HPLC Enantiomeric Separations of Pharmaceuticals Using Polar Organic Mobile Phases

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Agenda

- Background
- Benefits
- Mechanisms
- Separation Comparisons
- LC-MS Applications
- Optimization
- Screen Results
- Summary
- Conclusions

Background

Polar Organic Mode (POM):

- Astec CYCLOBOND™ (1989) (e.g. 95/5/0.3/0.2, CH_3CN/MeOH/HOAc/TEA)
  - Acetonitrile is a dominant solvent
  - Acid/base additives are to suppress ionization
  - Samples have at least 2 H-bonds capability
- Astec CHIROBIOTIC® (neutral molecules)
- Astec P-CAP, P-CAP-DP
- Cyclofructans
- Polysaccharides (e.g. ASTEC Cellulose DMP)

Polar Ionic Mode (PIM):

- Astec CHIROBIOTIC (2003) (e.g. 100/0.1/0.1, MeOH/HOAc/TEA)
  - Methanol is a dominant solvent
  - CSPs have ionic character
  - Acid/base additives promote ionic interactions for ionizable samples
  - ASTEC CHIROBIOTIC V2

Benefits of Polar Organic Mode (POM)

Selectivity

- Conformational changes of CSPs
- Different interaction mechanisms

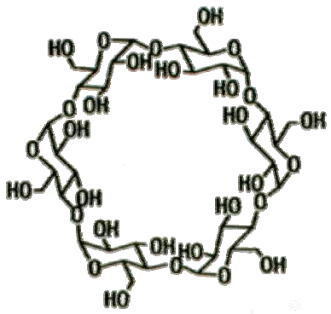
Sensitivity

- Less baseline noise in UV detection
- LC-MS compatible for biological samples

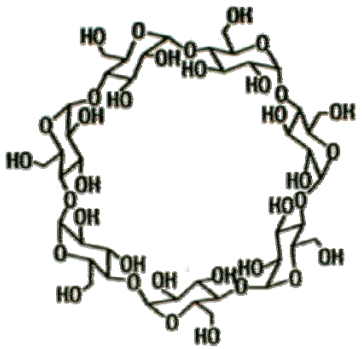
Solubility

- Easy sample prep
- Easy scale-up

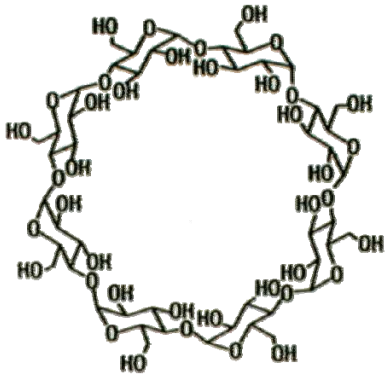
Mechanism 1: Astec CYCLOBOND CSPs



α-Cyclodextrin



β-Cyclodextrin

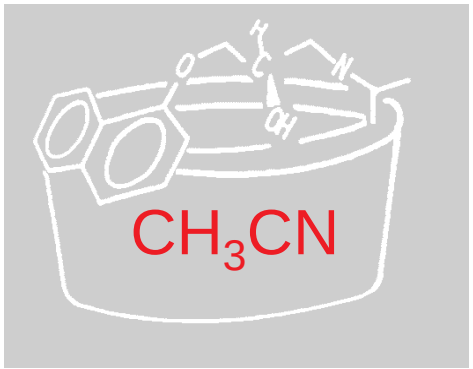


γ-Cyclodextrin

Reversed phase mode: the most hydrophobic portion of the molecule will form an inclusion complex with the cyclodextrin cavity.

Inclusion Complexation

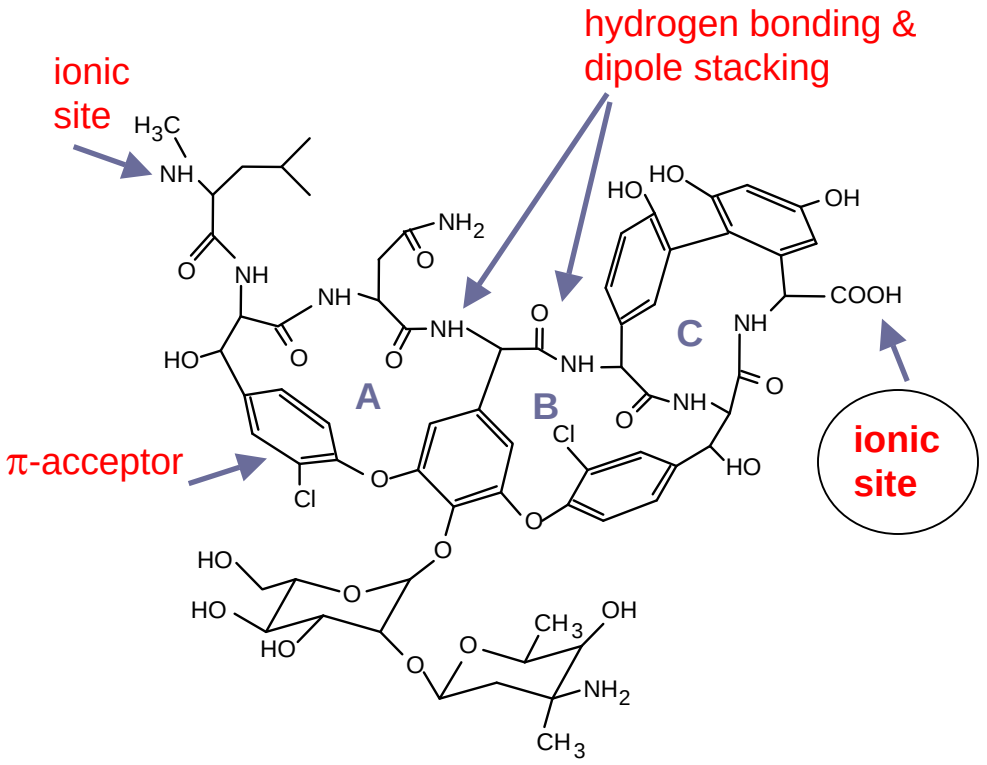
Polar organic mode: CH_3CN occupies the cavity, so the chiral molecule lies across the surface and interacts with the upper rim of the cyclodextrin ring



Surface Interactions

Mechanism 2: Astec CHIROBIOTIC CSPs

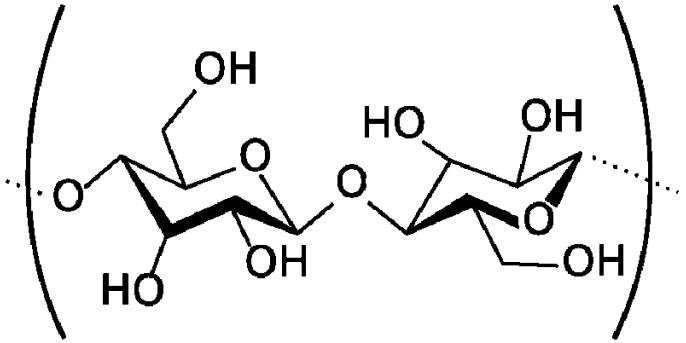
- Macrocyclic glycopeptides provide a multi-modal chiral surface capable of a wide variety of different interactions
- Subtle differences between them provide different, dominant retention mechanisms that lead to enantiomeric recognition
- Among these mechanisms, ionic interactions dominate for ionizable molecules
- A family of 6 columns
- Macrocyclic glycopeptide CSPs provide unique separations for polar, ionic molecules

