Advances in Method Development for Preparative Chiral Chromatography

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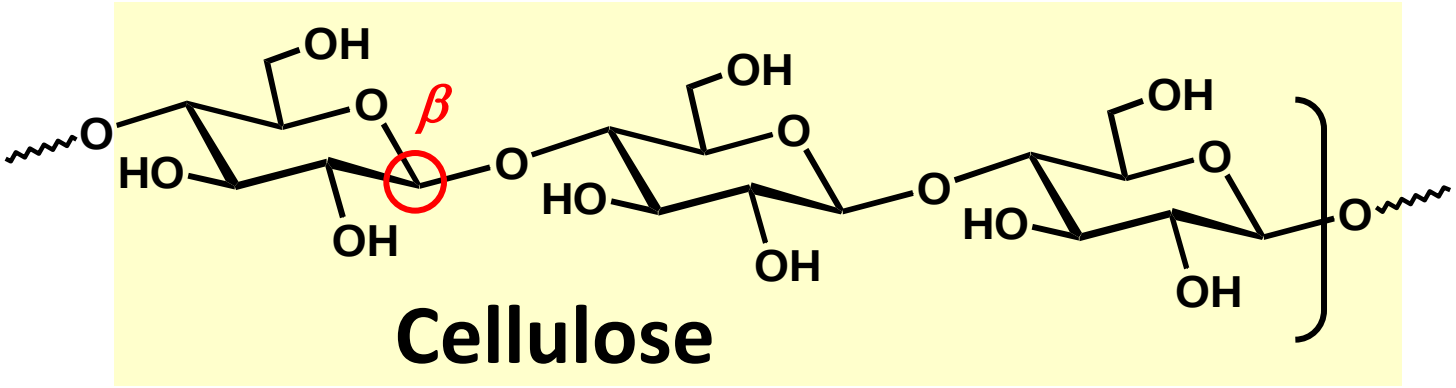
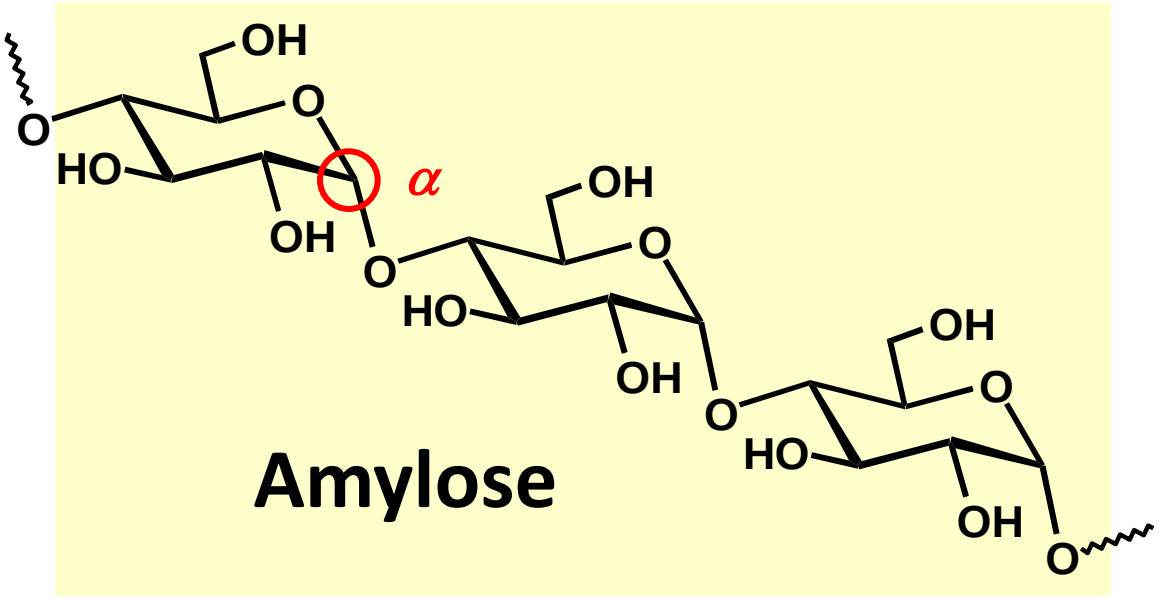
Key Requirements for High Productivity in Preparative Chiral Chromatography Which Guide Method Development

- Excellent **Solubility** of Compound in Mobile Phases
- Very Good **Selectivity** for the Pair of Enantiomers to be Separated
- High **Loading Capacity** of the Chiral Stationary Phase

Polysaccharides – Most Abundant Chiral Molecules in Nature



Cellulose



Amylose

When Derivatized and Coated or Immobilized on Silica, Polysaccharide-Based Chiral Stationary Phases:

- Work Very Well in Preparative Applications:
- Well Established **High Loading Capacity**
- Excellent Chance of Finding a Separation with **Very Good Selectivity** with a small group of well selected columns
- What about **Excellent Sample Solubility** of Compatible Mobile Phases

Mobile Phases for Polysaccharide - Based Chiral Stationary Phases

- Coated Columns
  - Alkane/Alcohols
  - MeOH, EtOH, IPA, Acetonitrile
- Immobilized Columns
  - All Organic Solvents

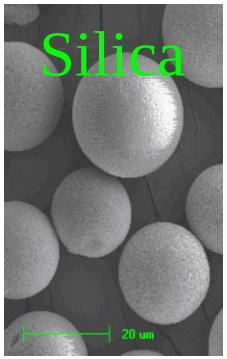
Real World Situation

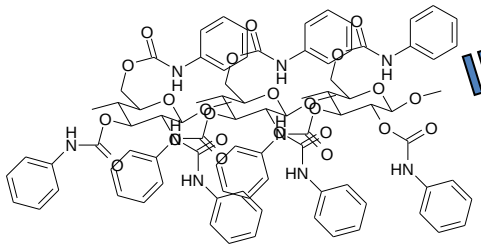
- Diuretic Compound for Small Scale Prep
- Normal Phase Method – Coated Cellulosic column and Hexane/Isopropanol Mobile Phase
- Sample is Only Soluble in Methanol, Acetonitrile, and maybe other Polar Organic Solvents
- **Impasse- No Good Way to Make this System Work**

