

Chiral Purification using Macrocyclic Glycopeptide CSPs with Simulated Moving Bed Technology

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Abstract

- Chirality has long been regarded as one of the critical issues in the discovery and drug design processes of the pharmaceutical and biotechnologies industries. During the early development stages, the pharmacokinetic aspects of the chiral drugs need to be addressed because each enantiomer can behave differently in terms of absorption, distribution, metabolism, and excretion in clinical studies. Therefore, any chiral molecules under development, small amounts of pure enantiomers are needed for such studies.
- LC chiral stationary phases (CSPs), made by bonding macrocyclic glycopeptides, have demonstrated wide chiral selectivity and excellent robustness since their introduction in 1995.

Abstract (contd.)

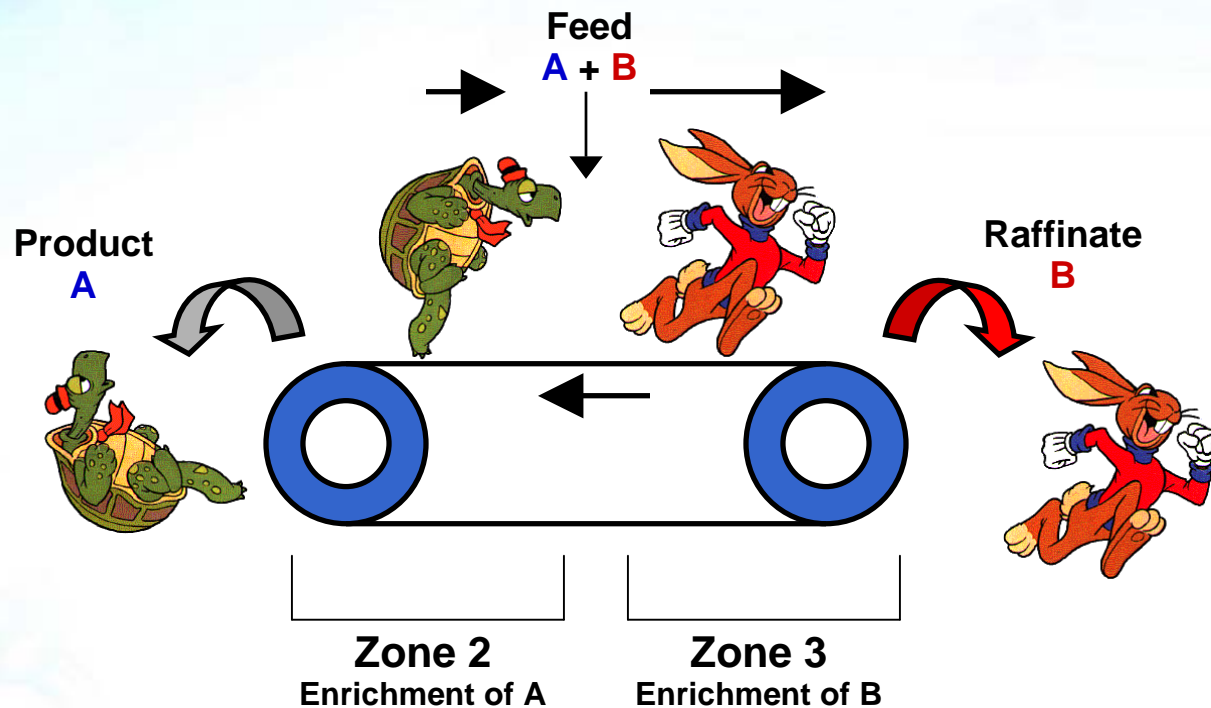
The advances of simulated moving bed (SMB) technologies have been successfully applied for many large-scale binary separations of sugars, hydrocarbons and other small molecules. Thus, a small scale, bench-top SMB would expand the use of this powerful separation technique in obtaining gram quantities of enantiomers in a very short period of time.

- This presentation will explore the methodology of utilizing macrocyclic glycopeptide CSPs in a bench-top SMB prototype system to purify chiral pharmaceutical drugs. Various mobile phase systems will be employed. The advantages of this approach over traditional batch preparative chromatography will be discussed in terms of overall throughput, purity of enantiomers, and the reduction of solvent waste. The ease of method development and ruggedness of this system plus the chiral stationary phases will also be demonstrated.

Success Factors for Chiral Prep Applications

- Selectivity
- Solubility
- Sample Recovery
- Solvent Composition/Consumption
- Throughput/Productivity
- Robustness

True Moving Bed (TMB) Chromatography



The switch time in Zones 2 and 3 must be greater than the residence time of B and less than the residence time of A.