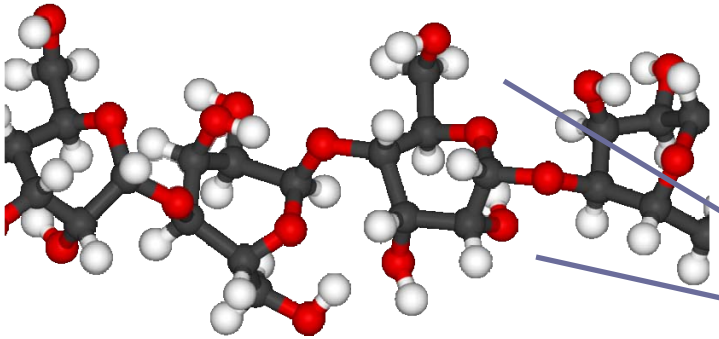


Vancomycin

(CHIROBIOTIC V2/V)

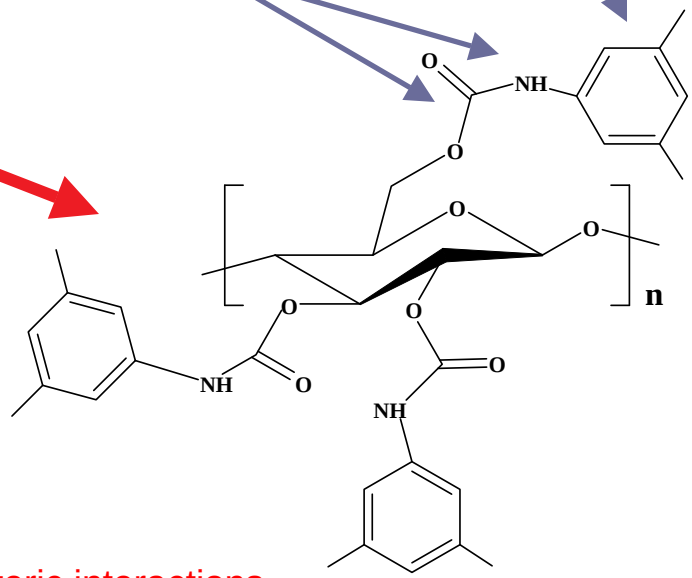
Mechanism 3: Cellulose DMPC Derivative





hydrogen bonding &
dipole stacking

π- π



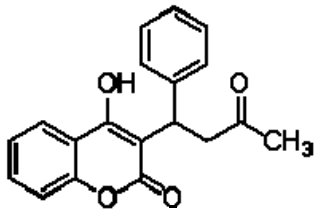
steric interactions

Cellulose, a linear polymer of D-glucose linked by β(1→4)-glycosidic bonds with several hundreds to over ten thousand units.

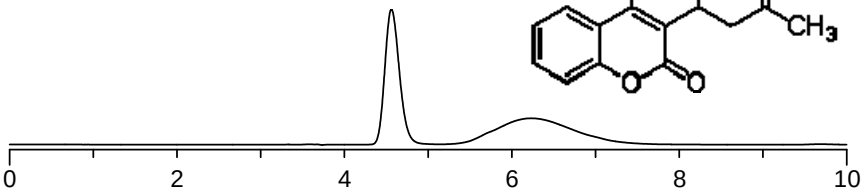
DMPC**,** 3,5-Dimethylphenyl carbamate derivatized cellulosic phase coated onto silica.

From NP to POM: Cellulose DMP (Warfarin)

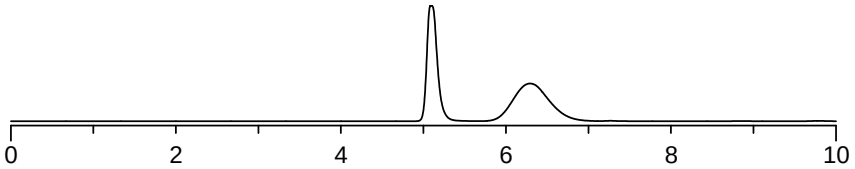
15 cm x 4.6 mm; Flow Rate: 0.5 mL/min; UV: 278 nm



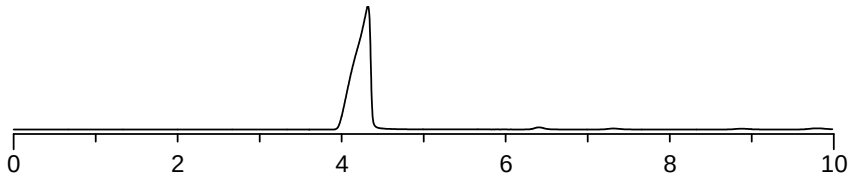
50/50/0.1, EtOH/Hexane/TFA
Selectivity: 2.44
Resolution: 1.61



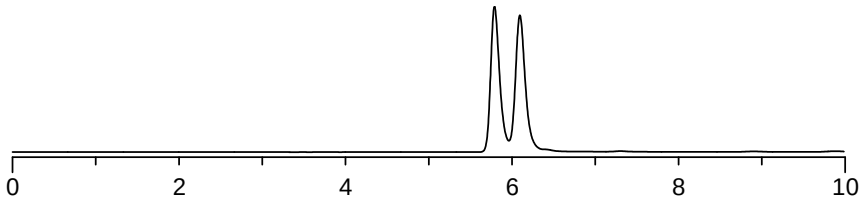
100/0.1, MeOH/TFA
Selectivity: 1.71
Resolution: 2.34



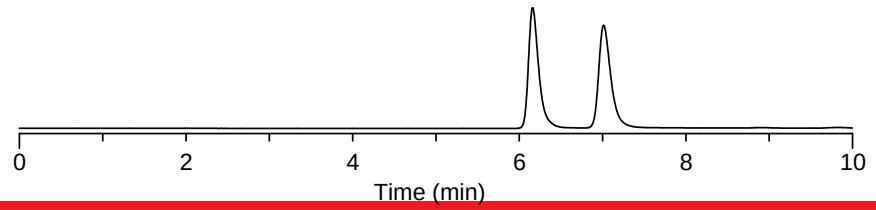
100/0.1, MeOH/DEA (or IPAmine)
Selectivity: 1.00
Resolution: N/A