Temptation – Dissolve Your Sample in a Good Solvent and Shoot as Much as Possible

- Negative Consequences
  - Coated Columns Destroyed by Forbidden Sample Diluents
  - Sample Crashes Out of Solution
    - Rapid Decline in Column Performance
    - Blocked Frits, High Pressure Drop
- Solution – Develop a Separation in Which the Mobile Phase is also a Good Sample Diluent

Immobilization Process





Polymer

Proprietary Immobilization Techniques

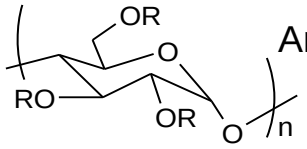
CSP

CHIRALPAK IA
CHIRALPAK IB
CHIRALPAK IC
CHIRALPAK ID
CHIRALPAK IE
CHIRALPAK IF

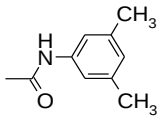
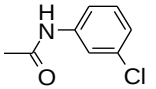
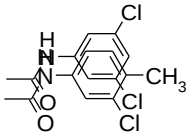
Advantages of Immobilized Polysaccharide Columns

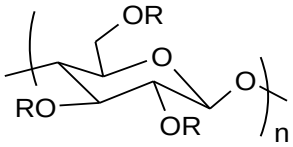
- Unique to Chiral Technologies
- Rugged
- Use with any organic solvent
- Very helpful for samples that are difficult to dissolve
- Choose solvents that are appropriate for reactive compounds
- Etc.

Screening Options: Second Generation Polysaccharide-derived CSPs

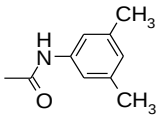
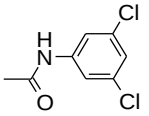


Amylose-based

CSP	Nature	-R
CHIRALPAK IA	Immobilized	
CHIRALPAK ID	Immobilized	
CHIRALPAK IE	Immobilized	
CHIRALPAK IF	Immobilized	



