SIMULATED MOVING BED (SMB) SEPARATION OF PHARMACEUTICAL ENANTIOMERS

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Authors investigated the separation of chiral racemic mixture - pharmaceutical enantiomers - in a laboratory scale SMB equipment. They chose the proper chiral chromatographic packing and eluent for the separation of SMB with the help of previous analytical HPLC investigation. This was followed by frontal adsorption-elution measurements on the chosen Chiralcel OD packing (20μm particle size) in n-hexane-IPA eluent at 20°C. The measurements of adsorption equilibrium (k' values) and NTP, HETP data were carried out with laboratory scale preparative HPLC equipment (L=25 cm, ID=1 cm). The parameter planning, previous estimation of SMB operation was carried out with KROM-N and SMB-KROM-N simulation programs. This was followed by the experimental study of the SMB preparative liquid chromatographic operation (laboratory scale open loop eluent circle 4 column (L=25 cm, ID=1 cm) equipment), and the comparison of the mathematical simulation with the results achieved in practice. The prescribed 99,9 % m/m purity for the „S" component of raffinate can be reached. At the optimum experiment the yield for „S" was over 99 %, the productivity was 62 mg S g^{-1} packing day^{-1} and the eluent consumption was 5,4 mL eluent mg^{-1} S.

Keywords: preparative liquid chromatography, simulated moving bed chromatography, pharmaceutical enantiomers, chiral chromatographic packing

Introduction

Nowadays almost half of the registered pharmaceutical products have chiral structure, accordingly they are of importance in pharmaceutical industry [1]. Pharmacologically, most often only one optical isomer has proper activity, while the other one is inactive, possibly toxic. As a consequence the optical purity of enantiomers have got significant importance. As the enantiomers have the same physicochemical features and they show different characters only in optically active surroundings, the separation of them is unachievable without chiral interactions. Chiral stationary phases can be produced by chiral selectors modifying the next natural materials (proteins, cyclodextrins, saccharides, antibiotics), synthetic polimers, and small, completely synthetic chiral compounds.

Nowadays the production and separation of enantiomer can be done by asymmetric catalysis, biotransformation, liquid-liquid extraction, capillary electrophoresis, membrane separation, crystallization, chromatography, and within chromatography: with capillary electrochromatography (CEC), supercritical fluid chromatography (SCF), gas chromatography (GC) and liquid chromatography (LC). That is thin-layer chromatography (TLC), countercurrent chromatography (CCC) and simulated moving bed chromatography (SMB). The advantage of the SMB method is that the procedure can be made continuously, the columns are completely used, the productivity and yield is higher, and the consumption of the eluent is lower compared to the batch chromatographic process. The disadvantage is the high investment cost, and the considerable sensitivity of the operational parameters.

In the traditional batch elution chromatography the sample is injected on the top of the column and the components get separated after a certain time by moving through the column forced by the mobile phase [2]. However, this process is not very efficient as during the chromatography only a small part of the whole stationary phase is used for separation. A possibility to improve the packing utilization, is given by the true moving bed chromatography (TMB) principle. This chromatographic process was first introduced in the late 1960s by the Universal Oil Product Company [3-5] and

was intensively investigated by Ching and co-workers [6-11]. According to this concept, not only the liquid phase is moving but the solid phase as well (TMB, *Fig. 1*). For example, at a given counter current liquid phase and solid phase velocities, the faster eluting compound (Raffinate, „B") moves forward in the liquid direction and the slower eluting compound (Extract, „A") moves in the opposite direction together with the solid phase. Under this condition, the full mass of the solid phase contributing to the separation is continuosly used, thus improving considerably the productivity of the system. Obviously, this principle is particularly suitable for a binary mixture, especially for racemates (optical isomer mixtures). However, it is technically difficult to move a solid phase, thus the solid phase movement is simulated (SMB, *Fig 2*). In fact, it is a continuous process, the system consists of a number of small columns arranged in series in practice, as shown schematically in *Fig 2*. As the inlet and outlet points are regularly changed (switching time), the net result is the same as it would be if the stationary phase were moving.

In the last decades simulated moving bed processes were widely spread in preparative chromatography on the field of high-purity materials production in the pharmaceutical, fine chemical and biotechnology industries. If materials are difficult to be separated ($\alpha \approx 1$) the process efficiency is disadvantegous. Otherwise if a component of a mixture was strongly adsorbed, the process would become uneconomical because a large volume of eluent should be used.

