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Less common applications of simulated moving bed chromatography in the pharmaceutical industry☆

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Abstract

Simulated moving bed (SMB) chromatography is often perceived in the pharmaceutical industry as chromatographic method for separating binary mixtures, like racemates. However, SMB can also be used for unbalanced separations, i.e. binary mixtures of varying compositions and multi-component mixtures. These less common application modes of isocratic SMB chromatography are exemplified for four different compounds (racemates and diastereomers) and discussed in view of the so-called 'triangle theory' from an industrial perspective.

Keywords: Simulated moving bed chromatography; Preparative HPLC; Chiral separation; Enantiomer separation; Chiral discrimination

1. Introduction

In the recent years simulated moving bed (SMB) chromatography on chiral stationary phases (CSPs), especially helical homochiral polymers derived from naturally occurring macromolecules, such as cellulose or amylose, has become an essential tool for the chromatographic resolution of racemates on a preparative scale [1]. The technology has shown in some cases distinct advantages over synthetic routes involving chiral or prochiral precursors and "classical" resolutions. The impact of enantioselective chromatography on the development of pharmaceuticals has been reviewed recently by Francotte [2,3] and others [4,5].

Efficient criteria for the optimal design of SMB systems have been developed, which allow one to account for the non-linear character of the involved adsorption equilibria and to optimize the productivity per kg CSP easily [6]. Following the so-called 'triangle theory' constraints on these criteria have been derived which allow for complete separation of a binary mixture following the Langmuir and the modified Langmuir isotherm [7] and the most general case of a bi-Langmuir multi-component adsorption isotherm [8]. Today, development, scale-up and optimization of SMB separations follow a straightforward protocol and can be performed within a few days.

In comparison to preparative HPLC chromatographic separations using SMB units show several distinct advantages, especially a lower solvent consumption and a lower inventory of chiral stationary phase. These time and cost-saving advantages prompted us to study possibilities to use SMB units not only for racemate resolutions, but also for more complex separation problems such as "unbalanced" (i.e. not 1:1 ratio of binary substrates) and multi-component mixtures. It should be emphasized that multi-component separations using the SMB technology are common practice in the petrochemical industry and that the Molex process pioneered by UOP as described by the first patent filed on SMB [9] in 1961 by Broughton and Gerhold is intended for the separation of linear and branched hydrocarbons. Today a number of industrial scale processes similar to the Molex are used on a 100,000 t/a scale. However, all of them use either zeolithes or ion-exchangers as stationary phases and do not employ high pressure liquid chromatography [10].

Various examples for pharmaceutical applications will be given for such less-common application modes, paying

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special attention to benefits to be gained in comparison to more conventional *modi operandi*. All purifications, about which we will report in the following paragraphs, are performed for investigational new drugs, which have not yet been submitted for patent application. Thus presently the structural information cannot be disclosed. However, we aim to present scenarios, which can be generalized and can easily be transferred to other chromatographic purifications.

2. Experimental

2.1. Equipment

The analytical HPLC unit used was an Agilent HP1090 system (Basel, Switzerland) consisting of a quaternary pump, auto sampler and a diode array detector.

Two Licosep 10 × 50 units, produced by Novasep (Pompey, France) were used for the pilot runs. A detailed description of the unit layout has been given recently [11]. It was equipped with eight NW-50 (non-jacketed) columns produced by Merck (Darmstadt, Germany) with a measured internal diameter of 48 mm. The column length can be adjusted up to a maximum of 120 mm. The columns can be self-packed easily.

2.2. Materials

The chiral stationary phases (CSPs) were obtained from Chiral Technologies Europe (Illkirch, France) as 20 μm bulk packing. All compounds submitted for purification were synthesized in the laboratories of CarboGen AG (Aarau, Switzerland). The solvents used as eluents were reagent grade or better quality and obtained from SDS, Peypin, France. Analytical in process control of extract and raffinate stream was performed employing the same stationary phase and eluent as for the preparative separation. Dilution with the eluent was employed when necessary.

2.3. Column packing and testing

Bulk CSPs were packed into eight NW-50 columns purchased from Merck (Darmstadt, Germany). Each column contained exactly 110.0 g dry mass of the stationary phase. The column volume was determined individually for all columns, which were tested with a preparative HPLC system provided by Knauer (Berlin, Germany), which consisted of a K-1800 pump with a 1000 mL/min pump head, a HPLC-Box and a K-2500 UV detector.

2.4. Determination of adsorption isotherms, Henry constants and start parameters for the separations

Analytical HPLC columns, containing various lots of Chiralpak AD or Chiralcel OD were installed into a HP1090 system (cf. Section 2.1) equipped with a Jasco CO-1560 oven (Omnilab, Mettmenstetten, Switzerland) and thermostated at the operating temperature of the SMB unit ±0.1 °C.

A simulation software called “softSMB” is supplied with the SMB and allows one to approximate the adsorption isotherms of binary mixtures and to optimize the operating parameters before starting the unit itself. Based on a few injections at increasing volume of a concentrated product solution on analytical HPLC columns filled with Chiralpak AD or Chiralcel OD the Novasep software package “softSMB” correlates through a curve-fitting procedure the equilibrium experimental results with a postulated modified Langmuir competitive isotherm (cf. Eq. (1)), which takes the form:

$$n_i = \lambda c_i + \frac{\bar{N}_i K_i c_i}{1 + \sum_{k=1}^{2} K_k c_k} \quad (1)$$

In this equation n_i and c_i are the adsorbed and the fluid phase concentration, respectively; λ is a dimensionless coefficient; K_i the equilibrium constant of the ith component, which accounts for the overload effects; the upper limit of n_i is given by the saturation capacity $\bar{N}_i$.

The Henry constants give the slope of a component’s adsorption isotherm under linear conditions, i.e. at infinitely small concentration:

$$n_i = H_i c_i \quad (2)$$

At low concentrations the modified Langmuir isotherm (cf. Eq. (1)) allows a calculation of the Henry constants:

$$H_i = \bar{N}_i K_i + \lambda \quad (3)$$

The ratio of the Henry constants is equal to the enantioselectivity α. It should be noted that the constants, which can be determined independently from simple experiments [12], are affected by variations in bed density and the resulting overall porosity ε^*.

2.5. SMB hardware and control software

Two different SMB units of identical layout were used for the separations. The Licosep 10 × 50 SMB units from Novasep (Pompey, France) are controlled by a central system composed of a Siemens PLC (type S7-300) and a personal computer as the user interface. The supervision software works under DOS or Windows 2000 and allows the full control of the unit parameters (valves, pumps, flow rates, pressures) when the unit is running or under test. All relevant parameters and data are continuously stored in files for quality control. The software allows an easy access to real time curves (flow rates, temperature, pressure). All measurements are transmitted from the Licosep 10 × 50 through a control board, containing the PLC and all required interfaces and supplies.

