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Enantiomer Separation of a Novel Ca-Sensitizing Drug by Simulated Moving Bed (SMB) – Chromatography

Ralf M. Devant and Rochus Jonas

Darmstadt, Merck KGaA, Department of Medicinal Chemistry

Michael Schulte and Andreas Keil

Darmstadt, Merck KGaA, Division Laboratory Products

Frederic Charton

Vandoeuvre-lès Nancy Cedex (France), NOVA*SEP*

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Dedicated to Prof. Dr. E. Winterfeldt on the Occasion of this 65. Birthday

Abstract. The continuous chromatographic separation by means of Simulated Moving Bed (SMB) – Chromatography was used to produce the enantiomers of 5-(1,2,3,4-tetrahydroquinoline-6-yl)-6-methyl-3,6-dihydro-1,3,4-thiadiazin-2-one (EMD 53986), a precursor of the novel Ca-sensitizing drug 5-(1-(3,4-dimethoxybenzoyl)-1,2,3,4-tetrahydroquinoline-6-yl)-6-methyl-3,6-dihydro-1,3,4-thiadiazin-2-one (EMD 57033). The (+)-enantiomer EMD 57033 is a potent Ca-sensitizing drug, whereas its (–)-antipode is a pure phosphodiesterase-(PDE)-inhibitor without any Ca-sensitizing activity. A chromatographic separation of the enantiomers was developed on two different chiral stationary phases: a cellulose-tri-(*p*-methyl-benzoate) phase and a polymeric silica based stationary phase. A process simulation software was used to calculate the starting parameters for the SMB-system. For both stationary phases the SMB-parameters were optimized and pure enantiomers were produced using a system with 8 columns of 26 mm internal diameter. A comparison of the purities and throughput achieved with both stationary phases is given.

Continuous processes for the separation of enantiomers are known for a long time. Already in 1941 Martin and Kuhn described a system which was able to enrich one enantiomer of mandelic acid esters [1]. However, the breakthrough of continuous chromatographic systems came in the 1960's, with the introduction of the Sorbex-Process for the separation of xylene-isomers on a multi-ton/year scale.

Allthough the advantages of this system design was evident, it was not used for the production of enantiomerically pure fine chemicals and pharmaceuticals because it was not possible to meet the demand for high enantiomeric purities of the separated compounds. The scaling down of the Sorbex process caused considerable problems due to the dead-volumes inside the system which ruined the purity of the products. In the 1990's, Separex [2] introduced a new series of SMB-systems called Licosep, which overcame these problems by a combination of special system design and control software. With the Licosep systems, the separation of enantiomers can be achieved with a purity of the products > 99% ee, which is a must for pharmaceutical drugs.

The Simulated Moving Bed (SMB) – Chromatography is a continuous chromatographic process which simulates a countercurrent movement of stationary phase and mobile phase. A continuous operating mode is achieved by continuously feeding the eluent and the mixture to be separated into the system and by continuous withdrawal of the separated compounds (raffinate and extract) until a steady state is reached which is

then invariant during the separation process. The feeding and the withdrawal of the eluent and compound flows is achieved by four individual pumps. The main flow of the eluent is recycled within the system by means of a fifth pump (recycling pump) which adjusts the mobile phase flow rate in the stationary phase (Fig. 1). Due to this setup only small amounts of fresh eluent have to be feeded into the system to compensate for the withdrawal of extract and raffinate (feed + fresh eluent = raffinate + extract) and therefore the eluent consumption is considerably lower as compared to a discontinuous, batchwise operation mode.

Although different theoretical models can be used for designing a SMB-process, the four zone model with an adsorption- and a desorption zone for each of the compounds to be separated conveniently allows the separation of a two component mixture *e.g.* the enantiomers of a racemate. The four zones are defined with respect to the inlets and outlets of the eluent and compound flows (Fig. 1).

