

High Throughput Screening For Chiral Selectivity by HPLC for Pharmaceutical Applications



T408108

Overview

This presentation will explore the chromatographic challenges of chiral separations from the drug discovery platform through all stages of drug development. Shortening the development time for the successful separation of a wide variety of racemates can be accomplished by first screening analytes using up to four CHIROBIOTIC phases and four CYCLOBOND phases in up to four different mobile phase types. Initial screening using this array of column/mobile phase protocols, and subsequent optimization of partial separations will depend upon whether analytes are chiral acid, base or neutral molecules. The outcomes of these screening and optimization methods will be discussed with respect to a broad spectrum of pharmaceutical compounds.

Application and CSP Versatility Needs

- Drug Discovery Platform – requires high throughput, high selectivity, good solubility and peak capacity for prep purifications
- Process Sciences - low cost, high capacity, long life
- Med/Tox/Clinical – high throughput, requires LC/MS compatible mobile phases and high sensitivity (LLOQ)
- Manufacturing – processing (biocatalysis), drug stability, dissolution analysis, requires aqueous compatibility, high sensitivity

Test Range of Racemic Switches- Representatives of Popular Chiral Drugs

- Basic:

- Analgesic: Methadone; Nefopam; (R,R)-Tramadol
- CNS stimulant: Methylphenidate
- Gastroprokinetic: Mosapride
- Anthelmintic: Oxamniquine
- Decongestant: (S,S)-Pseudoephedrine
- Antipsychotic: Thioridazine
- Muscle relaxant: Tolperisone
- Sedative: (S)-Zopiclone

Test Range of Racemic Switches- Representatives of Popular Chiral Drugs

- Basic:

- Bronchodilator: R(-)-Albuterol; Clenbuterol; R(-)-Epinephrine; (R,R)-Formoterol; Isoproterenol; Terbutaline
- Antihypertensive: (-)-Amlodipine; Lercanidipine; Metoprolol; Nicardipine; Propranolol; Sotalol
- Antifungal: Miconazole
- Anesthetic: S(-)-Bupivacaine
- Antihistaminic: (+)-Chlorpheniramine
- Antidepressant: (S)-Citalopram; Fluoxetine; Mianserin; Nefopam; (S,S)-Sertraline; Trimipramine

Test Range of Racemic Switches- Representatives of Popular Chiral Drugs

- Acidic:

- Antiinflammatory: S(+)-Ibuprofen; S(+)-Ketoprofen;
S(+)-Naproxen

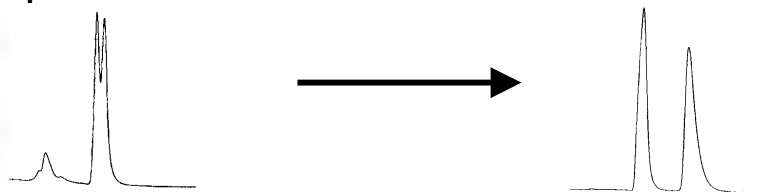
- Neutral:

- Antiulcerative: S(-)-Omeprazole
- Anxiolytic: Lorazepam; Oxazepam
- Pediculicide: cis-Permethrin
- Anticoagulant: S(-)-Warfarin

Modern Screening Strategy

Choose a small set of CSPs that:

1. are broad-based to increase chances of success for a whole range of molecular types
2. offer selectivity in a wide range of mobile phases for increased selectivity possibilities and sample solubilities
3. are complementary to each other in order to increase overall hit rate
4. can be quickly and easily optimized to baseline separation.



Interactions that Favor Chiral Selectivity

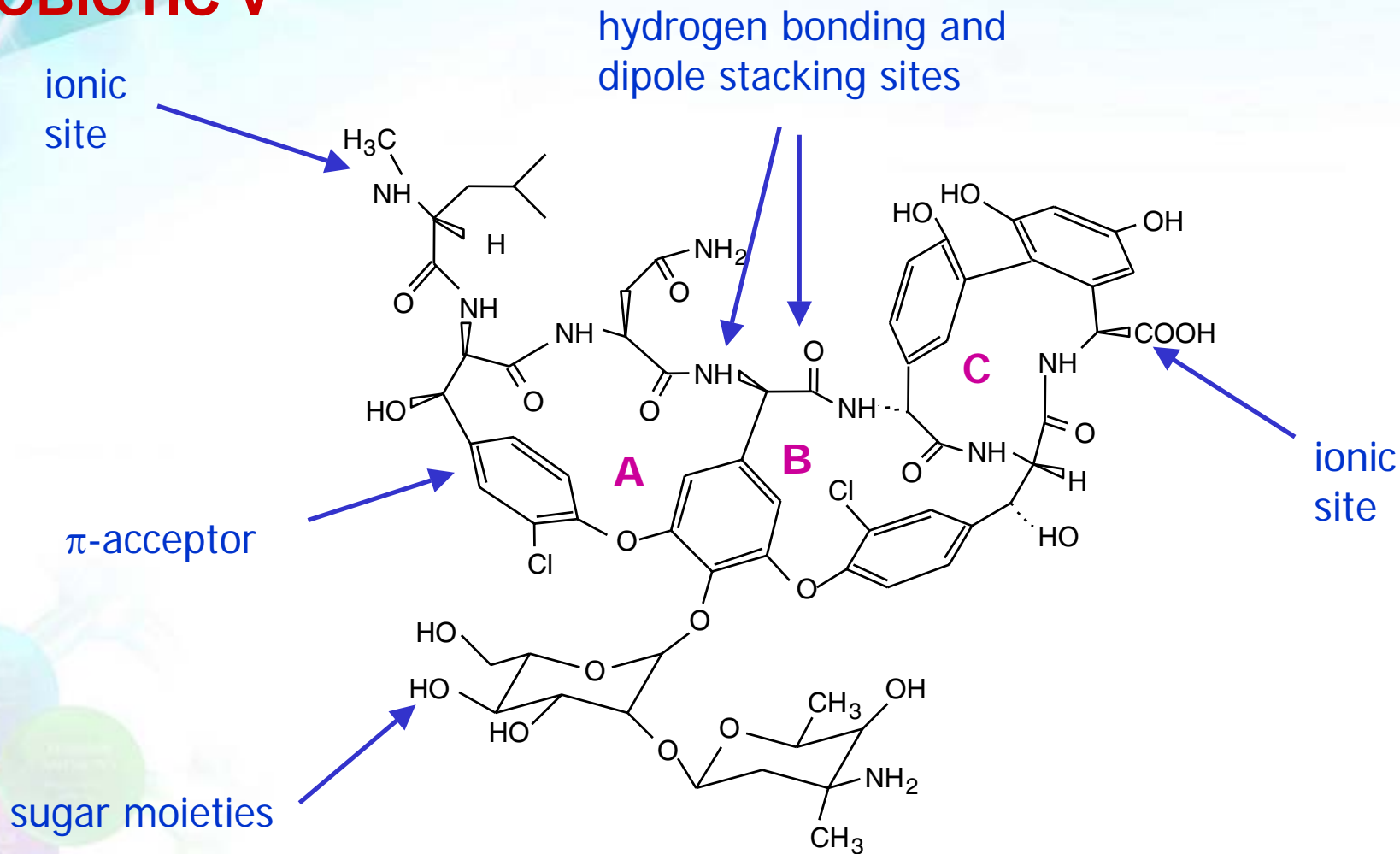
- Hydrogen Bonding
- π - π
- Ionic
- Dipole
- Steric
- Inclusion

General Characteristics CHIROBIOTIC™ HPLC Columns

- Broad applicability to many different classes/multiple interactive sites
- Covalently bonded to pure silica through >4 linkages
- Chiral selectivity in several mobile phase types **on same column**
- Stable to high flow rates and pressures
- No memory effect
- Stable to all commonly used organic solvents, including chlorinated