Cellulose-based

CSP	Nature	-R
CHIRALPAK IB	Immobilized	
CHIRALPAK IC	Immobilized	

Screening in HPLC

Primary Screen

CHIRALPAK IA
CHIRALPAK IB
CHIRALPAK IC
CHIRALPAK ID

Secondary Screen

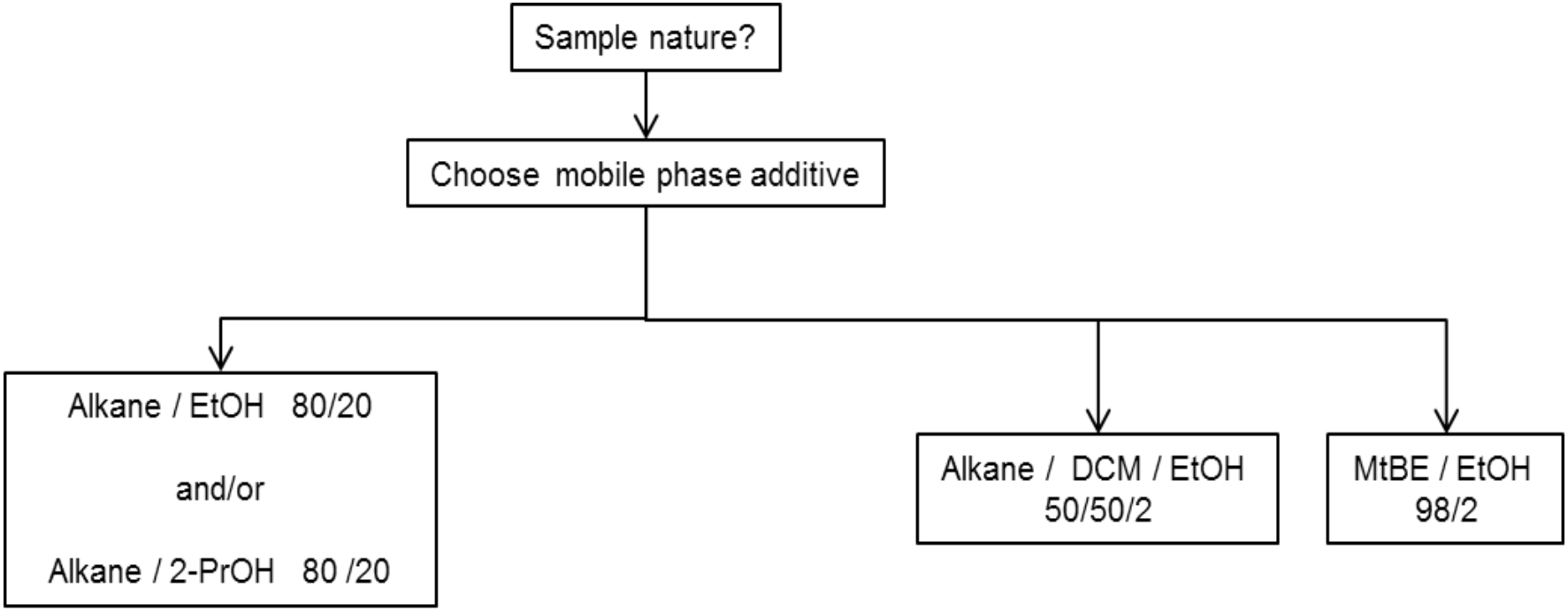
CHIRALPAK IE
CHIRALPAK IF

CHIRALPAK AD-H
CHIRALPAK AS-H
CHIRALPAK AY-H
CHIRALPAK AZ-H

CHIRALCEL OD-H
CHIRALCEL OJ-H
CHIRALCEL OZ-H
CHIRALCEL OX-H

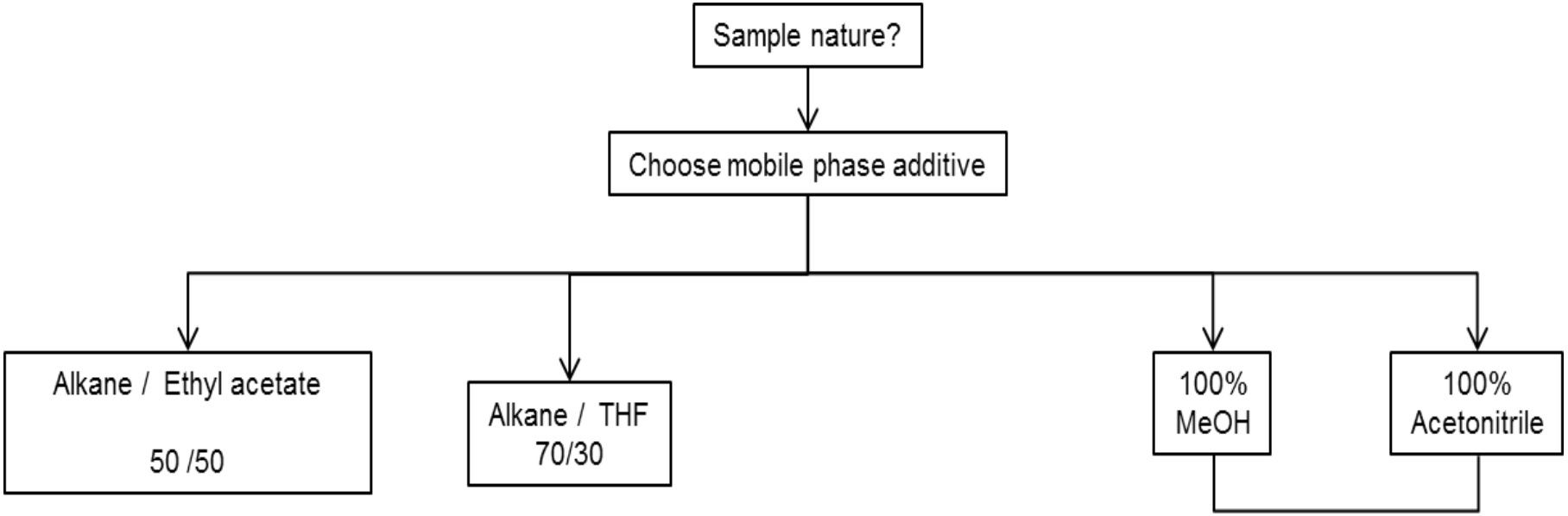
Primary Solvents used in HPLC - Organic

Immobilized CSPs



Secondary Solvents used in HPLC - Organic

Immobilized CSPs



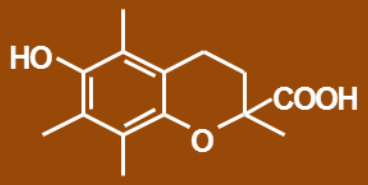
Method Optimization

Straightforward optimisation

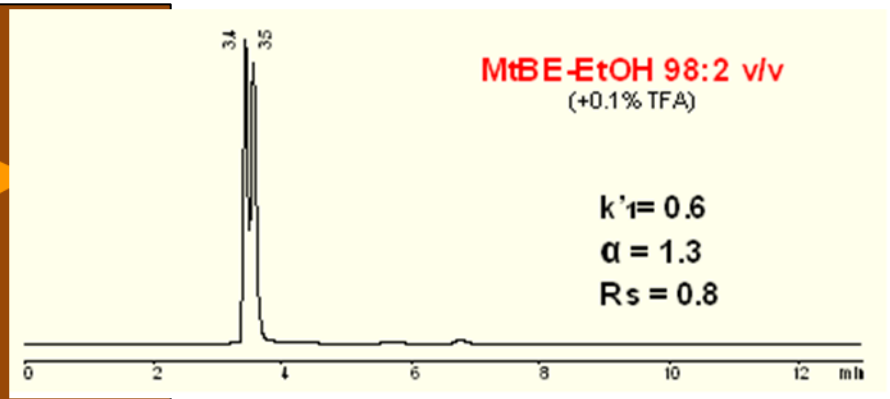
Primary solvent mixtures	Alkane-MtBE-EtOH
Starting condition	0:98:2
Optimisation range	80:20:0 to 0:40:60