100/0.1w%, MeOH/NH_4formate
Selectivity: 1.13
Resolution: 1.40

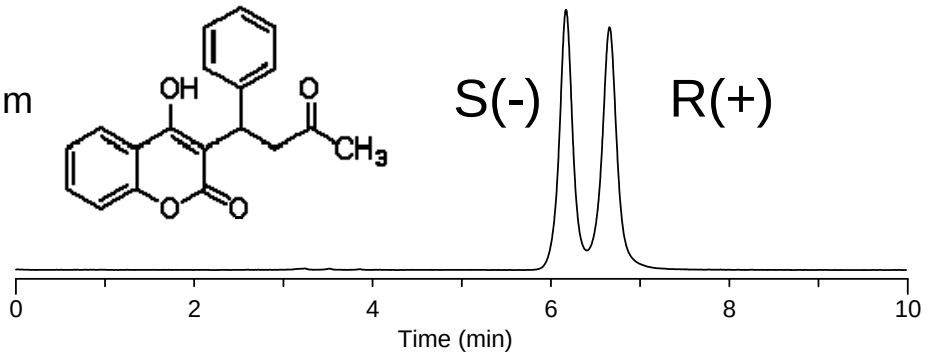


100/0.2/0.1, MeOH/HOAc/TEA
Selectivity: 1.31
Resolution: 3.71

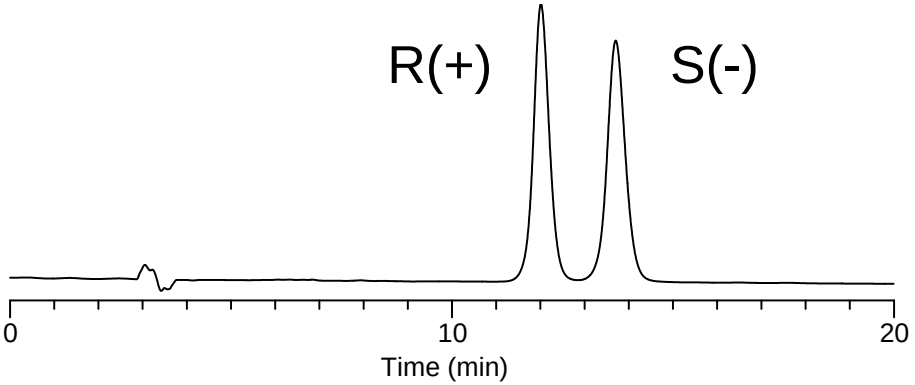


Separation Comparison: Warfarin

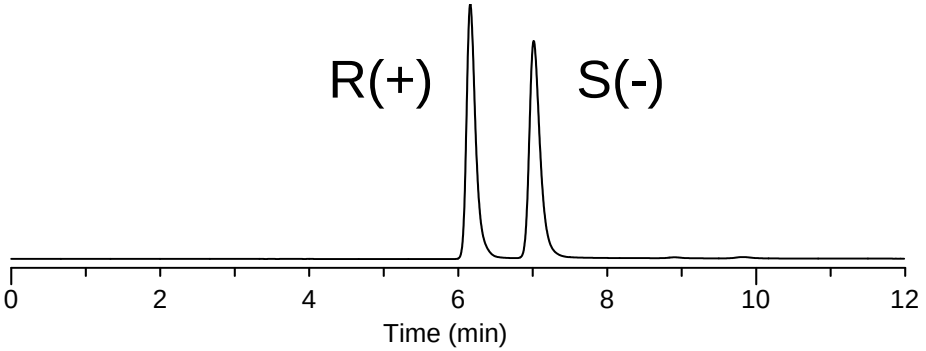
CYCLOBOND I 2000, 25 cm x 4.6 mm
100/0.3/0.2, CH_3CN/HOAc/TEA
Flow Rate: 1.0 mL/min, UV: 278 nm



CHIROBIOTIC V, 25 cm x 4.6 mm
30/70, CH_3CN/5 mM NH_4OAc, pH 4.1
Flow Rate: 1.0 mL/min, UV: 278 nm



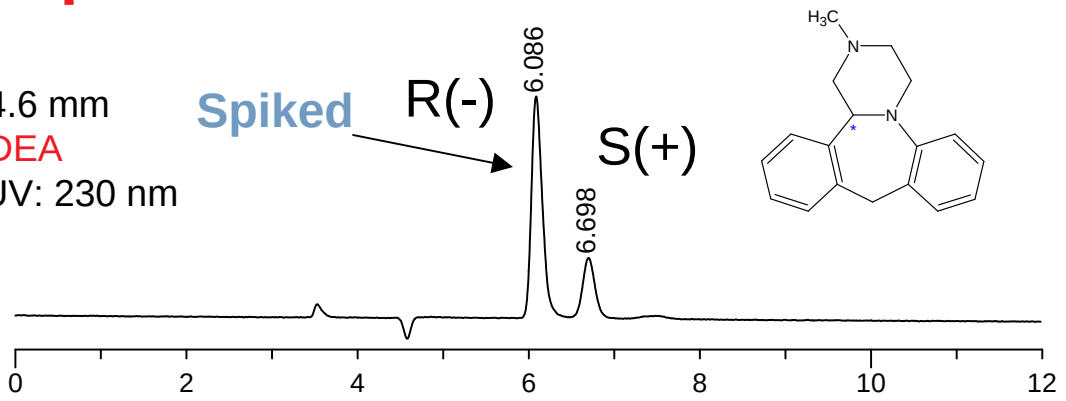
Cellulose DMP, 15 cm x 4.6 mm
100/0.2/0.1, MeOH/HOAc/TEA
Flow Rate: 0.5 mL/min, UV: 278 nm



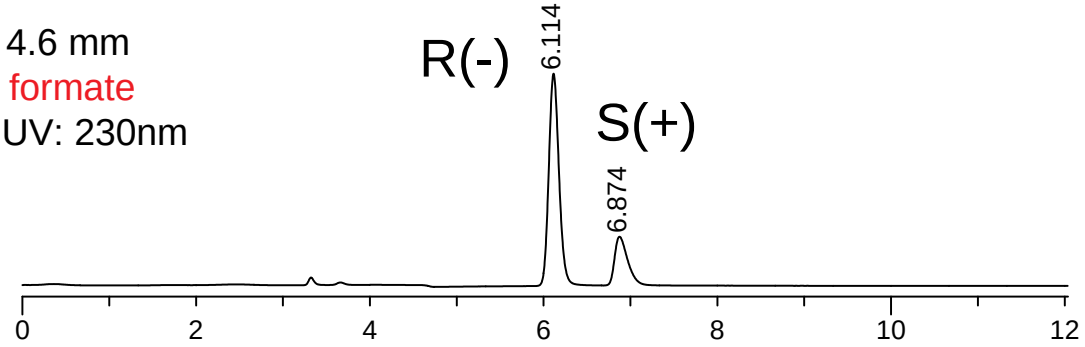
S-form more potent, anticoagulant

Separation Comparison: Mianserin

Cellulose DMP,15 cm x 4.6 mm
10/90/0.1, IPA/Heptane/DEA
Flow Rate: 0.5 mL/min, UV: 230 nm

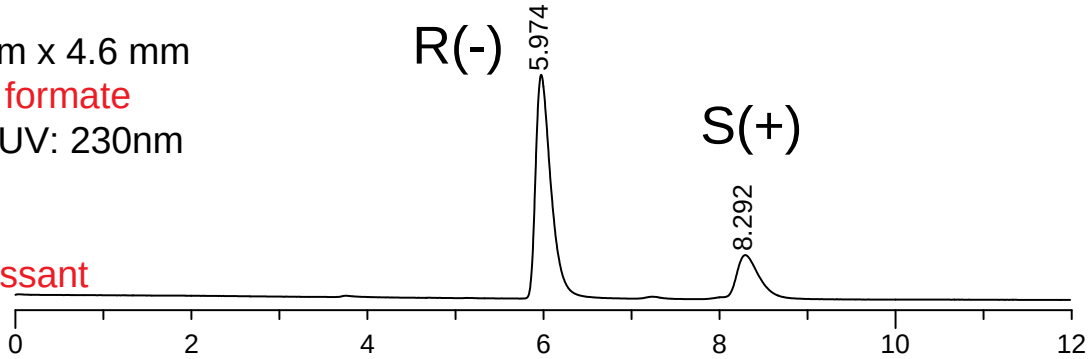


Cellulose DMP,15 cm x 4.6 mm
100/0.1w%, MeOH/NH_4 formate
Flow Rate: 0.5 mL/min, UV: 230nm



