Performance and Benefits of Macrocyclic Glycopeptide-Based CSPs in Enantiomeric Purification using Bench-Top Simulated Moving Bed (SMB) Technology

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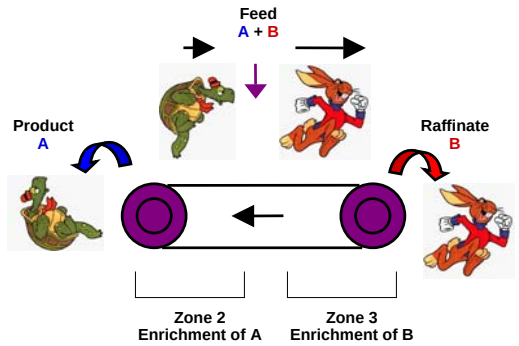
ABSTRACT

Chiral stationary phases (CSPs) comprising macrocyclic glycopeptides covalently bonded to high purity silica have significant benefits in preparative chiral separations, including wide enantioselectivity, use of mobile phases that can be optimized for maximum sample solubility, ability to retain and resolve polar and ionic compounds, and excellent robustness. When used in conjunction with a new bench-top simulated moving bed (SMB) instrument, the benefits of these CSPs are combined with the advantages of SMB for the rapid isolation of gram quantities of purified enantiomers.

This presentation will demonstrate the power of macrocyclic glycopeptide CSPs and the aforementioned bench-top SMB system to purify chiral pharmaceutical compounds in polar mobile phase systems that enhance the compound's solubility. The advantages of this approach over traditional single column batch-type preparative chromatography will be shown in terms of productivity, reduction of solvent waste and product recovery. The ease of method development from HPLC to SMB scales and the ruggedness of the CSPs and the instrument will also be demonstrated.

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

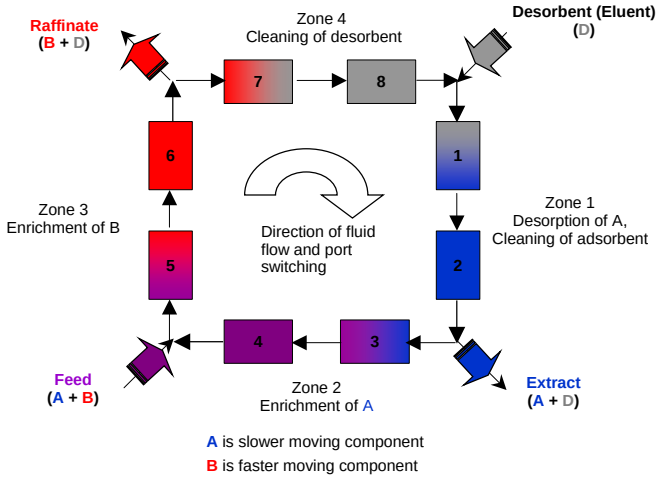
1989-present, large scale chiral purifications

- Novasep and Knauer etc.

Continuous countercurrent chromatography

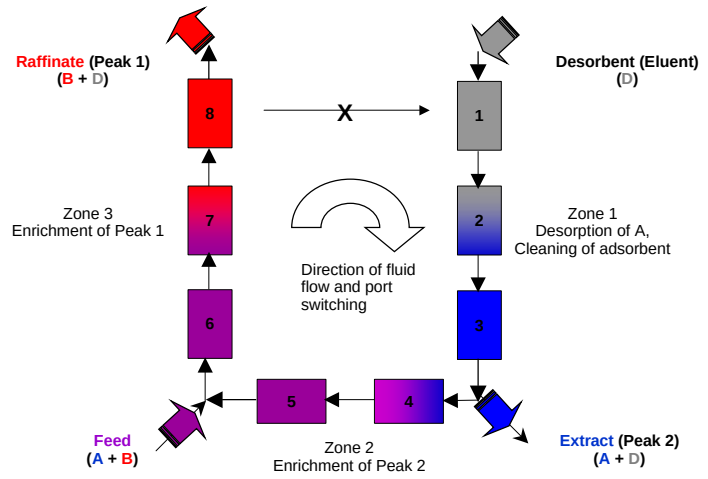
- 4/8/12/16 HPLC columns-in 4 or 3 zones
- Each column has 2 Inlets (Feed/Eluent), 2 outlets (Extract/Raffinate) and a connection valve between columns
- Continuous valves/ports switching at a fixed time interval in the direction of the eluent flow
- Binary separations