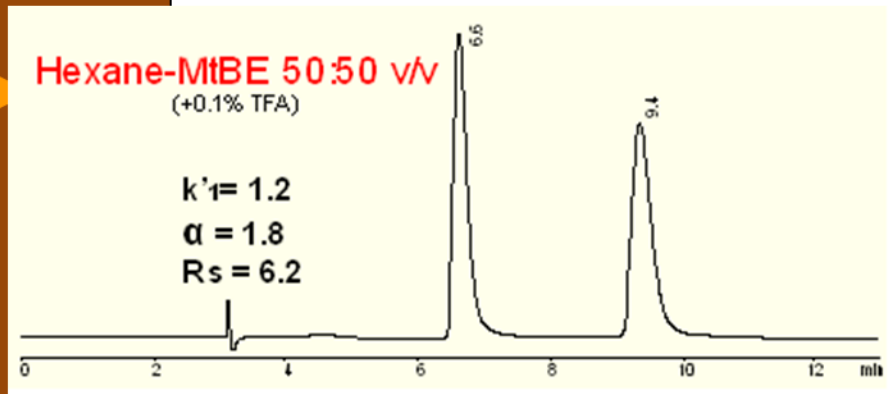
CHIRALPAK® IC

250x4.6mm (I.D.)
1.0ml/min
25° C



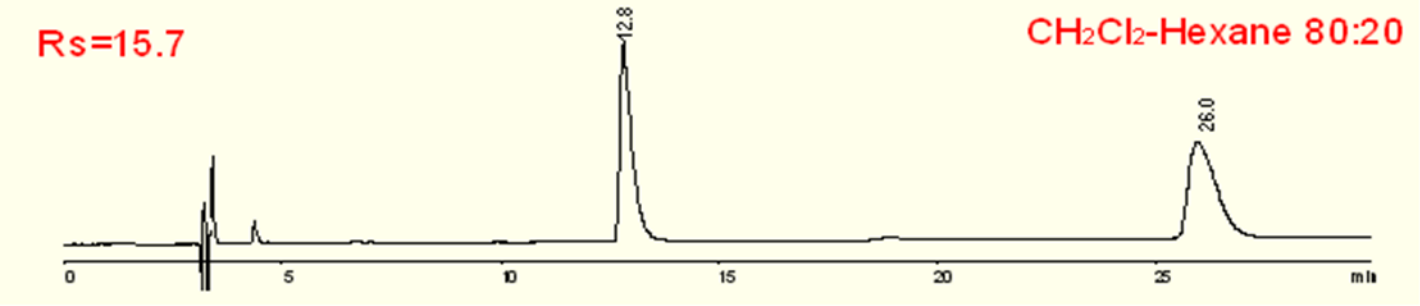
6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