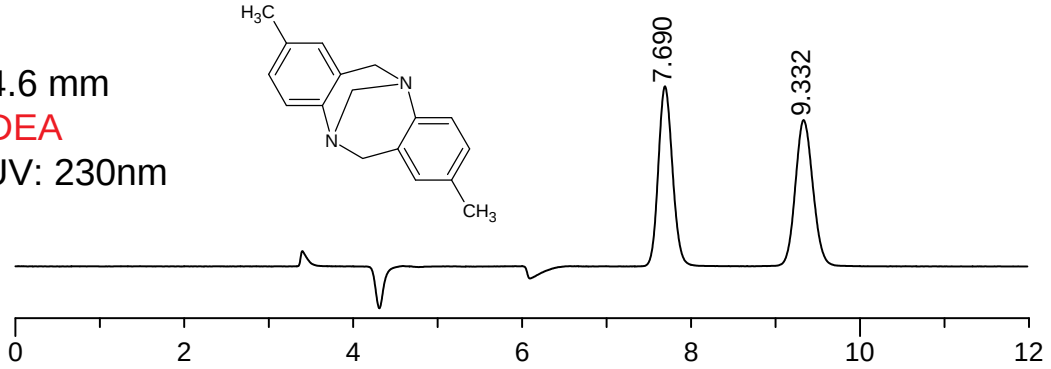
CHIROBIOTIC V2, 25 cm x 4.6 mm
100/0.1w%, MeOH/NH_4 formate
Flow Rate: 0.8 mL/min, UV: 230nm

S-active form, antidepressant

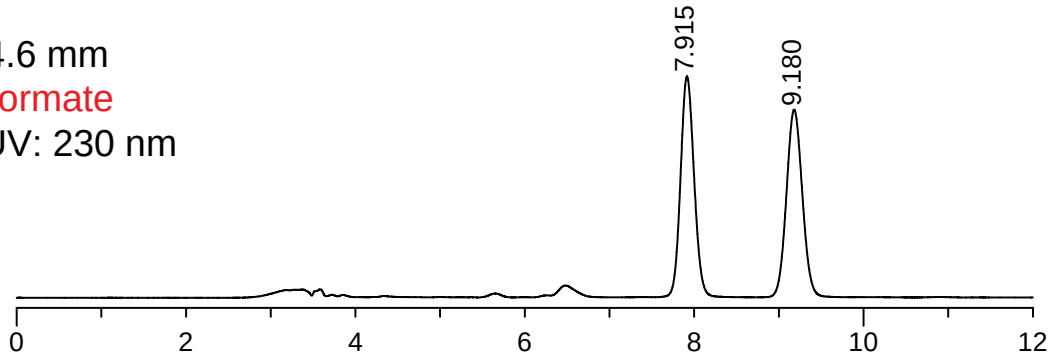


Separation Comparison: Tröger's Base

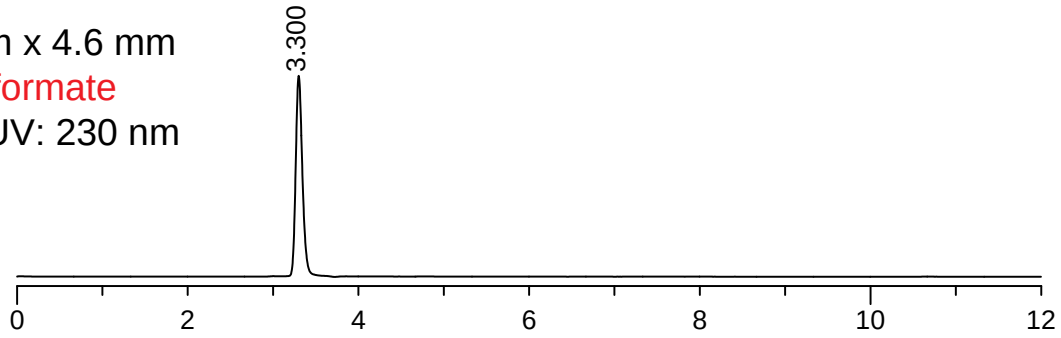
Cellulose DMP,15 cm x 4.6 mm
10/90/0.1, IPA/Heptane/DEA
Flow Rate: 0.5 mL/min, UV: 230nm



Cellulose DMP,15 cm x 4.6 mm
100/0.1w%, MeOH/NH_4 formate
Flow Rate: 0.5 mL/min, UV: 230 nm



CHIROBIOTIC V2, 25 cm x 4.6 mm
100/0.1w%, MeOH/NH_4 formate
Flow Rate: 1.0 mL/min, UV: 230 nm