Experimental

Examination of Adsorbent Packings

During examination Chiralcel OD-H, Chiralcel OJ, Chiralpak AD, Chiralpak IA, Chiralpak AS chiral packings were compared.

Selection of chiral chromatographic packings and eluents

The selection of the chiral stationary packings (CSP) and eluents carried out by a reference book published by DAICEL. These materials are coated or immobilised polysaccharide-derived CSPs.

The chiral stationary packings tested by analytical HPLC are the follows (Fig. 3):

- *Chiralcel OD-H*: Cellulose tris(3,5-dimethyl-phenylcarbamate), coated on a 5 µm silica support, particle size 5 µm,
- *Chiralcel OD*: Cellulose tris(3,5-dimethyl-phenylcarbamate), coated on a 20 µm silica support, particle size 5 µm,

- *Chiralcel OJ*: Cellulose tris(4-methylbenzoate), coated on a 20 µm silica support, particle size 20 µm,
- *Chiralpak AD*: Amylose tris(3,5-dimethyl-phenylcarbamate) coated on a 20 µm silica support, particle size 20 µm,
- *Chiralpak IA*: Amylose tris(3,5-dimethyl-phenylcarbamate) immobilized on a 5 µm silica support, particle size 5 µm,
- *Chiralpak AS*: Amylose tris[(S)-phenylethyl-carbamate] coated on a 5 µm silica support, particle size 5 µm.
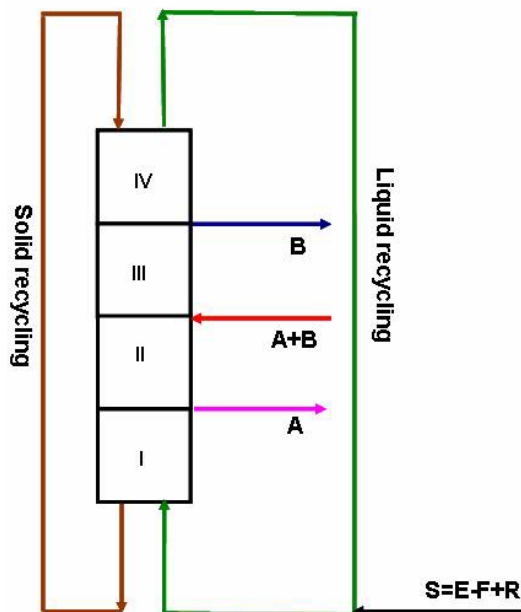
Fig. 1: Column disposition in the true moving bed chromatography (TMB)
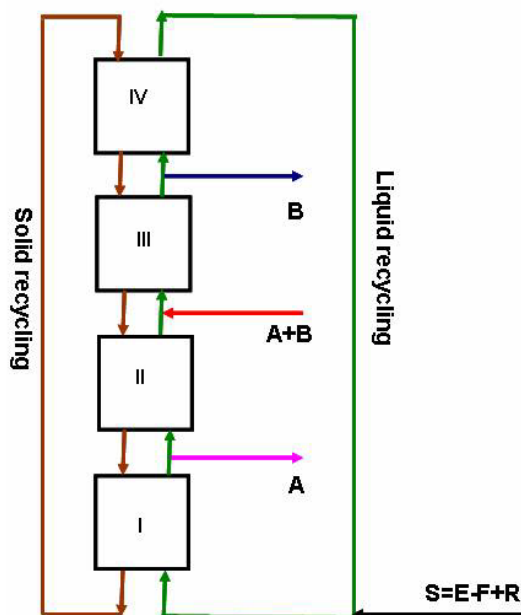
Fig. 2: Column disposition in the simulated moving bed chromatography (SMB), short columns in series
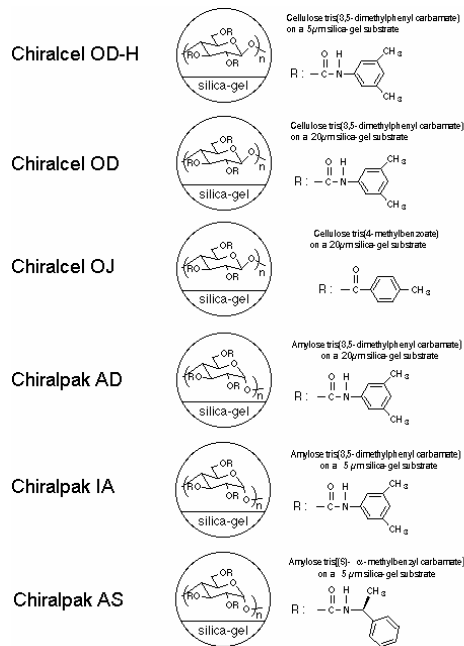
Fig. 3: Chiral packings tested by analitycal HPLC

The measurements were done by GILSON type analytical HPLC. Detection was carried out at 254 nm UV wavelength, at 20°C. As can be seen in *Table 1*, the n-hexane-IPA eluent and the Chiralcel-OD chiral stationary packing proved to be the most appropriate according to the selectivity ($\alpha = \alpha_S^R$), so this system was chosen for further examination.