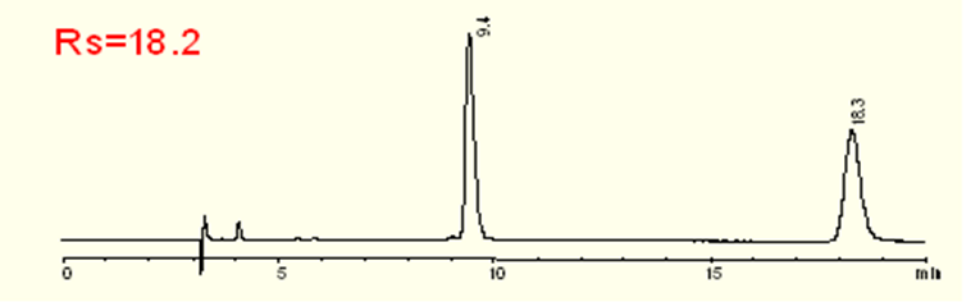




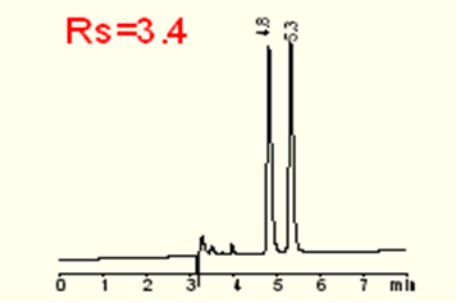
Method Optimization

Analytical application - Optimisation





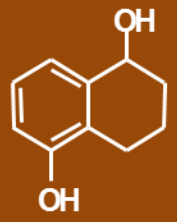
CH_2Cl_2 100%



CH_2Cl_2-MeOH 99:1

CHIRALPAK® IC

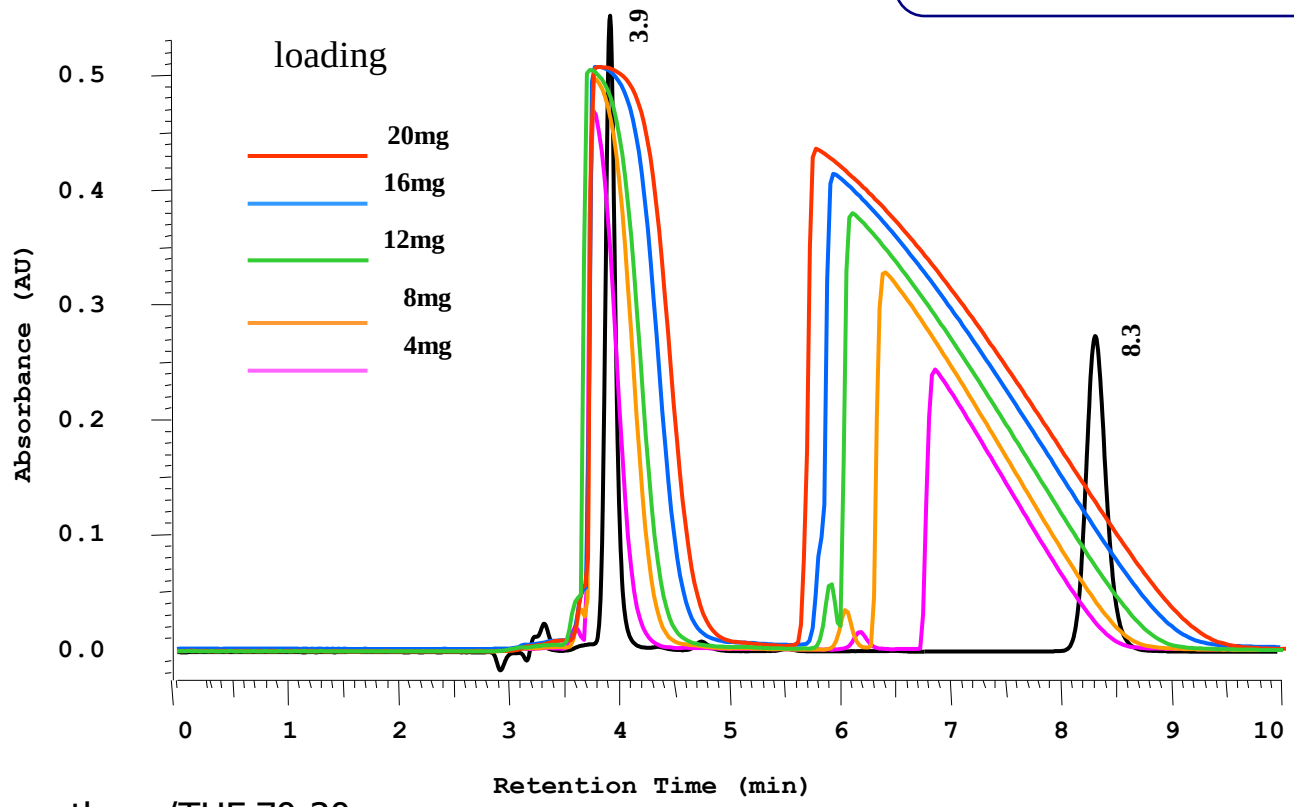
250x4.6mm (I.D.)
1.0ml/min
25° C



1,5-dihydroxy-1,2,3,4-tetrahydronaphthalene

Loading Study for EMD-53986

Estimated productivity:
2.8kg enantiomer/kg CSP/day



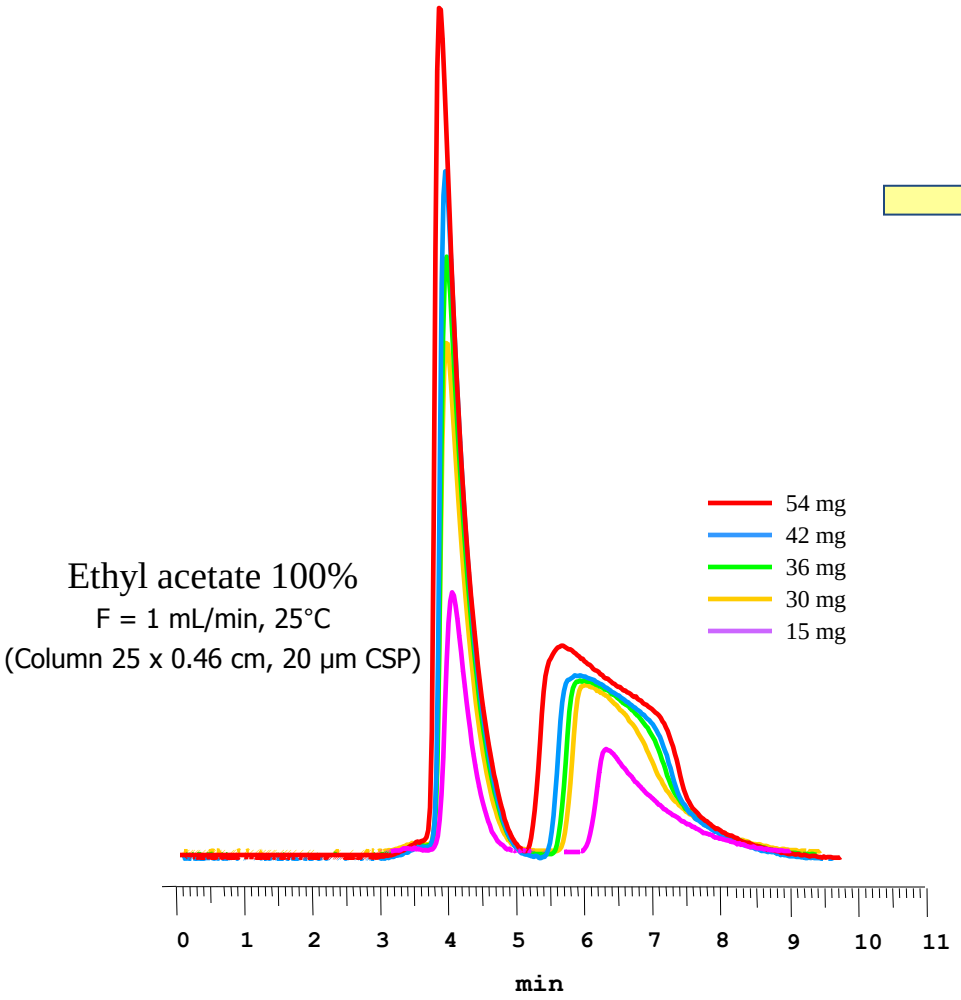
Dichloromethane/THF 70:30

F = 1 mL/min, 25°C

(Column 25 x 0.46 cm, 5 µm CSP)