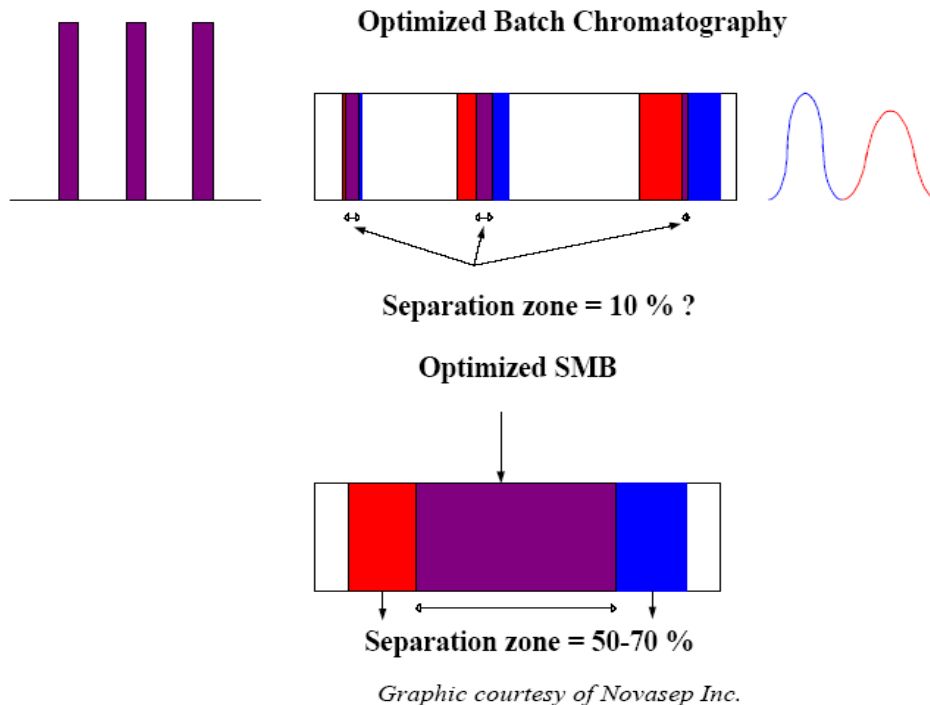
SMB Introduction

- 1961 Broughton and Gerhold (UOP), US patent
 - Petrochemical industry
 - Sugars, amino acids purification
- 1989-present, large scale chiral purifications
 - Novasep and Knauer etc.
- Continuous countercurrent chromatography
- 4/8/12/16 HPLC columns-in 4 or 3 zones
- Each column has 2 Inlets (Feed/Eluent), 2 outlets (Extract/Raffinate) and a connection valve between columns
- Continuous valves/ports switching at a fixed time interval in the direction of the eluent flow
- Binary separations

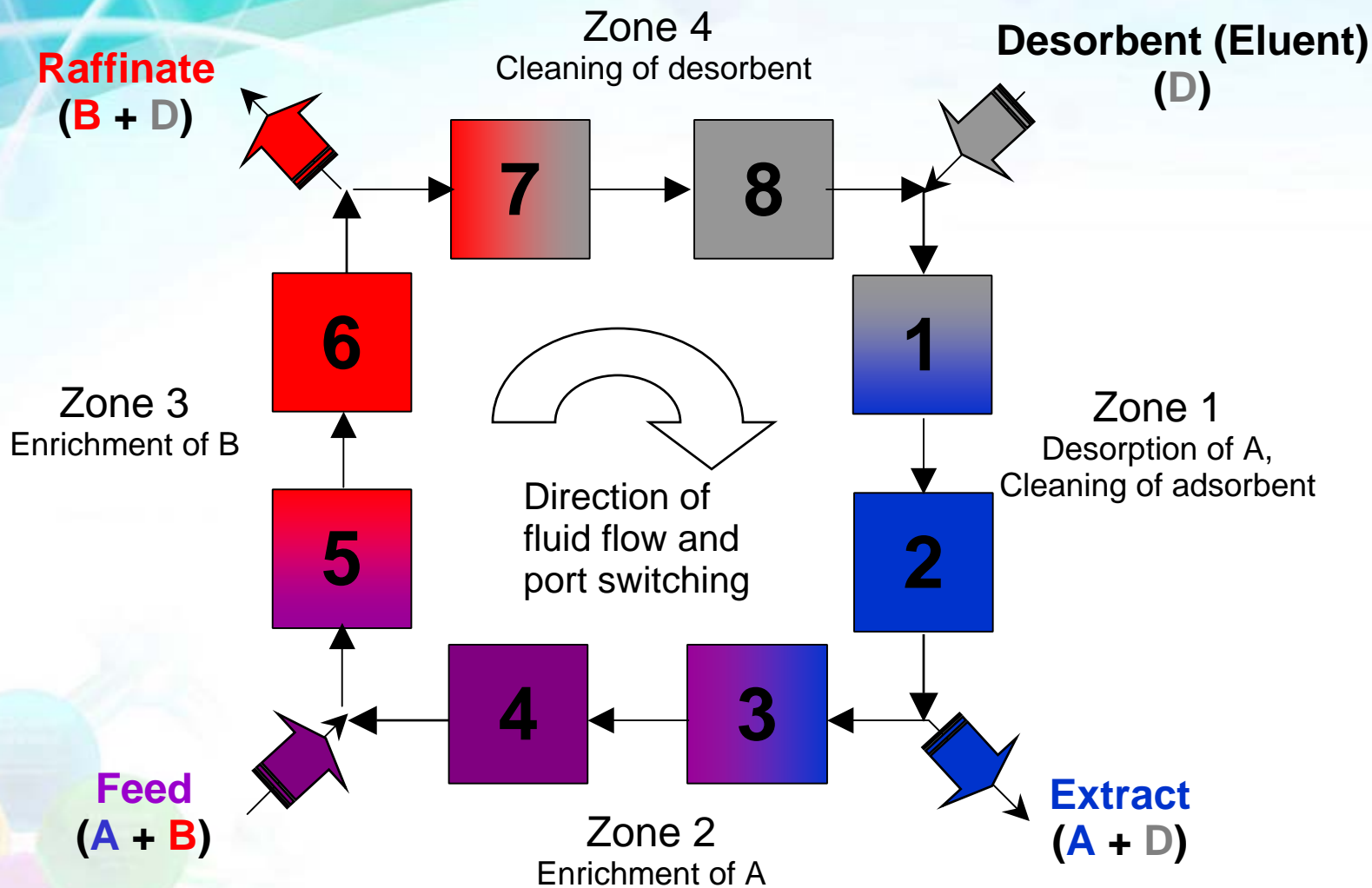
SMB Uses Chiral Stationary Phases More Efficiently