Determination of the Number of Theoretical Plates (NTP) – Height of Equivalent Theoretical Plate (HETP) and Selectivity (α_S^R)

The definition of the equilibrium data of „S"(B) and „R"(A) optical isomers was carried out on SUPELCO product preparative HPLC column (I.D. = 1 cm, L = 25 cm), filled with Chiralcel OD (particle size: 20μm) packing, with the aid of a column-packing vibrator, at 20°C. Air was removed from the column by means of 95:5 (v/v) n-hexane-IPA and an LMIM D-167 pump. A Rheodyne injection valve with 100μL loop was connected to the column inlet and a Waters UV detector to the outlet, where we monitored the signal of optical isomers. The elution residence-time curve was recorded by UV spectrophotometer (λ = 254 nm). The sample was 541/BK chiral racemic mixture soluted in eluent in 50 mg mL^{-1} concentration, from which 100μL was injected into the column. The eluent was n-hexane-IPA used in three different volumetric ratio with five different volume flow rates. The results of the measurements are shown in *Table 2*.

The residence time curve was evaluated by the triangulation method determining σ and t_R values:

$$k' = \frac{t_R - t_0}{t_0} \qquad \alpha_S^R = \frac{t_{R_R} - t_0}{t_{R_S} - t_0} = \frac{k'_R}{k'_S}$$

$$NTP = \left(\frac{t_R}{\sigma}\right)^2 \qquad HETP = \frac{L}{NTP}$$

Frontal Adsorption - Elution Measurements

The Langmuir constants and the data of the adsorption isotherm were calculated from the k' values:

$$K_{B(S)} = k'_{B(S)}\frac{\varepsilon}{1-\varepsilon} \qquad K_{A(R)} = k'_{A(R)}\frac{\varepsilon}{1-\varepsilon}$$

$$\varepsilon = 0.67\frac{mL\,liquid}{mL\,column} \qquad \rho_{BULK} = 0.6\frac{g\,packing}{mL\,column}$$

$$a_{B(S)} = K_{B(S)}\frac{1-\varepsilon}{\rho_{BULK}} \qquad a_{A(R)} = K_{A(R)}\frac{1-\varepsilon}{\rho_{BULK}}$$

$$b_{B(S)} = b_{A(R)} \quad \text{(computative data)}$$

$$a_B^* = 5.3366\frac{mL\ liquid\ free\ volume}{g\ packing}$$

$$b_B = 0.016\frac{mL\ liquid\ free\ volume}{mg\ B\ component}$$

$$a_A^* = 6.4405\frac{mL\ liquid\ free\ volume}{g\ packing}$$

$$b_A = 0.016\frac{mL\ liquid\ free\ volume}{mg\ A\ component}$$

Thus enough information was assembled to do the computer simulations (with KROM-N Software) of the frontal adsorption-elution. The data input to the software is shown on *Table 3*. Hereby there was a possibility to compare the simulation and the laboratory measurements. The laboratory measurements were performed with the column used before. Air was removed from the column by means of 95:5 (v/v) n-hexane-IPA at 20°C, directed downwards by means of an LMIM-D167 piston pump. During the frontal adsorption a 10 mL of 50:50 (m/m) mixture of chiral mixtures S+R (total conc. 2-5-10 g L^{-1}) was applied downwards to the column at a flow rate 2-2,5-5 mL min^{-1} at 20°C. Feeding of the mixture S and R was stopped after 10 mL and pure eluent (95:5 and 93:7 n-hexane-IPA) was pumped into the column at a flow rate of 2-2,5-5 mL min^{-1} (*Table 4*). The eluent was collected in sample collectors and concentration analysed on-line by UV spectrophotometer. The concentration of the given samples were measured by analytical HPLC. The frontal adsorption-desorption simulation and laboratory measurement results are shown in (*Fig. 4.*).