Structure of Vancomycin CSP

CHIROBIOTIC V



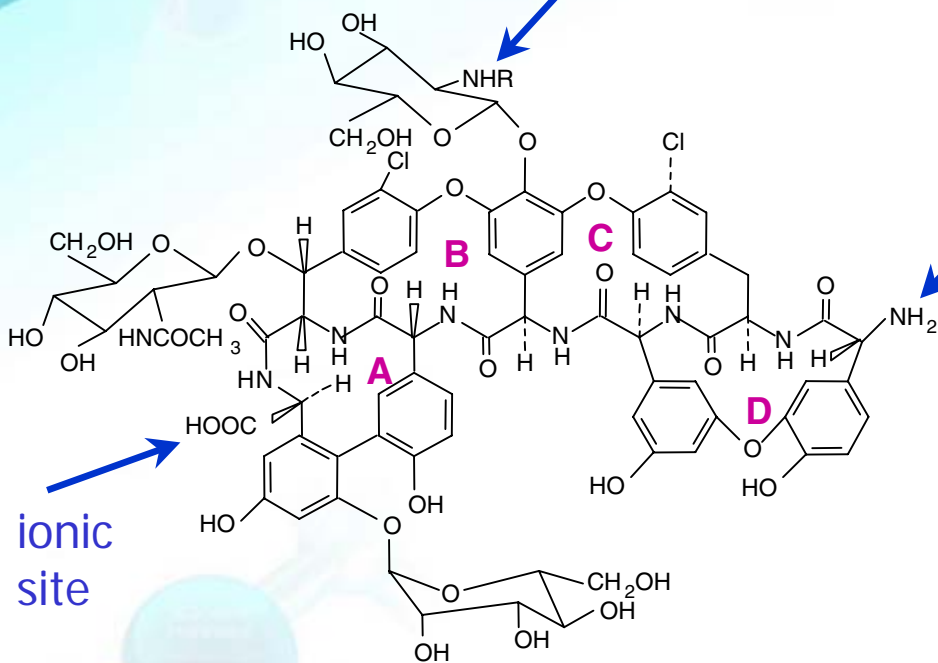
A, B, C are inclusion pockets (weak)

Structure of Teicoplanin CSP

**Teicoplanin,
CHIROBIOTIC T**

→ Key sites

sugar and alkyl chain

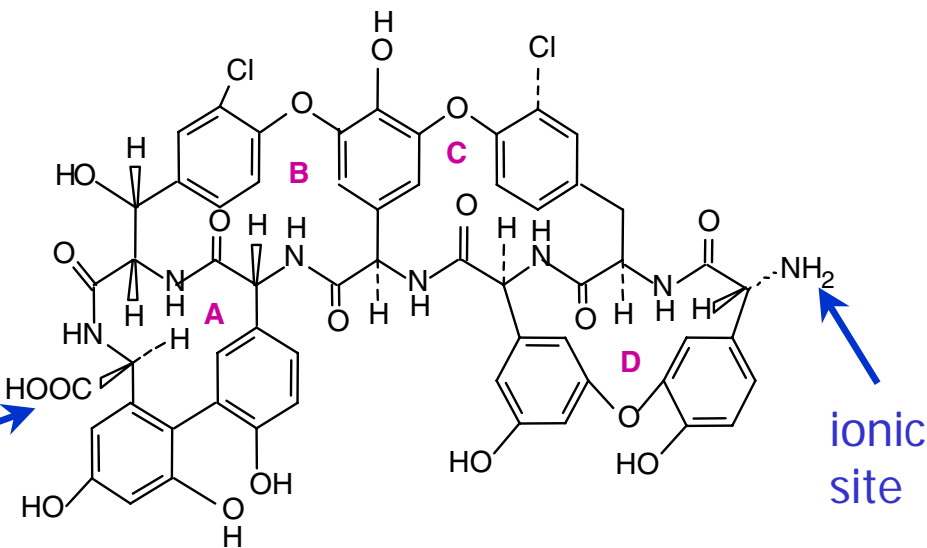


ionic site

**Teicoplanin Aglycone,
CHIROBIOTIC TAG**

ionic site

ionic site



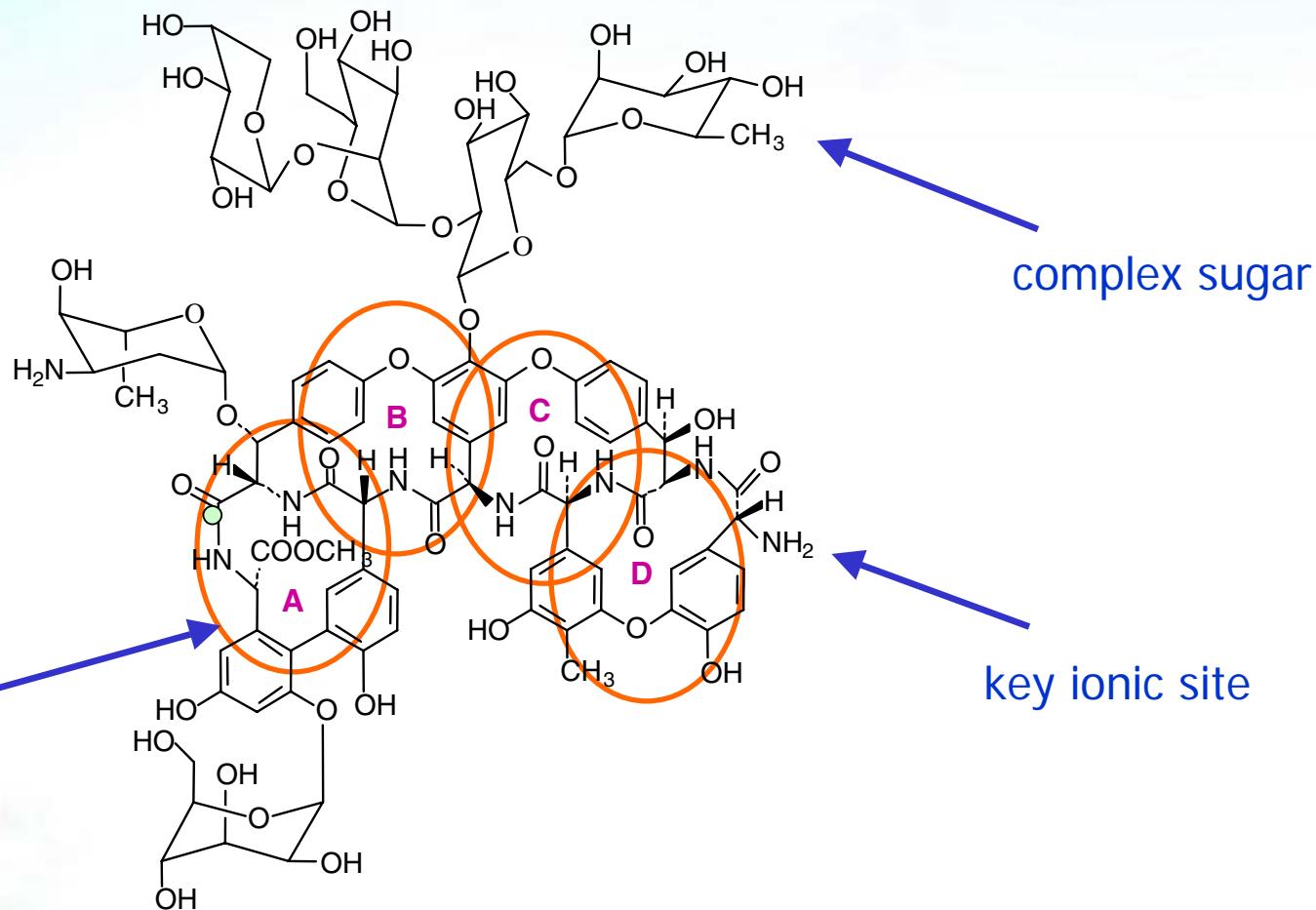
ionic site

CHIROBIOTIC V2 and CHIROBIOTIC T2

- Extensions of the CHIROBIOTIC V2 and T2 created by changing the position of several linkages and the chain length used to anchor the ligand.
- These changes can **enhance** the **selectivity** and **capacity** of these phases mainly in the polar ionic and polar organic modes, and sometimes in reversed phase.

Proposed Structure of Ristocetin A CSP

CHIROBIOTIC R



inclusion cavities

complex sugar

key ionic site

Questions to Consider

- What mobile phase types favor certain interactions?
- How do you manipulate these interactions to achieve the highest selectivity?
- How do you set up an effective screening system?
- What are the anticipated results?

