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Comparison of the specific productivity of different chiral stationary phases used for simulated moving-bed chromatography

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Abstract

The use of preparative chromatography for the separation of enantiomers is now a well established technique. With the technical setup of simulated moving bed-chromatography significant improvements can be achieved regarding throughput and eluent consumption. To find the optimum separation system, the determination of some parameters with analytical chromatographic methods is sufficient. This article gives a procedure to optimize the separation conditions for a precursor of the novel Ca-sensitizing drug (EMD 53998) on four different chiral stationary phases. This analytical procedure led to a maximum specific productivity for the desired enantiomer of 430 g enantiomer/d·kg chiral stationary phase.

Keywords: Chiral stationary phases, LC; Simulated moving-bed chromatography; Enantiomer separation; Preparative chromatography

1. Introduction

Since the production of pure enantiomers of a pharmaceutical compound in a short time and with very high enantiomeric purity is more and more necessary for the pharmaceutical industry [1], the use of continuous process chromatography is recognized as a very versatile tool to face this challenge. From the process chromatography modes the use of simulated moving bed (SMB) chromatography is one of the most suitable ways to produce pure enantiomers because of its ability to be designed and used in a very economic way [2].

Unlike analytical separation of enantiomers, the separation has to be carried out in a solvent with a high solubility for the racemate. Therefore it is absolutely necessary to choose the right combination of suitable chiral stationary phase (CSP) and optimum mobile phase for a given separation.

There is almost no possibility of establishing a process with sufficient economy experimentally, meaning by way of trial and error. With only the advantages of modelling of non-linear chromatographic behaviour and a few experiments, selected from the results of the modelling study and the experience of the user, an optimum separation process can be designed.

A route to find such an optimum process is described.

2. Experimental

Analytical HPLC system: Merck–Hitachi L-6200 Intelligent pump; Merck–Hitachi L-4000 UV–Vis-detector, Merck–Hitachi D-2500 Chromato-Integra-

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tor; sample injection system: Rheodyne 7125; SMB-system: Separex Licosep 12-26; simulation software: Separex HELP; mobile phases: methanol, ethanol and ethyl acetate were of Merck LiChrosolv quality; analytical stationary phases were slurry-packed into 125×4 mm I.D. Hibar steel columns, preparative stationary phases were vacuum packed into 300×26 mm Superformance glass columns.

2.1. How to choose a CSP/eluent combination

Since the first CSPs became available in the 1980s many more have made their way onto the market and a lot more were described in the literature [3]. Taking into account the variety of different solvents which are suitable as mobile phases for chromatography, a multitude of combinations of CSPs and eluents has to be screened to find the optimum combination for each single separation problem (Table 1).

It is obvious that a screening procedure has to focus first on the discovery of a CSP that is able to separate the racemate. This is still a field of experience and knowledge, also there are first screening routines established to automate this procedure. Once a suitable CSP with a maximum α value is found, the choice of the best eluent has to be made. This choice is forced by the fact that a solvent with a maximum solubility for the racemate has to be found, and limited by the stability of some of the CSPs against some eluents.

At this point a CSP with the best α value and a solvent with the best solubility for the racemate to separate are known. But are they the best of all possible combinations? It is clearly known from the literature and our experience that different CSPs have very different properties with different solvents. Saturation capacity and the mass transfer kinetics in particular are highly affected by the CSP/eluent combination. So the definition of the best suitable combination is difficult and we need some rigorous methodology. The solution of this problem is in the modelling/simulation of non-linear chromatography.

2.2. The methodology: modelling of multicomponent chromatography

The behaviour of a chromatographic system is governed by three basic phenomena:

(1) The adsorption thermodynamics, described by equilibrium isotherms which give the composition in the stationary phase versus the composition in the mobile phase when equilibrium is reached, at given temperature. For each different CSP the individual enantioselective saturation capacity can be derived from the isotherms.

(2) The column hydrodynamics, i.e., the properties of the flow through the porous medium.

(3) The mass transfer kinetics.

To model multicomponent chromatography means associating a mathematical equation with each of these three basic phenomena. The way to solve this problem we have reported previously [4].

For this difficult task simulation software is now available to calculate the operating conditions of a SMB. The software in fact solves numerically the mass balance equations written over a column slice. To be accurate it is absolutely necessary to take into account the non-linear effects related to the adsorption thermodynamics.

The methodology is then the following:

(1) Measuring of some basic data characterizing the system studied. The main data to be measured are the adsorption isotherms.

(2) Using the previous data and running the simulation software. This presents operating conditions and performances to the user.