Solubility in mobile phase: 45 g/L

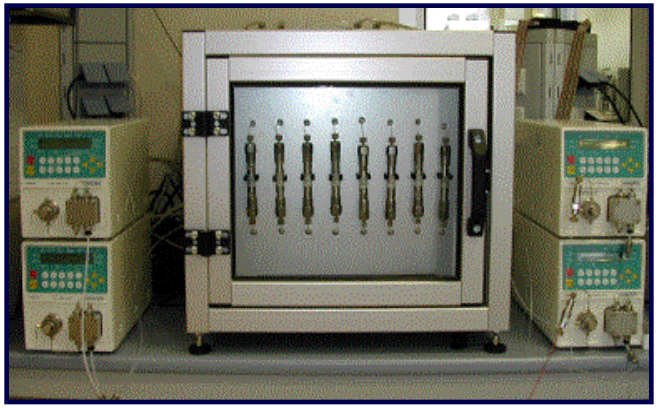
Glutethimide



Solubility in mobile phase: 300 g/L

Productivity:
> 11 kg enantiomer/kg CSP/day

Productivity demonstrated
under SMB conditions

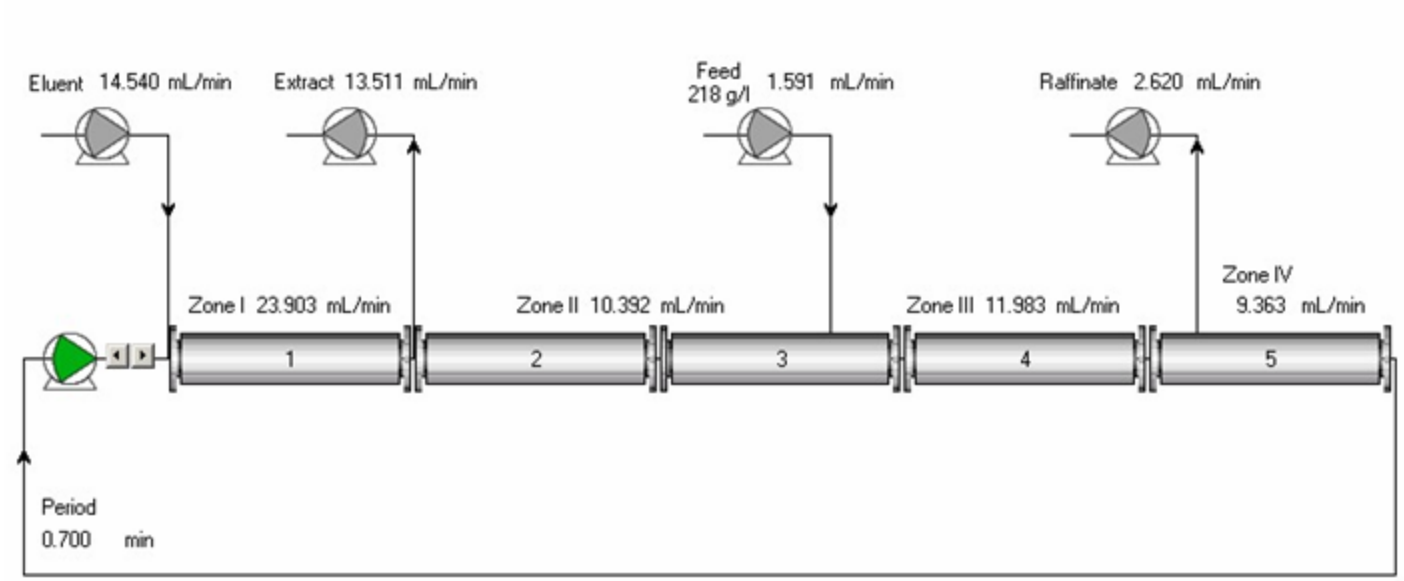


Preparative Chromatography

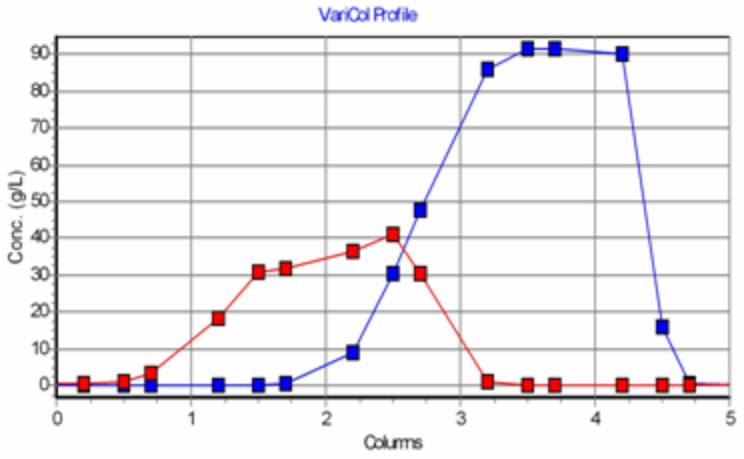
HPLC (batch)

SMB (continuous)

