

ERNST FREUND
STEFANIE ABEL
ELKE HUTHMANN
JÖRG LILL



Ernst Freund

Chiral chromatography in the early phases of pharmaceutical development

ABSTRACT

For fast access to pure enantiomers during early pharmaceutical development, several chromatographic methods are available. The reasons for using apparently expensive technology at an early stage will be discussed. The individual techniques; SMB (Simulated Moving Bed), preparative HPLC and SFC (Supercritical Fluid Chromatography) are briefly described and illustrated by 3 case studies. The different methods are compared and their strengths and limitations are discussed. The article concludes with a short outlook on possible developments in this specific sector of the pharmaceutical industry.

INTRODUCTION

Today new chiral drugs are mainly marketed as single enantiomers (1). As the access to pure enantiomers by synthesis or crystallisation can be very laborious, preparative chromatography on chiral stationary phases (CSPs) (2), in the following discussion termed "chiral chromatography", is an attractive alternative. Chromatography is a simple, fast and broadly applicable technique to access larger amounts of both enantiomers in high yield and chiral purity. There are two main reasons for the use of (chiral) chromatography in early development phases. First there are considerable costs and time associated with developing an enantioselective synthesis. Given that many APIs (active pharmaceutical ingredients) fail in toxicology studies or in the early clinical phases, there is no return for the additional work performed. Secondly, if the API is successful, the use of chromatography to produce pure material in a shorter

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timeframe could potentially reduce development time. Faster time to market generates additional revenue of hundreds of millions of dollars from a potential blockbuster.

The Drug Substance material need for the early phases of API development depends on its activity and is typically in the range of hundreds of grams in the toxicology phase. In the clinical phases where the drug is administered to increasingly larger groups of volunteers or patients; multi kilogram amounts of the API are usually needed. The circumstances of the actual separation problem will determine, which of the following chromatographic techniques will be chosen.

SMB is a multi column process based on a simulated counter current movement of liquid and stationary phase, which is achieved by valve switching. These results in a continuous separation with high productivity ideally suited for binary mixtures (3). This technique is available up to a scale of >100 tons per year. The need for expensive CSP and the solvent consumption is strongly reduced compared to the corresponding HPLC separation. For HPLC the biggest benefits are the straightforward and fast method development, scale-up and the easy instrumentation. In addition multiple compounds (e.g. impurities) can be separated and solubility problems of the racemates can be circumvented as different solvents can be chosen for feed and elution.

In SFC (4) supercritical CO₂ plays the role of the weak eluent comparable to the considerably more expensive and flammable n-heptane in normal phase chromatography. The racemates are dissolved in a suitable organic solvent for injection and eluted with a mixture of CO₂ and preferably the same solvent

used for the feed solution. The product fractions are obtained as concentrates in the organic modifier, thus eliminating the need for further expensive downstream processing. The low viscosity of the carbon dioxide allows high flow rates even on columns with small particle size and the fast diffusion of the solutes leads to narrow peaks.

In the following case studies typical problems encountered in early pharmaceutical development and their possible solutions are presented and discussed. For reasons of confidentiality the compound structures cannot be disclosed.

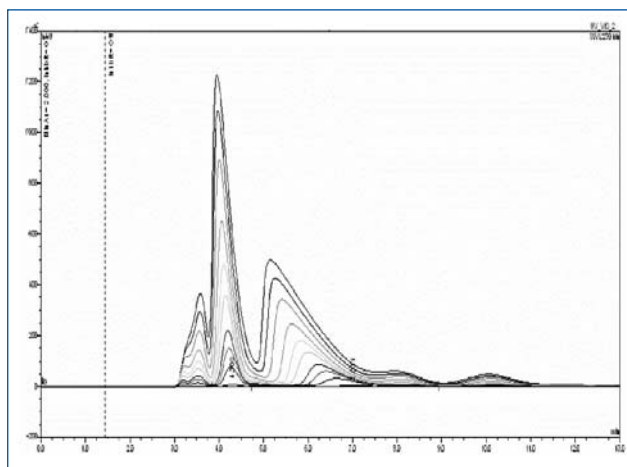


Figure 1. Chromatograms of overloaded injections necessary for method development of SMB and preparative HPLC separations

CASE STUDIES

In the first case study, approx. 9 kg of a racemic pharmaceutical intermediate had to be separated into its enantiomers. A chiral purity of ≥ 99 percent a/a HPLC was desired for both enantiomers. Based on the amount of racemate to be separated it was decided to use the SMB. After screening and optimisation the best separation was found on the Chiralpak IC stationary phase using acetonitrile as eluent. From the overloaded injections (Figure 1) the simulation software Lico-HELP 6.6 calculated the starting parameters for the later SMB separation. As a consequence of the chosen robust separation parameters, the target enantiomer fulfilled the specification from the start of the separation and after short optimisation also the non-target enantiomer was obtained with the desired purity. The separation was performed at room temperature on a Licosep 10-50 SMB system equipped with 8 columns in a 2-2-2-2 configuration. Each column was packed with 110 g of Chiralpak IC 20 mm.