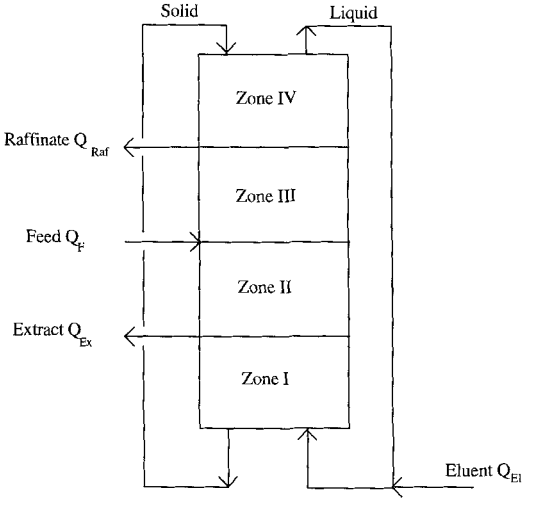

Fig. 1 Scheme of a four zone countercurrent adsorption process, showing the definition of the four zones with respect to the inlet (eluent and feed) and outlet (extract and raffinate) lines and the countercurrent of mobile and stationary phase.

In order to realise the countercurrent system, the stationary phase has to be divided into separate chromatographic columns which are connected in a cyclic series. Since a four zone model was used we chose two columns in each zone which adds up to a total of eight columns. Each column head is equiped with valves which allow the connection to the four lines: eluent, feed and the two separated compounds raffinate and extract. (Fig. 2). The simulation of the countercurrent movement of stationary phase and mobile phase is realized by shifting the inlet (eluent and feed) and outlet (raffinate and extract) lines after a given time (Δt) by one column in the direction of the mobile phase thus simulating the movement of the stationary phase in the opposite direction. After a complete cycle the four lines reach again their initial positions. To run the SMB system requires a set of starting parameters which are obtained by calculation with a process simulation software. Running the system for several cycles allows the system to reach a steady state characterized by an internal concentration profile which is invariant in time. Determination and analysis of this internal profile allows the optimization of the system performance.

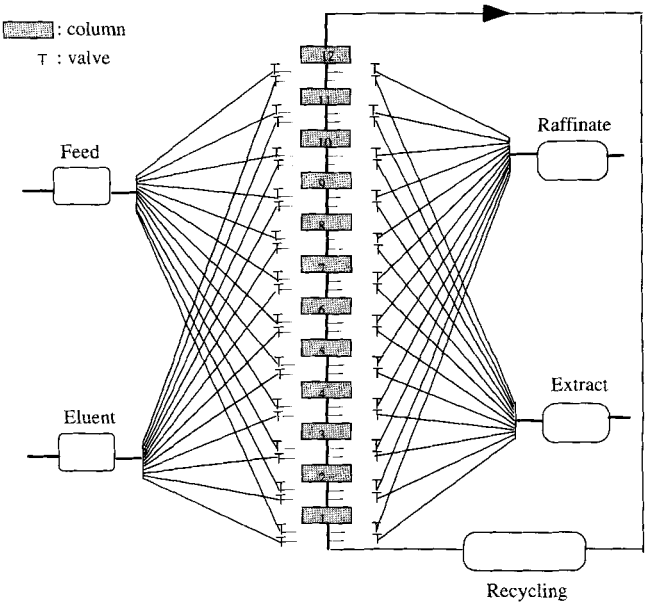

Fig. 2 LICOSEP 12–26 system, consisting of five pumps (eluent, feed, extract, raffinate and recycling) and 8–12 columns which are connected to each pump.

EMD 57033 and its Synthesis Intermediate EMD 53986

5-(1-(3,4-dimethoxybenzoyl)-1,2,3,4-tetrahydroquinoline-6-yl)-6-methyl-3,6-dihydro-1,3,4-thiadiazine-2-one (EMD 57033) is the first compound of a new class of cardiotonic agents that shows a highly interesting dual mechanism of action. The (+) - enantiomer EMD 57033 (Fig. 3) is a potent Ca-sensitizing drug, whereas the (–)-enantiomer is a pure phosphodiesterase-(PDE)-inhibitor devoid of any Ca-sensitizing activity [3]. The two enantiomers were initially prepared via kinetic resolution of a diastereomeric camphanic acid derivative [4]. However, for the preparation of larger amounts the chromatographic enantiomer separation of the synthesis intermediate 5-(1,2,3,4-tetrahydroquinoline-6-yl)-6-methyl-3,6-dihydro-1,3,4-thiadiazine-2-one EMD 53986 (Fig. 4, [5]) turned out to be a more convenient process.