Polar Organic Mode-Cellulose DMP

100/0.1, MeOH/DEA
Flow Rate: 0.5 mL/min
UV: 230 nm

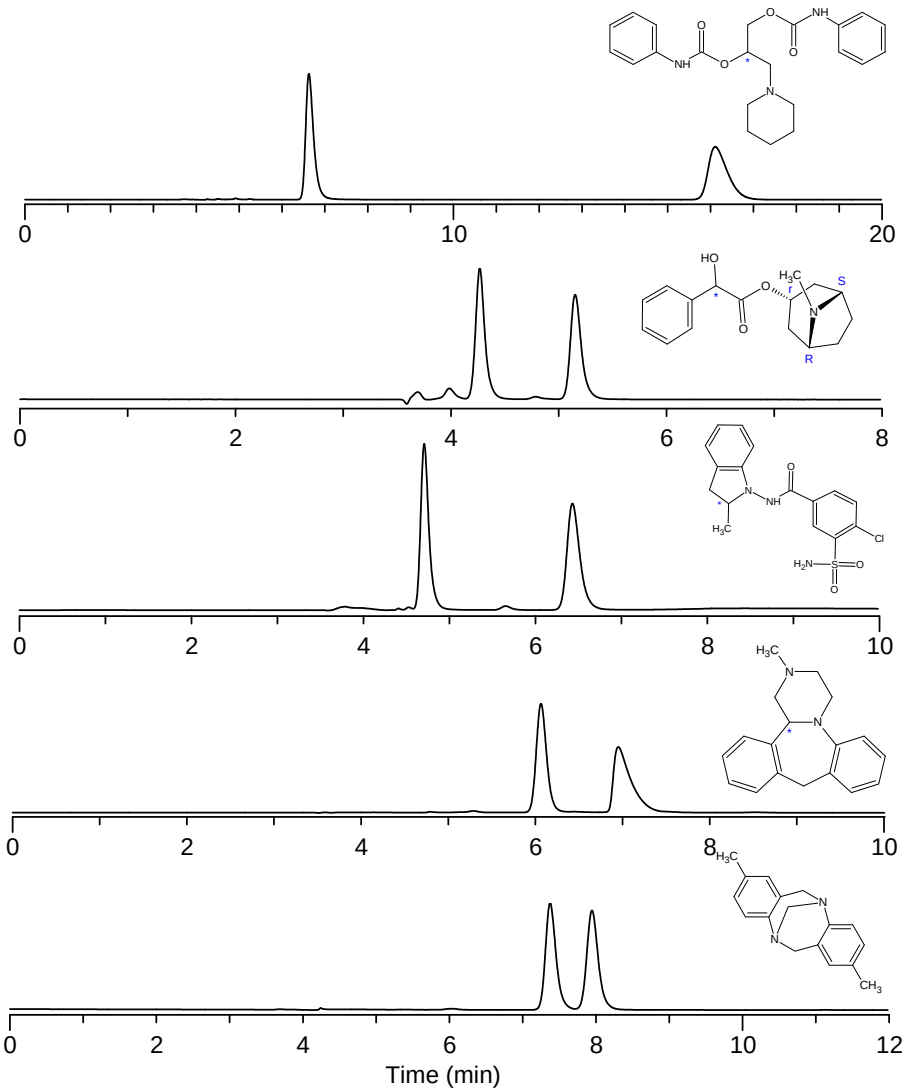
Diperodon

Homatropine

Indapamide

Mianserin

Tröger's base



Polar Organic Mode-Cellulose DMP

100/0.1w%, MeOH/NH_4Formate (LC-MS compatible)
Flow Rate: 0.5 mL/min
UV: 230 nm

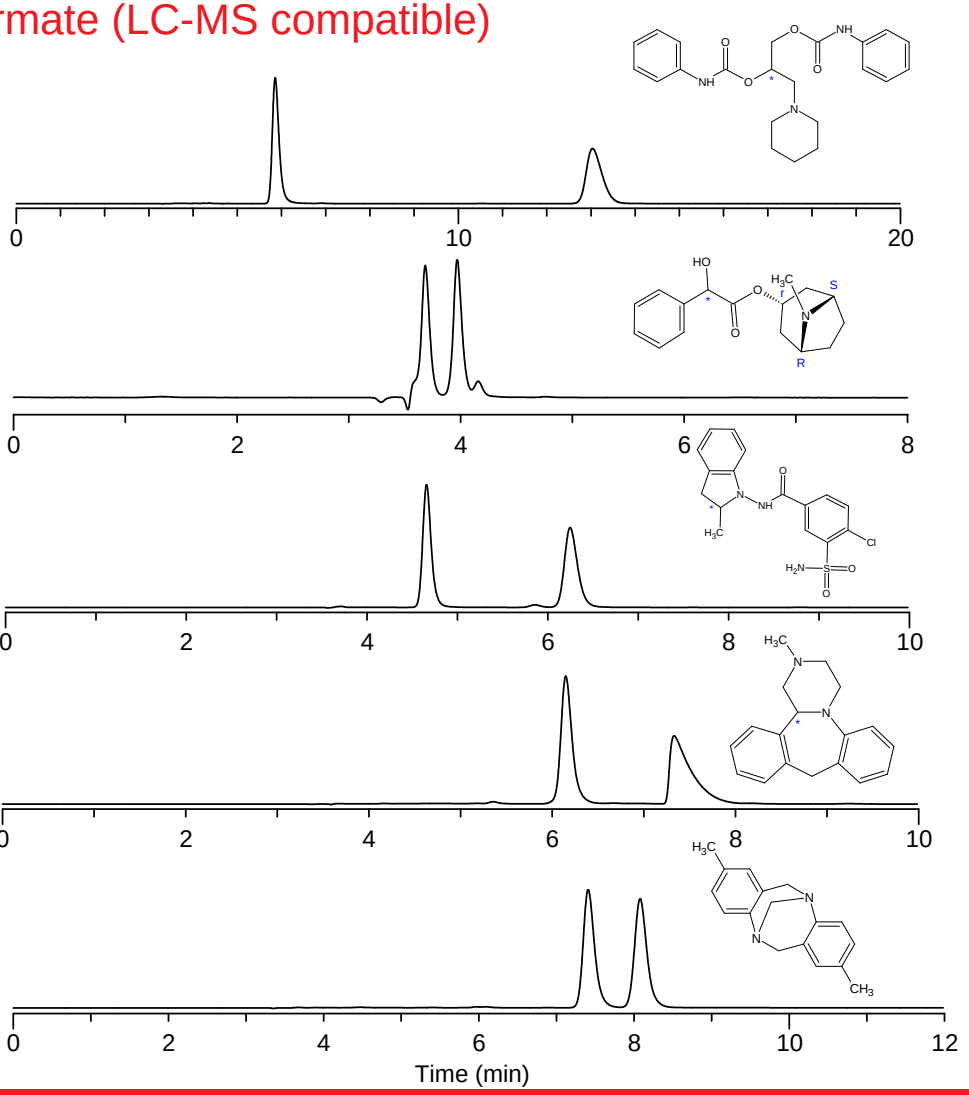
Diperodon

Homatropine

Indapamide

Mianserin

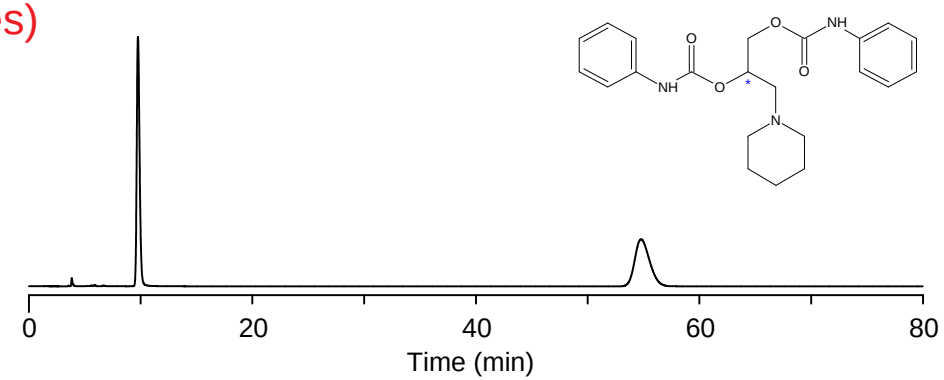
Tröger's base



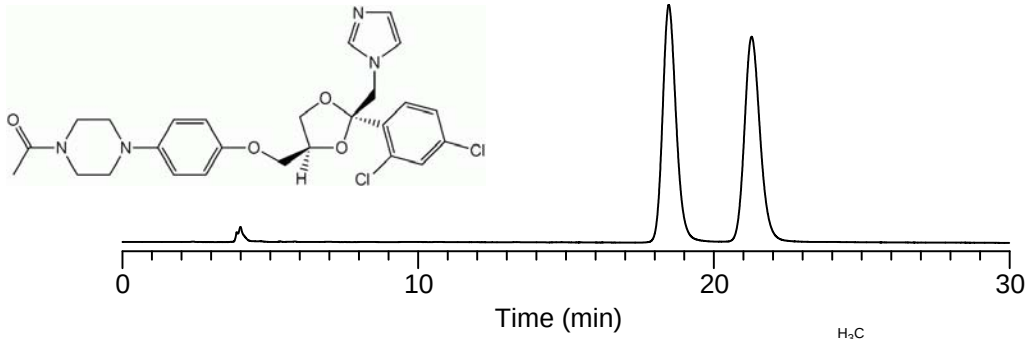
Polar Organic Mode-Cellulose DMP

100% CH_3CN (No additives)
Flow Rate: 0.5 mL/min
UV: 230 nm

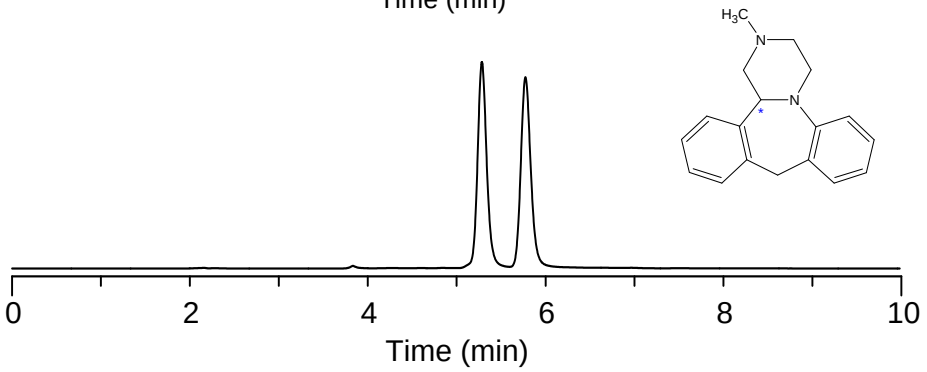
Diperodon



Ketoconazole



Mianserin