3. Results and discussion

3.1. Design of operating conditions for a SMB separation

Simulated moving bed units are complex systems, whose operation requires the choice of several parameters, e.g. the flow rates in the four sections (1, ..., 4) of such a unit, the period after which the in- and outlet ports are switched and the feed composition. Neglecting axial dispersion and mass-transfer resistance an 'equilibrium theory' model can be used to design optimal operating conditions and to explain experimental results, when the equivalency between the true moving bed and SMB is exploited. This "triangle theory" allows an easy graphical description of the internal flow-rates and the switch time, which both determine the flow-rate ratios, m_j, defined in Eq. (4).

$$m_j = \frac{Q_j t^* - V\varepsilon^*}{V(1-\varepsilon^*)} \quad (j = 1, \ldots, 4) \tag{4}$$

Here, Q_j, $j = 1, \ldots, 4$, are the volumetric flow rates in sections 1–4 of the SMB, ε^* is the overall void fraction of the columns, t^* the switch time, and V the single column volume. The isotherm parameters (cf. Section 2.4) and the feed composition allow one to define several regions in an 'operating parameter plane' spanned by the flow rate ratios m_j in the central sections of the SMB unit (m_2, m_3) as drawn in Fig. 1 for linear adsorption behavior.

Various areas in this plane can be distinguished. A triangular region describes an area where the flow-rates in sections 2 and 3 of the SMB lead to a complete separation. This triangle is determined through adsorption isotherms and concentration of the two species to be separated, and the two Henry constants (see Eq. (3)) H_1 and H_2 of the two compounds [13]. Above this triangle a region is found where only the extract stream (more retained component(s)) is pure, and on the left side of this triangle a region is located where only the raffinate stream (less adsorbed component(s)) is pure. Between the regions of pure raffinate and extract (above the vertex of the triangle) another region is located, where neither stream is pure. The area under the diagonal of the m_2, m_3 plane has no physical meaning. The interested reader is referred to the literature where the 'triangle theory' is explained and applied in great detail [6–8]. The area of complete separation becomes larger if the difference between the Henry constants is great and becomes smaller at lower loading capacities ($\bar{N}_i$) and higher feed concentrations.

Sections 1 and 4 are not described by the m_2, m_3 plane, but have to fulfill certain criteria, like m_2 and m_3, in order to provide pure outlet streams as summarized below (Eq. (5)):

$$\begin{aligned} &H_2 < m_1 \\ &H_1 < m_2 < m_3 < H_2 \\ &m_4 < H_1 \end{aligned} \tag{5}$$

The requirements on zones 1 and 4 can be visualized by a plot of the m_1, m_4 plane (cf. Fig. 2). Only flow rate ratios, which lie in the gray area in Fig. 2 will allow for complete regeneration of mobile and stationary phase. If the operating point is located below the area of regeneration, the stronger adsorbed component will not be desorbed from the stationary phase in zone 1. It will be carried over to section 4 and eventually section 3 and pollute the raffinate stream. If the operating point lies to the right of the gray area the flow rate in section 4 will be too high for adsorbing the less retained component completely in this zone and, as a consequence, the extract will be polluted.

If the constraints on all flow rate ratios are fulfilled, a complete separation of the two components can be expected. In practice sufficient safety margins must be applied, i.e. m_1

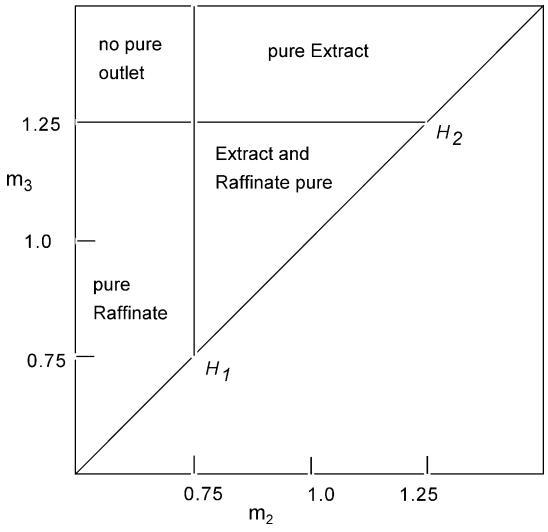

Fig. 1. Regions in the m_2, m_3 plane with different separation regimes in terms of purity of the outlet streams, for a system described by a linear isotherm with $H_1 = 0.75$, $H_2 = 1.25$.

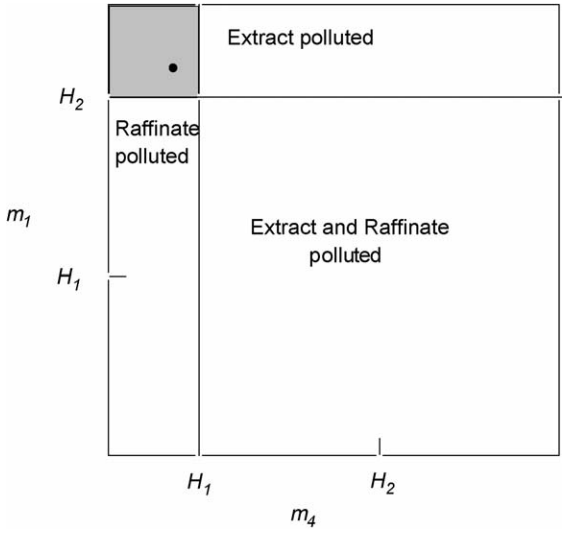

Fig. 2. Regions in the m_1, m_4 plane with different separation regimes in terms of purity of the outlet streams, for a system described by a linear isotherm; (●) exemplary operating point for complete regeneration.

should be at least 10% higher than H_2 and m_4 should be at least 10% lower than H_1.

3.2. Unbalanced two component separations

A multitude of reports on racemate resolution via the SMB technology have been published during the last 10 years [14–36]. However, only in a few cases mixtures with unequal amounts of two components have been separated [37]. Therefore some general considerations on non-balanced (i.e. not 50:50) crude feed-stocks will be summarized in the following. These feedstocks can originate either from stereoselective reactions leading to enantiomerically enriched materials, or from reactions leading to a diastereomeric enrichment, like crystallizations with chiral acids or bases. In some cases it will also be necessary to purify non-racemic solutions obtained during optimization or malfunctions of "regular" SMB chromatographic runs, where racemates are resolved on chiral stationary phases (CSPs).

3.2.1. Case study I: 1 versus 1 and 1 versus 10

An example of the latter case is given in Fig. 3. A racemic mixture (called in the following compound A) with a chemical purity of 82% and a sodium chloride content of approximately 30% was separated on a 7.5 kg scale using Chiralpak AD as stationary phase and a solvent mixture consisting of acetonitrile/IPA/DEA (95:5:0.2, v:v:v) as eluent.