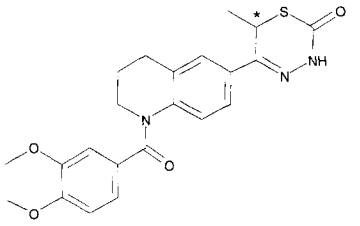

Fig. 3 Chemical structure of 5-(1-(3,4-dimethoxybenzoyl)-1,2,3,4-tetrahydroquinoline-6-yl)-6-methyl-3,6-dihydro-1,3,4-thiadiazine-2-one (EMD 57033).

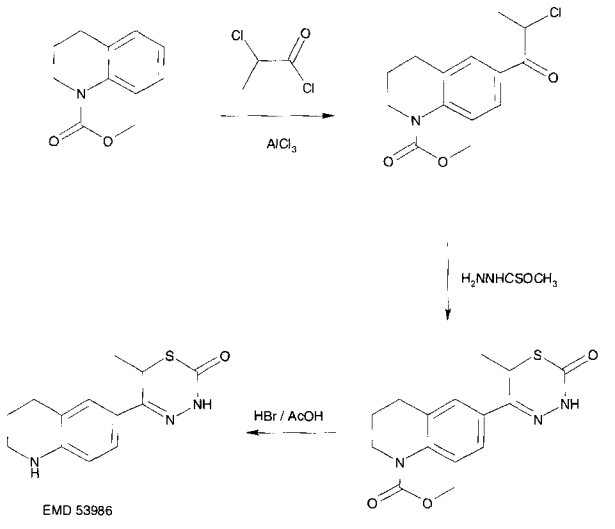

Fig. 4 Synthesis of 5-(1,2,3,4-tetrahydroquinoline-6-yl)-6-methyl-3,6-dihydro-1,3,4-thiadiazine-2-one (EMD 53986).

Results and Discussion

Routine screening for chromatographic enantiomer separation of the synthesis precursors of EMD 57033 revealed, that EMD 53986 can be nicely separated on several chiral stationary phases. Two CSP's were selected as being suitable for a preparative scale separation on a SMB system.

Separation of the enantiomers, with an α-value of 1.8, was achieved on a CSP with cellulose-tri-(*p*-methyl-benzoate) as the chiral selector (Fig. 5). The CSP was prepared by suspension polymerization which leads to a stationary phase consisting of Cellulose beads [6].

Baseline separation was also found on a stationary phase with a poly-[*N*-acryloyl-aminoacid-ester] as the chiral selector (Chiraspher™ Type). This CSP could separate the racemate in 100% THF as the mobile phase. However, due to its unfavourable properties (*e.g.* peroxide formation), THF is not a preferred mobile phase for preparative chromatography. Fortunately, also with

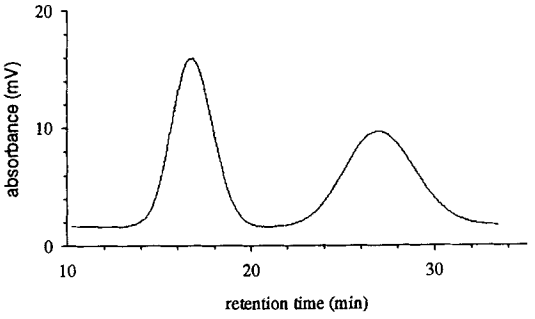

Fig. 5 Separation of 5-(1,2,3,4-tetrahydroquinoline-6-yl)-6-methyl-3,6-dihydro-1,3,4-thiadiazine-2-one (EMD 53986) on cellulose-tri-(*p*-methyl-benzoate); column dimensions: 125 × 4 mm; mobile phase: methanol; flow rate: 0.8 ml/min; detection: UV 254 nm.