3-Point Chiral Interactions on Multi-modal CHIROBIOTIC™ Phases

The most plausible interaction forces in descending order of strength under different mobile phase conditions:

1. **Polar Ionic Mode (ionizable compounds only)**
 - A. Ionic
 - B. Hydrogen Bonding
 - C. Steric/ π - π
2. **Polar Organic/Normal Phase Mode (neutral compounds)**
 - A. Hydrogen Bonding
 - B. π - π
 - C. Steric/Dipole
3. **Reversed Phase Mode (all compounds)**
 - A. Ionic
 - B. Hydrogen Bonding
 - C. Steric/Inclusion/Hydrophobic

Comparison of Four Basic Mobile Phase Types for Glycopeptide CSPs

Ionizable Analytes;

PIM Composition:

Methanol + Acid + Base (100+0.1+0.1, v/v/v)
or Methanol + Volatile Ammonium Salt (100+0.1% v/w)

Neutral Analytes;

NP Composition:

Polar + Nonpolar (EtOH or IPA+Heptane)

Neutral-Polar Analytes;

POM Composition:

Polar/Nonpolar (MeOH or EtOH or ACN or combinations, eg MeOH/ACN)

All Analytes;

RP Composition:

Organic + Aqueous Buffer (ACN+TEAA or ACN +NH₄OAc)

Chiral Screening Protocol

1. Mobile Phase Composition:

No.	Mobile Phase	Composition (v/v)
1	MeOH/20mM NH ₄ OAc, pH 4.0	20/80
2	MeOH/20mM NH ₄ OAc, pH 6.0	20/80
3	ACN/20mM NH ₄ OAc, pH 4.0	30/70
4	ACN/20mM NH ₄ OAc, pH 6.0	30/70
5	MeOH/HOAc/TEA (PIM)*	100/0.1/0.1
6	ACN/MeOH/HOAc/TEA (POM)**	95/5/0.3/0.2
7	EtOH/Heptane	30/70
8	EtOH	100

*PIM: Polar Ionic Mode, CHIROBIOTIC

** POM: Polar Organic Mode, CYCLOBOND

Chiral Screening Protocol

2. Run Table:

No	Column Type	1	2	3	4	5	6	7	8
I	CHIROBIOTIC V2	y		y		y		y	y
II	CHIROBIOTIC T	y	y		y	y		y	y
III	CHIROBIOTIC R		y		y	y		y	y
VI	CHIROBIOTIC TAG	y	y		y	y		y	y
V	CYCLOBOND I 2000	y		y			y		y
VI	CYCLOBOND DNP	y		y			y	y	y
VII	CYCLOBOND DMP	y		y			y	y	y
VIII	CYCLOBOND HP-RSP	y		y	y		y		y

Note: Each CSP favors certain molecular types.

New Updated Version
astec



CHIRAL METHOD DEVELOPMENT SCREEN CHIROBIOTIC & CYCLOBOND PHASES

1. COLUMN INSTALLATION

CHIROBIOTIC™ columns are shipped in methanol. Before starting to use a new column, wash with 20 mL HPLC grade methanol at 1 mL/min. The column test standard, 5-methyl-5-phenylhydantoin, can be injected at this stage.

CYCLOBOND™ columns are shipped in IPA and should be washed with 30 mL HPLC grade water at 0.8 mL/min before starting the method development screen.

2. MOBILE PHASE CHOICE

No.	Mobile Phase	Composition (% v)
REVERSED PHASE MODE:		
1	MeOH/20mM NH ₄ OAc, pH 4.0	20/80
2	MeOH/20mM NH ₄ OAc, pH 6.0	20/80
3	ACN/20mM NH ₄ OAc, pH 4.0	30/70
4	ACN/20mM NH ₄ OAc, pH 6.0	30/70
POLAR IONIC MODE®:		
5	MeOH/HOAc/TEA*	100/0.1/0.1
POLAR ORGANIC MODE:		
6	ACN/MeOH/HOAc/TEA	95/5/0.3/0.2
	If not progressing to normal phase, wash with MeOH at this stage to test and store the column.	100% MeOH
NORMAL PHASE MODE:		
7	EtOH/Hexane (or heptane, isohexane)	30/70
8	Washing cycle	100% EtOH
9	Column storage: CHIROBIOTIC CYCLOBOND	100% MeOH 100% IPA

* Use salts (NH₄O₂CCF₃ for bases, NH₄OAc for acids) when developing methods for prep.

3. COLUMN CHOICE AND RUN TABLE

Select your choice of columns from the list below. For a 6-column switching system, we recommend CHIROBIOTIC V2, T, R and CYCLOBOND I 2000, DNP and HP-RSP.

No.	Column Type (250x4.6mm)	1	2	3	4	5	6	7	8
I	CHIROBIOTIC V2	y		y		y		y	y
II	CHIROBIOTIC T	y	y		y	y		y	y
III	CHIROBIOTIC R		y		y	y		y	y
IV	CHIROBIOTIC TAG	y	y		y	y		y	y
V	CYCLOBOND I 2000	y		y			y		y
VI	CYCLOBOND I 2000 DNP	y		y			y	y	y
VII	CYCLOBOND I 2000 DMP	y		y			y	y	y
VIII	CYCLOBOND I 2000 HP-RSP	y		y	y		y		y

4. RUN CONDITIONS

Flow Rate:	1.0 mL/min.
Equilibration Time:	25 minutes
Run Time:	25 minutes
Temperature:	Ambient
Detector:	UV - 230nm
Sample:	1 mg/mL in MeOH

Notes

- The recommended protocol assumes the use of 250 x 4.6mm columns. For 100 x 4.6mm columns, use the same conditions at 0.5 mL/min.
- It is permissible to run straight from the reversed phase to the polar ionic mode®, and from the polar ionic mode to normal phase without an intermediate solvent wash.
- If any screening run results in a retention time less than 5 minutes, reduce the strength of the mobile phase and re-run. Aim for retention times from 5 to 20 minutes. In reversed phase mode reduce organic component, in polar ionic mode® or polar organic mode reduce acid/base concentration. Retention times can be later reduced in the optimization process.
- If a separation occurs in the polar ionic mode®, for a neutral molecule, change to 100% organic solvent (i.e. MeOH, EtOH or ACN).
- If the compound does not elute in reversed phase, increase the organic content to 40%. In the polar ionic mode®, increase the acid/base concentration up to 1.0/1.0. In the polar organic mode for the CYCLOBOND columns, increase the MeOH concentration up to 10%.

5. OPTIMIZATION PROCEDURES

Polar ionic mode® (CHIROBIOTIC phases only)	<ul style="list-style-type: none"> Test alternative acid/base ratios (generally higher acid for basic molecules, higher base for acidic molecules) To change acid/base to a volatile salt, use ammonium trifluoroacetate for basic compounds and ammonium acetate for acidic compounds at a concentration of 0.1wt%, adjust accordingly. Ammonium formate may be used as a compromise for both acidic and basic compounds.
Polar organic mode	<ul style="list-style-type: none"> Eliminate MeOH Test alternative acid/base ratios
Reversed phase mode	<ul style="list-style-type: none"> Test smaller pH changes Change organic to THF, ACN, MeOH Change buffer type and buffer concentration Change temperature
Normal phase mode	<ul style="list-style-type: none"> Change EtOH concentration

6. OPTIMIZING FOR MS DETECTION

CHIROBIOTIC: Use salts, as in Step 5, when using the polar ionic or polar organic modes.

CYCLOBOND: Use NH₄OH to replace TEA in polar organic mode, to lower concentration by 50 to 75%.
Both Phases: Use ammonium acetate or ammonium formate when using in reversed phase.

7. RETESTING YOUR METHOD DEVELOPMENT COLUMNS

To ensure the selectivity performance of CHIROBIOTIC columns, periodically test with 5-methyl-5-phenylhydantoin in 100% MeOH. For testing CYCLOBOND columns, please refer to your CYCLOBOND Handbook.

ADVANCED SEPARATION TECHNOLOGIES

World Headquarters: 37 Leslie Court, Post Office Box 297, Whippany, NJ 07981 USA Tel: (973) 428-9080 Fax: (973) 428-0152
 E-mail: info@astecusa.com www.astecusa.com

UK and Ireland Sales Office: 1 Blake Street, Congleton, Cheshire CW12 4DS UK Tel: +44 (0) 1260 276276 Fax: +44 (0) 1260 290067
 E-mail: info@asteceuro.com www.asteceuro.com

CHIROBIOTIC Screening Hits Leading to Baseline Separations

Compounds	Columns*	Compounds	Columns*
Albuterol	T2/T/TAG	Ketoprofen	R/V2/V
Amlodipine	V2/V	Lercanidipine	V2/V
Bupivacaine	V2/V	Lorazepam	T/TAG/T2
Chlorthalidone	T	Methylphenidate	V2/V/T/T2
Citalopram	V2/V	Metoprolol	T/T2
Clenbuterol	T2/T	Mianserin	V2/V/T2/T
Fluoxetine	V2/V	Mosapride	V2/V
Formoterol	T2	Naproxen	V2/V
Ibuprofen	V2/V	Nefopam	V2/V
Isoproterenol	T2/T/TAG	Nicardipine	V2/V

* CHIROBIOTIC: V/V2/T/T2/TAG/R

Note: Compounds from racemic switch list.