After evaporation to dryness of the target stream, 4.29 kg (48 percent, 100 percent a/a HPLC) of the target enantiomer was obtained. The second enantiomer was obtained in two portions: 3.26 kg (36.4 percent, 99.9 percent a/a HPLC) and 0.52 kg (5.0 percent, 86.1 percent a/a HPLC). The productivity was 4.1 $\text{kg}_{\text{rac}}/\text{d}/\text{kg}_{\text{CSP}}$ with a solvent consumption of 101 $\text{L}/\text{kg}_{\text{rac}}$. For the corresponding HPLC separation on the same CSP a best case productivity of approx. 1.7 $\text{kg}_{\text{rac}}/\text{d}/\text{kg}_{\text{CSP}}$ with a solvent consumption of 400 $\text{L}/\text{kg}_{\text{rac}}$ was estimated based on the chromatograms from the overloaded injections.

In the second case study one diastereomer had to be isolated from 45.5 kg of the corresponding mixture of a pharmaceutical intermediate containing two stereocenters. The specification for the target enantiomer was > 99.5 percent a/a HPLC chiral purity and a total content of < 1 percent a/a HPLC for the three undesired isomers (Figure 2 a). As the target enantiomer eluted last the SMB could be used for this multi-component separation. The optimised separation employed Chiralcel OD as stationary phase and n-heptane:ethanol=80:20 (v:v) as eluent. For the high observed selectivity, the free solubility of the mixture and the good loading capacity of the stationary phase, the highest productivity so far obtained in our lab was achieved in this case. The SMB separation was performed in 5 days and after evaporation of the product streams 4.91 kg (45 percent, 99.9 percent a/a HPLC) of the target enantiomer and 33.6 kg (36.9 percent, 97.4 percent a/a HPLC) of the mixture of the remaining three diastereoisomers were obtained. The productivity was 11.5 $\text{kg}_{\text{mix}}/\text{d}/\text{kg}_{\text{CSP}}$ with a solvent consumption of 31 $\text{L}/\text{kg}_{\text{mix}}$. The corresponding HPLC separation would have an estimated productivity of 5.6 $\text{kg}_{\text{mix}}/\text{d}/\text{kg}_{\text{CSP}}$ with a solvent consumption of 120 $\text{L}/\text{kg}_{\text{mix}}$.

One drawback of the SMB as a binary separation technique is that the chemical purity of the two product streams cannot be adequately controlled. Impurities cannot be removed but they will co-elute with one or both enantiomers. For this second separation, late eluting impurities were co-eluting with the target (Figure 2b). A use test on the next stage, however, showed that the material was of sufficient quality for the subsequent conversion.

For the analysis of the SMB process a fast In-Process-Control method had to be developed. Resolution of the cis-enantiomers was not required, however, the trans-isomers had to be resolved and separated from the two cis-enantiomers. In contrast to the HPLC method (Figure 3a) the SFC method employing gradient elution (Figure 3b) was considerably shorter.

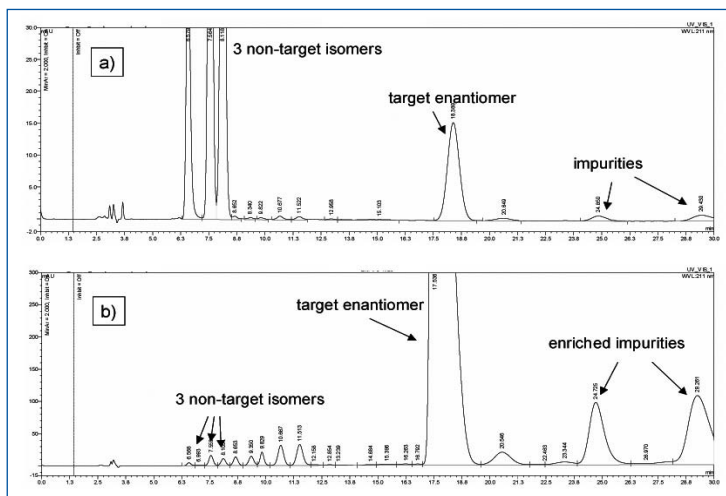


Figure 2. HPLC chromatograms of case study 2: a) crude mixture b) isolated target enantiomer