100% ethylacetate as the mobile phase a separation with an α-value of 12 could be achieved. This α-value was by far too high to be used for an enantiomer separation on a SMB-system. The long retention time of the second enantiomer would cause a drastic increase in eluent consumption and leads to unfavourable economical properties of the separation. Therefore the retention times were decreased by adding 5% ethanol. With this eluent system an α-value of 3.30 was obtained (Fig. 6).

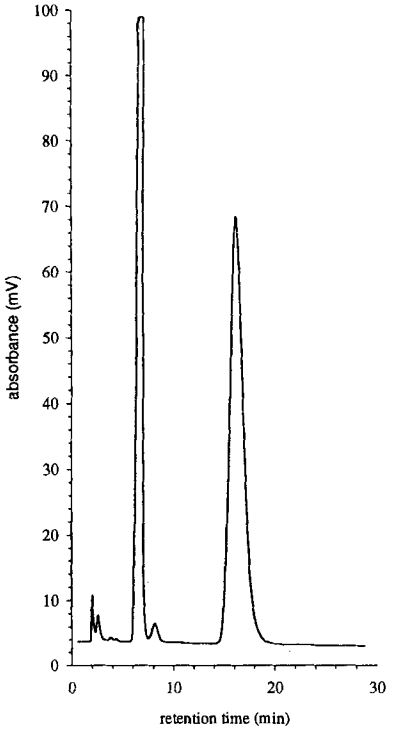

Fig. 6 Separation of of 5-(1,2,3,4-tetrahydroquinoline-6-yl)-6-methyl-3,6-dihydro-1,3,4-thiadiazine-2-one (EMD 53986) on a poly-[*N*-acryloyl-aminoacid-ester] chiral stationary phase; column dimensions: 125×4 mm; mobile phase: ethylacetate/ethanol 95/5; flow rate: 1.0 ml/min; detection: UV 254 nm

Running a separation on a SMB-system under optimized conditions requires the simultaneous adjustment of 6 independent parameters (5 flow rates and 1 valve switching time). It is evident that such a complex system cannot be effectively operated by experiments based on trial and error. For the determination of initial values for the system parameters, and their further optimization during the running separation process, it is necessary to estimate the adsorption isotherms of the compounds to be separated. With this data and a simulation software (HELP in the case of the Separex systems) it is possible to calculate proper starting parameters.

Different methods for determining adsorption isotherms are described in the literature, each of them having its advantages and disadvantages [7]. A quick and convenient method is the injection of increasing amounts and determination of the isotherms from the retention times and peak shapes of the different compounds [8]. This method known as Elution by characteristic points (ECP) [9] can be used when a baseline separation of the two enantiomers with a high plate number N can be achieved.

To determine the adsorption isotherms of the enantiomers of EMD 53986 on the poly-[*N*-acryloyl-aminoacidester] CSP, samples with different concentrations of the racemate were injected into an analytical HPLC system. Three resulting chromatograms are overlayed to show the changes in retention times and peak form (Fig. 7).