As mentioned above the adsorption isotherm governs the shape of the triangular region of complete separation under overloaded conditions. The competitive adsorption isotherms for compound A have been determined using the procedures developed by Novasep; it was found that the experimental data fits well into the modified Langmuir competitive

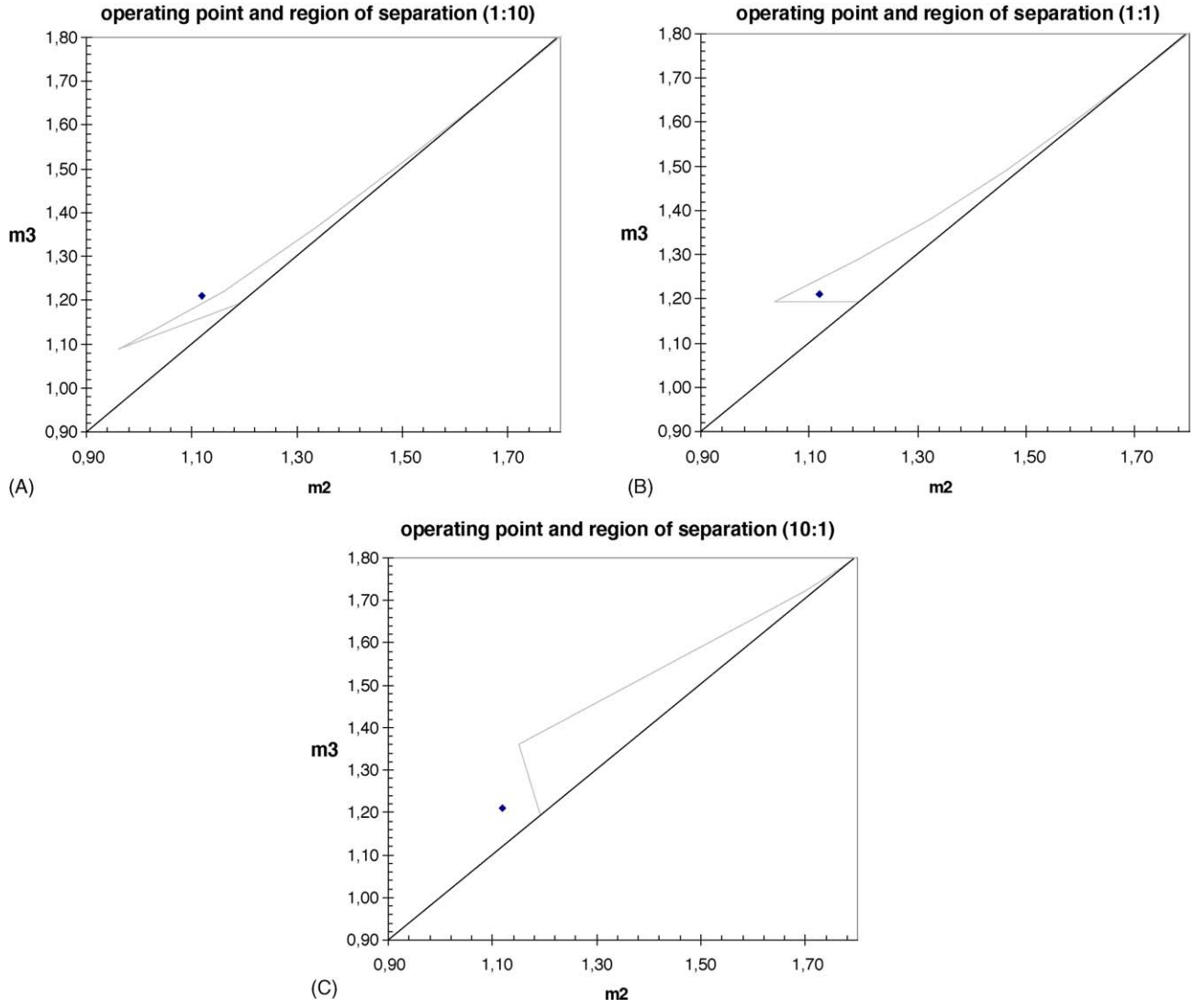

Fig. 3. Separation of compound A; regions in the m_2, m_3 plane with different separation regimes in terms of purity of the outlet streams. Predicted region of complete separation for three different scenarios: (——) for isotherm parameters: $\lambda = 0.8$, $NK_1 = 0.392$, $NK_2 = 1.076$, $\bar{N}_i = 20$; (A) $c_1 = 6.44$ g/L, $c_2 = 57.96$ g/L; (B) $c_1 = c_2 = 32.2$ g/L; (C) $c_1 = 57.96$ g/L, $c_2 = 6.44$ g/L; (♦) operating point.

isotherm model. The model can be written as described in Eq. (1).

It should be noted that for a Langmuirian behavior the triangle under non-linear conditions becomes compressed to the lower left part of the m_2, m_3 diagram as can be seen in Fig. 3B for a racemic mixture of compound A. However, the adsorption isotherm determined by the Novasep software "soft-SMB" does not agree in all cases with the experimental results, which necessitates experimental fine-tuning. After optimization the target enantiomer (Raffinate) could be obtained in sufficient enantiomeric purity (i.e. >98% ee). Since the desired overall yield of the target enantiomer could not be met after the first pass through the SMB unit it was decided to concentrate the (enriched) out-of-specification material and to submit the concentrated solution again to enantioselective chromatography.

Fig. 3A and C shows how the shape of the triangle changes when the more and less retained enantiomer are abundant to 90%, respectively. For comparison the operating point of the first separation (ratio 1:1) has been maintained in the graphics. In Fig. 3A the region of complete separation is even more compressed than for the original case (1:1) and the operating point, which used to lie inside the triangle, is now in the region of pure extract. Obtaining two pure outlet streams will be quite a challenge in this case, whereas it can be expected that a feedstock already enriched in the more retained enantiomer will be found in enantiomerically pure form in the extract when the same operation point is used. In Fig. 3C the operating point is far to the left of the triangle and would result in pure raffinate. In order to obtain the more retained enantiomer out of a depleted mixture of enantiomers significant changes in the operating conditions would be needed.

The productivity of the racemate separation was ~818 g racemate/day/kg CSP with an eluent consumption of 346 L/kg crude/day. Overall 23% of the target enantiomer with an ee of >99.6% and a chemical purity of 91.6% could be isolated in the given time frame. It seems worth mentioning that the low overall yield of this separation has its reason in the chemical purity of the starting material (i.e. 82% area HPLC, see above) and the fact that the overall yield was determined after an additional extraction step of the crude target enantiomer after chromatography.

During early scale-up experiments compound A was also separated via preparative HPLC (column size: 230 mm × 100 mm ID). The productivity for the preparative HPLC was 50 g/day/kg CSP with an eluent consumption of 430 L/day. As expected the performance of the SMB technology is superior to preparative HPLC and the use of this technology leads to lower eluent consumption.

