Study of a Simulated Moving Bed (SMB) Preparative Liquid Chromatographic Appliance and Technology

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Abstract
The simulated moving bed (SMB) technique is one of the newest method among the preparative chromatographic technologies. This technique is widely used in pharmaceutical industry, in analytical chemistry, in separating biotechnology and anywhere else, where other methods fail to achieve high purity in the product. We can optimize the efficiency of the separation with changing the operation parameters. The main goal is to get the highest quality along the highest productivity.

Introduction
For the laboratory tests we make the plans of a 4-columned equipment; each column is 25 cm long and the inner diameter is 1 cm. We separated two steroid isomers on YMC S-50 silica-gel adsorbent. We analyzed the effect of the concentration changing of the fresh eluent to the efficiency of the separation. The quality and other specific physical data of the product was determined by gas-chromatographic measurements. Using the collected results we created a mathematical model and a computer program for this SMB method. The model is using the physical and chemical data of the chemicals, and the theoretical plate number of the column. These data were determined by other classical measurement techniques.

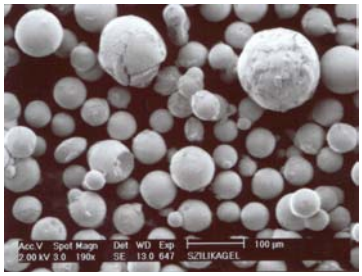
Methodology
SMB is a continuous liquid chromatographic separation technique using multiple columns, to achieve a quasi-stationary concentration profile while we change the flowing of the steady-phase the opposed moving eluent and the point of the inflow and outflow step-by-step.

Investigation of adsorbent charge
We determined the specific surface and pore size distribution of the YMC S-50 silica-gel with ASAP-2000 equipment (**Table 1.**) and we made scanning electron microscopy photo (**Fig. 1.**).

Table 1. Determine of BET specific surface

Adsorbent	YMC S-50
Surface of BET	798.63 m^2/g
BJH pore volume $1.7nm < d_{pore} < 300nm$	1.318 cm^3/g
Average pore size of BJH	5.4026 nm

Fig. 1. SEM photo of adsorbent



Equilibrium measurements

Determination of the acetone's and „A" and „B" steroid's (**Fig.3.**) adsorption equilibrium data (**Fig.2.**) were done in a dichloromethane-silica-gel system.
The measurement was done by the Gedeon Richter Rt. on a YMC S-50 silica-gel, acetone, dichloro-methane system at 20°C temperature. The examination made by analytical HPLC with multistage frontal saturation.

Fig.2. Adsorption equilibrium of the acetone

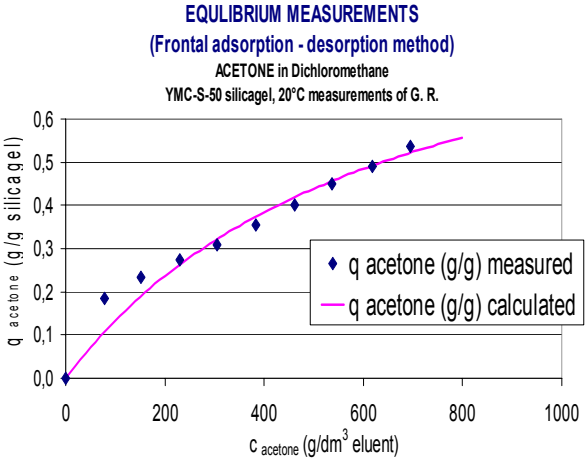
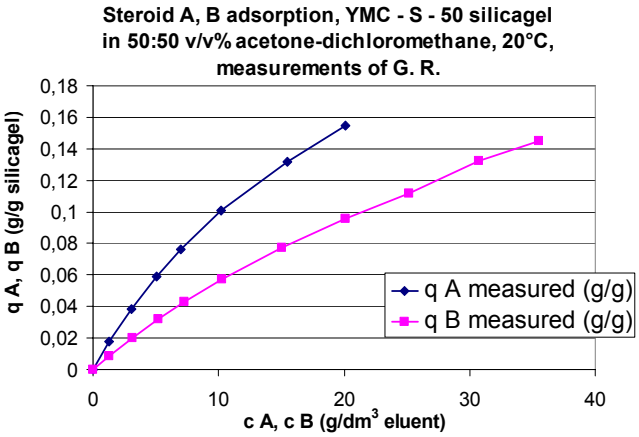