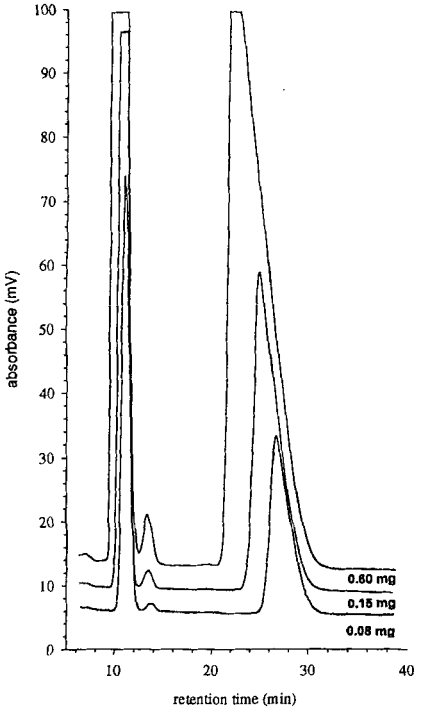

Fig. 7 Injection of 1.5/3.0/12.0 mg of EMD 53986 on a poly-[*N*-acryloyl-aminoacidester] chiral stationary phase; injection volume: 200 µl; column dimensions: 125×4 mm; detection UV 254 nm, mobile phase: ethyl-acetate/ethanol 95/5; flow rate: 0.5 ml/min (The impurity eluting at 13.0 min causes no problems in the SMB separation since it is eluted with the raffinate, which is the non desired enantiomer)

The resulting chromatograms show a decrease of the retention times and a slight decrease of the α-value. The detailed results are shown in figure 8.

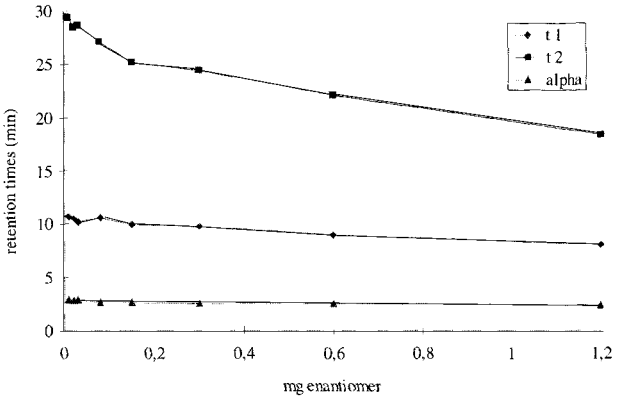

Fig. 8 Injection of increasing amounts of of 5-(1,2,3,4-tetrahydroquinoline-6-yl)-6-methyl-3,6-dihydro-1,3,4-thiadiazine-2-one (EMD 53986) on a poly-[*N*-acryloyl-aminoacidester] chiral stationary phase.

The adsorption isotherms of the enantiomers of EMD 53986 on cellulose-tri-(*p*-methyl)-benzoate CSP were determined by the adsorption–desorption method, where the column is equilibrated with a solution of the racemate followed by the determination of the amount of solute which was stored on the column. The procedure was repeated for solutions with different defined concentrations (Fig. 9). Equation 1 allows to calculate the amount of solute in the stationary phase:

$$\overline{C}_i = (Q_i - \varepsilon \times V \times C_i) / (1 - \varepsilon) \times V \qquad (1)$$

with: Q_i = material stored on the column (mg)
V = column volume (ml)
ε = external porosity
C_i = concentration in mobile phase (g/l)
$\overline{C}_i$ = concentration in stationary phase (g/l of CSP)

In order to describe the chromatographic behaviour of the enantiomers the set of $\overline{C}_i/C_i$- values were fitted to an appropriate isotherm model. For most racemate separations the modified Langmuir isotherm (Eq. 2) is suitable.

$$\bar{C}_i = \bar{k}_i \cdot C_i + \frac{\bar{N} \cdot \tilde{K}_i \cdot C_i}{1 + \sum_j \tilde{K}_j \cdot C_j} \quad (2)$$

with: $\bar{C}_i$ = stationary phase concentration of component *i*
$\bar{k}_i$ = linear term (non-selective adsorption)
C_i = mobile phase concentration of component *i*
$\bar{N}$ = saturation capacity of the packing
$\tilde{K}_i$ = adsorption constant