3.3. Three component separations via SMB

Separation of more than two components is a task often encountered in pharmaceutical industry. A broad range of feed stocks falls under this category, ranging from racemates which contain an undesired impurity to compounds containing unreacted material from a previous step to racemates which show after reaction or storage significant amounts of an undesired third (side-)product.

3.3.1. Case study II: three component separations – beneficial scenarios

It is obvious that when the desired compound is eluting either first or last (cf. Fig. 4, **1** or **3**) it can be collected in raffinate or extract, respectively.

In the first case it is required that both non-desired components are retained in zone 2 of the SMB and are collected in the extract stream (cf. Fig. 4, left). Therefore the operating point for a system characterized by a linear adsorption behavior and $H_1 < H_2 < H_3$ should be selected in a way that these conditions are fulfilled:

$$\begin{aligned} &H_3 < m_1 \\ &H_1 < m_2 < m_3 < H_2 \\ &m_4 < H_1 \end{aligned} \tag{6}$$

For a system where the target compound is eluted last (i.e. peak **3**) the non-desired components are collected in the raffinate (cf. Fig. 4, right). The separation in zones 2 and 3 of the SMB requires that the most retained component is kept in zone two, while the less retained species are carried by the mobile phase up-stream, i.e. into the direction of the raffinate. However, in zone 1 all components must be desorbed and in zone 4 all components must be adsorbed by the chiral stationary phase. These conditions lead to a set of Henry

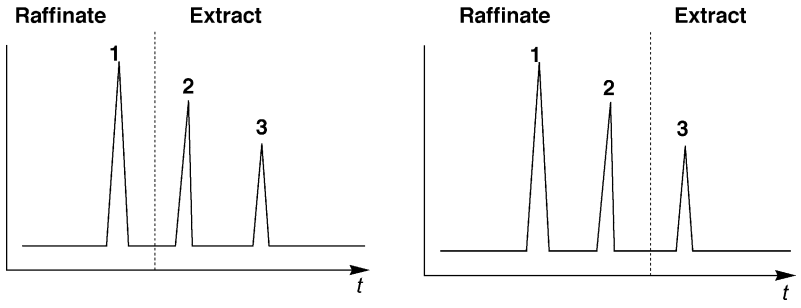

Fig. 4. Binary separation of three components (**1**, **2**, **3**) via SMB, target compound is eluting either first or last and can be collected in raffinate or extract.

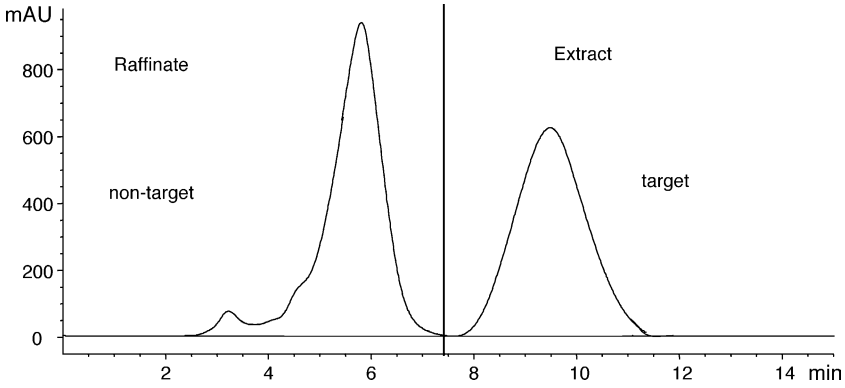

Fig. 5. Separation of compound B on Chiralpak AD, eluent *n*-heptane/IPA (60:40, v:v); the vertical line in the HPLC trace indicates the division between raffinate and extract. The impurities are eluted in the raffinate before and under the non-target diastereomer.

constants and m_j values for linear conditions as described below:

$$H_3 < m_1$$
$$H_2 < m_2 < m_3 < H_3 \quad (7)$$
$$m_4 < H_1$$

Despite several successful multi-zone SMB applications in Academia [38–40] and low pressure applications [41,42], currently no commercial SMB unit can be used for collection of more than two product streams. Therefore separations where the target compound elutes in the middle of two peaks require the application of at least two unit-operations, e.g. crystallization and chromatography, or two-step chromatographic purification. An example for this "unwanted" scenario is given below (cf. *case study III—unwanted scenario*, compound C).

In practice linear adsorption isotherms are seldom observed and the internal flow rates of the SMB unit must be adapted to the non-linearity of the adsorption isotherms.

A mixture of diastereomers (called in the following compound B) was separated on a 3.0 kg scale using Chiralpak AD as stationary phase and a solvent mixture consisting of *n*-heptane/IPA (60:40, v:v) as eluent. The target diastereomer was eluted last, while an impurity and the non-target diastereomer eluted first and second, respectively (cf. Fig. 5). The productivity of the separation was 755 g crude/day/kg CSP with an eluent consumption of 519 L/kg crude/day, overall 40% of the target diastereomer with a de of >99% could be isolated from the crude compound B. The operating points had to be moved into the region of pure extract (see Fig. 6), since the compound exhibited an unusual adsorption behavior, which could not be predicted in enough detail using a modified Langmuir model as proposed in Eq. (1).

No protocol for crystallization or chromatographic purification on achiral media could be developed in our hands during the given time frame of four weeks for method development, separation and isolation of the target diastereomer.

3.3.2. *Case study III: three component separations – unwanted scenario requiring two separation steps*

Especially during the first phases of the drug development cycle racemates, which can contain significant amounts of side products, are submitted for chromatographic resolution. The SMB technology can be applied in a straightforward manner to isolate first or last eluting components. However, when the target is eluted "in the middle of the chromatogram" it can become necessary to use either batch HPLC or to resort to subsequent SMB runs. A scenario for the latter approach is given in Fig. 7.

A racemic mixture (called in the following compound C) was separated twice on a 100 g scale via preparative HPLC using Chiralpak AD as stationary phase and a solvent mix-

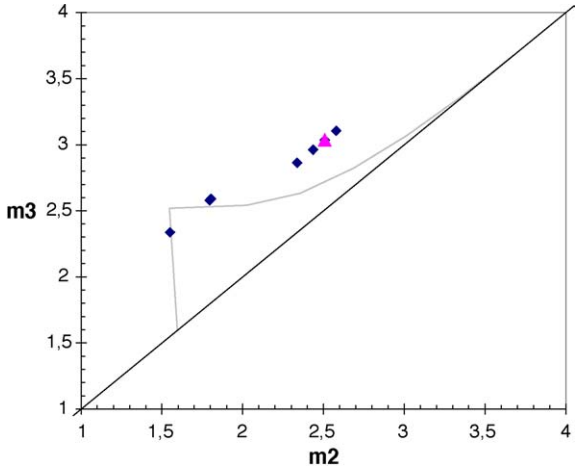

Fig. 6. Separation of compound B; regions in the m_2, m_3 plane with different separation regimes in terms of purity of the outlet streams. Predicted region of complete separation: (——) for isotherm parameters: $\lambda = 1.5$, $NK_1 = 0.0953$, $NK_2 = 2.174$, $\bar{N}_i = 30$; $c_1 = c_2 = 30.0$ g/L; (♦) operating points for optimization, (▲) final operating point.