In the third case study an HPLC separation of 1.06 kg of a racemic amino acid precursor is described. The optimised method on Chiralcel OD employed n-heptane:ethanol=85:15 (v:v) as eluent (Figure 4), in which the racemate showed a good solubility of 200 g/L. The separation was performed at room temperature on an ID=10 cm column packed with 1.0 kg of Chiralcel OD 20 mm. As the remaining impurities eluted in a short time frame, stacked injections were performed reducing the run time from 9 to 6 minutes. This saved approx. 30 percent of solvent and increased the throughput by the same amount. After evaporation of the target fraction 413 g (39 percent, 98.0 percent a/a HPLC) of the first eluting enantiomer were obtained. The productivity was 1.4 $\text{kg}_{\text{rac}}/\text{d}/\text{kg}_{\text{CSP}}$ with a solvent consumption of 480 $\text{L}/\text{kg}_{\text{rac}}$. The SMB simulation software predicted a productivity of approx. 3.0 $\text{kg}_{\text{rac}}/\text{d}/\text{kg}_{\text{CSP}}$ and a solvent consumption of 90 $\text{L}/\text{kg}_{\text{rac}}$ for the corresponding SMB separation.

RESULTS AND DISCUSSION

SMB is superior to HPLC with respect to productivity for its operating principle. This is well reflected by the three case studies where factors of 2-3 for the productivity and factors of 4-5 for the solvent consumption in favour of SMB are found. Case study 1 shows an extraordinarily high productivity for a separation, where both enantiomers are collected at a high purity. Case study 2 is a rare case of a multi-component

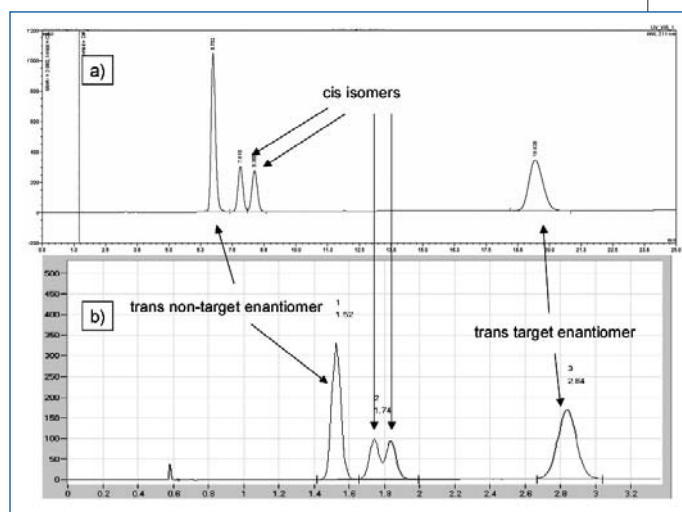


Figure 3. Case study 2: a) Chiralcel OD-H 250x4.6 mm, 5 μm , n-heptane:ethanol=95:5 (v:v), 1 mL/min at 40°C b) Chiralpak AS-H 250x4.6 mm, 5 μm , CO_2 :methanol=90:10 (v:v) for 1 min, ramp up to 80:20 during 2 min then re-equilibrate during 2 min, 5 mL/min at 35°C and 100 bar

separation showing an exceptional productivity. Most of the SMB separations so far performed in our lab reached productivities of 0.8-3 kg_{rac}/d/kg_{CSP} with typical solvent consumptions of 100-300 L/kg_{rac}. Recent results with new CSPs indicate that these values may still be considerably improved. The SFC analysis shown in figure 3 is approximately 5 times faster than the corresponding HPLC separation with a sufficient selectivity and resolution. The same factor would be obtained comparing the productivity of the SFC and HPLC separation on the same column size. As 5 mm particles are generating too much backpressure on larger HPLC systems (10-20 μm CSPs are being used) the higher efficiency also favours SFC conditions for a preparative separation.

In the described HPLC separation this technique was preferred as only a small amount of racemate had to be separated and the target enantiomer was required fast. The development, optimisation and execution of the separation was achieved within two weeks. The productivity is high for a HPLC separation thanks to the good selectivity at low retention times, the high solubility of the racemate and also the fact that the target enantiomer was eluting first. Thanks to the high purity of the racemate – no impurities had to be separated – the productivity could be increased by using stacked injections.

It is important to note, that the described separations are not completely optimised. As there is considerable time pressure and a high recovery is usually desired, the production runs are often started after the productivity is satisfying. Each optimisation run costs time and potentially produces material, which would need reprocessing. This is the case for all techniques but most true for SMB, where the optimisation is performed on scale with the production system.

CONCLUSIONS AND OUTLOOK

The case studies show, that SMB and preparative HPLC are mature technologies, which are used in a regulated environment for the generation of pure enantiomers for several years now. SFC is a younger complementary technology, with an increasing significance for its benefits like speed and separation efficiency.