Batch vs. SMB

- **Batch process requires >10,000 plates to achieve baseline separation**
- **SMB uses <500 plates and works with very little separation.**

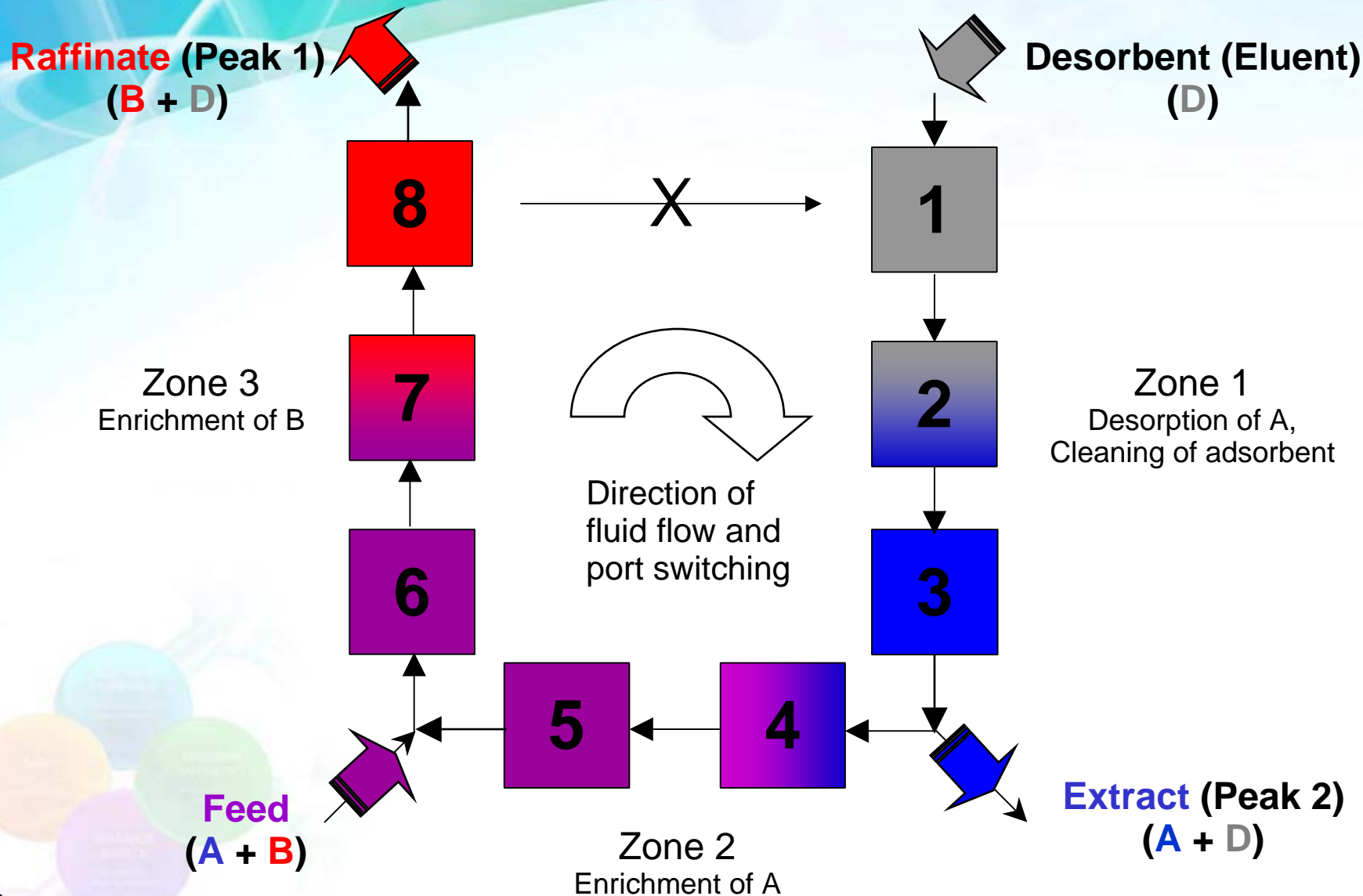


Classical 2-2-2-2 SMB Configuration



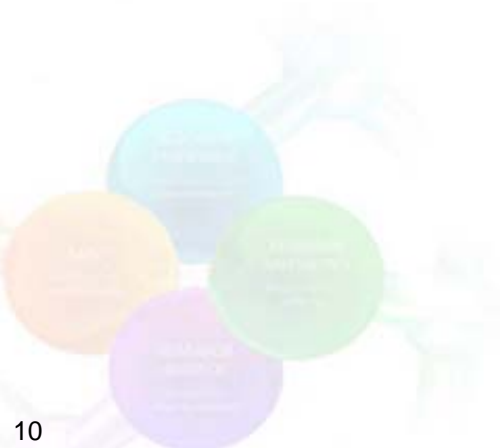
A is slower moving component
B is faster moving component

Modified 3-2-3 Configuration for Chiral Application



Advantages of 3 Zones for Small-Scale SMB

- No need to recycle desorbent
- Flow of Zone 3 into Zone 1 prevented
- Maximizes options for configuration in Zones 1-3
- Simplifies flow rate optimization



Semba Octave Chromatography System

- A versatile **bench-top** multi-column chromatography system
- Capable of performing SMB and other continuous automated separation protocols
- Suitable for milligram to gram scale purification
- Chiral or protein purification



 **Semba**[®]
Biosciences

Key Features

- Eight column positions, accommodates a variety of column sizes
- Proprietary valve block design minimizes dead volume
- Four available inlet and four available outlet channels per column, plus shut-off between columns
- Up to 270 psi operating pressure
- Four independently-controlled pumps
- Non-metallic flow path, compatible with chemical and biological samples and solvents
- Easy-to-use software interface for valve and pump control



Key Measurements to Determine SMB Conditions

- Column properties
 - Single column volume V
 - Extra-column dead volume V^D
 - Retention time of inert tracer at flow rate $Q = t_0$
- Sample properties
 - Analyte resolution, solubility, viscosity