Cellulose DMP: NP→POM→NP→POM

Dimension: 15 cm x 4.6 mm
Flow Rate: 0.5 mL/min
Temperature: 25 ° C
UV: 254 nm,
Samples: *trans*-stilbene oxide (NP)/mianserin (POM)

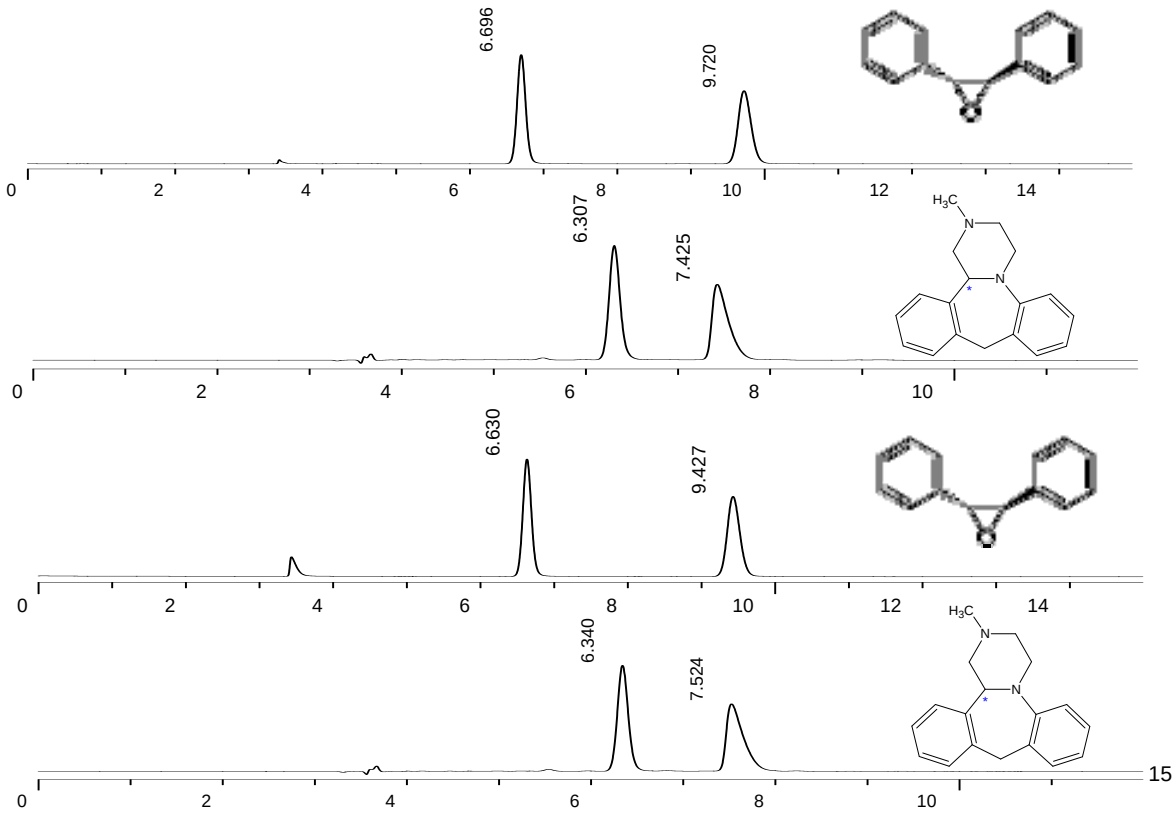
NP: 90/10, Heptane/IPA
POM: 100/0.1w%, MeOH/NH_4formate

NP:
P:17 bar
N eff (P1): 15896
Selectivity: 1.92
Resolution: 11.28

POM:
P:23 bar
N eff (P1): 13736
Selectivity: 1.39
Resolution: 4.19

NP:
P:17 bar
N eff (P1): 15583
Selectivity: 1.87
Resolution: 10.60

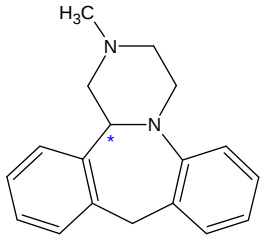
POM:
P:23 bar
N eff (P1): 13878
Selectivity: 1.40
Resolution: 4.30

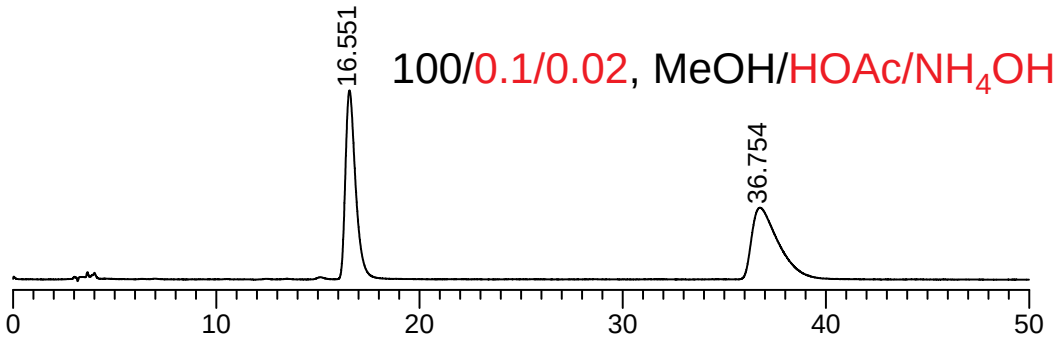


Optimization: CHIROBIOTIC (Acid/Base Ratio Effect)