2.2.1. Determination of adsorption isotherms

To determine the adsorption isotherm of a compound, different methods are suitable. If, in the case of a chiral separation, a baseline separation can be obtained, the determination of isotherms can be done by some very easy methods.

Starting from an analytical type injection the adsorption isotherms are derived from the injection of increasing amounts of the solution to be separated using the method known as peak maxima method. When the amounts injected are increased, the retention time of the peak maxima are shifted towards lower values. These values are related to the amount injected and to the adsorption isotherms through well-known mathematical equations [5].

Fitting together experimental retention times and calculated retention times allows one to reach the adsorption isotherm parameters.

In most cases the adsorption isotherms of enantio-

Table 1
Suitable combinations of CSP and eluents □

CSP	Eluent											
	n-Heptane	*n*-Hexane	Dioxane	Tetrahydro-furan	Methyl *tert.*-butyl ether	ETAT	Toluene	EtOH	MeOH	2-PrOH	CH_3CN	Water
Microcrystalline cellulose-triacetate/-tribenzoate								□	□			□
Porous cellulose-ester beads	□	□	□	□				□	□	□		
Cellulose ester + carbamates coated on silica	□	□	□	□				□	□	□		□
Polyacrylamide ester beads	□	□	□	□	□	□	□	□	□	□		
Polyacrylamide ester/silica-composites	□	□	□	□	□	□	□	□	□	□	□	□
Brush-type-CSP (Pirkle-type)	□	□	□	□	□	□	□	□	□	□	□	□
Cyclodextrin CSP								□	□	□		□
Ligand-exchange CSP												□
Crown ether CSP								□	□	□	□	□

Table 2
Experimental results of the study for determination of adsorption isotherms

	Cellulose-(*p*-methyl)tribenzoate beads	Poly(N-acryloyl-amino acid ester)silica CSP	Cellulose-(*p*-methyl)-tribenzoate coated on silica (Chiralcel OJ)	Amylose-(3,5-dimethyl)-phenyl-carbamate coated on silica (Chiralpak AD)
Column dimensions (mm)	125×4	125×4	125×4	125×4
Eluent	Methanol 100%	Ethyl acetate–ethanol (95:5)	Ethanol 100%	Ethanol 100%
Temperature (°C)	25	25	25	25
Particle diameter (μm)	20–30	10	20	20
Maximum solubility (g racemate/l)	8	13	8	8
Pressure drop (1.5 ml/min)	2	18	23	19
Number of plates N (1.5 ml/min)	43	521	185	130
k' (first enantiomer)	5.97	8.23	4.78	6.14
Selectivity α (1.5 ml/min)	1.84	2.82	1.98	3.10
Enantioselective saturation capacity (g/l)	50	10	12.5	12.5

mers can be accurately described by a modified competitive Langmuir isotherm equation written as:

$$\bar{C}_i = \lambda \cdot C_i + \frac{\bar{N} \cdot \bar{K}_i \cdot C_i}{1 + \sum_j \bar{K}_j \cdot C_j}$$

where C_i, $\bar{C}_i$ are respectively the concentrations of i in the liquid and solid-phases at equilibrium.

Besides the information given by the recorded chromatograms it is only necessary to determine the data given in Table 2.

2.3. The separation studied

For a comprehensive study of different CSP the separation of the experimental drug EMD 53986 was chosen. EMD 53986 is a precursor of the novel Ca-sensitizing drug EMD 53998, which exhibits strongly different pharmacological behaviour for the two enantiomers [6]. This compound could be separated on a variety of CSP of a different type, including amylose and cellulose derivatives coated on silica, bulk polymeric cellulose derivatives and polyacrylamide–amino acids bonded on silica (Fig. 1a–d).

As an example for the determination of adsorption isotherms by the retention time method, the chromatograms of the separation of EMD 53986 on an amylose-tris(3,5-dimethylphenylcarbamate) CSP (Chiralpak AD) are shown in Fig. 2a–c.

3. Results

The series of experiments necessary for the determination of the adsorption isotherms was done for the four suitable CSPs. The results are summarized in Table 2.

Some interesting results can be obtained from the experimental data in Table 2: with a comparable α value for all four CSPs of >1.8, the enantioselective saturation capacity is the highest for the Cellulose-(*p*-methyl)tribenzoate beads, because of their total porous structure, with the highest amount of chiral selector per mass unit. On the other hand the number of plates is the lowest for this CSP. As a consequence the columns for a SMB system equipped with this CSP have to be longer than for other stationary phases. This leads to unfavorable pressure drop and slower internal flow-rates.