 - Retention time of A at flow rate $Q = t^R_A$
 - Retention time of B at flow rate $Q = t^R_B$
 - Loading studies (expecting nonlinear adsorption isotherms)

Key Parameters for SMB Conditions

- Switch time: t^*
- External flow rates: $Q_{\text{Eluent}} + Q_{\text{Feed}} = Q_{\text{Extract}} + Q_{\text{Raffinate}}$
- 3-zone internal flow rates – simple to calculate
 - $Q_1 = Q_{\text{Eluent}}$
 - $Q_2 = Q_1 - Q_{\text{Extract}}$
 - $Q_3 = Q_2 + Q_{\text{Feed}}$

Henry Constants Determination

$$H_i = [(t_i^R - t_o)/t_o] \times [\varepsilon/(1 - \varepsilon)]$$

(Selectivity = H_2/H_1)

HPLC RUN

t_i^R = retention time of component i (small pulse)

t_o = retention time of inert tracer

ε = overall void fraction of column = $t_o * Q/V$,

where V = column volume, Q = flow rate

Words into Math-Equilibrium Theory

$$q_i = H_i c_i \rightarrow t_{Ri} = t_M [1 + (1 - \epsilon_{total}) / \epsilon_{total} H_i] \quad [Nicoud, 1992]$$

$$t_{A1} \leq t_{switch}$$

$$t_{B2} \leq t_{switch} \leq t_{A2}$$

$$t_{B3} \leq t_{switch} \leq t_{A3}$$

$$t_{switch} \leq t_{B4}$$

Equilibrium Theory: under ideal conditions separation depends on generalized flow rate ratios in the 4 zones

$$m_j = \frac{Q_j^{SMB} t_{switch} - V \epsilon_{total}}{V(1 - \epsilon_{total})}$$

$$H_A < m_1 < \infty$$

$$H_B < m_2 < H_A$$

$$H_B < m_3 < H_A$$

$$m_4 < H_B$$

H_i : Henry coefficient – linear adsorption coefficient

m_j : flow rate ratio in zone j

Q_j^{SMB} : volume flow rate in SMB zone j

Migliorini, Morbidelli et al. (2)

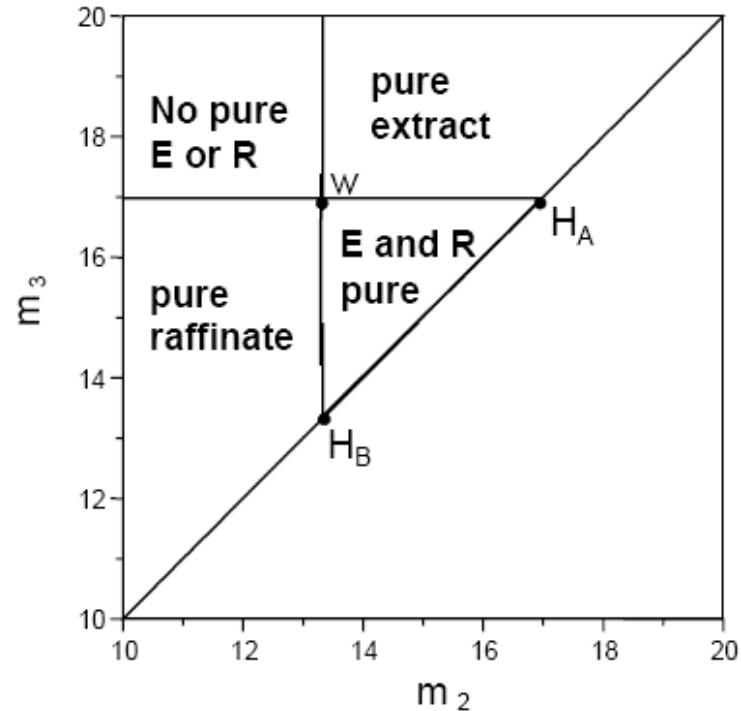
m_2, m_3 -plane for Linear Adsorption Isotherms