CHIROBIOTIC Screening Hits Leading to Baseline Separations con't

Compounds	Columns*	Compounds	Columns*
Omeprazole	R	Trimipramine	V2/V
Oxamniquine	V2/V	Warfarin	V2/V
Oxazepam	T/T2/TAG		
cis-Permethrin	TAG		
Propranolol	T/T2/TAG		
Pseudo-ephedrine	T2		
Sotalol	T/T2		
Terbutaline	T2/T/TAG		
Thalidomide	V2/V		
Tolperisone	V2/V		

*** CHIROBIOTIC: V/V2/T/T2/TAG/R – 80% hit rate!**

What Represents a Hit?

For CHIROBIOTIC phases hit is defined as $\alpha > 1.0$ i.e., some sign of chiral recognition. This usually means the opportunity for a baseline separation.

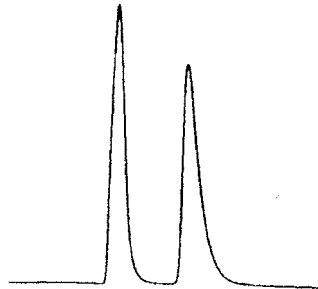
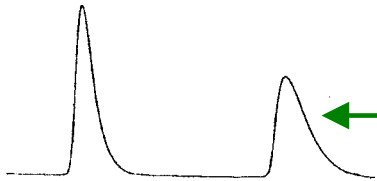
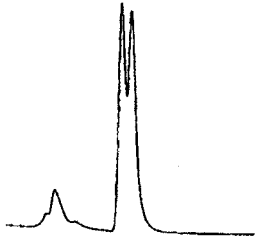
For CYCLOBOND phases α has to be at least 1.2.

From hit list we pick the column with the shortest R_t and greatest α for optimization. Typically, the Polar Ionic Mode (PIM) wins over all others except where the Reversed Phase (RP) offers solutions to solubility issues.

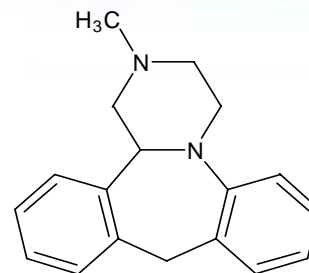
Advantages of Polar Ionic Mode

- Non-aqueous polar mobile phase
- Broad selectivity
- High efficiency
- Easy manipulation
- Short analysis time
- Low pressure, long column life
- MS compatible
- Low solvent volatility
- Excellent for prep
- Complementary to normal phase separations on polysaccharide CSPs

Importance of Acid/Base Ratios In PIM

Example	CHIROBIOTIC V
Mobile Phase	MeOH/Acid/Base
100/0.1/0.1 Peak 1 – 6.2 min. Peak 2 – 7.4 min. Ratio: 1:1	
100/0.15/0.05 Peak 1 – 10.4 min. Peak 2 – 14.5 min. Ratio: 3:1	
100/0.05/0.15 Peak 1 – 3.4 min. Peak 2 – 3.6 min. Ratio: 1:3	

Mianserin



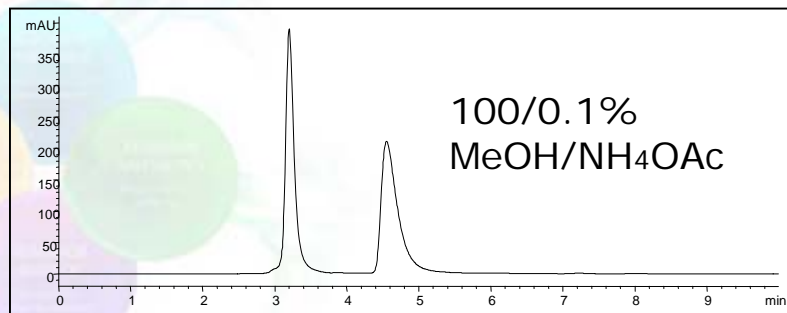
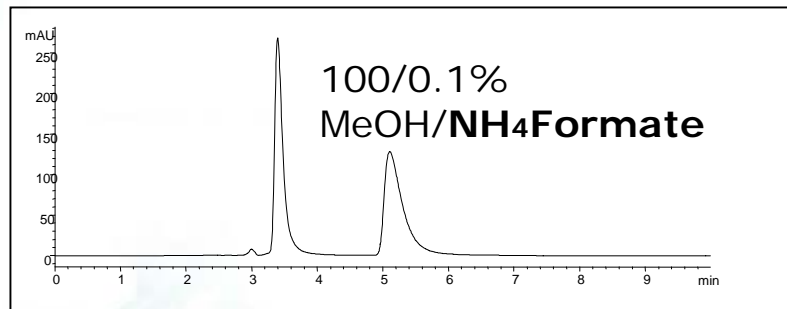
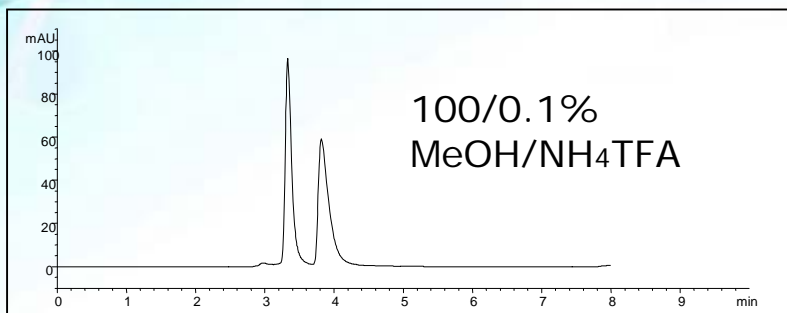
Nitrogen on Mianserin group is NH(+), COOH on V is (-)

Nitrogen on Mianserin group as free amine, but COOH on V is fully charged: weak ionic interaction

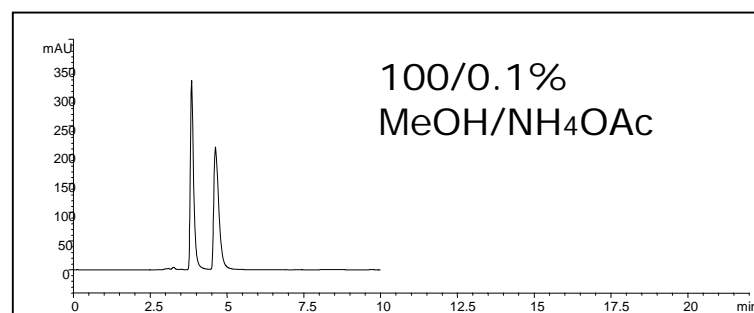
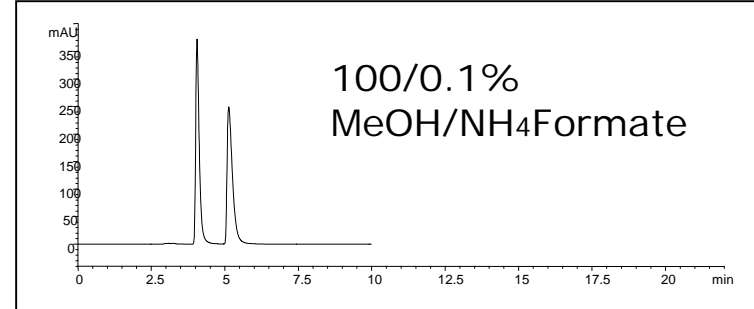
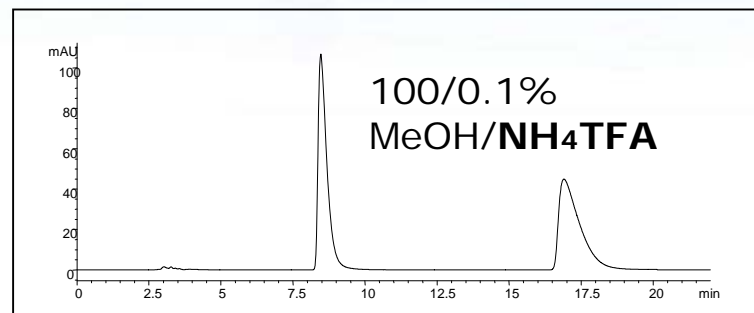
Note:
short
retention
times