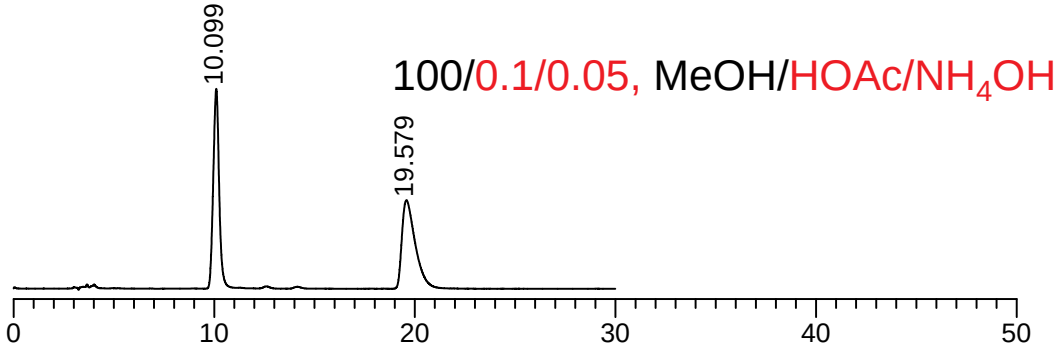
Mianserin

CHIROBIOTIC V2
25 cm x 4.6 mm
1 mL/min
UV: 230nm

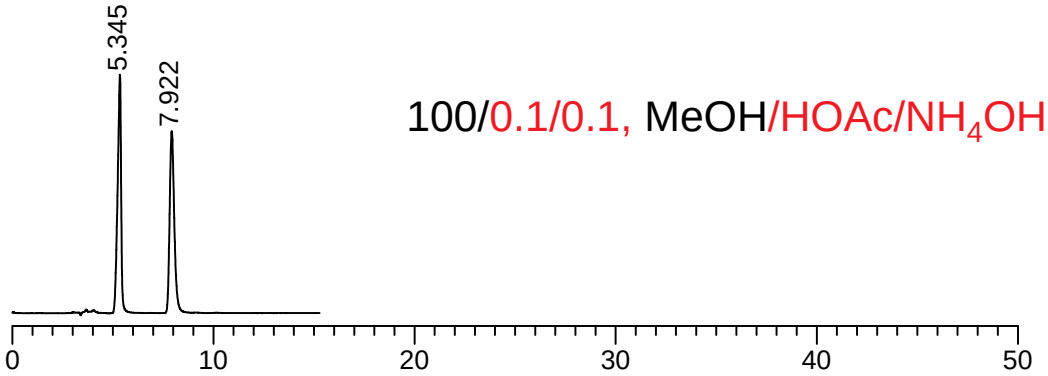




100/0.1/0.02, MeOH/HOAc/NH_4OH



100/0.1/0.05, MeOH/HOAc/NH_4OH

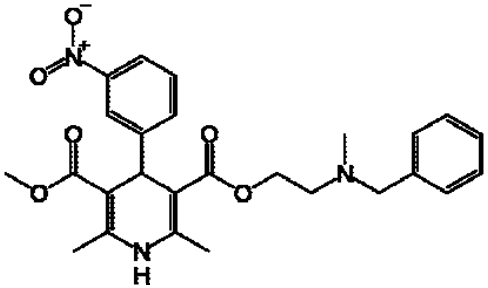


100/0.1/0.1, MeOH/HOAc/NH_4OH

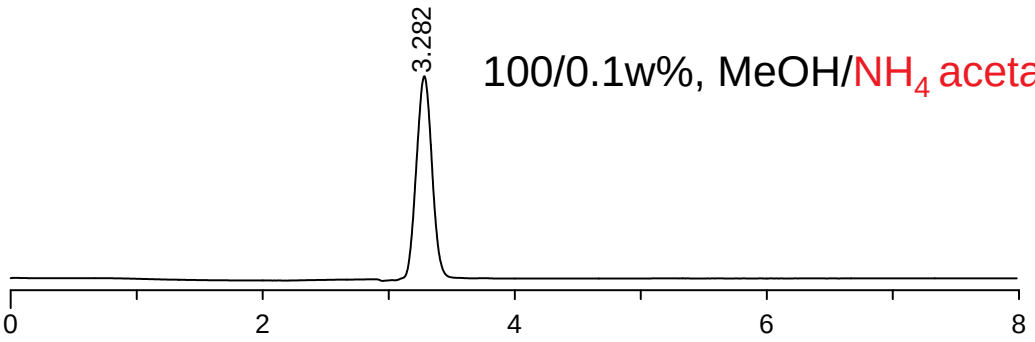
Optimization: CHIROBIOTIC (Salt Effect)

Nicardipine

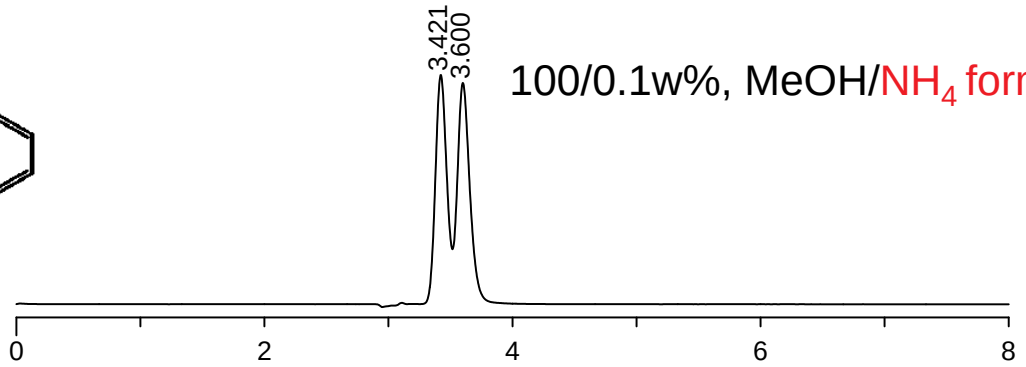
CHIROBIOTIC V2
25 cm x 4.6 mm
1 mL/min
UV: 230 nm