$$m_3 > m_2$$

$$H_B < m_2 < H_A$$

$$H_B < m_3 < H_A$$

$$m_j = \frac{Q_j^{SMB} t_{switch} - V \epsilon_{total}}{V(1 - \epsilon_{total})}$$



W = optimal operating point = maximal productivity

Practical Effects of Parameters (m_2/m_3) Adjustment

Eluent flow/Switch time

Increase

Decrease



Extract flow rate

Increase

Decrease

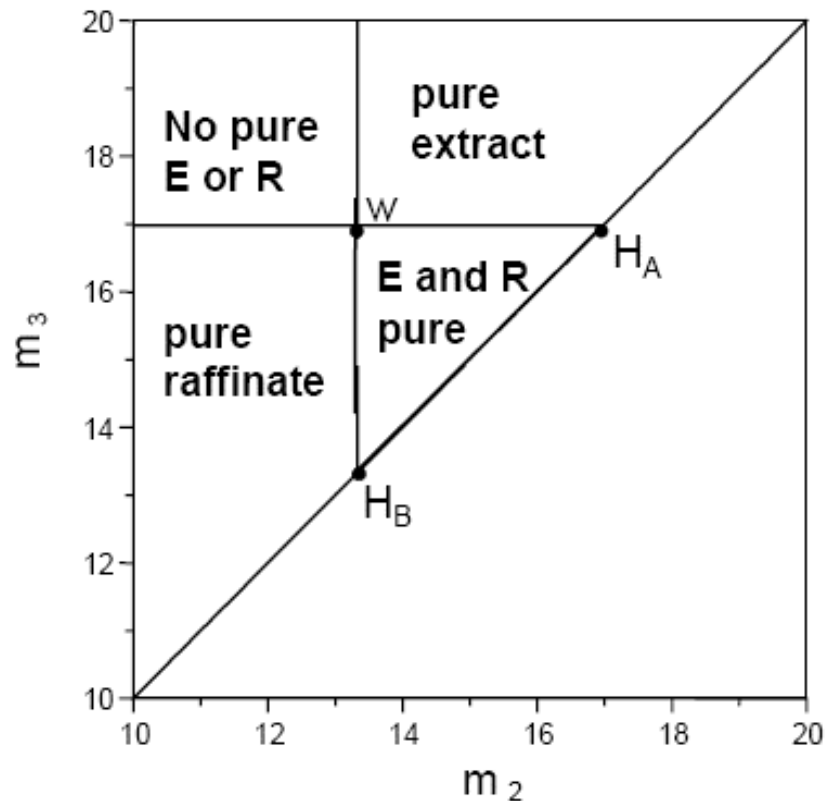


Feed flow rate

(Affecting m_3 only)

Increase

Decrease



$$m_j = \frac{Q_j^{SMB} t_{switch} - V \epsilon_{total}}{V(1 - \epsilon_{total})}$$

Sample Overload: Langmuirian or anti-Langmuirian?