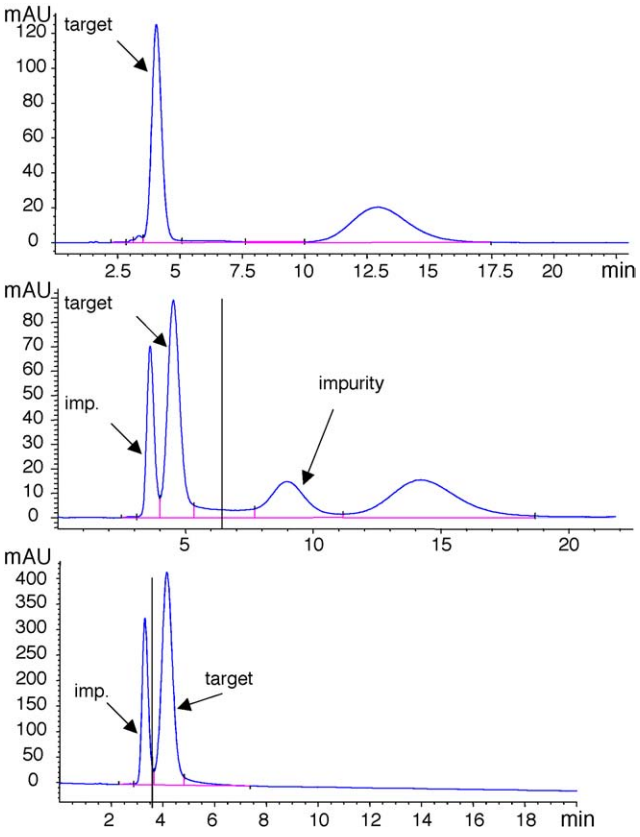

Fig. 7. Separation of compound C on Chiralpak AD, eluent: ethanol/DEA (100:0.1, v:v). Top trace: racemic compound C, middle trace: racemic compound C and two side products, lower trace: enantiomerically pure compound C with diastereomeric impurity eluting in front. Vertical lines in the two lower HPLC traces indicate the division between raffinate (left) and extract (right).

ture consisting of ethanol/DEA (100:0.1, v:v) as eluent. The target enantiomer was eluted first, while the non-target enantiomer eluted second (cf. Fig. 7, top trace). The productivity of the separation was 177 g racemate/day/kg CSP with an eluent consumption of 1926 L/kg crude/day, overall 45% of the target enantiomer with an ee of >99.9% could be isolated. When a larger amount of the compound was synthesized a significant over-reduction of an aromatic moiety in the molecule was observed. This side reaction resulted in two enantiomeric compounds, which eluted before and after the target compound (cf. Fig. 7, middle trace). In order to obtain the target enantiomer it became necessary first to remove the non-desired enantiomer and one of the new species via SMB chromatography and then to subject the pre-purified target enantiomer (cf. Fig. 7, lower trace) to a second SMB chromatographic step. The first separation step allowed for a specific productivity of 1.6 kg racemate/day/kg CSP with an eluent consumption of 185 L/kg crude/day, the second separation step allowed for a specific productivity of 2.4 kg racemate/day/kg CSP with an eluent consumption of 40 L/kg crude/day. The overall yield was 26% of the target enantiomer with an ee of >99.9%.

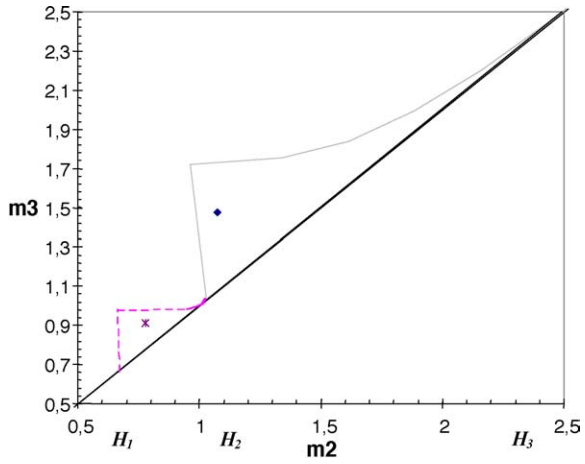

Fig. 8. Separation of compound C; regions in the m_2, m_3 plane with different separation regimes in terms of purity of the outlet streams. Predicted regions of complete separation: (——) for isotherm parameters: $\lambda = 0.9$, $NK_1 = 0.129$, $NK_2 = 1.802$, $\bar{N}_i = 40$; $c_1 = c_2 = 24.75$ g/L; (♦) operating point for enantiomer separation; (- - -) for isotherm parameters: $\lambda = 0.6$, $NK_1 = 0.069$, $NK_2 = 0.428$, $\bar{N}_i = 100$; $c_1 = c_2 = 24.75$ g/L; (✕) operating point for separation of enantiomer and impurity.

It should be noted that the specific productivity of the SMB separations is by a factor of 10 higher than observed for preparative HPLC, which easily justifies the two chromatographic runs in the SMB mode. SMB was also the method of choice for the purification of three more batches (without the reduction side product) subsequently submitted for enantioselective chromatography.

Fig. 8 shows the regions of complete separation for the chromatogram in Fig. 7 (middle trace). Two triangular regions can be observed, one for the separation of the target enantiomer and early eluting impurity (raffinate) from the later eluting impurity and non-target enantiomer (extract); the second (much smaller) for the separation of the two former compounds. The first operating point in the triangle with the basis H_2 and H_3 allowed to obtain streams with a purity of >99.9% in the extract and of 99.8% in the raffinate. The second separation—described by the triangle with the basis H_1 and H_2 yielded an extract purity of >99.9% (target enantiomer) and a raffinate purity of 97.8%.

The separation of compound C shows that the applicability of SMB is by far larger than often assumed. If a solvent/stationary phase system can be found, which allows for sufficient selectivity it can be used also for separations,

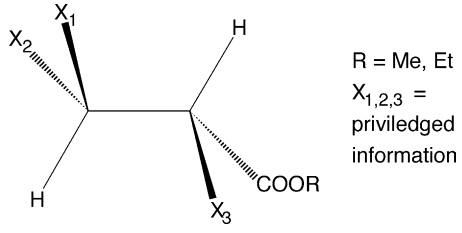

Fig. 9. Generalized structure of compound D.