Figure 5: Productivity estimations for enantiomer separation of glutethimide

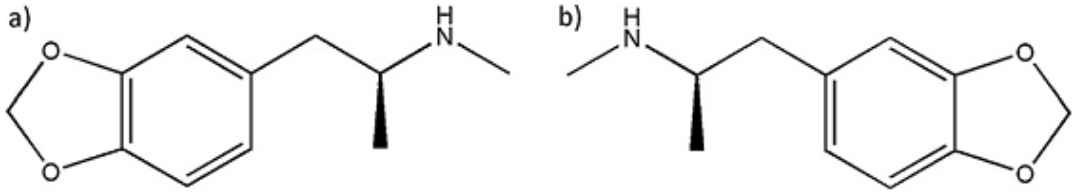


CSP: CHIRALPAK IA (20 µm)
Mobile phase: Ethyl acetate 100%
Temperature: 25°C



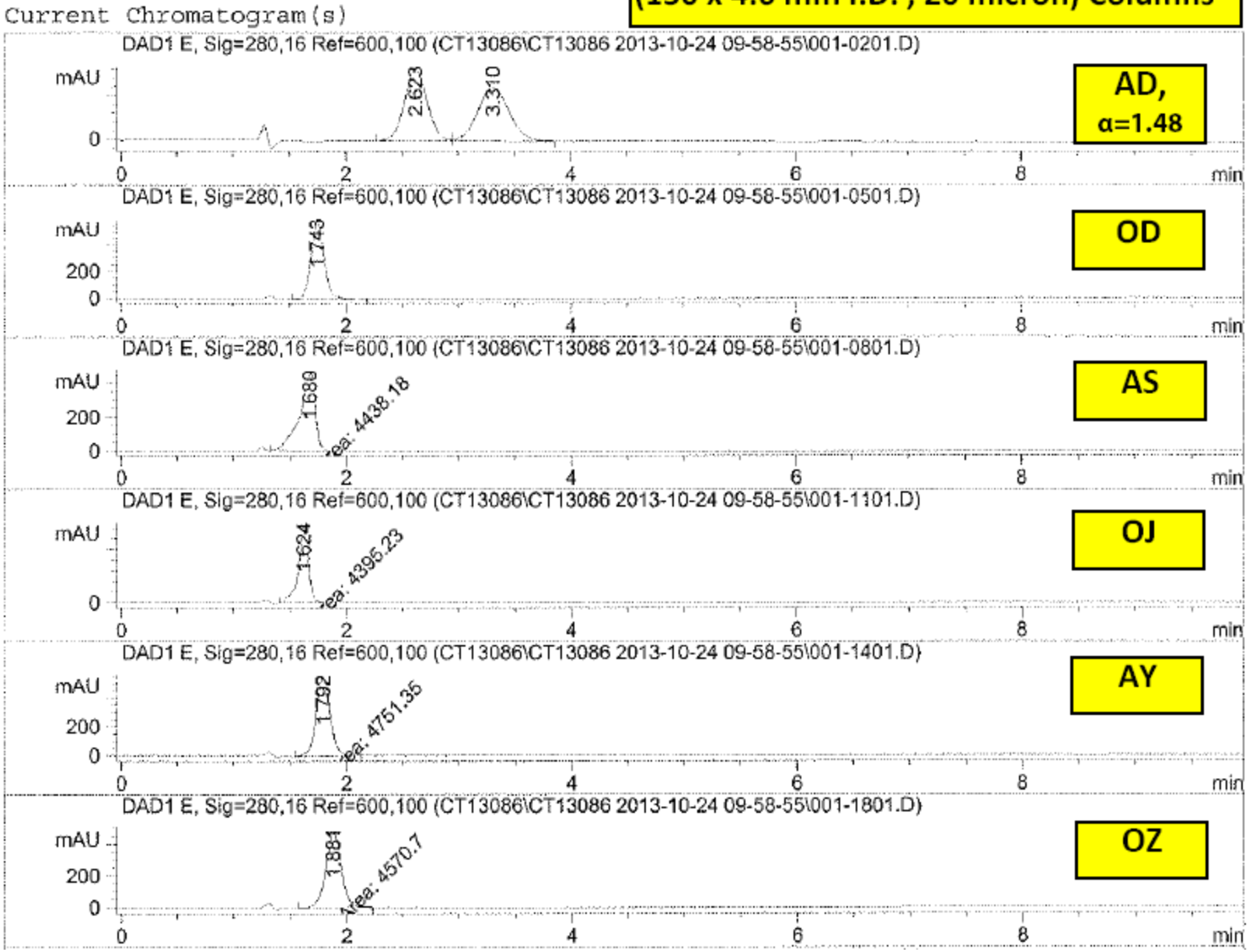
MDMA (3,4-methylenedioxy-methamphetamine) or “Ecstasy,” is a well-known drug of abuse that has become popular at raves due to its stimulant action and ability to induce a feeling of euphoria and intimacy. MDMA is a chiral compound due to the asymmetric center. Although both enantiomers show different pharmacologic activities, stereoselective metabolism and body disposition, it is consumed as a racemate.

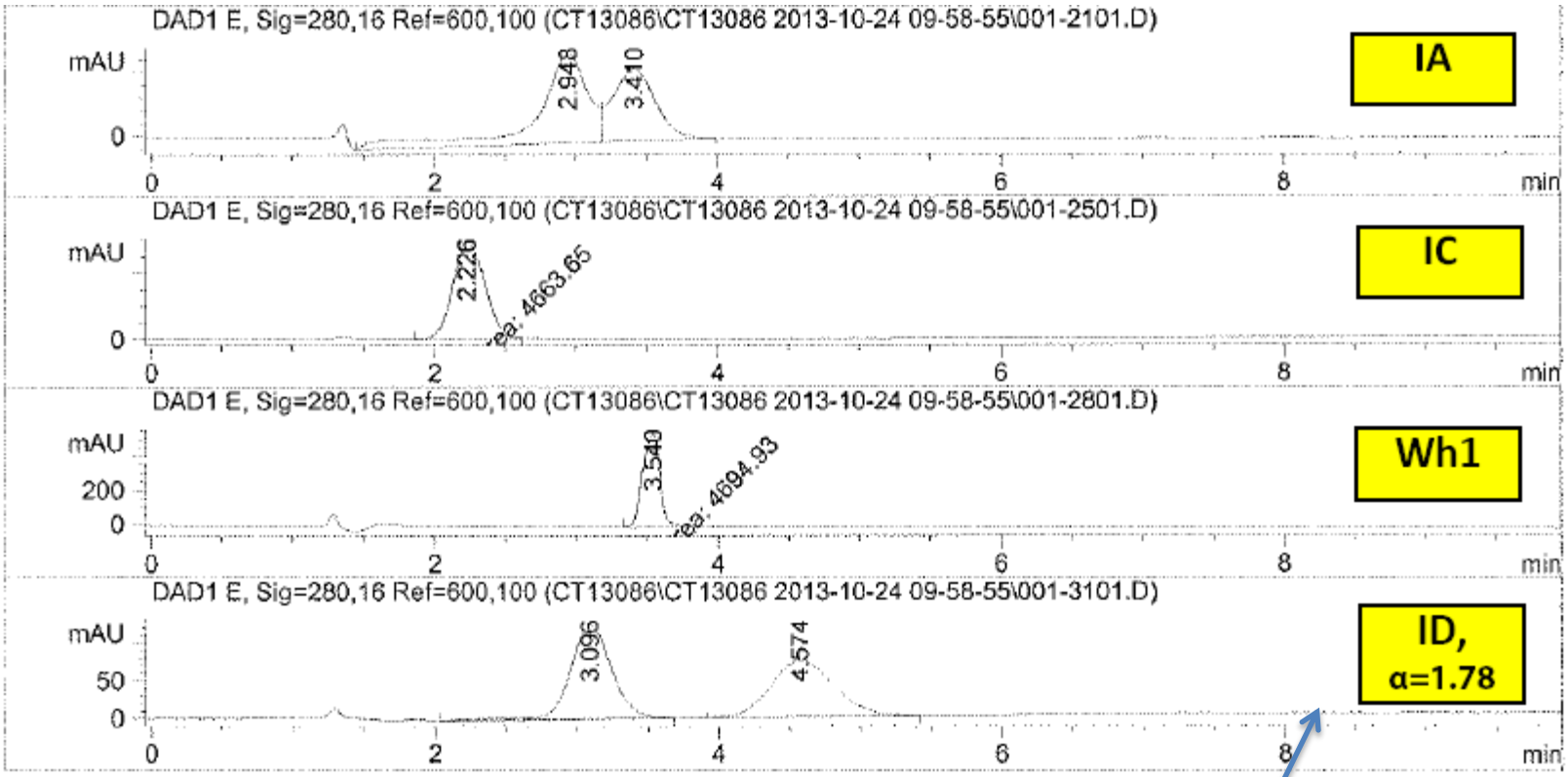
Studies have shown that (S)-(+)-MDMA is more active than the (R)-(−)-MDMA on central nervous system and contributes more to the serotonergic degeneration associated with MDMA consumption[1,2,3]. Besides that, while (R)-(−)-MDMA showed to be responsible for oxidative damage in rats liver, the (*S*)-(+)-MDMA preserves the liver against oxidative effects[4].