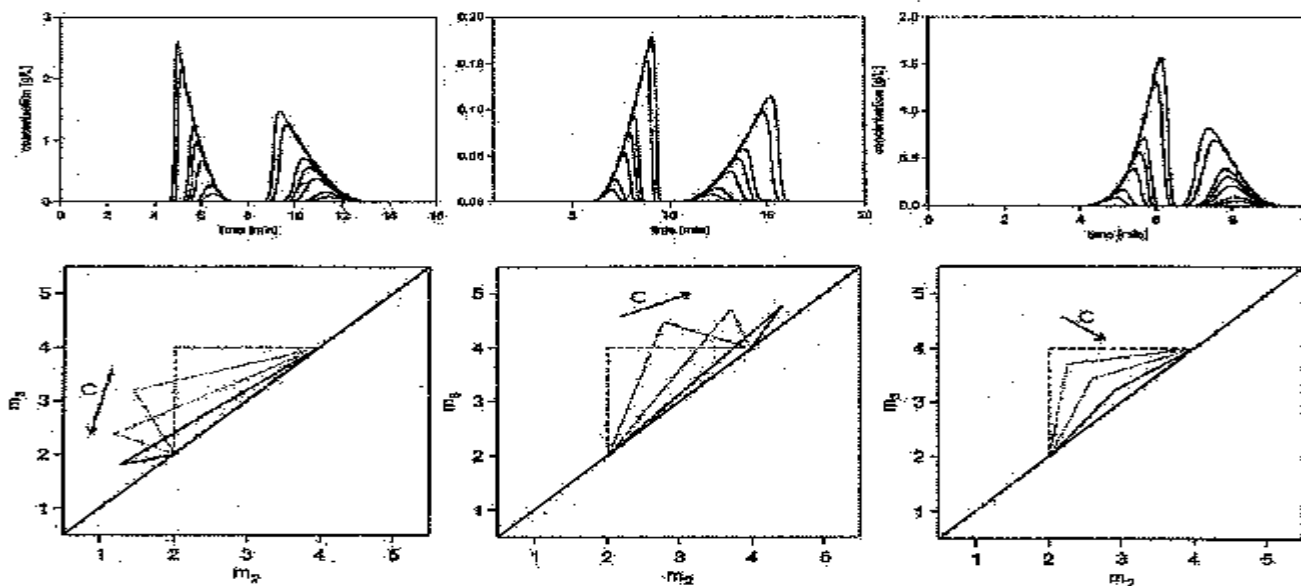
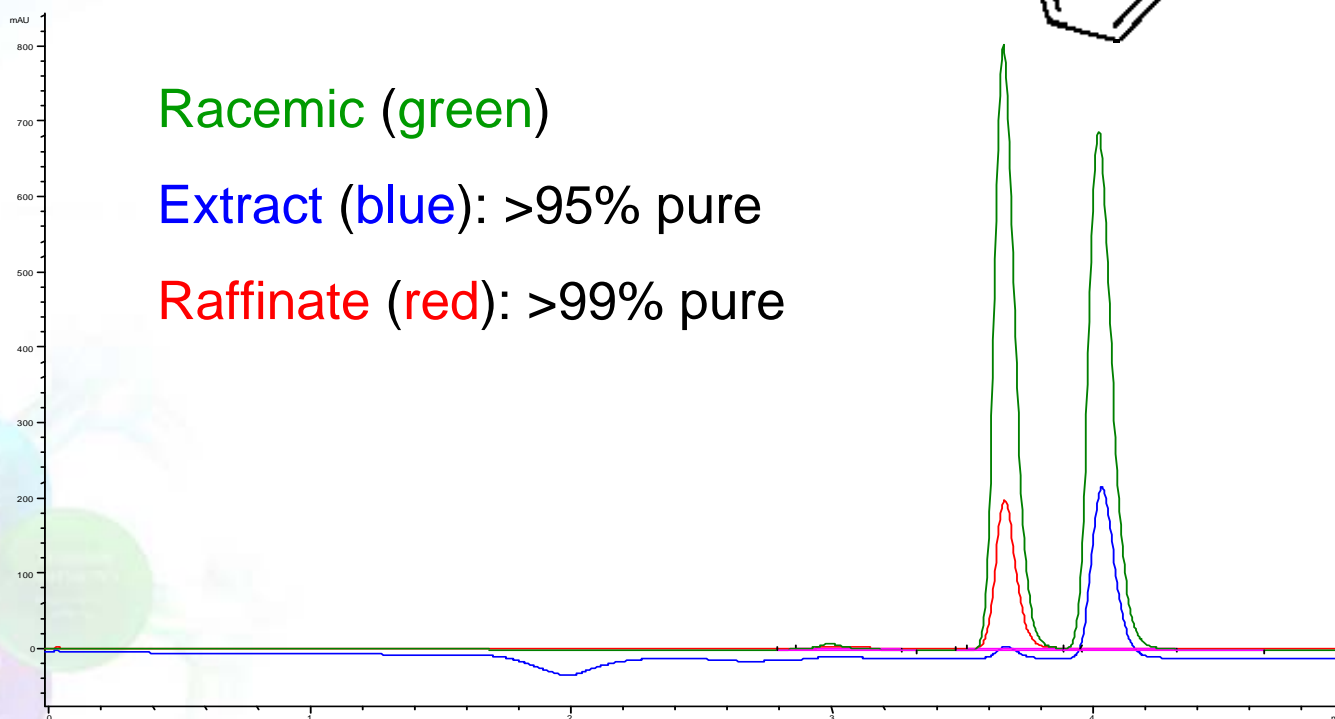
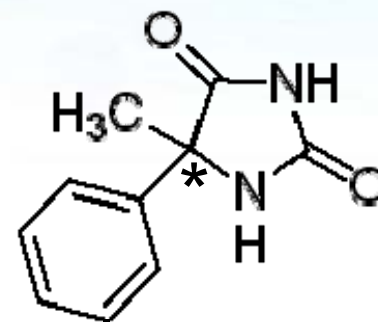


Fig. 7.27 Qualitative trends: peak shapes from loading studies and corresponding separation regions in the m_1 , m_2 plane; left: Langmuirian behavior; middle: Anti-Langmuirian behavior; right: Langmuirian for the second eluted enantiomer, Anti-Langmuirian for the first eluted enantiomer.