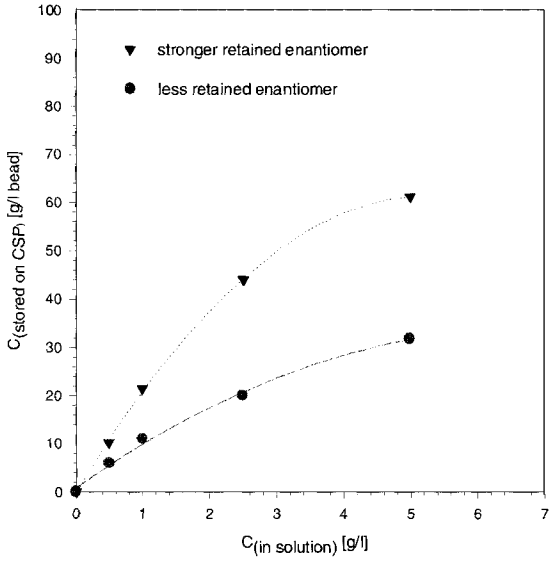

Fig. 9 Adsorption isotherms of the enantiomers of 5-(1,2,3,4-tetrahydroquinoline-6-yl)-6-methyl-3,6-dihydro-1,3,4-thiadiazine-2-one (EMD 53986) on the cellulose-tri-(p-methyl)-benzoate chiral stationary phase.

Using the adsorption isotherms and the appropriate isotherm model as the input, the process simulation software calculates initial values for the 6 system parameters. After running the system for several cycles, a steady state was reached and the internal concentration profile was determined. (Figure 10a–c). Since a SMB system with 8 columns was used, a complete cycle consists of 8 periods. At the end of each period the inlet and outlet lines are switched by one column. The concentration profile was determined over a complete cycle by taking samples in the middle of each period through a 6-port valve. The samples were analyzed by HPLC for the concentration of both enantiomers (Table 1, initial values). Based on this internal concentration profile the simulation software calculates optimized system parameters. The two steps, determining the concentration profile and calculating optimized system parameters can be repeated until the required product purities are