It's remarkable that the separation of "S" and "R" optical isomers are favourable at small total contrantrations compared to the high ones.

The estimation of the SMB measurements with computer simulations

The simulations were calculated by SMB-KROM-N software. The model of the software uses the physical and chemical data of chemicals, the number of theoretical plates (NTP), volumetric flow rates, adsorption equilibrium data, switching time, etc. published by Morbidelli [12] and his partners. The software of the simulation solves the differential equations by the numerical method of finite differencies [13].

Determination of Morbidelli Parameters

On the bases of a theoretical method assuming independent adsorption and linear isotherms, published by Massimo Morbidelli [12] and his team, the right values of volumetric stream can be well estimated.

Values of distribution quotient:

$$K_B = \frac{a_B^* \cdot \rho_H}{(1-\varepsilon)} = 9.703 \frac{\text{mL free volume liquid}}{\text{mL solid silicagel}}$$

$$a_B^* = 5.3366 \frac{\text{mL free volume liquid}}{\text{mL silicagel}}$$

$$K_A = \frac{a_A^* \cdot \rho_H}{(1-\varepsilon)} = 11.71 \frac{\text{mL free volume liquid}}{\text{mL solid silicagel}}$$

$$a_A^* = 6.4405 \frac{\text{mL free volume liquid}}{\text{mL silicagel}}$$

By the Morbidelli criteria the next relations must be true for producing pure „S" (B component) and „R" (A component) isomers:

$$11.71 = K_A < m_I$$
$$9.703 = K_B < m_{II} < K_A = 11.71$$
$$9.703 = K_B < m_{III} < K_A = 11.71$$
$$m_{IV} < K_B = 9.703$$

The following parameters were chosen for the purpose of measurement, because these parameters accomplish Morbidelli criteria providing adequate results during simulations. Further data can be seen in the next chapter.

F = 0.3 mL min^{-1}
E = 4.4 mL min^{-1}
R = 4.4 mL min^{-1}
S = 12 mL min^{-1}
LR OUT = 3.5 mL min^{-1}
D = S + REC = 12 mL min^{-1}
T = 10 min (switching time)
L = 25 cm (column length)

$$A_f = \frac{D^2 \pi}{4}$$

Values of Morbidelli parameters:

$$m_I = \frac{\frac{D}{A_f}T - L\varepsilon}{L(1-\varepsilon)} = 16.4989 > 11.7108$$

$$9.7028 < m_{II} = \frac{\frac{D-E}{A_f}T - L\varepsilon}{L(1-\varepsilon)} = 9.7049 < 11.7108$$

$$9.7028 < m_{III} = \frac{\frac{D-E+F}{A_f}T - L\varepsilon}{L(1-\varepsilon)} = 10.1681 < 11.7108$$

$$9.7028 < m_{III} = \frac{\frac{D-E+F}{A_f}T - L\varepsilon}{L(1-\varepsilon)} = 10.1681 < 11.7108$$

Data input to the SMB-KROM-N software

Number of components: k = 2
Column inner diameter: I.D. = 1 cm
Column length: L = 25 cm
Number of columns: N = 4
Free volume coefficient: EPS = 0.67 mL liquid free volume mL^{-1} column
Bulk density: ρ_{BULK}=0.6 g packing mL^{-1} column
Feed: F = 0.3 mL min^{-1}
Fresh eluent: S = 12 mL min^{-1}
Extract: E = 4.4 mL min^{-1}
Raffinate: R = 4.4 mL min^{-1}
Recycling: REC = 0 mL min^{-1}
Langmuir constants: as given above
Feed concentration: c_B^F = 2.5 mg B component mL^{-1} liquid
c_A^F = 2.5 mg A component mL^{-1} liquid
Number of Theoretical Plates: NTP = 200 per 25 cm column
Switching time: 10 min
Calculation time: 400 min