216 | 7.4 Common Applications of Enantioselective HPLC Using the SMB Technology

Experimental 1. HPLC Results

column: Astec CHIROBIOTIC™ V2, 25 cm x 4.6 mm, 5 μm
mobile phase: 100% MeOH
flow rate: 1.0 mL/min.
sample: 5-methyl 5-phenylhydantoin (2 mg/mL)



Racemic (green)

Extract (blue): >95% pure

Raffinate (red): >99% pure

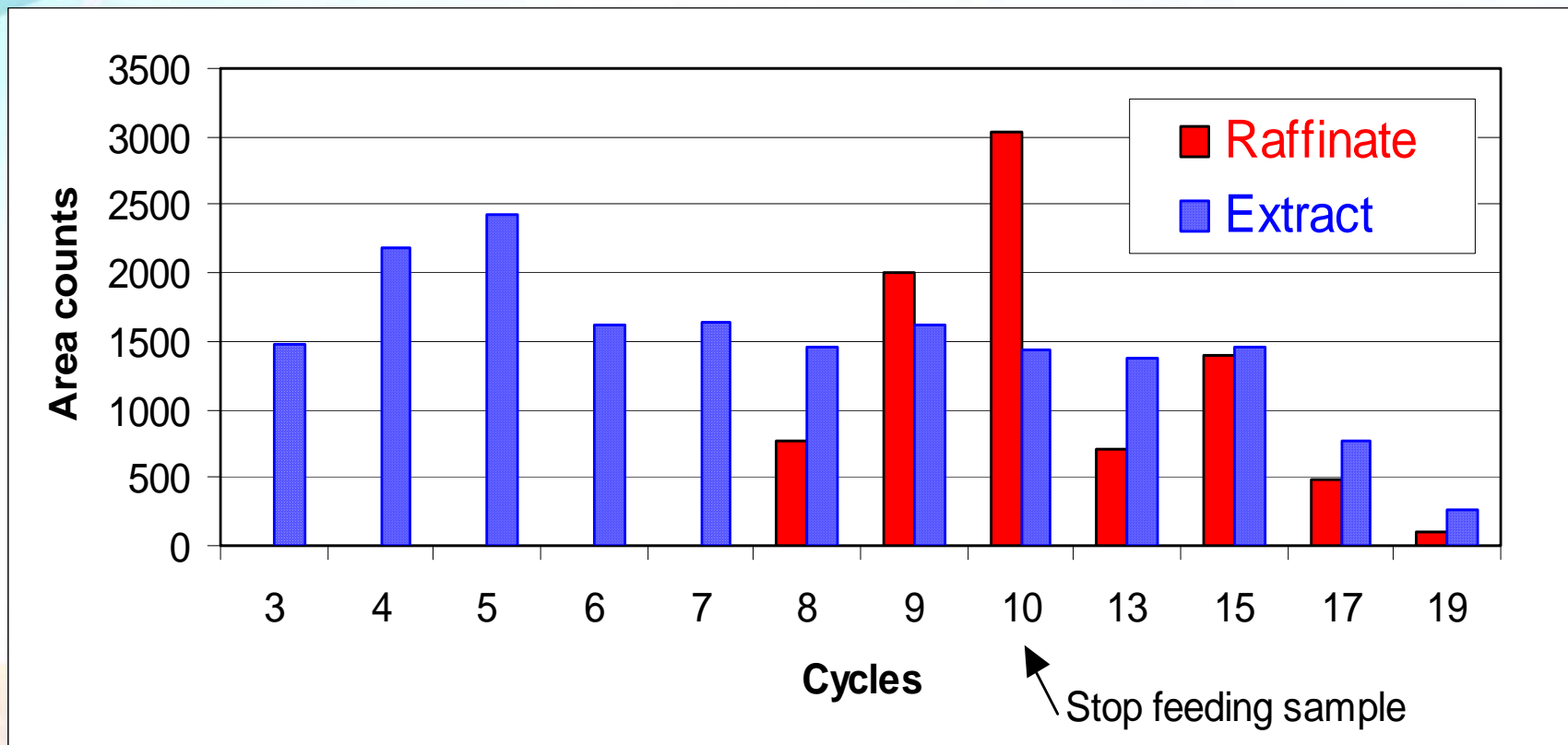
Experimental 1. SMB Results

- Compound: 5-methyl 5-phenylhydantoin (Selectivity = 1.39)
- Eluent: 100% CH₃OH
- Column (x8): Astec CHIROBIOTIC V2, 5 cm x 10 mm, 15 μm particles
- Henry constant A: 0.80
- Henry constant B: 1.11
- Recovery: 90%
- Results:

Concentration	Feed Flow	Productivity	Purity
10 mg/mL	0.15 mL/min.	20 mg/hr. each enantiomer	Raffinate: 99.8% Extract: 95.7%
20 mg/mL	0.12 mL/min.	35 mg/hr. each enantiomer	Raffinate: 99.5% Extract: 93.5%
60 mg/mL	0.15 mL/min.	60 mg/hr. Extract only	Raffinate: 65% Extract: 93.0%