For the poly(N-acryloyl amino acid ester)silica CSP the solubility in the suitable mobile phase is the highest, which gives some advantages for this CSP.

3.1. SMB Parameters

Starting with the results from the analytical study, the SMB parameters were determined by means of modelling and simulation of non-linear chromatography. For this purpose the very powerful simulation software HELP was used.

The results of the modelling study led to the SMB parameters summarized in Table 3. With this data it is possible to choose the optimum combination of CSP and eluent for a preparative separation of EMD 53986.

The details of the preparative separation process onto the SMB system are given in another publication [7].

Comparing the results for the four different stationary phases some conclusions can be derived from Table 3:

(1) All four CSPs are suitable to produce the desired enantiomer in a purity of >99%, even though there are some differences in the performances of the stationary phases.

(2) The specific eluent consumption is comparable for all phases. The lowest eluent consumption is obtained for the Chiralcel OJ phase because this phase shows the shortest retention times for the enantiomers. This shows clearly that a short retention time with a sufficient α value leads to a favorable economic situation.

On the other hand the period time for the cellulose-(*p*-methyl)tribenzoate beads is the longest, because of the highest k_2' value for this phase, but this does not affect the productivity. Because of the low plate number the column length for the cellulose-(*p*-methyl)tribenzoate beads is larger, and consequently a higher amount of stationary phase is needed. Also the flow-rates for this CSP are the lowest, due to limited mass transfer kinetics of this phase. As a consequence the specific productivity is the highest for the Chiralpak AD column: this column has a

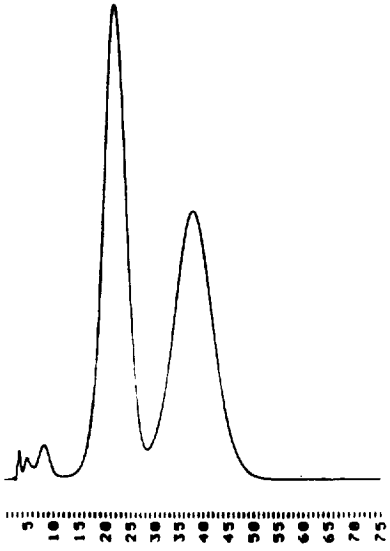

figure 1 - a

figure 1 - b

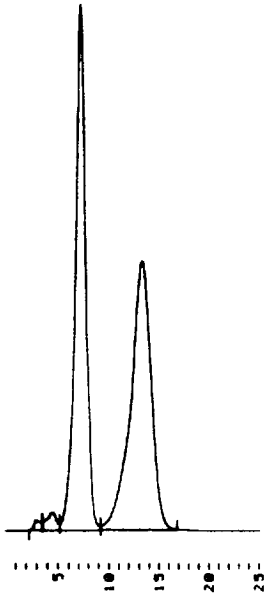

figure 1 - c

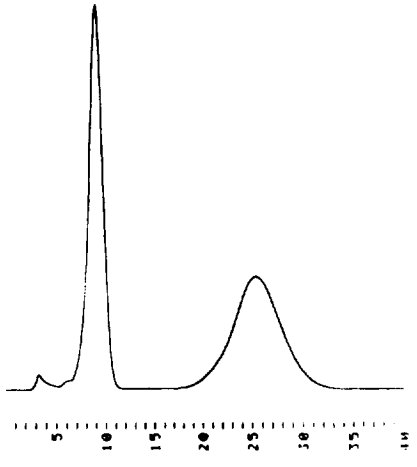

figure 1 - d

Fig. 1. (a) Separation on cellulose-(*p*-methyl)tribenzoate beads; mobile phase, methanol; flow-rate, 0.5 ml/min. (b) Separation on poly(N-acryloyl-amino acid ester)silica CSP; mobile phase, ethyl acetate–ethanol (95:5); flow-rate, 1.5 ml/min. (c) Separation on cellulose-(*p*-methyl)tribenzoate coated on silica (Chiralcel OJ); mobile phase, ethanol; flow-rate, 0.5 ml/min. (d) Separation on amylose-(3,5-dimethyl)phenyl carbamate coated on silica (Chiralpak AD); mobile phase, ethanol; flow-rate, 0.5 ml/min.