Salt Effects in the Polar Ionic Mode

**Atrolactic acid,
CHIROBIOTIC T2**



**Mianserin
CHIROBIOTIC V2**



Reversed Phase Mode: Advantages

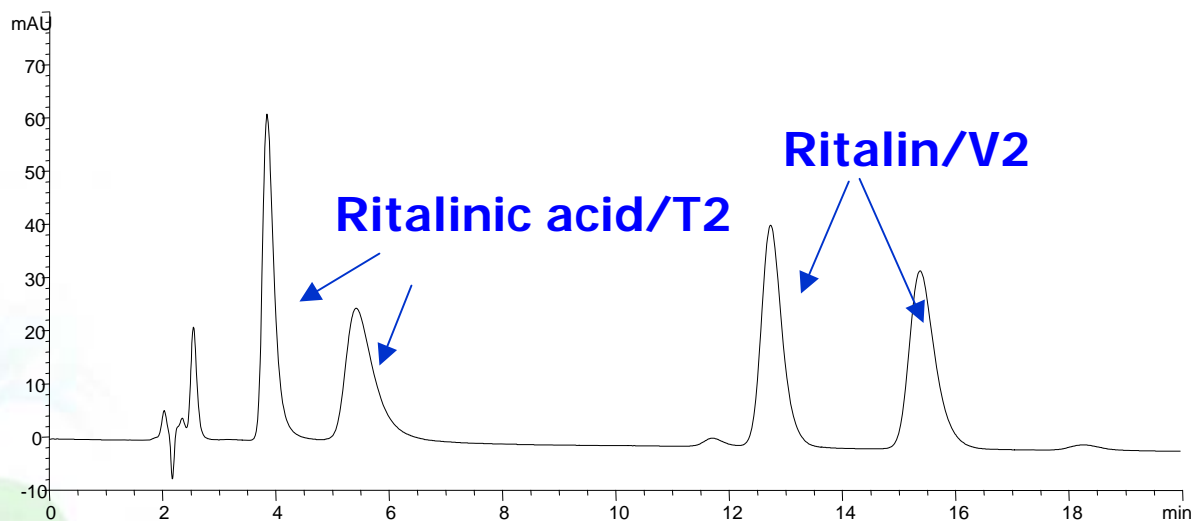
- Broad selectivity
- U-shaped curve for composition vs k'/α
- MS compatible mobile phase
- Applicable to dissolution analysis, biocatalysis

Column Coupling: Separation of Ritalin and Its Metabolite

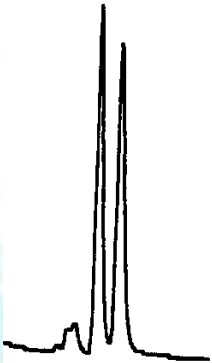
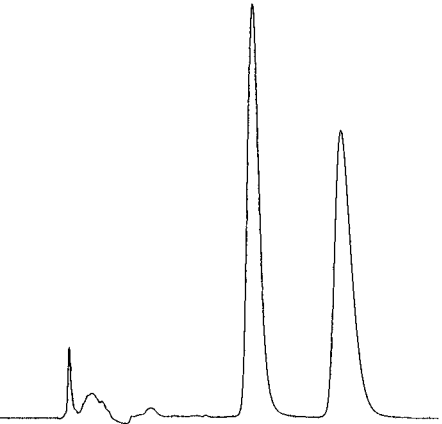
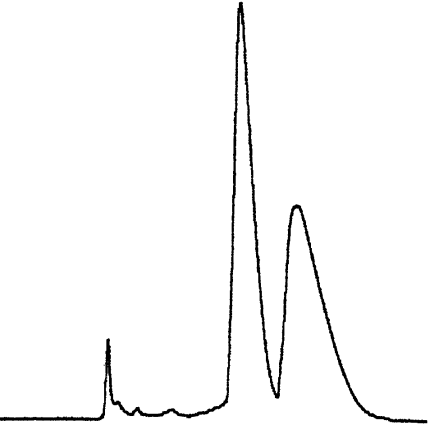
Column: **CHIROBIOTIC T2 (20x4.0mm) +**
CHIROBIOTIC V2 (150x4.6mm)

Mobile Phase: **93/7: MeOH/20 mM NH₄OAc, pH 4.1**

Flow Rate: **1.0 mL/min**



Example of Improved Resolution and Capacity for a Basic Compound

Analytical Separation on CHIROBIOTIC V, 5 μ m 250x4.6mm	Analytical Separation on CHIROBIOTIC V2, 5 μ m 250x4.6mm	Preparative Separation on CHIROBIOTIC V2, 5 μ m 250x21.2mm
Peak 1 - 10.51 min. Peak 2 - 11.53 min. $\alpha = 1.13$ 	Peak 1 - 8.83 min. Peak 2 - 11.76 min. $\alpha = 1.50$ 	Peak 1 - 10.22 min. Peak 2 - 12.54 min. 
100/0.2/0.1:MeOH/AcOH/TEA 0.9 mL/min. Inj. 2.0 μ L (5mg/mL)	100/0.5/0.5:MeOH/AcOH/TEA 1.0 mL/min. Inj. 10.0 μ L (5mg/mL)	100/0.2/0.1:MeOH/AcOH/TEA 15.0 mL/min. Inj. 140mg (in 2mL MeOH)

Racemic Switches: Optimized Conditions CHIROBIOTIC Phases

Final Results

Compounds	Mobile Phase	Column	k1'	α	Rs
Albuterol	100/0.1w%, MeOH/NH ₄ TFA	T2	2.5	1.33	2.8
Amlodipine	100/0.1w%, MeOH/NH ₄ TFA	V2	3.2	1.11	1.5
Bupivacaine	100/0.1w%, MeOH/NH ₄ TFA	V2	0.8	1.40	2.5
Citalopram	100/0.1w%, MeOH/NH ₄ TFA	V	4.8	1.12	1.5
Clenbuterol	100/0.1w%, MeOH/NH ₄ TFA	T2	2.1	1.26	2.5
Fluoxetine	100/0.1w%, MeOH/NH ₄ TFA	V2	1.5	1.27	2.5
Formoterol	100/0.6/0.4, MeOH/HOAc/TEA	T2	2.6	1.20	1.5

Racemic Switches: Optimized Conditions

CHIROBIOTIC Phases con't

Final Results

Compounds	Mobile Phase	Column	k ₁ '	α	Rs
Ibuprofen	10/90, THF/20mM NaCitate, pH6.3	V	0.8	1.24	1.5
Isoproterenol	100/0.1/0.1, MeOH/HOAc/TEA	T2	5.5	1.33	3.3
Ketoprofen	20/80, MeOH/0.1% TEAA, pH 6.0	R	2.6	1.60	3.0
Lercanidipine	100/0.02w%, MeOH/ NH ₄ TFA	V	1.0	1.18	1.5
Lorazepam	100 % MeOH	T	0.4	4.01	11
Methylphenidate	100/0.1w%, MeOH/NH ₄ TFA	V2	2.4	1.53	4.0
Metoprolol	100/0.1w%, MeOH/NH ₄ TFA	T	4.5	1.14	2.0
Mianserin	100/0.1w%, MeOH/NH ₄ Formate	V2	0.5	1.86	2.8

Racemic Switches: Optimized Conditions

CHIROBIOTIC Phases con't

Final Results

Compounds	Mobile Phase	Column	k1'	α	Rs
Mosapride	30/70, ACN/0.1% TEAA, pH 4.1	V	3.0	1.32	3.3
Naproxen	10/90, THF/NaCitrate, 20mM, pH6.3	V	1.4	1.22	1.5
Nicardipine	100/0.02w%, MeOH/NH ₄ TFA	V	0.7	1.74	4.5
Oxamniquine	100/0.1w%, MeOH/NH ₄ TFA	V2	2.7	1.23	2.2
Oxazepam	100% MeOH	T	0.5	4.31	12
cis-Permethrin	99.5/0.5, Hex/EtOH	TAG	4.6	1.22	2.5
Propranolol	100/0.1w%, MeOH/NH ₄ TFA	T	4.2	1.14	1.9
Pseudo-ephedrine	100/0.1w%, MeOH/NH ₄ TFA	T2	2.7	1.13	1.7

Racemic Switches: Optimized Conditions

CHIROBIOTIC Phases con't

Final Results

Compounds	Mobile Phase	Column	k ₁ '	α	Rs
Sotalol	100/0.2/0.1, MeOH/HOAc/TEA	T	3.5	1.12	1.5
Terbutaline	100/0.1w%, MeOH/NH ₄ TFA	T2	2.2	1.92	7.0
Thalidomide	100% MeOH	V	0.7	3.44	7.0
Tolperisone	100/0.1w%, MeOH/NH ₄ TFA	V2	1.8	2.33	2.7
Trimipramine	100/0.1w%, MeOH/NH ₄ TFA	V2	1.7	1.28	2.3
Warfarin	40/60, EtOH/0.1% TEAA, pH 4.1	V	1.3	1.45	3.0

CYCLOBOND Cyclodextrin Phases

- **CYCLODEXTRIN** phases are complementary to **CHIROBIOTIC** phases addressing certain structural issues like anti-fungal agents, primary and secondary amines (HP-RSP) and other issues like reversal of elution order.
- For a comprehensive review of applications see CYCLOBOND handbook, 7th edition, pages 30- 46.