Fig.3. Adsorption equilibrium of the „A" and „B" steroid



We used the following Langmuir constants during the calculations:

$$a^*_{Acetone} = 1.543 \frac{cm^3\ liquid\ free\ volume}{g\ silicagel} \qquad b_{Acetone} = 0.001517 \frac{cm^3\ liquid\ free\ volume}{mg\ aceton}$$

$$a^*_{B} = 10.36 \frac{cm^3\ liquid\ free\ volume}{g\ silicagel} \qquad b_{B} = 0.02675 \frac{cm^3\ liquid\ free\ volume}{mg\ B}$$

$$a^*_{A} = 22.55 \frac{cm^3\ liquid\ free\ volume}{g\ silicagel} \qquad b_{A} = 0.06640 \frac{cm^3\ liquid\ free\ volume}{mg\ A}$$

Determination of the Number of Theoretical Plates (NTP) and Height of Equivalent Theoretical Plate (HETP)

We poured YMC S-50 silica-gel into the SUPELCO chromatographic column (D_{in}=1 cm, L= 25cm) with a charge loader vibrator. The mass of the charge is 8 g. The air was removed from the column by a LMIM D-167 pump and an acetone:dichloromethane (1:1) mixture. We put Rheodyne injection cock on the input of the column with 100 µl loop. We connected a Waters UV detector to the output of the column, where we detected the changing of the impulses of the 0.2 %v/v acetophenone in elunet and dichloromethane on 300 nm wavelength. We evaluated the retention time density function with the triangle method:

$$NTP = \left(\frac{t_R}{\sigma}\right)^2$$ (**Fig.4.**)

NTP Number of Theoretical Plates
t_R retention time
σ scatter

$$HETP = \frac{L}{NTP}$$ (**Fig.5.**)

HETP High of Equivalent Theoretical Plate
L charge length

Fig.4. NTP measurement

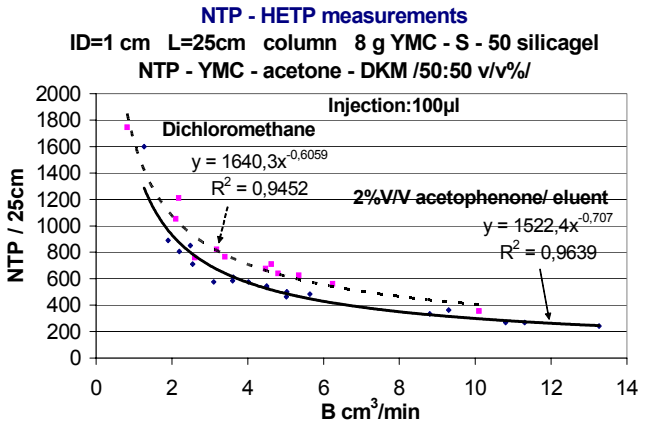
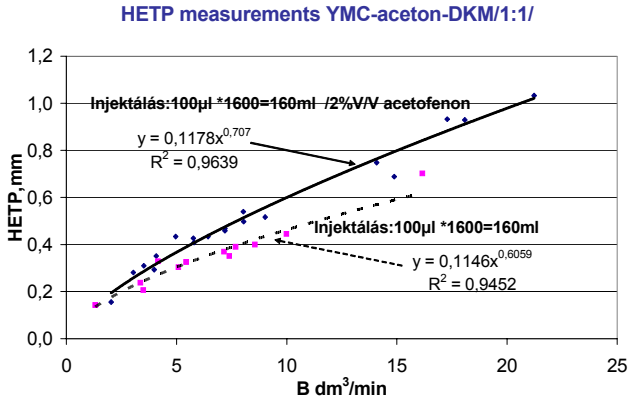