Classical 4-zone (2-2-2-2) Configuration



A is slower moving component
B is faster moving component

Modified 3-2-3 Configuration for Chiral Application



Semba Octave Chromatography System

- A versatile small footprint system capable of performing SMB and other continuous automated separation protocols
- Suitable for grams scale purification
- 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 shutoff between columns
- Non-metallic flow path, compatible with chemical and biological samples and solvents
- Up to 270 psi operating pressure
- Chiral or protein purification

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)

Henry Constants Determination

$$H_i = [(t^R_i - t_0)/t_0] \times [e/(1- e)]$$

(Selectivity = H_2/H_1)

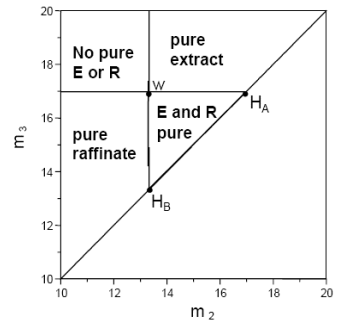
HPLC RUN

t^R_i = retention time of component i (small pulse)
t_0 = retention time of inert tracer
e = overall void fraction of column = t_0*Q/V,
where V = column volume, Q = flow rate

m_2, m_3-plane for Linear Adsorption Isotherms

Equilibrium Theory → $$m_j = \frac{Q_j^{SMB} t_{switch} - V\varepsilon_{total}}{V(1-\varepsilon_{total})}$$

$$m_3 > m_2$$
$$H_B < m_2 < H_A$$
$$H_B < m_3 < H_A$$



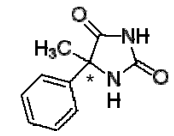
W: optimal operating point = maximal productivity

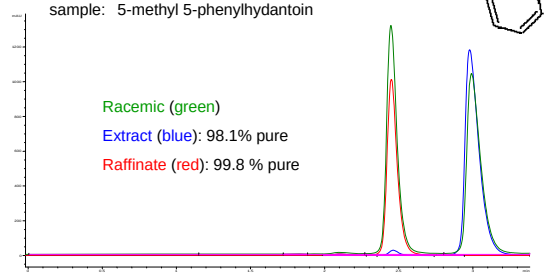
SMB Experimental Results

Selectivity vs. Productivity		
CHIROBIOTIC™ V2, 5 cm x 10 mm, 15 µm; 25 g CSP total		
Sample A: 5-methyl 5-phenylhydantoin	**Productivity**	**Purity**
Eluent: 100% MeOH Henry constants A/B: 0.80/1.11; Selectivity: 1.39	35 mg/hr Each enantiomer	Raffinate: 99.5% Extract: 93.5%
Sample B: tolperisone		
Eluent: 100/0.1/0.1, MeOH/HOAc/TEA Henry constants A/B: 3.66/4.96; Selectivity: 1.36	30 mg/hr Each enantiomer	Raffinate: 75.2% Extract: 94.5%
CHIROBIOTIC T, 5 cm x 10 mm, 15 µm; 25 g CSP total		
Sample A: 5-methyl 5-phenylhydantoin	**Productivity**	**Purity**
Eluent: 100% MeOH Henry constants A/B: 0.72/1.33; Selectivity: 1.85	70 mg/hr Each enantiomer	Raffinate: 99.8% Extract: 98.1%
Sample B: ketorolac (2-3-3 configuration)		
Eluent: 100/0.1w%, MeOH/NH_4Formate Henry constants A/B: 0.76/1.61; Selectivity: 2.12	75 mg/hr Each enantiomer	Raffinate: 99.8% Extract: 98.8%

HPLC Purity Tests from Collections

column: CHIROBIOTIC T, 10 cm x 4.6 mm, 5 µm
mobile phase: 100% MeOH
flow rate: 0.6 mL/min.
sample: 5-methyl 5-phenylhydantoin





CONCLUSIONS

Bench-top SMB is a viable option for grams quantity chiral purification with quick turnaround time

Polar mobile phase design on 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 alleviate some sample solubility issues
- CHIROBIOTIC columns are very rugged and reproducible

Compared to batch chromatography, SMB provides greener process

- Less solvent consumption
- Less sample recovery time
- Higher productivity/throughput