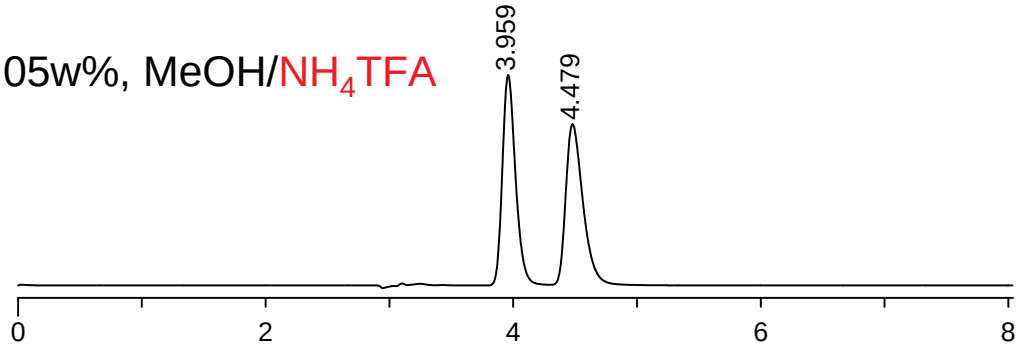
100/0.1w%, MeOH/NH_4 acetate



100/0.1w%, MeOH/NH_4 formate



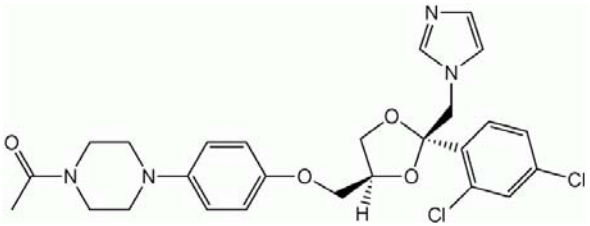
100/0.05w%, MeOH/NH_4TFA



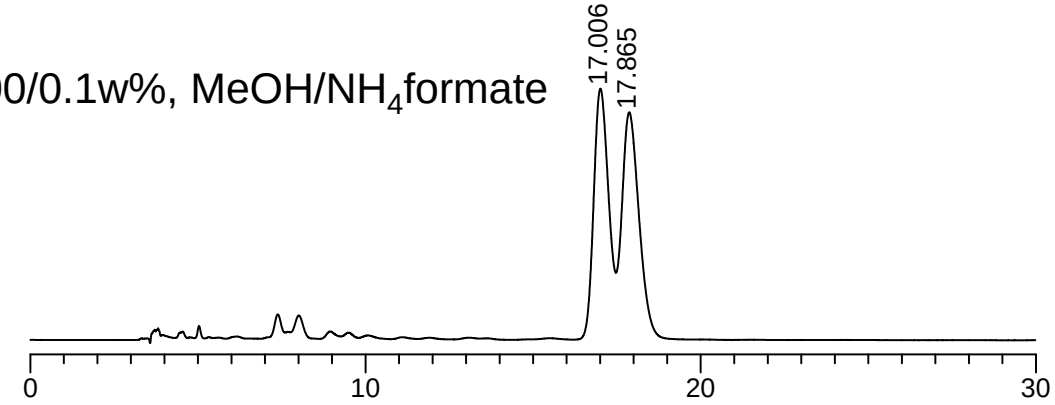
Optimization: Polysaccharides (Solvent Effect)

Ketoconazole

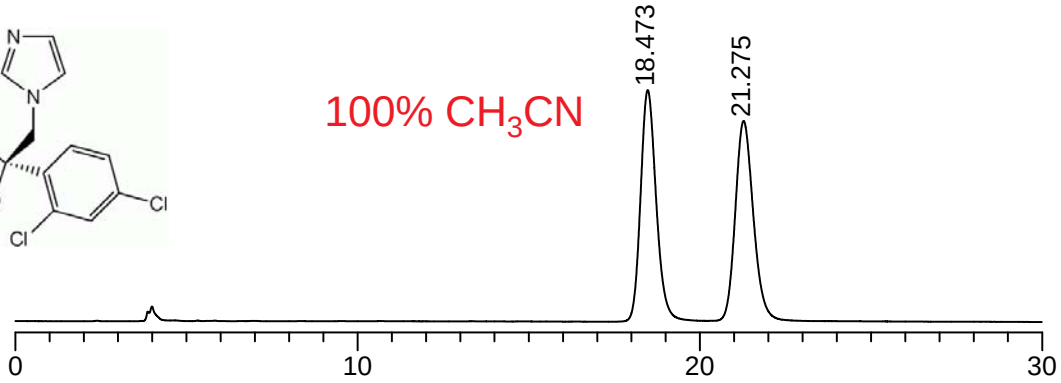
Cellulose DMP
15 cm x 4.6 mm
0.5 mL/min
UV: 230 nm



100/0.1w%, MeOH/NH_4formate



100% CH_3CN



Full Screen Results-1

10/90/0.1, IPA/Heptane/DEA

100/0.1w%, MeOH/NH_4 formate

Basic Pharmaceuticals	Cellulose DMP Normal Phase k_1/Selectivity	Cellulose DMP Polar Organic Mode k_1/Selectivity	CHIROBIOTIC V2 Polar Ionic Mode k_1/Selectivity
Atropine	0.06/1.33	0.18/1.00	3.54/1.00
Bupivacaine	0.86/1.00	0.23/1.00	0.31/1.34
Citalopram	2.75/1.14	0.26/1.00	2.37/1.12
Clenbuterol	1.34/1.00	0.03/1.00	1.02/1.22
Diperodon	No elution	0.73/3.89	0.66/1.00
Disopyramide	1.65/1.07	0.11/1.02	1.08/1.14
Esmolol	3.36/1.57	0.09/1.25	1.34/1.12
Fluoxetine	1.09/1.08	0.07/1.02	2.00/1.24
Homatropine	2.40/1.62	0.08/2.04	0.13/1.00
Hydroxyzine	1.16/1.23	0.40/1.10	0.71/1.00
Indapamide	No elution	0.37/2.27	0.26/1.00
Ketamine	0.80/1.14	0.48/1.00	0.27/1.00
Ketoconazole	No elution	4.31/1.06	0.31/1.00

Full Screen Results-2

Basic Pharmaceuticals	Cellulose DMP Normal Phase k_1/Selectivity	Cellulose DMP Polar Organic Mode k_1/Selectivity	CHIROBIOTIC V2 Polar Ionic Mode k_1/Selectivity
Mefloguine	1.59/1.19	0.07/1.00	2.86/1.36
Methocarbamol	No elution	0.30/1/35	1.08/1.00
Methoxypheamine	0.86/1.21	0.07/1.00	1.52/1.16
Metoprolol	1.25/2.66	0.08/1.38	1.22/1.12
Mianserin	0.79/1.23	0.96/1.26	0.65/1.98
Ofloxacin	No elution	1.91/1.13	No Elution
Ondansetron	No elution	1.62/1.07	1.02/1.00
Promethazine	0.58/1.05	0.47/1.00	1.76/1.68
Propranolol	2.36/2.22	0.16/1.24	1.60/1.16
Ritalin	0.66/1.09	0.16/1.00	1.32/1.45
Thalidomide	No elution	1.20/1.00	0.47/2.97
Tolperisone	0.41/1.00	0.27/1.00	1.14/1.24
Troger's base	0.78/1.22	1.33/1.28	0.18/1.00

Summary

Macrocyclic glycopeptides and polysaccharide CSPs can be complementary to one another using polar organic mobile phases

Suggested Sample Screen: 100/0.1w%, MeOH/NH_4 formate

- Astec CHIROBIOTIC V2 and T (TAG)
- Astec Cellulose DMP and “AD”-type phases
- Other CSPs
  - Different derivatives of polysaccharides
  - Immobilized polysaccharides
  - Astec P-CAP (adds 50-70% CH_3CN)
  - Cyclofructans (adds 30-50% CH_3CN)
  - Cinchona alkaloid ion exchange CSP (adds 30-50% CH_3CN)
  - Others

Conclusions

- Polar organic mobile phases provide additional opportunities for chiral selectivity should other types of mobile phases fail
- PIM/POM provide easy sample preparation for polar/ionizable compounds
- No memory effect (quick equilibration)
- LC-MS compatible mobile phases
- Easy scale-up for prep purification
- Straight-forward optimization steps