Fig.5. Determination of HETP



The simulation data of the frontal adsorption – desorption

(Calculation KROM – N software with solvent adsorption – desorption)

Input data of the software

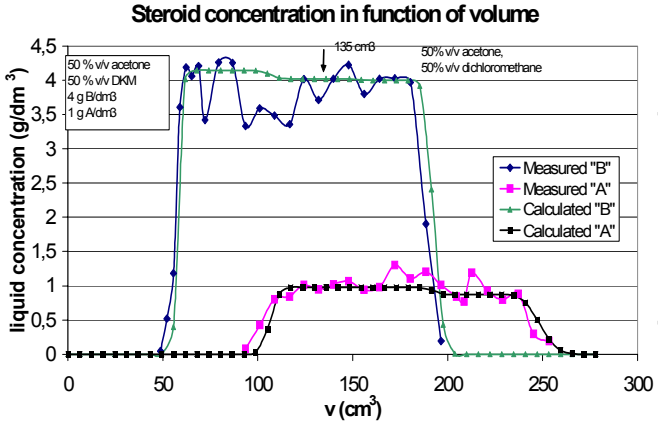
Number of components:	k = 3
Column inner diameter:	ID=0.735 cm
Column length:	L=42 cm
Free volume coefficient:	EPS= 0.8018 cm^3 liquid free volume/ cm^3 column
Feed:	B=2.47 cm^3/min
Bulk density:	ROH= 0.4045 g silicagel/cm^3 column
Langmuir constants:	*(same as on page 2)*
Sample feeding time:	55 min (135.85 cm^3)
Sample concentration:	$c_{Acetone}$=396 mg/cm^3
	c_B=4 mg/cm^3
	c_A=1 mg/cm^3
Eluent concentration:	$c_{Acetone}$=396 mg/cm^3
	c_B=c_A=0 mg/cm^3
	NTP=400
End of elution time:	112 min
Printing time:	2.5 min

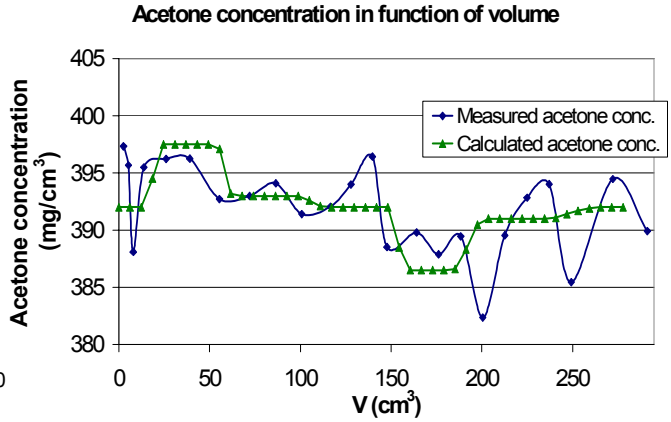
Frontal adsorption-desorption measurement compare with simulation

We poured YMC S-50 silica-gel into the SUPELCO chromatographic column (D_{in}=1 cm, L= 25cm) with a charge loader vibrator. The air was removed from the column by a LMIM D-167 pump and an acetone:dichloromethane (1:1) mixture.

During the frontal adsorption we fed 5 g (A+B)/dm^3 (B ≈ 80 m/m%, A ≈ 20m/m%) mixture at 20°C with a 2.47 cm^3/min volume flow rate into the upper part of the column. We finished the feeding of „A" and „B" steroid after 55 min (≈ 135 cm^3 liquid), then lead pure eluent (1:1 v/v% acetone:dichloromethane) with a 2.85 cm^3/min volume flow rate into the column. We determined the concentration of the samples with analytical gaschromatographic measurement (**Fig.6**. and **Fig.7.**).

Fig.6. and **Fig.7.** Frontal adsorption-desorption measurement of the steroids and acetone





Planning (Fig.8.) and making SMB equipment (Fig.9.)