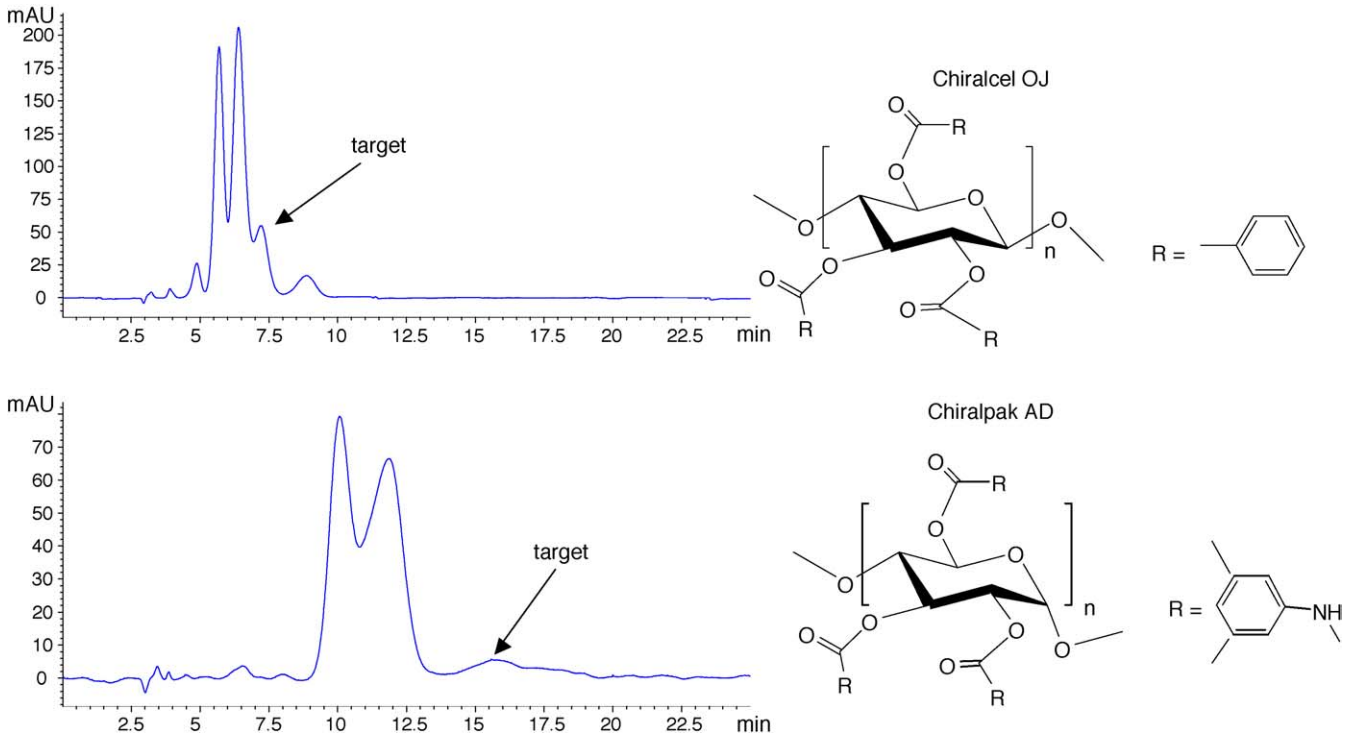

Fig. 10. Separation of compound D on Chiralcel OJ (top trace) and Chiralpak AD (bottom trace).

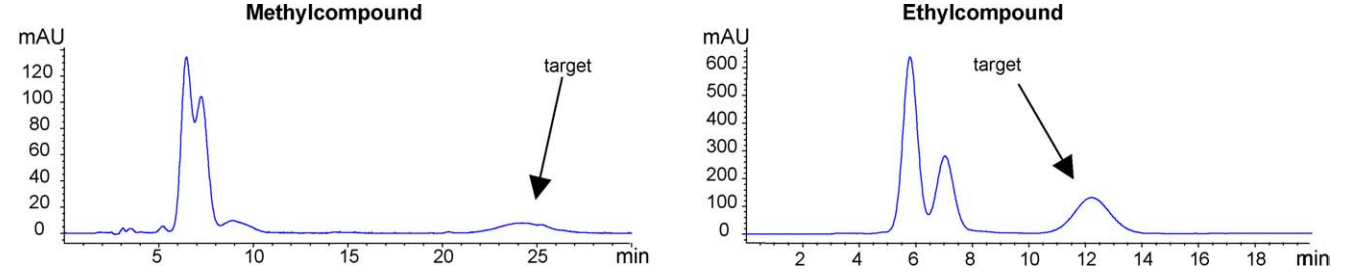

Fig. 11. Separation of compound D on Chiralpak AD (left methyl ester, right ethyl ester), eluent: methanol/ethanol (50:50, v:v) for the ethyl ester, ethanol/hexane (80:20, v:v) for the methyl compound. An arrow indicates the target *R*,*S* enantiomer.

which are less common than the typical racemate resolution.

3.4. Multi-component separations via SMB

Chemical development in the pharmaceutical industry is often driven by the objective to be "faster on the market". Therefore sometimes procedures from medicinal chemistry have to be transferred under high time pressure to larger scale, especially during the preclinical phase of the compounds, when only some 100 g are needed for toxicological studies. While no concessions to safety are acceptable, low yields and in some cases lower purity can be accepted for the time being, also under the perspective that more than 90% of all chemical entities never reach the first clinical phase.

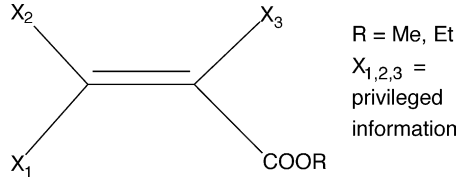

Fig. 12. Elimination product of compound D.

3.4.1. Case study IV: multi-component separations – complex scenarios

Compound D (cf. Fig. 9) is one of these very fast candidates in the drug development and was obtained in a six-step synthesis. Since compound D bears two chiral carbon atoms four diastereomers are formed following a non-stereoselective pathway for synthesis. However, only one of the enantiomers leads to the desired active pharmaceutical ingredient. Initial studies showed that the elution of the target enantiomer as isolated peak was not possible

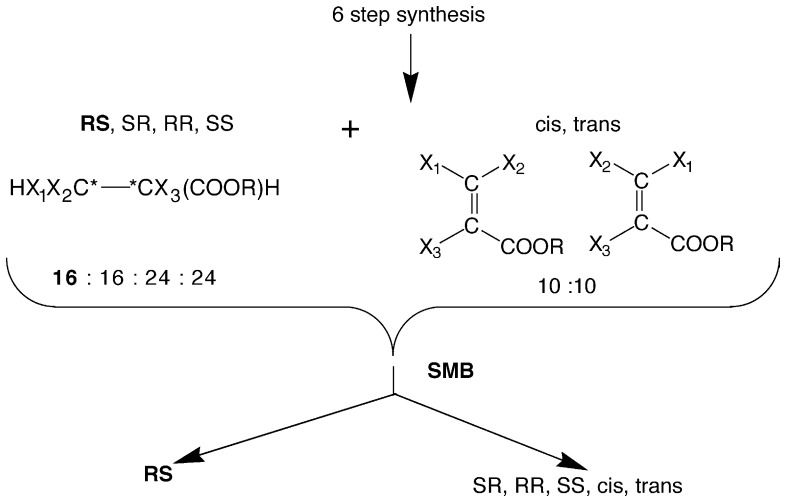

Fig. 13. Overall reaction scheme and distribution of compounds into extract and raffinate. The *R*,*S* enantiomer is eluted last and collected in the extract, all other compounds are collected in the raffinate.