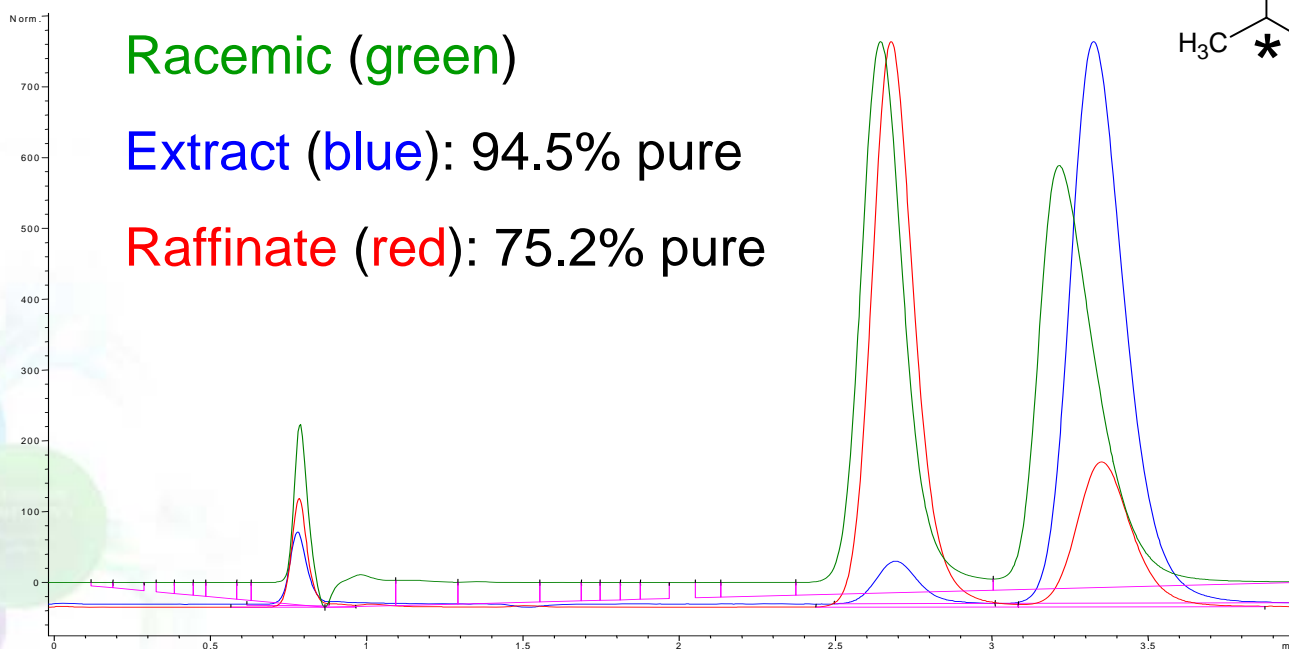
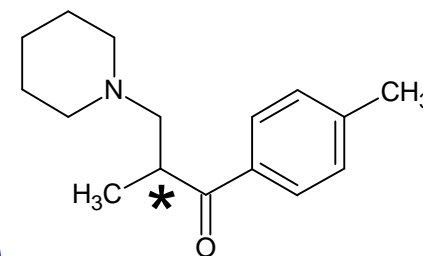
- Throughput of batch chromatography (stacked injections) is 1.5 mg/g CSP/hr.

Typical Concentration Profile from Collections



Experimental 2. HPLC Results

- Sample: Tolperisone (2 mg/mL)
- Mobile Phase: 100/0.1/0.1, MeOH/HOAc/TEA
- Column: Astec CHIROBIOTIC V2, 3 cm x 4.6 mm, 5 μm
- Flow Rate: 0.5 mL/min.



Racemic (green)

Extract (blue): 94.5% pure

Raffinate (red): 75.2% pure

Experimental 2. SMB Results

- Compound: Tolperisone (Selectivity = 1.36)
- Eluent: 100/0.1/0.1 (v/v/v), MeOH/HOAc/TEA
- Column (x8): Astec CHIROBIOTIC V2, 5 cm x 10 mm, 15 μm
- Henry constant A: 3.66
- Henry constant B: 4.96
- Recovery: 80%
- Results:

Concentration	Feed Flow	Throughput	Purity
2.5 mg/mL	0.2 mL/min.	0.7 mg/g CSP/hr.	Raffinate: 99.9% Extract: 98.0%
10 mg/mL	0.2 mL/min.	3 mg/g CSP/hr.	Raffinate: 75.2% Extract: 94.5%

- Throughput of batch chromatography (stacked injections) is 2 mg/g CSP/hr.

Conclusions

- Bench-top SMB is a viable option for small scale (grams quantity) chiral purification with quick turnaround time
- Mobile phase design on Astec CHIROBIOTIC CSPs is suitable for SMB application
 - Both polar organic and polar ionic mode provide good separation with high efficiency and low pressure drop
 - Consisting of 100% methanol, these two mobile phase types do not have sample solubility issues
 - Astec CHIROBIOTIC columns are very rugged and reproducible
- Compared to batch chromatography, SMB provides greener process
 - Less solvent consumption
 - Less sample recovery time
 - Higher throughput

Acknowledgements

- Semba Biosciences
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References

1. E. Huthmann and M. Juza, J. of Chromatography (2005) 1092, 24-35.
2. C. Migliorini *et al*, AIChE J., (2002) Vol. 48, No. 1, 69-77.