We assembled the four-columned, four-sectored, open eluent loop SMB preparative chromatographic equipment from the following parts:

- Stand from stainless steel
- 4 pieces Preparative HPLC column, stainless steel, D_{in}= 10mm, L= 250mm (SUPELCO)
- 5 pieces Four-way cock stainless steel (VALCO)
- 4 pieces Liquid distributor 4-way
- 4 pieces Liquid distributor 1+6-way
- Open-close cock, stainless steel, high-pressure (VALCO)
- 4 pieces Gilson type HPLC pump
- 2 pieces non-return valve, stainless steel, high-pressure
- 10 m acid-resistant capillary

Fig.8. The flowchart of SMB

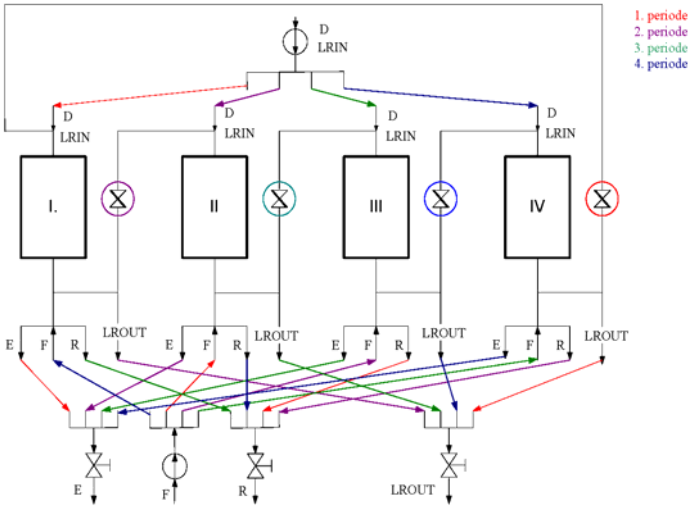


Fig.9. The SMB equipment

The simulation data of the gradient SMB

SMB – KROM – N Software with solvent adsorption – desorption

Input data of the software

Number of components:	k = 3
Column inner diameter:	ID=1 cm
Column length:	L=25 cm
Number of columns:	N=4
Free volume coefficient:	EPS= 0.8018 cm^3 liquid free volume/ cm^3 column
Feed:	F=1.5 cm^3/min
Fresh eluent:	S=7.3 cm^3/min
Extract:	E=6.2 cm^3/min
Raffinate:	R=2.6 cm^3/min
Bulk density:	ROH= 0.045 g silicagel/cm^3 column
Langmuir constants:	*(same as on page 2)*
Feed concentration:	$c^F_{acetone} = 396 \frac{mg\ acetone}{cm^3\ liquid}$

$$c^F_B = 4 \frac{mg\ B}{cm^3\ liquid} \qquad c^F_A = 1 \frac{mg\ A}{cm^3\ liquid}$$

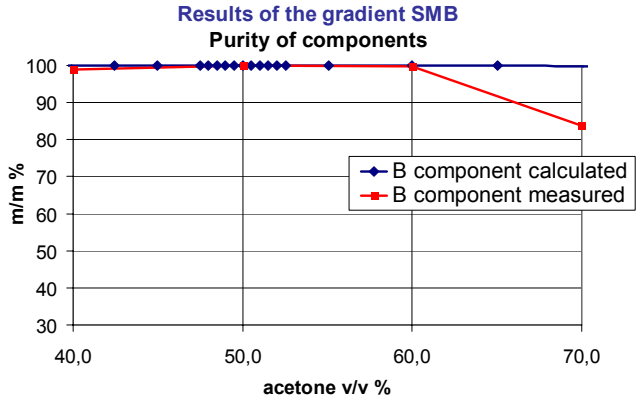
Fresh eluent concentration:

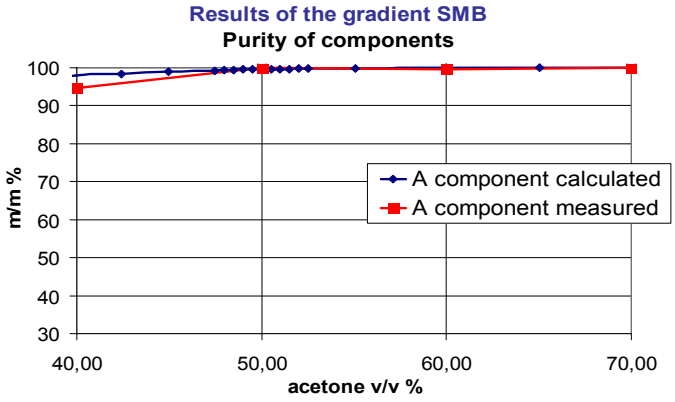
$$c^S_B = c^S_A = 0 \frac{mg\ B\ or\ A}{cm^3\ liquid}$$

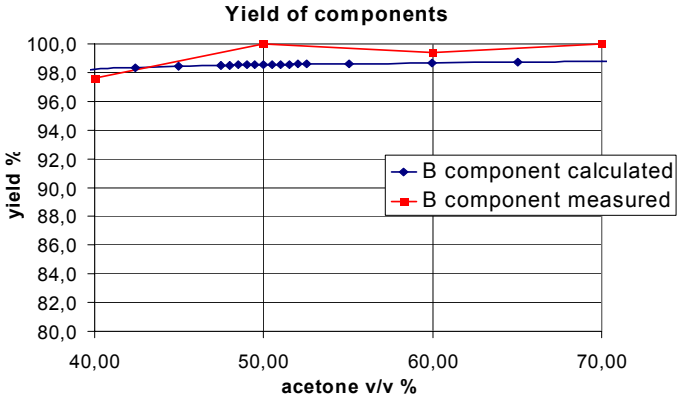
Measurements	SMB 7	SMB1	SMB5	SMB 6
Acetone v/v %	40	50	60	70
$c^S_{Acetone}$ (mg acetone/cm^3 liquid)	316.8	396	475.2	554.4

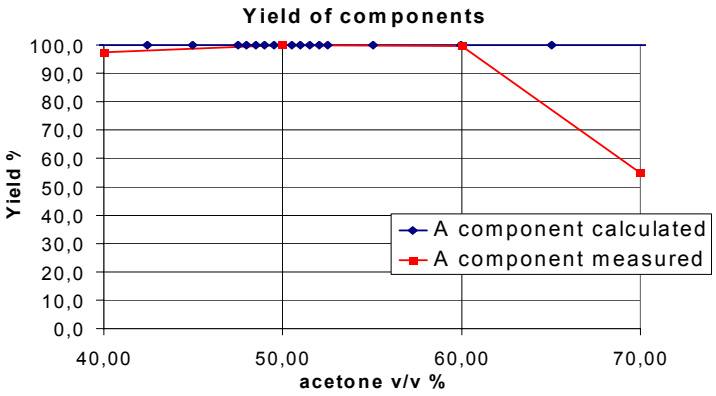
Number of Theoretical Plates:	NTP=200 for a 25 cm column
Switching time:	22.5 min
Calculation time:	585 min

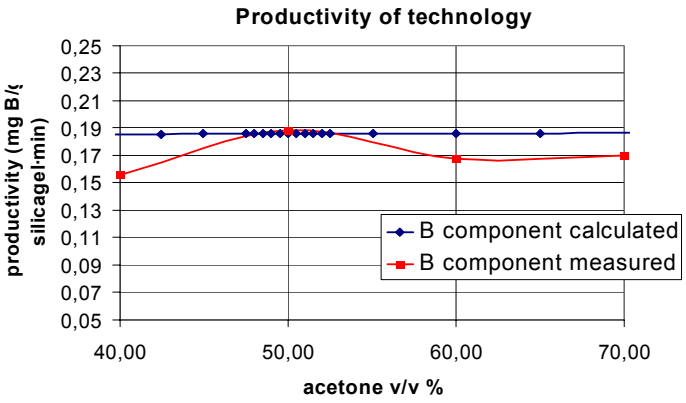
The results of the gradient SMB measurements compare with simulation