SMB Equipment Planning, Construction and Installation

The SMB preparative liquid chromatographic equipment with four columns, four sectors and open eluent circle was constructed in the Central Mechanical Workshop of the University of Veszprém (*Figs. 5 and 6*). During installation the four preparative liquid chromatographic columns (I.D. = 10 mm, L = 250 mm) were filled with Chiralcel-OD packing by the vibration method (~30 min filling time). The column packing density was 0.6 g mL^{-1}, the free volume factor was 0.67. Each column was filled with approximately 11.77 g packing. Stainless steel frits (2μm) were placed at the top and the bottom of each column. Before measurements air was removed with eluent.
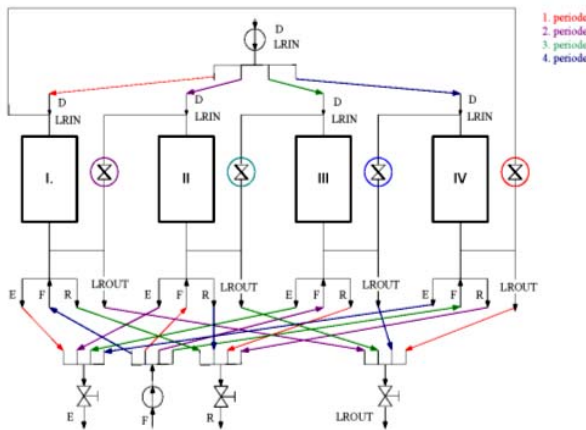
Fig. 5: Photograph of the SMB equipment

Fig. 6: The block diagram of the SMB equipment with four columns, four sectors, and open eluent cycle

SMB measurements

Out of the 22 executed simulations the best ones were chosen, according to which four measurements were done with the laboratory scale 1:1:1:1 column configuration, open eluent circle SMB equipment of the Department of Chemical Engineering Processes. The conditions and the results of the SMB measurements are included in *Table 5* and in the graphs (*Fig.7*).

Results

Measurement Results of SMB Compared to Simulation Results

Columns of the four-column SMB equipment, previously equilibrated at 20°C with 95:5 (v/v) n-hexane-IPA as eluent, were used to separate a racemic mixture of 2.5 g L^{-1} isomer A (R) and 2.5 g L^{-1} isomer B (S) in the same solvent mixture. The mixture to be separated was fed at the top of column III. up to 10 min at a flow rate of 0.3 mL min^{-1}. (The other volumetric ratios: E = 4.4 mL min^{-1}, R = 4,4 mL min^{-1}, fresh eluent: 12 mL min^{-1}). It was followed by switching columns were exchanged according to the SMB process and the eluent was not recirculated. Flow rates were controlled by digital balances with the help of computer during the 10 min switching time.

Fig. 7 shows the eluent consumption, productivity, purity and yield for component "S" in the raffinate fraction at quasi-stationary state.

Our conclusion on the bases of laboratory measurements is that the SMB-KROM-N software is very adaptable to optimize SMB operation.

The prescribed 99,9 % m/m purity for the „S" component of raffinate can be reached. At the optimum experiment the yield for „S" was over 99 %, the productivity was 62 mg „S" g^{-1} packing day^{-1} and the eluent consumption was 5,4 mL eluent mg^{-1} „S".