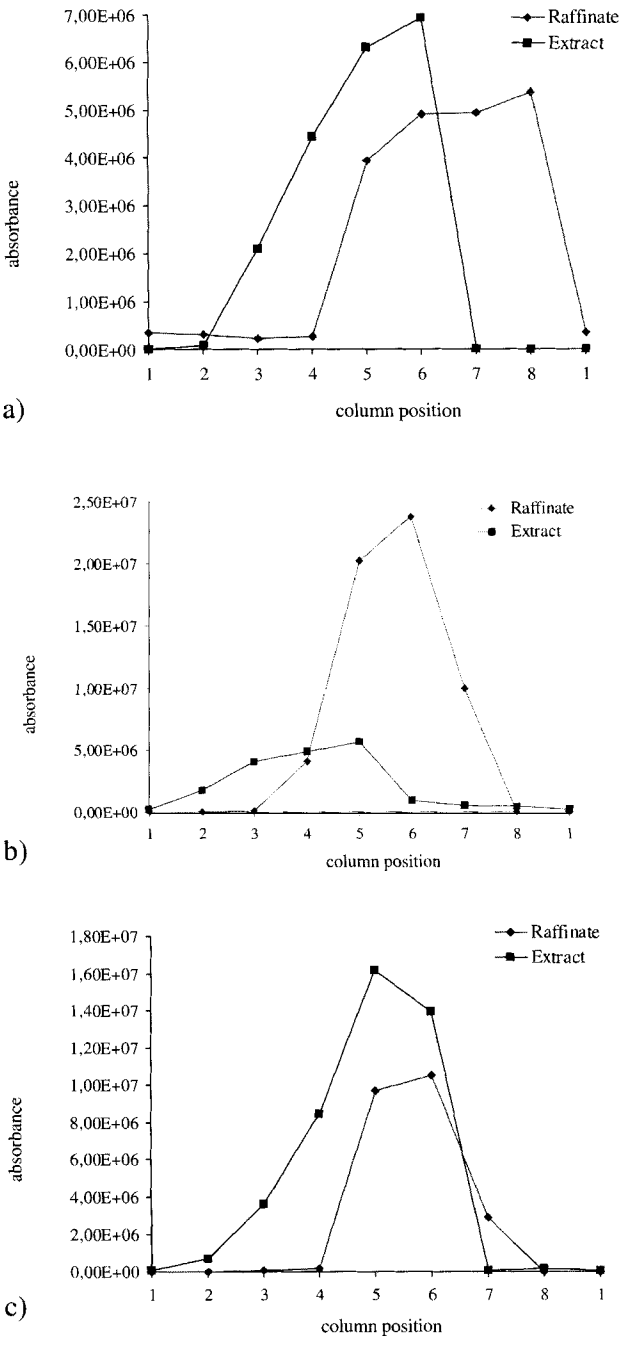

Fig. 10 a) Concentration profile of the enantiomers of EMD 53986 on a Celluloseester bead chiral stationary phase (initial values of the system parameters). b) Concentration profile of the enantiomers of EMD 53986 on a Poly-[*N*-acryloyl-aminoacid ester] chiral stationary phase (initial values of the system parameters). c) Concentration profile of the enantiomers of EMD 53986 on a Poly-[*N*-acryloyl-aminoacidester] chiral stationary phase (optimized values of the system parameters).

Table 1 Comparison of the system parameters for the separation of EMD 53986 on two different chiral stationary phases. Cellulose-tri-(*p*-methyl-benzoate) and -poly-[*N*-acryloyl-aminoacid-ester]

	Cellulose-tri-(*p*-methyl-benzoate)		Poly-[*N*-acryloyl-aminoacidester]	
	initial values	optimized values	initial values	optimized values
recycling flow rate (ml/min)	48.94	46.50	32.0	31.95
feed flow rate (ml/min)	5.66	5.65	1.84	1.66
feed concentration (g/l)	7.5	5.0	12.0	12.0
extract flow rate (ml/min)	26.28	24.47	20.64	18.63
raffinate flow rate (ml/min)	5.89	7.75	4.0	6.70
eluent flow rate (ml/min)	26.51	26.57	22.80	23.67
switching time (min)	16.36	15.50	6.33	7.03
purity of extract	16 : 84	2 : 98	3.3 : 96.7	< 0.5 : 99.5
purity of raffinate	90 : 10	> 99.5 :0.5	96.5 : 3.5	86.9 : 13.1

Table 2 Comparison of Celluloseester-Beads and Poly-[*N*-acryloyl-aminoacidester] as chiral stationary phases for the separation of EMD 53986

	Cellulose-tri-(*p*-methyl-benzoate)	Poly-[*N*-acryloyl-aminoacidester]
Column dimension (mm)	8 × (100 × 26)	8 × (54 × 26)
Amount of stationary phase (g)	210	90
Processing of Feed (g Racemate/d)	40.7	30.0
Eluent consumption (l/g Enantiomer)	2.28	2.43
Specific Productivity (g Enant./d × kg CSP)	98.6	166.6

reached. Since mainly the Ca-sensitizing (+)-enantiomer is of interest, the system parameters for the separation of the precursor EMD 53986 were optimized for a high enantiomeric excess of the extract (Table 1, optimized values).

Table 2 shows a comparison of the performance of the SMB system with the two different chiral stationary phases. Using the silica bonded Poly-[*N*-acryloyl aminoacidester] as the chiral stationary phase, a preparative separation of EMD 53986 was performed for a period of over two month.

Conclusions

From the enantiomer separation of the racemate EMD 53986 with SMB-chromatography on two different chiral stationary phases, some interesting results can be derived:

- It was possible to achieve high purities on different chiral stationary phases. However, the purity was higher for the silica-bonded Poly-[*N*-acryloyl-aminoacidester]phase.
- Although the eluent consumption per g of enantiomer was nearly the same for both CSPs, the specific productivity was much higher for the silica-bonded phase.

These results lead to some requirements for the CSPs that are suitable for SMB-chromatography:

- The CSP should have a high capacity rather than a very high selectivity.
- The CSP should be stable against a wide variety of mobile phases, because the compounds which are to be separated have to be dissolved in the mobile phase. A different feed-solvent would crucially influence the internal concentration profile in the steady state.
- The SMB process is designed for long production periods which renders the separation of large amounts of enantiomers possible. Therefore the CSP should have a high long term stability.