Most Productive CYCLOBOND Phases

- **CYCLOBOND I 2000**
- **CYCLOBOND I 2000 HP-RSP (highest hit rate)**

Primarily basic chiral compounds have been resolved on the RSP and to a lesser extent both neutrals and acidics. All successful separations have been in the reversed phase mode with the organic component in the range of 5-40%.
- **CYCLOBOND I 2000 DMP (second most productive CSP in this line)** This is a π -basic phase.
- **CYCLOBOND I 2000 DNP (new addition)**

This is a π - acidic phase that has demonstrated separations not previously possible on any cyclodextrin phase.

CYCLOBOND Screening Hits Leading to Baseline Separations

Compounds	Columns*	Compounds	Columns*
Chlorpheniramine	β -CD	Sertraline	HP-RSP/ β -CD
Chlorthalidone	HP-RSP/ β -CD	Thioridazine	β -CD
Epinephrine	HP-RSP	Thalidomide	DNP
Methadone	HP-RSP	Tramadol	DMP
Miconazole	HP-RSP	Warfarin	β -CD
Nefopam	DNP/HP-RSP	Zopiclone	β -CD
Omeprazole	DMP		
Oxazepam	DNP		
Propranolol	β -CD		
Pseudo-ephedrine	β -CD		

* CYCLOBOND I 2000: β -CD/HP-RSP/DNP/DMP – 40% hit rate!

Note: Compounds from racemic switch list.

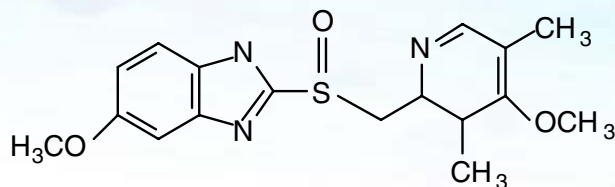
Racemic Switches: Optimized Conditions CYCLOBOND Phases

Final Results

Compounds	Mobile Phase	Column	k ₁ '	α	Rs
Chlorpheniramine	10/90, ACN/0.1% TEAA, pH4.1	β-CD	4.7	1.14	1.5
Chlorthalidone	15/85, ACN/10mM NH ₄ OAc, pH4.0	HP-RSP	1.4	1.22	1.6
Epinephrine	25/75, ACN/20mM NH ₄ OAc, pH4.1	HP-RSP	7.1	1.16	2.0
Methadone	20/80, ACN/ 10mM NH ₄ OAc, pH 3.6	HP-RSP	1.5	1.24	1.7
Miconazole	25/75, ACN/10mM NH ₄ OAc, pH 4.0	HP-RSP	4.1	1.18	1.6
Nefopam	25/75, ACN/20mMNH ₄ OAc,pH 4.1	DNP	0.5	1.52	2.2
Omeprazole	100/0.4/0.1, ACN/HOAc/NH ₄ OH	DMP	3.3	1.41	2.4
Sertraline	30/70, ACN/20mM NH ₄ OAc, pH4.1	HP-RSP	5.4	1.19	1.7
Thioridazine	20/80, ACN/0.1% TEAA, pH4.1	β-CD	6.9	1.21	2.5
Tramadol	20/80, ACN/20mM NH ₄ OAc, pH5.5	DMP	1.6	2.20	2.8
Zopiclone	95/5/0.3/0.2,ACN/MeOH/HOAc/TEA	β-CD	1.0	1.13	1.5

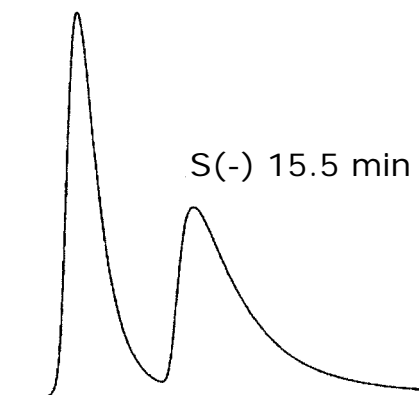
Options from Results of Dual Screen

Omeprazole (Nexium)



CHIROBIOTIC R (NP)

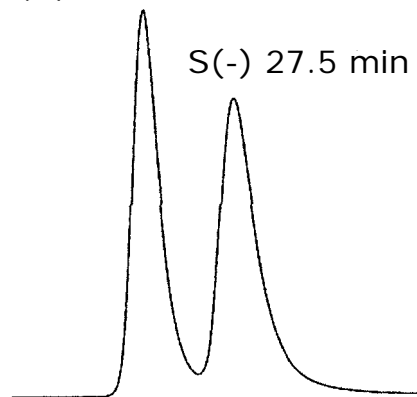
R(+) 13.4 min



40/60: EtOH/Heptane

CHIROBIOTIC R (RP)

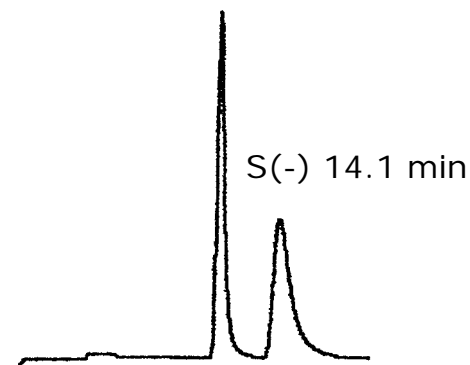
R(+) 24.5 min



30/70: MeOH/10mM
NH₄OAc, pH 4.1

CYCLOBOND I 2000 DMP (POM)

R(+) 10.8 min



100/0.4/0.1:
ACN/HOAc/NH₄OH

Statistics

- Of forty racemic switch drugs tested in this study:
 - The success rate for CHIROBIOTIC phases is 80%.
 - The success rate for CYCLOBOND phases is 40%.
 - Eight compounds can be separated by both types (20%).
 - Resolution range:
 - CYCLOBOND Phases: 1.5 to 2.8
 - CHIROBIOTIC Phases: 1.5 to 11.0

Conclusions

1. A successful high throughput screening system consisting of 4 CHIROBIOTIC phases and 4 CYCLOBOND phases has been devised employing 8 mobile phases that cover all mobile phase types.
2. The polar ionic mode offers excellent opportunities for LC/MS and preparative applications.
3. The CYCLOBOND CSPs are complementary to the CHIROBIOTIC CSPs.
4. A 100% hit rate for 40 divergent racemic switches was obtained with a 20% overlap and resolutions from 1.5 to 11.0.

Conclusions cont'd

- There are complementary effects within the same class of CSPs: CHIROBIOTIC V2/T/TAG/R and CYCLOBOND I 2000 β -CD/DNP/DMP/HP-RSP were chosen due to their higher success rate.
- Reversal of elution order was frequently observed within either CHIROBIOTIC or CYCLOBOND CSPs and also between these two types of CSPs.
- Most applications can be converted into LC/MS compatible methods.
- Greater than 90% of basic pharmaceutical drugs can be baseline-separated by CHIROBIOTIC V2/V/T2/T in polar ionic mode or reversed-phase mode.

Application Areas

- LC-MS
- Amino Acids/Peptides
- Biocatalysis
- Preparative

CASE STUDY

- Fluoxetine methods development

Column Screening Procedure

Enantiomeric selectivity for samples is examined through a primary method development protocol:

- Columns:

- CHIROBIOTIC™
 - V2
 - T
 - TAG
- CYCLOBOND™
 - B-CD
 - DNP
 - HP-RSP

- Mobile Phases:

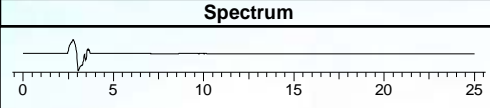
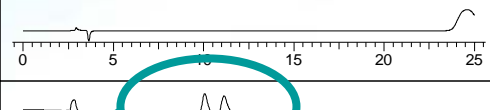
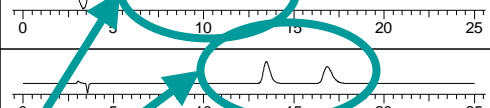
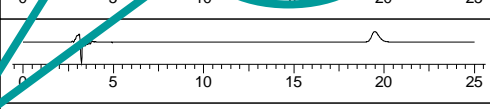
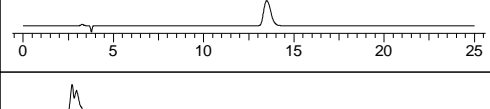
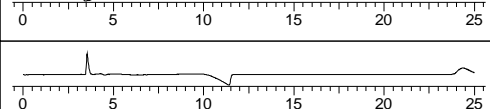
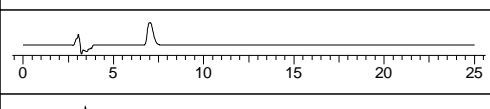
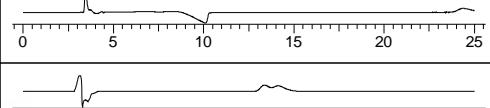
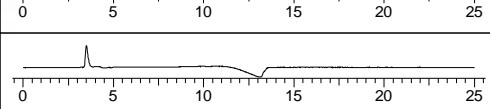


- Polar-Ionic Mode (PIM)
 - 100:0.1:0.1, methanol:acetic acid:triethylamine
- Reversed-Phase (RP)
 - 70:30, 20 mM ammonium acetate (pH 4.0):acetonitrile
- Polar-Organic Mode (POM)
 - 95:5:0.3:0.2, acetonitrile:methanol: acetic acid:triethylamine.