In general there are probably three major non-chromatographic factors, which will determine the choice of the separation technique. In the early phases of drug development the pure enantiomers are required fast. Then the batch size and finally the total costs, which are composed of processing costs and investment costs play a major role. The productivity of the separation itself is obviously another factor, which influences the selection of the separation technique. Productive separations as described in the case studies above can just be achieved if the two key parameters, selectivity and solubility are optimally matching. A good first estimation of a separation can be made after a screening of CSPs, determination of the solubility and performing overloaded injections. This work can usually be done within one to two weeks.

Preparative HPLC and SFC systems are normally used for separations of less than 10 kg of racemate. SFC is superior to HPLC but the investment costs are considerably higher as an additional SFC system for the method development is needed, whereas analytical HPLC systems are omnipresent.

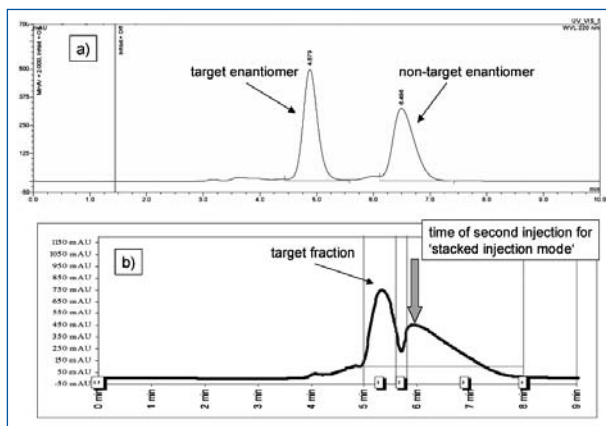


Figure 4. Case study 3: a) Chiralcel OD 250x4.6 mm, 20 μm, n-heptane:ethanol=85:15 (v:v), 1 mL/min at RT b) Preparative separation on a 10 cm ID column with a loading of 5.6 g racemate after linear scale up

Additionally HPLC systems can also be used for the separation of highly polar compounds under reversed phase conditions.

The most powerful separation technology is SMB, which is reflected by some commercial drugs, where the manufacturing process involves SMB separations (5). However, the investment costs are highest and SMB is restricted to binary separations. An elegant solution to avoid high investment costs

for the chromatography systems is outsourcing, which is common practice especially in early phases of drug development. Then no investments into technologies, which are probably just rarely used is necessary. In addition the optimum process for a given separation problem can be applied, guaranteeing minimal processing costs.

In the future SFC is expected to become more dominant for enantiomer separations, depending also on the technical progress, which will be achieved with these systems. The SMB technology will continue to be used as it has proven to be reliable and economic at all scales. The latest generation of small scale systems has been significantly improved regarding the instrumentation (6). These measures should further increase the reliability and lower the operation costs. Automated control systems will help to optimise the productivity and the robustness of the SMB separations (7).

Most likely the biggest progress in chiral chromatography will be made on the field of the stationary phases. Some patents of the most successful CSPs have expired and new manufacturers are appearing. This therefore exerts pressure for new developments. Several recent projects from CARBOGEN AMICIS indicate that with some new chiral stationary phases, very high productivities can be reached. However, the method development will become more challenging as a consequence of the wider choice of columns and tolerated solvent systems. Even if chromatography on chiral stationary phases is too expensive for the fine chemicals sector, for the early development of APIs it is a perfect fit. It is a universal and fast approach, which saves development time and costs. With technological progress, the costs per kg of pure enantiomer will drop and this could potentially open up new fields of application for chiral chromatography.

REFERENCES AND NOTES

1. S.K. Branch, G. Subramanian, *Chiral Separation Techniques a Practical Approach*, Wiley-VCH, Weinheim, pp. 319-342 (2001).
2. E.R. Francotte, *Journal of Chromatography A*, **906**, pp. 379-397 (2001).
3. A. Rajendran, G. Paredes et al., *J. of Chromatogr. A*, **1216**, pp. 709-738 (2009).
4. L.T. Taylor, *J. of Supercritical Fluids* **47**, pp. 566-573 (2009).
5. S. Abel, M. Juza, *Chiral Separation Techniques a Practical Approach*, Wiley-VCH, Weinheim, pp. 203-274 (2007).
6. A. Seidel-Morgenstern, L.C. Kessler et al., *Chemie Ingenieur Technik* **80**(6), pp. 725-740 (2008).
7. C. Langel, C. Grossmann et al., *J. of Chromatogr. A*, in press.

ERNST FREUND*, STEFANIE ABEL,
ELKE HUTHMANN, JÖRG LILL

*Corresponding author
CARBOGEN AMICIS AG
Schachenallee 29
Aarau, CH-5001, Switzerland

Even if chromatography on chiral stationary phases is too expensive for the fine chemicals sector, for the early development of APIs it is a perfect fit