Print of window 38: Current Chromatogram(s)

CT13083, 3 mg/ml in EtOH, 5 uL;
ACN/DEA 100:0.1, 1.5 mL/min;
(150 x 4.6 mm I.D. , 20 micron) Columns

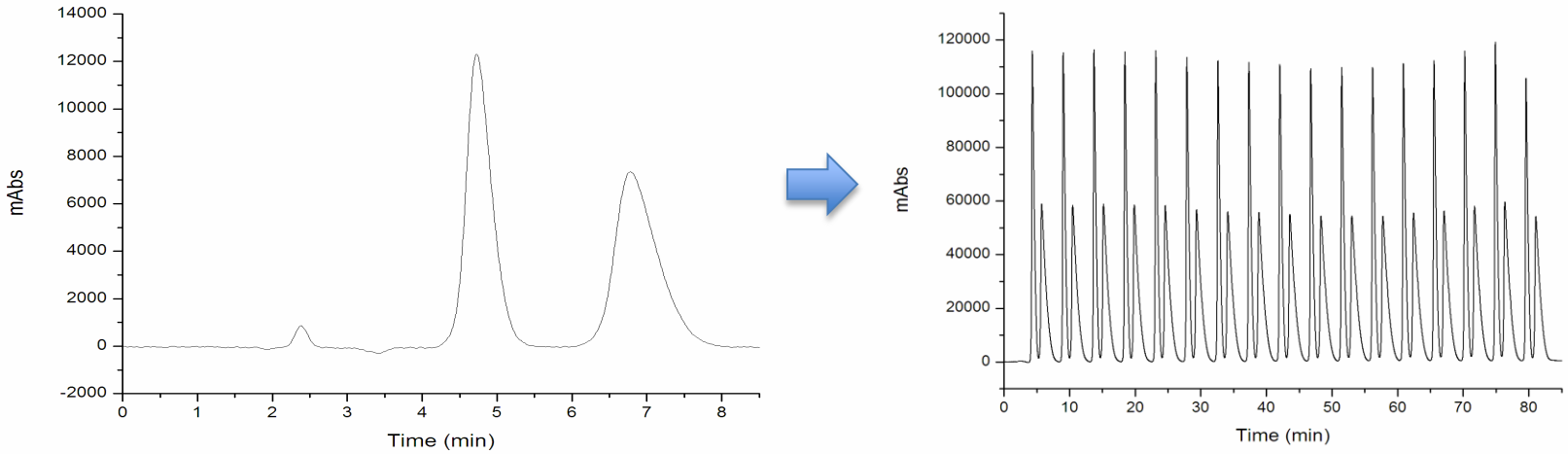




Column chosen

MDMA racemate (135 mg) was separated by a total of 27 injections (158.20 minutes). 63.10 mg of the first (+)- enantiomer and 64.00 mg of the second (-)-enantiomer was obtained, resulting in a recovery higher than 93% for both enantiomers.

Alpha 1.75
Resolution 3.5



Results obtained from separation of 135 mg of MDMA on CHIRALPAK ID (1.0 x 15 cm; 20 µm), Acetonitrila/DEA (0.1%), 5.0 mL/min, 270 nm, injection volume 500 µL.

First enantiomer	Enantiomer ratio	99.9%
Mass (mg)	61 mg	
Production rate (mg/day)	572 mg/day	
Recovery	93.5%	
Second enantiomer	Enantiomer ratio	99.9%
Mass (mg)	64 mg	
Production rate (g/day)	581 mg/day	
Recovery	94.8%	

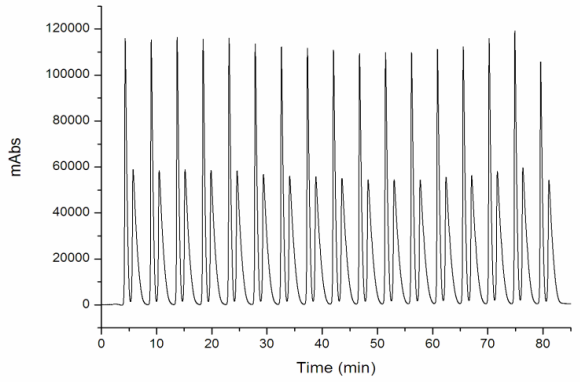


Table 1. Process parameters

Preparative separation parameters	Values
Enantiomeric ratio (%)	Higher than 99.9
Mean mass collected (mg)	63.55
Mean production rate (mg per day)	577
Mean recovery (%)	94.1
Productivity	**0.17 kg enantiomer/kg CSP/day**

Advantages of Immobilized Polysaccharide Columns

- Unique to Chiral Technologies
- Rugged
- Use with any organic solvent
- Very helpful for samples that are difficult to dissolve
- Choose solvents that are appropriate for reactive compounds
- Excellent for Development of High Productivity Preparative Separations