With the help of experimental and theoretical optimization of SMB process (switching time decrease, feed concentration increase, volumetric flow-rate change, gradient methods application, column number increase, column configuration change etc.) markedly can be improve the specific values of the SMB process. The investigation is in progressive state at the University of Veszprém and at the Gedeon Richter LTD.
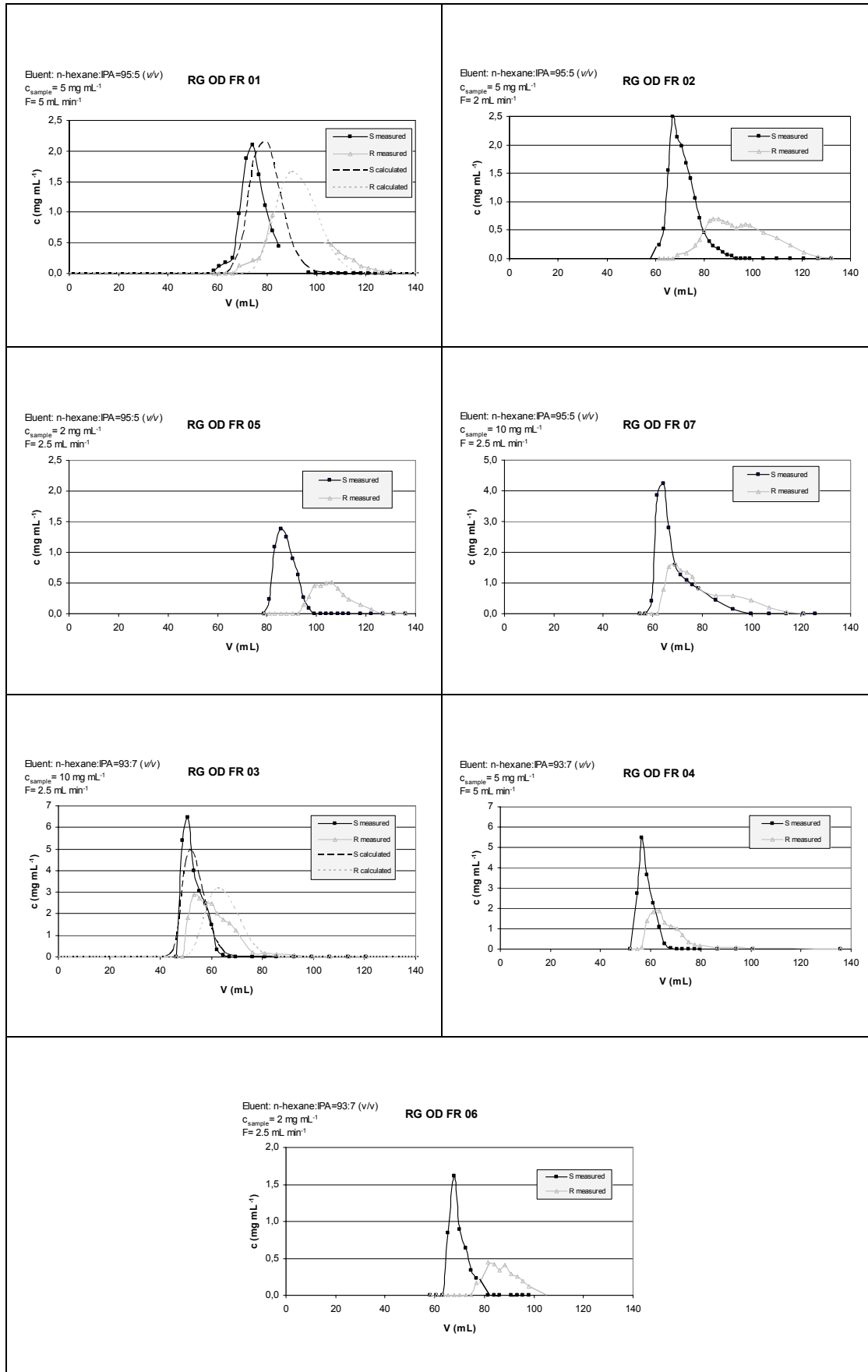
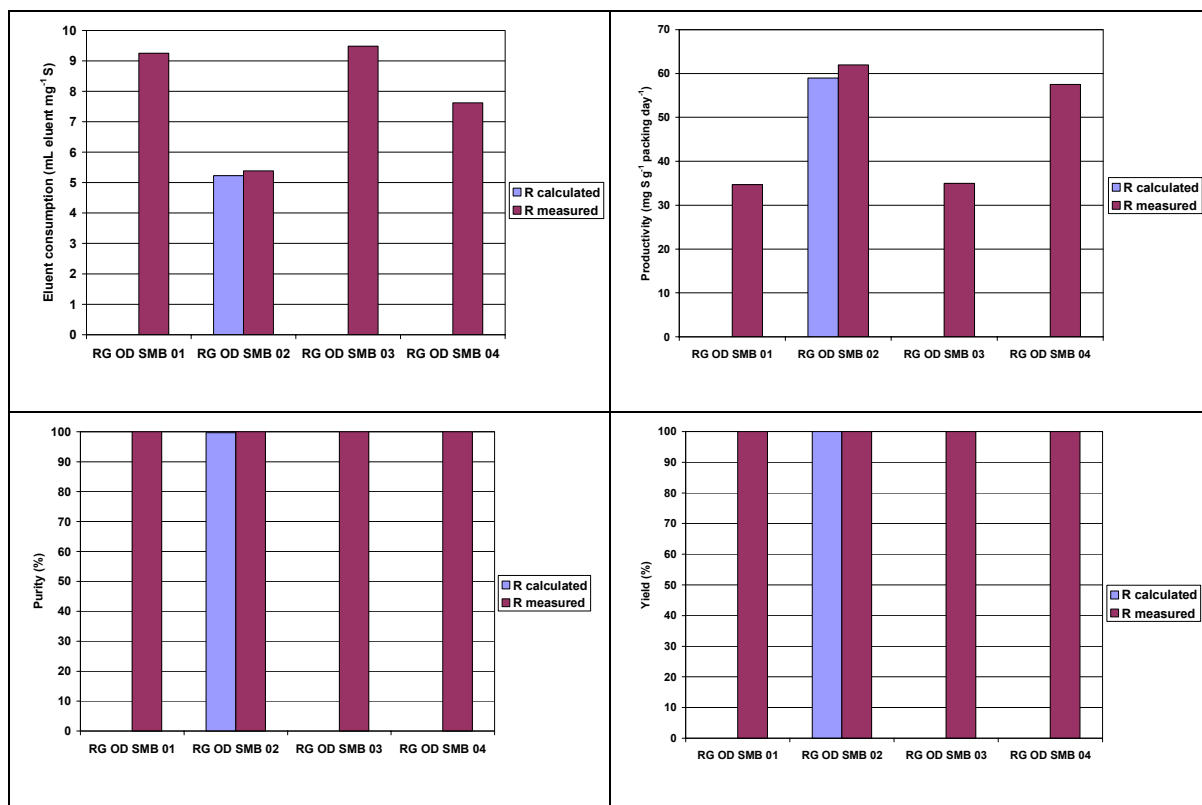*Fig. 4:* The results of frontal adsorption-desorption simulation and laboratory measurements