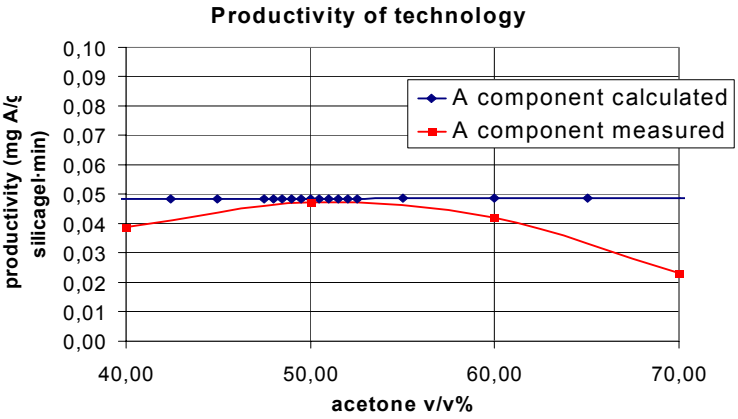


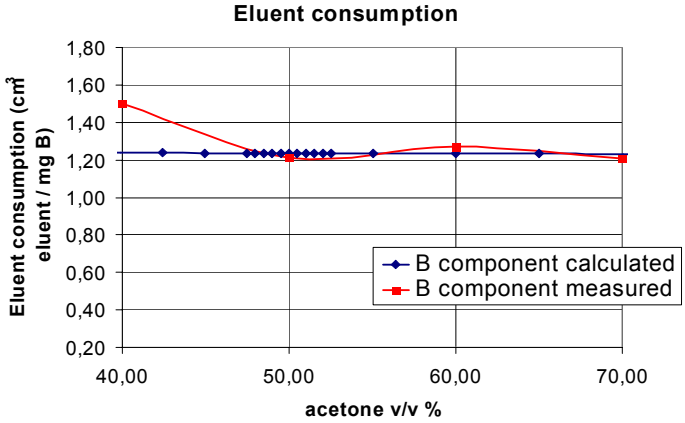


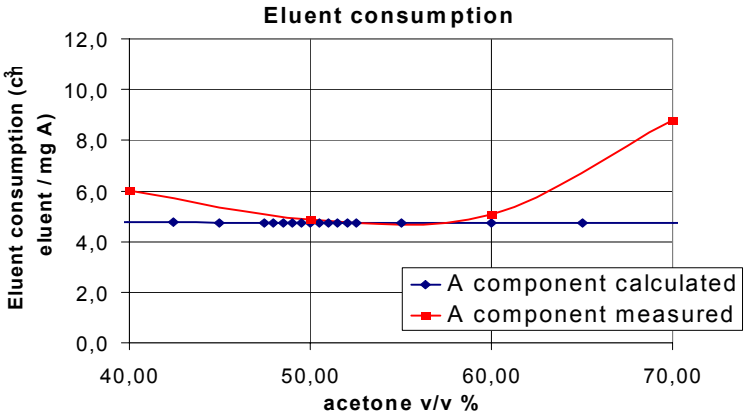












Results and Conclusions

Changing the acetone concentration of the fresh eluent between 40…70 % v/v in DKM according to the Morbidelli triangle the extract and raffinate concentrations change as well.
Over 60 % v/v acetone in dichloromethane the „B" in raffinate is under 99.9% m/m required purity, under 45 % v/v acetone in dichloromethane the „A" in extract is under 99.9% m/m required purity.
The optimum concentrations are between 45-60 % v/v acetone in dichloromethane.
The new computer programmes (KROM-N, SMB-KROM-N with solvent adsorption-desorption) gave proper results at the working point (50-50 % v/v acetone in dichloromethane). The difference between the measured and calculated concentrations in dichloromethane until 40% v/v or increasing up-to 70% v/v in dichloromethane.
The conclusion is that the authors have to modify the model parameters in the next future.

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