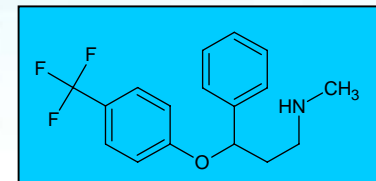
The columns and mobile phases have been selected through experience to cover the greatest number of compounds with the fewest number of injections (80/20 rule).

Results of Fluoxetine Column Screen

(+/-)-Fluoxetine underwent the primary screening protocol and yielded positive results.

CHIROBIOTIC V2 in both RP and PIM provided excellent selectivity

Spectrum	Column	mode	elution
	CHIROBIOTIC TAG	RP	No Elution
	CHIROBIOTIC TAG	PIM	No Separation
	CHIROBIOTIC V2	RP	Separation
	CHIROBIOTIC V2	PIM	Separation
	CHIROBIOTIC T	RP	No separation
	CHIROBIOTIC T	PIM	No Separation
	Cyclobond I 2000	RP	No Retention
	Cyclobond I 2000	POM	No Separation
	Cyclobond 2000 HP-RSP	RP	No Separation
	Cyclobond 2000 HP-RSP	POM	No Separation
	Cyclobond 2000 DNP	RP	Separation
	Cyclobond 2000 DNP	POM	Unknown



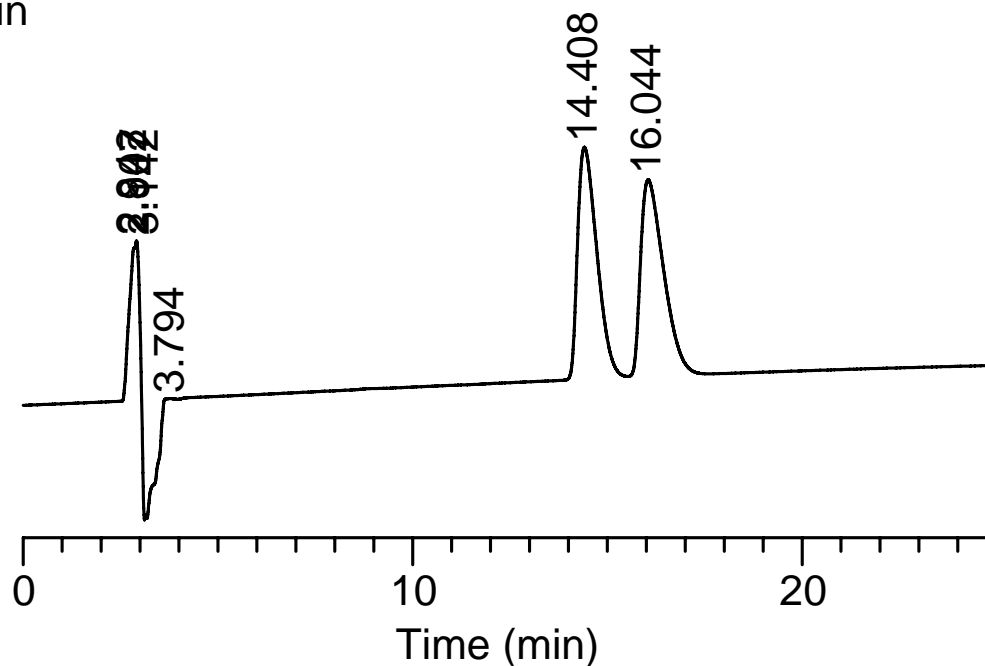
• Screening results are viewed in a tabular form for easy review and comparison.

Reversed-Phase Results

Subsequent to screening, positive results are then confirmed on an additional instrument.

Conditions:

Column: CHIROBIOTIC V2, 250 x 4.6 mm, 5 μ m particles
Mobile Phase: 70:30, 20 mM NH_4OAc (pH 4): ACN (Reversed-Phase Mode):
Sample: 1 mg/mL (racemic)
Flow Rate: 1 mL/min
UV: 230 nm



Method Optimization in Reversed-Phase Mode

- Organic modifier type and composition can dramatically effect retention and selectivity

- Later studies showed that methanol acted as a better organic modifier than ACN in the case of fluoxetine.

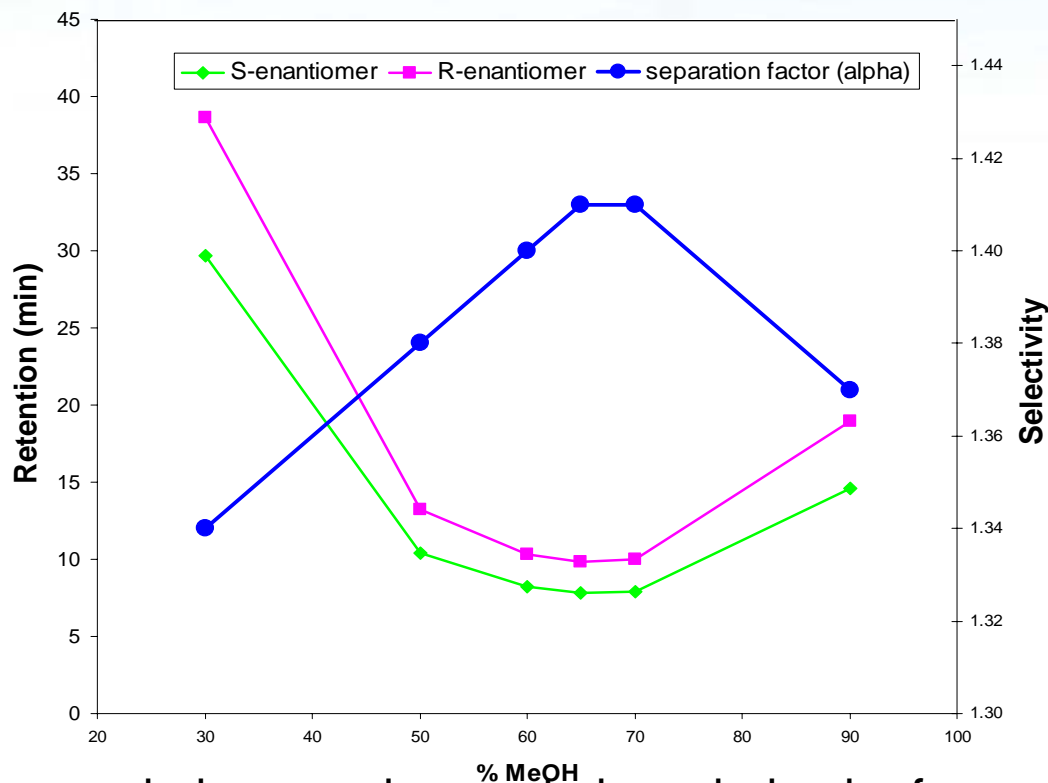
- Other parameters to consider:

- Temperature
- Flow rate
- Organic type
- pH
- Buffer

- The graph shows both reversed-phase and normal-phase behavior for fluoxetine on CHIROBIOTIC V2 as a function of methanol composition

- selectivity is optimal between 65% and 70% methanol
- no further optimization required in this case

Retention Behavior of Fluoxetine in Reversed-Phase Mode



Optimization in the Polar-Ionic Mode

The following steps may be followed to enhance the separation results from column screening:

- **Choose the most appropriate acid and base modifiers.**
 - Then modify the relative concentrations and ratio of the chosen acid/base combinations.
- **Change the flow rate.**
 - Lower flow rates often result in higher resolution in this particular mode.
- **Change temperature.**
 - Lower temperature often results in higher resolution.
 - Higher temperatures can reduce tailing, decrease retention and, in a number of cases, reverse elution order.
- **Evaluate CHIROBIOTIC V or other pairs of CHIROBIOTIC phases.**
 - Use the exact same mobile phase conditions that have been established.
 - May enhance resolution