Table 1
Comparison of multi-component SMB separations

Compound	CSP/mobile phase	Productivity of sepn. (g/kg CSP/day)	Yield (%)	Purity (%), ee (%)
Ethyl *R*,*S*	Chiralpak AD MeOH/EtOH 50:50	1005	90	76.4 (100)
Methyl *R*,*S*	Chiralpak AD EtOH/Hex 80:20	760	83	59.0 (100)
Methyl (wrong enantiomer) *S*,*R*	Chiralpak AD MeOH	1123	84	86.2 (100)

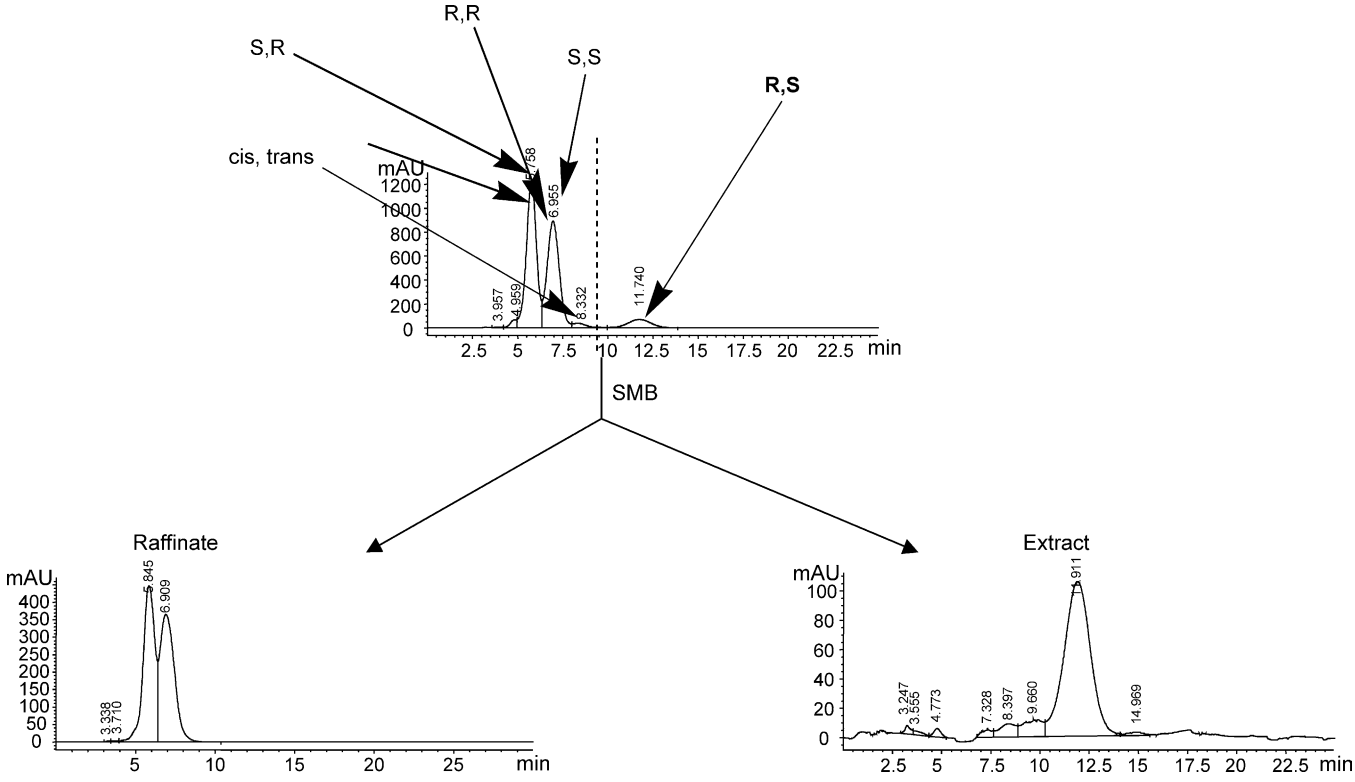

Fig. 14. Simultaneous separation of ethyl ester of compound D in non-target compounds (non-target enantiomer, diastereomers and two elimination products) and the target enantiomer on Chiralpak AD.

on Chiralcel OJ (cf. Fig. 10, top trace), but, after a thorough screening of commercially available stationary phases in combination with various mobile phases a simultaneous resolution of diastereomers and enantiomers could be obtained (cf. Fig. 10, bottom trace).

The target enantiomer (either methyl or ethyl ester with *R,S* configuration) (cf. Fig. 9) could be obtained as the last eluting peak on Chiralpak AD (cf. Fig. 11) using methanol/ethanol (50:50, v:v) as eluent.

The high selectivity for the separation allows to perform the resolution at 35 °C, thus decreasing k' values of the compounds and the viscosity of the mobile phase.

During scale-up of the reaction it was observed that two new side products were formed, namely the elimination products (*cis* and *trans*), whose chemical structure is shown in Fig. 12.

Overall six major components were present in the crude material submitted for enantioselective HPLC (cf. Fig. 13). The simultaneous resolution represents a shortcut to the conventional two step approach often employed for such problems, resolving first diastereomers and side products via crystallization or chromatography on achiral media, followed by an enantioselective purification employing HPLC on a CSP (or other methods). Such a one-step purification – necessitating the use of a CSP – is a time effective process requiring less unit operations than other resolution protocols and uses efficiently the stationary phase when the SMB technology is applied. However, it requires one to "binarize" the separation, i.e. to develop the separation in a way that only the target enantiomer elutes in one of the outlet streams of the SMB.

The first batch of compound D (1.63 kg, ethyl ester) had a chemical purity of 76.4% and displayed a solubility of 40 g/L in the eluent (methanol/ethanol, 50:50, v:v). All non-target compounds (non-target enantiomer, diastereomers and two elimination products) are baseline separated from the target enantiomer, which accounted to about 16% of the crude material (cf. Fig. 14).

The triangle theory was applied as described (cf. compounds A–C) for designing and optimizing the separation of compound D. For brevity only the results of the separations are summarized.

