot to withdraw samples from the system. The samples were collected at each half-time period, and after 40 full cycles of continuous operation. The experimental performance parameters were determined by analysis of the extract and raffinate samples collected during the whole cycle 40 (cyclic steady-state). The better purity performance was obtained for a switch time interval of t* = 2.87 min, corresponding to a solid flow rate in the TMB of

11.65 mL min^{-1}. For this value, purities as high as 94.5 % in the extract and 98.9 % in the raffinate were obtained with good recoveries. A productivity of 68 g of racemic mixture processed per day and per liter of bed was achieved. The corresponding solvent consumption, was 1.2 L g^{-1} racemic mixture.

The steady state TMB model was used to simulate the SMB operation by predicting its performance and its internal concentration profiles. Model parameters are the solid/fluid ratio $(1-\varepsilon)/\varepsilon = 1.5$, Pe = 2000 and $\alpha = 34.44$ corresponding to k = 0.1 s–1. The ratios between fluid and solid velocities in the TMB are: $\gamma_I = 6.31$, $\gamma_{II} = 4.00$, $\gamma_{III} = 4.47$, and $\gamma_{IV} = 3.55$. The predicted extract purity is 95.2 %; for the raffinate the purity predicted is 99.1 %. [6]

k = mass transfer coefficient
Pe = peclet number
ε= bed porosity
t*= switch time interval
γ_i = ratio between fluid and interstitial velocities in zone 'i'

The first example of chiral separation using SMB was published by Negawa and Shoji, where a Chrialcel OD resin was used to separate 1-phenylethanol enantiomers. The results showed that the SMB process increased the productivity 60-fold and reduced the eluent consumption 86-fold, when compared to batch chromatography. [7]

M. A. G. Santos, V. Veredas, I. J. Silva Jr., C. R. D. Correia, L. T. Furlan and C. C. Santana conducted an experimental separation of

Table 2: Results of SMB experimental runs

Experimental Conditions	Stream flow rate (mL/min)					C_F (g/L)	Switch time (min)	Purity	
	F	D_1	D_{total}	Ex	Raf			Ex	Raf
1	0.15	0.85	0.37	0.32	0.31	5	30	95.1	98.8
2	0.18	1.1	0.38	0.47	0.43	2.5	25	97.7	100.0
3	0.18	1.1	0.38	0.47	0.43	1.5	25	100.0	100.0
4	0.1	1.1	0.37	0.44	0.39	5	25	95.7	99.0

F= feed D = Eluent Ex = extract phase
Raf = raffinate phase C_F = concentration of drug in the feed

the enantiomers of racemic drug ketamine under various conditions using SMB chromatography. The results obtained are as in table 2. [12]

There are many other instances which have proved SMB chromatography to be a valuable asset in the resolution of single enantiomeric drugs.

Praziquantel, an anthelmintic drug, was purified by Ching et al. [7] A chiral epoxide 1a,2,7,7a-tetrahydro-3- methoxynaphth-(2,3b)-oxirene was separated from its enantiomer using cellulose triacetate (CTA) as the stationary phase and methanol as the eluent by Nicoud et al. [7]

Many chiral systems have been tested using SMB since 1992, and these SMB applications to chiral separation have already been reviewed by two different groups, Schulte et al. and Juza et al.[10]

Advantages of SMB

SMB is operated in continuous mode, which is always desirable for large scale manufacture.

In chiral HPLC, large amounts of solvent are required to elute out the components. In SMB, the solvent is recycled and a minimal amount is used. This agrees with the principles of economy as well as green chemistry.

SMB calculations are performed using computer-aided numerical calculations. The possible outcomes are easily observed by software, thereby cutting down costs and time of trials.

At larger scales, the savings in both solvent and chiral stationary phases make SMB technology the correct choice.

Coupling non-stereoselective synthesis with a SMB process is an economical way to produce both enantiomers with high purity and recovery. [8]

SMB processes can be more economical at commercial scale than any other option.

Since 1998, UCB Pharma of Belgium and Daicel Chemical of Japan, two major pharmaceutical companies, have been using SMBs to produce multi-tons of pure enantiomers from two different chiral drugs. In 1999, Novasep (Vandoeuvre- lès-Nancy, France) installed a production-scale SMB for chiral separation at Aerojet Fine Chemicals (Sacramento, CA, USA).[10]

8. Further Advances

Improved CSPs: A procedure to optimize the SMB separation conditions by choosing the best chiral stationary phase/ eluent combination have been recently proposed by Schulte et al. [13] Research to obtain better CSPs continues unabated in the chemical industries. Some of the existing CSPs have been modified (cyclodextrins and polysaccharides) and some new CSPs have been synthesized (cellulose derivatives). Microcrystalline cellulose triacetate and cellulose tribenzoate have in some cases been used for chiral SMB separations as neat, non-coated particles. However, they are inconvenient to pack, have a restricted chemical stability and show low efficiency. To overcome these limitations, a family of derivatized cellulose and derivatized amylase CSPs (group B) has been produced. These chiral polymers are not covalently attached to the support but are coated on a silanized, wide-pore silica gel. [14] Pirkle phases have been developed, which show π-interactions. They can be used for resolutions in reverse phase chromatography

too. [14] Efforts to synthesize CSPs suitable for enantioseparation of a specific class of compounds have simplified separations of antibodies, amino acids.