Fig. 7: Results of 4 measurements and 1 simulation: eluent consumption, productivity, purity and yield for component "S" in the raffinate

Eluent	Concentration	Packing				
	[% v/v]	Chiralcel OD-H	Chiralcel OJ	Chiralpak AS	Chiralpak AD	Chiralpak IA
n-hexane:IPA	70:30		α=1			
n-hexane:IPA	80:20	**α=1.17**	α=1.196			
n-hexane:IPA	90:10	**α=1.173**	α=1		α=1	
n-hexane:IPA	97.5:2.5					α=1.06
n-hexane:IPA	95:5	**α=1.19**	α=1			α=1.03
n-hexane:Et-OH	95:5	α=1.122				
n-hexane:IPA:AcN	80:10:10		α=1			
AcN	100			α=1.31	α=1	
AcN-Me-OH	80:20			α=1	α=1	
Ethanol	100				α=1	
n-hexane:MTBÉ	80:20	$k'_{1,2}>20$				
n-hexane:MTBÉ	60:40	$k'_{1,2}=6.10$				
n-hexane:MTBE:Et-OH	(60:40)+5% Et-OH	$k'_{1,2}=1.086$				
n-hexane:IPA						α=1.08
n-hexane:Met-OH	99:1					α=1.12
n-hexane:Et-OH	99:1					α=1.11
n-hexane:DKM	75:25					α=1.03
n-hexane:IPA:EtOH	95:2.5:2.5	α = 1.15				
n-hexane:IPA:MetOH	95:2.5:2.5	α = 1.05				
n-hexane:EtOH:MetOH	95:2.5:2.5	α = 1.05				

Table 1: HPLC measurements on different charges

Eluent: n-hexane:IPA=95:5 [% v/v]

B [mL min^{-1}]	t_0 [sec]	$t_{R,1}$	k'_1	4σ [s]	NTP	HETP [mm]	$t_{R,2}$	k'_2	4σ [s]	NTP	HETP [mm]	α(k'_2/k'_1)	Pressure [psi]	Pressure [bar]
2.5	316	1902	5.027	378	405	0.617	2 532	7.023	510	394	0.634	1.397	42	3.0
5	158	912	4.779	192	361	0.693	1068	5.768	264	262	0.955	1.207	66	4.6
10	79	468	4.932	108	300	0.832	576	6.300	150	236	1.060	1.278	110	7.7
15	53	306	4.817	78	246	1.015	378	6.186	108	196	1.276	1.284	168	11.8
20	39	228	4.779	66	191	1.309	282	6.148	90	157	1.592	1.286	240	16.9
30	26	168	5.388	80	155	1.614	210	6.985	90	87	2.870	1.296	336	23.6

Eluent: n-hexane:IPA=90:10 [% v/v]

B [mL min^{-1}]	t_0 [sec]	$t_{R,1}$	k'_1	4σ [s]	NTP	HETP [mm]	$t_{R,2}$	k'_2	4σ [s]	NTP	HETP [mm]	α(k'_2/k'_1)	Pressure [psi]	Pressure [bar]
2.5	316	1248	2.954	258	374	0.668	1 530	3.848	336	332	0.754	1.302	30	2.1
5	158	612	2.878	126	377	0.662	732	3.639	192	233	1.075	1.264	48	3.4
10	79	303	2.840	72	283	0.882	366	3.639	102	206	1.214	1.281	100	7.0
15	53	204	2.878	56	212	1.177	242	3.601	80	146	1.708	1.251	144	10.1
20	39	152	2.853	48	160	1.558	182	3.613	72	102	2.445	1.267	210	14.8
30	26	102	2.878	40	104	2.403	120	3.563	72	44	5.625	1.238	336	23.6