As a general conclusion it could be shown that SMB-Chromatography is a powerful tool for the production of enantiomerically pure compounds on a large scale within a short development time.

Experimental

The analytical HPLC systems consists of a Merck–Hitachi D-6200 Intelligent Pump, a Merck–Hitachi D-4200 UV-VIS-detector, a Merck-Hitachi D-2500 Chromato-Integrator and a Rheodyne 7125 injection system.

The SMB-system used was a Licosep 12×26 system (NOVA*SEP*, Vandœuvre-lès-Nancy), equipped with 8 Superformance™ glass columns of 26 mm internal diameter and variable length. The withdrawal of samples was performed through a 6-port valve.

For analytical HPLC the chiral stationary phases were slurry-packed at 300 bar into 125×4 mm stainless steel columns

Merck Hibar™, with isopropanol as the slurry liquid. For the use in the SMB-system the stationary phases were vacuum packed into 8 Merck Superformance™ glass columns, using the mobile phase of the separation as the slurry liquid. The retention times of the enantiomers to be separated were tested on each individuell column. The differences in retention times were lower than 5%.

The cellulose-tri-(*p*-methyl-benzoate) beads were prepared according to literature 5 and the diploma thesis of A. Delp, Darmstadt, 1991 [10].

The poly-[*N*-acryloyl-aminoacidester]-CSP was a kind gift of R.Grosser, BAYER AG, ZF-TVG 5, Leverkusen.

All mobile phases were from Merck and at least of chromatography grade. They were filtered before use to avoid particels in the SMB-system.

Determination of adsorption isotherms

Elution by the characteristic point (ECP)- method: Solutions with different concentrations of the racemate to be separated were prepared, up to the solubility limit of the racemate. 20 µl of each of the solutions were injected into the analytical HPLC-system equiped with the analytical HPLC-column of the dimensions 125 mm length × 4 mm i.d. Chromatograms were recorded and used for further estimation of the adsorption isotherm.

Adsorption-Desorption-method: The analytical HPLC-column was flushed with a solution of known concentration, until equilibrium was reached. Than the column was bypassed and the system cleaned with fresh eluent. After reconnecting the column the amount of racemate stored on the column was rinsed off with fresh eluent which was collected in a measuring flask. After filling up to a defined volume the concentration of the two enantiomers in the solution was determined by analytical HPLC of an aliquot sample. The procedure was repeated with 4 solutions of different concentrations.

In-process control of the SMB-system: For the determination of an internal concentration profile, samples were withdrawn from the system at halftime of each period through the 6-port-valve. The samples were analysed with the analytical system described above and correlated with their respective column position. The raffinate and extract were collected over a whole cycle and the purities of the enantiomers was analysed.

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[5] Europ. Pat. 0294647 (25.5.1988): Chem. Abstr. 120223-14-5; Analytical Data for 5-(1,2,3,4-tetrahydroquinoline-6-yl)-6-methyl-3,6-dihydro-1,3,4-thiadiazine-2-one EMD 53986 (racemate): *m.p.* 174–175 °C. – MS (*m/z*): 261 [M⁺]. – ^{1}H NMR (200 MHz, DMSO-D_6): δ 1.48 (d, 3H, CH_3), 1.80 (m, 2H, CH_2), 2.70 (m, 2H, CH_2), 3.22 (m, 2H, CH_2), 4.57 (q, 1H, CH), 6.20 (1H, NH), 6.45 (1H, arom.), 7.63 (2H, arom.). – (+) enantiomer: *m.p.* 154–155 °C. $[\alpha]_D^{20} = +1009.8°$(*c* = 1, methanol).

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Address for correspondence:
Dr. Ralf M. Devant
Merck KGaA
Department of Medicinal Chemistry
Frankfurter Str. 250
D-64271 Darmstadt
Email: devant@merck.de