Improvement of Softwares used to Model the SMB: SMB uses synchronous shifting of inlet and outlet streams between successive columns arranged in series. A new continuous chromatographic separation technique has been developed which uses asynchronous shifting, called the VARICOL process. This has been shown to separate enantiomers of similar purity as the SMB technique, but with fewer columns. [15]

Studies to determine competitive isotherms are currently being carried out in order to develop equations that can represent systems with higher concentrations and thereby improve the productivity of the SMB unit in the future.

Use of Supercritical Fluids as Mobile Phases: Supercritical fluids have a distinct advantage over other organic solvents in chromatography. They have reduced mass transfer resistance, excellent solvent properties and they simplify the recovery of the solute. [16]

The SMB process combined with supercritical fluid chromatography (SFC) gave good results for separation of 1,1'-bi-2-naphthol enantiomers on Kromasil CHI-DMB and Chiralcel OJ phases.[17]

Conclusion

FDA guidelines paved a way for companies to extend their patents for marketed racemic drugs. In May 1992, it issued a policy stating: "Now that technological advances (large scale chiral

separation procedures or asymmetric syntheses) permit production of many single enantiomers on a commercial scale, it is appropriate to consider what FDA's policy with respect to stereoisomeric mixture should be".[8] Several assays have been developed using which the pharmacokinetic and pharmacodynamic properties of each enantiomer individually as well as in a mixture of various proportions can be evaluated. This data is used to determine whether the racemic mixture or the single enantiomer is appropriate to be used as a drug. It was proposed in 1997 that single enantiomers be accepted as New Drug Molecules with 5 year marketing exclusivity. These policy decisions by the FDA were the driving force for chiral switches and the commercial development of chromatographic processes such as simulated moving bed (SMB) technology.

Since 2000, many companies have been reported to carry out racemic switches, Forest Labs, Astra Zenaca, Celgene and Sepracor amongst others. Erstwhile racemates are licensed, patented and marketed as single enantiomers. This has expanded the single enantiomer drug market.

Large-scale chromatographic separations were limited mainly due to the high cost of the adsorbent, the high dilution of products, and the large amounts of mobile phase needed. With the introduction of the SMB technology, large-scale separations can now be carried out under cost-effective conditions. SMB technology is now an established technique for continuous chromatographic manufacture pure enantiomeric forms of the drugs and other pharmaceuticals. SMB chromatographic technique can provide the answer to the problem of economic resolution of chiral drugs.

References

1. McConathy J., Ph.D., and Owen M., Ph.D., Stereochemistry in drug action, Primary Care Companion J Clin Psychiatry, volume 5, 2003, 70–73.
2. Nguyen L., He H., Pham-Huy C., Chiral Drugs. An Overview, International journal of Biomedical Sciences, volume 2 no. 2, June 2006, 85-100.
3. C&EN COVER STORY October 1, 79, 2001, pg. 40
4. Murakami H., From racemates to single enantiomers – chiral synthetic drugs over the last 20 Years, Topics in Current Chemistry, 269 – Novel Optical Resolution Techniques, 273-299.
5. Nogradi M., Stereoselective Synthesis: A Practical Approach WILEY-VCH, Weinheim (Federal Republic of Germany), 1995, 27-28.
6. Rodrigues A., Pais L., Design of SMB Chiral separations using the concept of separation volume, Separation Science and Technology, 39, No. 2, 2004, 245–270.
7. Vaidya N., Diastereomeric crystallisation – the "classical" chiral technology Innovations in Pharmaceutical Technology, December 2001.
8. Subramanium G., Chiral Separation Techniques, WILEY-VCH, Weinheim (Federal Republic of Germany), 2001, 221-254.
9. G Cox, Chiral Technologies Inc.
10. Xie Y., Koo Y. and Wang N., Preparative chromatographic separation: simulated moving bed and modified chromatography methods, Biotechnol. Bioprocess Eng., 6, 2001, 363-375.
11. Allington R., Freitag R., Modern Advances in Chromatography, Springer - Verlag Berlin Heidelberg, 2002, 211-232.
12. Santos M., Veredas V., Silva Jr. I., Correia C., Furlan L., Santana C., Simulated moving bed adsorption for separation of racemic mixtures, Brazilian Journal of Chemical Engineering, 21, 1, January-March 2004, 127-136.
13. Schulte M., Ditz R., Devant R., Kinkel J., Charton F., J.Chromatogr. A 769, 1997, 93.
14. Juza M., Mazzotti M., Morbidelli M., Simulated moving-bed chromatography and its application to chirotechnology, Trends in Biotechnology, 18, March 2000, 108-118.
15. Ludemann-Hombourger O., Nicoud R., Bailly M., The "VARICOL" Process: A New Multicolumn Continuous chromatographic process, Separation Science andTechnology, 35, Issue 12 September 2000, 1829 – 1862.
16. Speight J., The chemistry and technology of petroleum, Talyor and Francis Group, Boca Raton, Florida pg 275.
17. Bojarski J., Aboul- Enein H., Ghanem A., What's New in