Eluent: n-hexane:IPA=80:20 [% v/v]

B [mL min^{-1}]	t_0 [sec]	$t_{R,1}$	k'_1	4σ [s]	NTP	HETP [mm]	$t_{R,2}$	k'_2	4σ [s]	NTP	HETP [mm]	α(k'_2/k'_1)	Pressure [psi]	Pressure [bar]
2.5	316	1008	2.194	210	369	0.678	1 218	2.859	249	383	0.653	1.303	0	0.0
5	158	492	2.118	102	372	0.672	588	2.726	138	290	0.861	1.287	0	0.0
10	79	242	2.067	58	279	0.898	288	2.650	78	218	1.146	1.282	54	3.8
15	53	162	2.080	46	198	1.260	192	2.650	58	175	1.426	1.274	108	7.6
20	39	120	2.042	40	144	1.736	142	2.599	58	96	2.607	1.273	168	11.8
30	26	81	2.080	35	88	2.835	94	2.574	40	88	2.829	1.238	306	21.5

Table 2: The parameters and the results of the elution measurements

Number of components:	k = 2
Column inner diameter:	I.D. = 1 cm
Column length:	L = 25 cm
Free volume coefficient:	EPS = 0.67 mL liquid free volume mL^{-1} column
Bulk density:	ρ_B= 0.6 g packing mL^{-1} column
Feed:	F = 2-2.5-5 mL min^{-1}
Langmuir constants:	as given above
Sample feeding volume:	10 mL
Sample concentration:	$c_B^F = 2-5-10$ mg B component mL^{-1} liquid $c_A^F = 2-5-10$ mg A component mL^{-1} liquid
Number of Theoretical Plates:	NTP = 200
Calculation time:	70 min

Table 3: Data input for the KROM-N software

Identifier	Feed [mL min^{-1}]	c$_{sample}$ [mg mL^{-1}]	Eluent, (*v/v*) n-hexane-IPA
RG OD FR 05	2.5	2	95:5
RG OD FR 02	2	5	95:5
RG OD FR 01	5	5	95:5
RG OD FR 07	2.5	10	95:5
RG OD FR 06	2.5	2	93:7
RG OD FR 04	5	5	93:7
RG OD FR 03	2.5	10	93:7

Table 4: The parameters of the frontal adsorption-elution measurements

RG OD SMB 01-04 measurements					
Identifier	RG OD SMB 01	RG OD SMB 02		RG OD SMB 03	RG OD SMB 04
Eluent	n-hexane:IPA = 93:7 (v/v)				
Sample	5 g racemic mL^{-1} in n-hexane:IPA = 95:5 (v/v)			5 g racemic mL^{-1} in n-hexane:IPA = 93:7 (v/v)	
Switching time (min)	10	10		10	7,5
D	11,55	12,01		11,93	15,76
E	4,29	4,29		5,06	6,59
F (mL min^{-1})	0,23	0,30		0,33	0,50
R	3,59	3,91		4,22	6,01
LR OUT	3,72	3,72		2,94	3,64
"S" isomer	Raffinate	simulation	Raffinate	Raffinate	Raffinate
c$_S$ (mg mL^{-1})	0,3477	0,0468	0,5702	0,2983	0,3442
Eluant consumption (mL eluent mg^{-1} S)	9,25	5,23	5,39	9,48	7,62
Productivity (mg S g^{-1} packing day^{-1})	34,69	59,00	61,96	34,98	57,50
Purity (%)	100,00	99,66	100,00	100,00	100,00
Yield (%)	100,00	99,95	100,00	100,00	100,00

Table 5: The conditions and the results of the SMB measurements

SYMBOLS

ε – free volume coefficient [mL liquid free volume mL^{-1} column]

F – volume flow rate of eluent [mL min^{-1}]

t_0 – dead time [min]

t_R – retention time [min]

k' – retention factor

S(–) – the „S", L-isomer, bonds weakly to adsorbent (B)

R(+) – az „R", D-isomer, bonds stronger to adsorbent (A)

α_S^R – separation factor

NTP – number of theoretical plates

HETP – height of theoretical plates [mm]

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