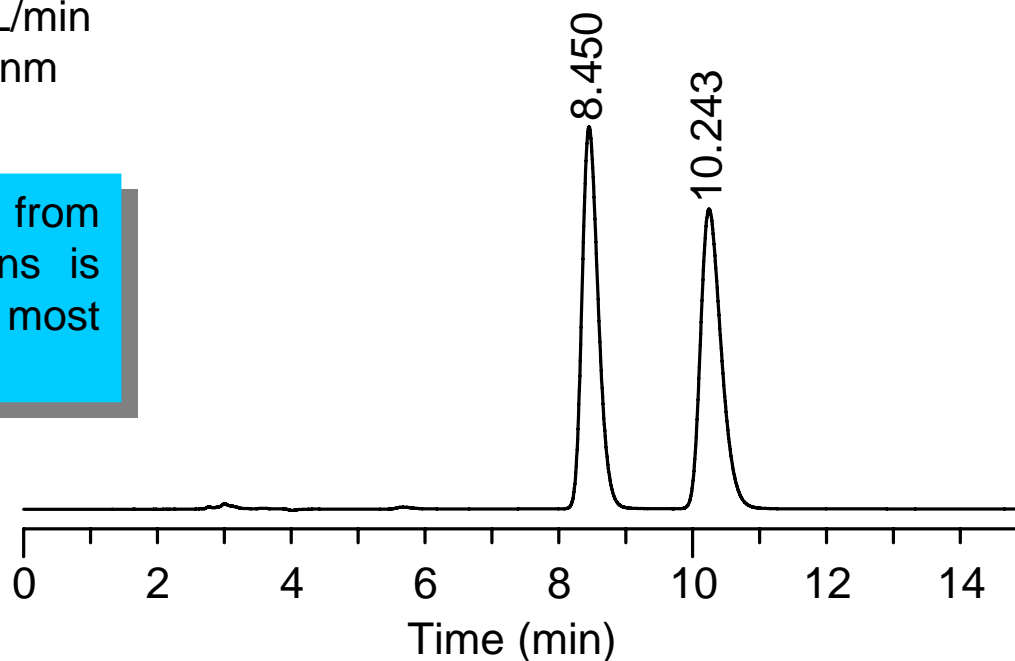
Polar-Ionic Results

In the case of fluoxetine, polar-ionic mode using CHIROBIOTIC V2 was shown to provide the greatest selectivity.

Conditions:

Column: CHIROBIOTIC V2, 250 x 4.6mm, 5 µm particles
Mobile Phase: 100/0.1/0.1, MeOH/HOAc/TEA (Polar-Ionic Mode, PIM)
Sample: 1 mg/mL (racemic)
Flow Rate: 1 mL/min
UV: 230 nm

The selectivity gained from the screening conditions is usually sufficient for most applications.



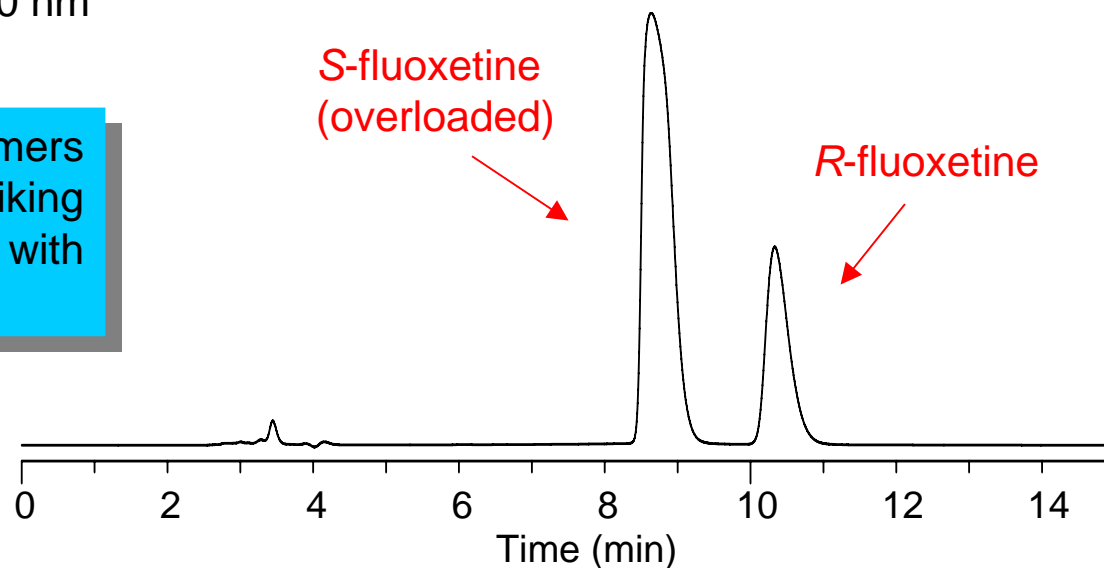
Conversion to LC-MS-Compatible Mobile Phase

To demonstrate the ability to use LC-MS-appropriate modifiers TEA was removed, and the separation was performed using ammonium formate.

Conditions:

Column: CHIROBIOTIC V2, 250 x 4.6mm, 5 μ m particles
Mobile Phase: 100/0.1w%, MeOH/NH₄Formate (PIM)
Flow Rate: 1mL/min
Sample: S/R ratio: 4/1 in MeOH
UV: 230 nm

The R/S enantiomers were identified by spiking the racemic sample with additional S-form.



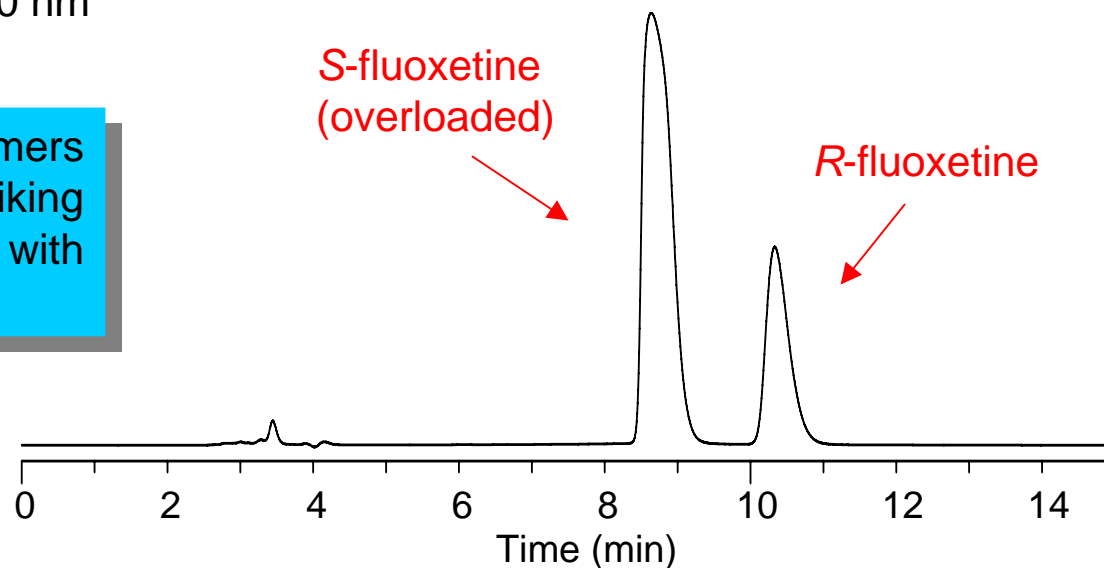
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Conditions:

Column: CHIROBIOTIC V2, 250 x 4.6mm, 5 μ m particles
Mobile Phase: 100/0.1w%, MeOH/NH₄Formate (PIM)
Flow Rate: 1mL/min
Sample: S/R ratio: 4/1 in MeOH
UV: 230 nm

The R/S enantiomers were identified by spiking the racemic sample with additional S-form.



Conclusions

- A primary chiral screen of racemic fluoxetine demonstrated baseline separation of the enantiomers.
- CHIROBIOTIC™ V2 in both reversed-phase and polar-ionic modes provided excellent resolution.
- Both reversed-phase and polar-ionic systems provide LC-MS compatible separations.
- Column screening is demonstrated to be an efficient approach to chiral method development.
 - CHIROBIOTIC and CYCLOBOND columns provide complementary mixtures of interaction mechanisms that allow for general use as chiral selectors.



Thank you for attending our seminar.

We welcome your
comments and suggestions.