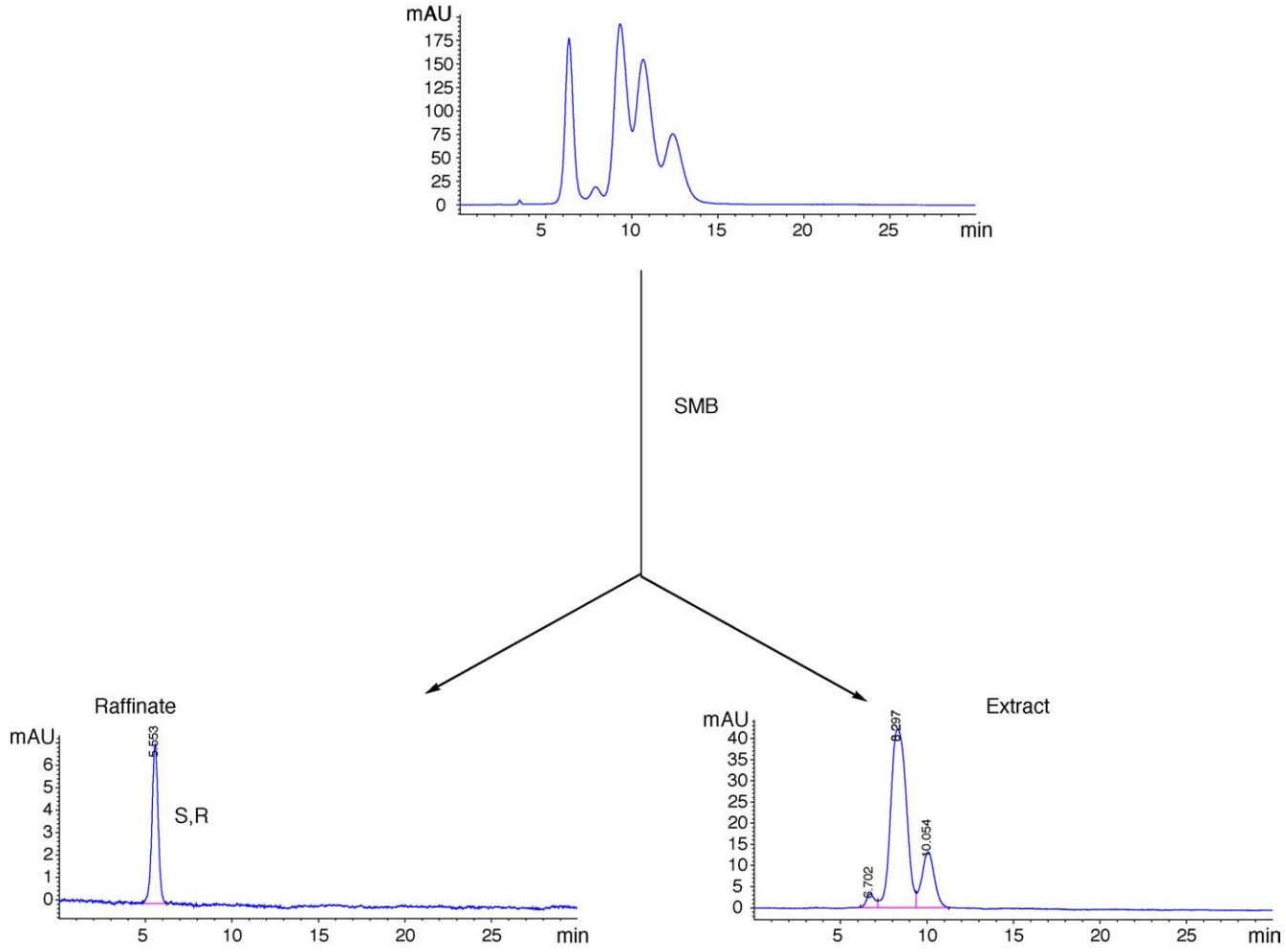

Fig. 15. Isolation of the non-target enantiomer (*S,R* methyl ester of compound D) on Chiralpak AD and methanol as eluent.

A total of 0.24 kg (overall yield: 90%) of the target enantiomer (*R*,*S* configuration (ethyl ester); ee >99.99%) were obtained using 380 L of eluent/kg crude compound D. This result is equivalent to a productivity of 1.0 kg crude/day/kg CSP.

The *R*,*S* methyl ester of compound D (2.13 kg with a chemical purity of 74.7%) could be separated in a similar way by employing ethanol/hexane (80:20, v:v) as eluent and Chiralpak AD as stationary phase (cf. Fig. 11, left chromatogram). Overall 0.26 kg (overall yield: 83%) of the target enantiomer (ee >99.99%) were obtained using 276 L of eluent/kg crude compound D. This result is equivalent to a productivity of 0.76 kg crude/day/kg CSP.

In view of the low overall yield for the *R*,*S* methyl ester of compound D it was decided to use the enantiomer of the target compound for formulation studies. It was possible to isolate the *S*,*R* enantiomer out of the raffinate stream of the first separation (1.26 kg with a chemical purity of 64.3%) by changing the eluent from ethanol/hexane (80:20, v:v) to methanol (100%) (cf. Fig. 15). Overall 0.24 kg (overall yield: 84%) of the non-target enantiomer (ee >99.99%) were obtained using 307 L of eluent/kg crude compound D. This result is equivalent to a productivity of 1.12 kg crude/day/kg CSP.

Table 1 summarizes the results of the three multi-component separations of compound D.

After two years, it was decided to stop all research activities devoted to finding an enantioselective synthesis for compound D as a result of the successful isolation of the target enantiomer(s) of compound D via multi-component SMB.

4. Conclusion

The separations of compounds A–D exemplify the potential the SMB technology has for resolving non 1:1 racemates and complex mixtures of structurally similar compounds in the pharmaceutical industry. If a chromatographic system can be designed that allows for the elution of the target compound as first or last peak, a straightforward approach ("cut through the chromatogram") leads directly to the desired isomer without prior isolation of diastereomers or racemate. Thus the SMB technology is not limited to racemate resolution or binary separations, but has a far greater potential for accelerating the drug development process than generally appreciated. Even two subsequent SMB separations can be faster and require less solvent than conventional preparative HPLC separations if the target compound is eluted between non-desired impurities.

However, it should be noted that a thorough screening of CSP/mobile phase combinations is a prerequisite for extending the use of the SMB principle to these less common applications. The triangle theory, developed 10 years ago by Mazzotti et al. [7], has become an indispensable tool to improve understanding of SMB units and for developing SMB separations in the pharmaceutical industry.

5. Nomenclature

c	mobile phase concentration
H	Henry constant
K	adsorption equilibrium constant
m	flow rate ratio, defined by Eq. (4)
n	adsorbed phase concentration
N	saturation capacity
Q	volumetric flow rate
t^*	switch time in a SMB unit
V	volume of a single column in a SMB unit

Greek letters

ε^*	overall void fraction of the bed
λ	linear coefficient of the modified Langmuir isotherm given by Eq. (1)

Subscripts

i	component index
j	section index

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