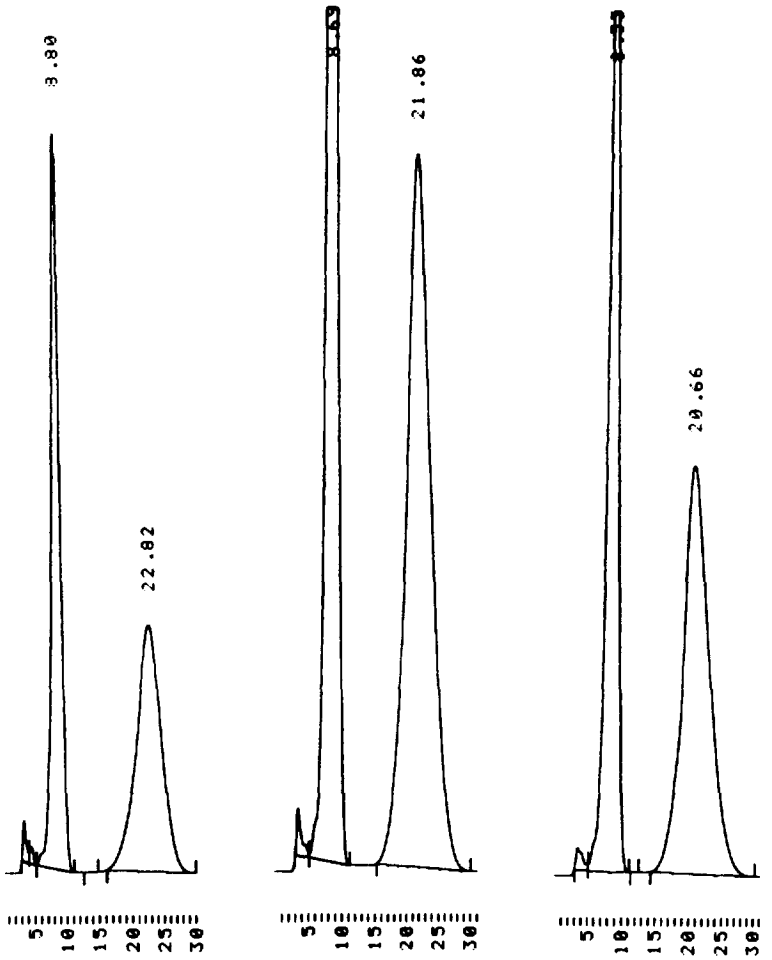

Figure 2 - a Figure 2 - b Figure 2 - c

Fig. 2. Separation of different amounts of EMD 53986 on Chiralpak AD. Column dimension, 125×4 mm; mobile phase, ethanol; flow-rate, 0.5 ml/min; detection, UV 254 nm; amount injected, 50 μl; concentrations, (a) 1.1 mg/ml, (b) 3.15 mg/ml, and (c) 7.26 mg/ml.

moderate capacity but allows high internal flow-rates because of good mass transfer kinetics.

4. Conclusions

From this comprehensive study some very important conclusions can be derived.

A lot of parameters which affect the performance of a CSP are to be taken into account in order to optimize a separation: (1) selectivity; (2) number of plates; (3) saturation capacity; (4) particle size; (5) retention; (6) solubility; (7) viscosity.

To focus only on one or some of these parameters when establishing a separation may be misleading, e.g., to choose the CSP with the highest saturation capacity would have given the lowest specific productivity for the separation studied.

As demonstrated, a lot of parameters are involved which may be favorable or unfavorable for one CSP. As a consequence it is difficult to choose the

Table 3
SMB parameters

SMB-parameters	Cellulose-(*p*-methyl)tribenzoate beads	Poly(N-acryloyl-amino acid ester)silica CSP	Cellulose-(*p*-methyl)tribenzoate coated on silica (Chiralcel OJ)	Amylose-(3,5,-dimethyl)phenylcarbamate coated on silica (Chiralpak AD)
Column dimensions (mm)	8×(100×26)	8×(54×26)	8×(50×26)	8×(50×26)
Particle diameter (μm)	20–30	20	20	20
Amount of stationary phase (g)	210	90	100	100
Feed concentration (g racemate/l)	5.0	12.0	6.0	6.0
Flow-rates (ml/min):				
Recycling	46.50	63.90	70.0	90.0
Feed	5.65	3.32	9.5	10.0
Extract	24.47	37.26	42.0	68.0
Raffinate	7.75	13.40	9.0	10.0
Eluent	36.57	47.34	41.5	68.0
Period time (min)	15.50	7.03	1.5	2.1
Purity desired enantiomer (%)	>99	>99	>99	>99
Eluent consumption (l/g enantiomer)	2.28	2.43	1.8	2.6
Specific productivity (g enantiomer/d kg CSP)	98.6	333.2	410	430

optimum combination only from experience. The key tool to make an optimum choice is the modelling and simulation of the separation process. This strategy has a lot of advantages: it is fast, requires only small amounts of products and is reliable and versatile.

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