

Chiral Method Development Techniques for Analytical Preparative HPLC



Method Development Techniques

Method 1 - Fast

- Column coupling enables simultaneous multi-column screening

CHIROBIOTIC R/V/T

Method 2 - Comprehensive

- Generic screening methods for fast chiral method development with switching valves for:
 - A. 9 columns, 9 mobile phases
 - B. 4 columns on 5-6 mobile phases

Features of Phases Used in the Screening Process

- ❖ Phases can be used effectively with more than one mobile phase type. (Multimodal)
- ❖ CHIROBIOTIC phases (100x4.6mm) R,V & T can be coupled for fast screening. Provides 9 screens in 150 minutes.
- ❖ Generic screen offers broad selectivity.

Typical Mobile Phase Types for Chiral Separations on CHIROBIOTIC Phases

- ✓ Polar Ionic Mode
 - MeOH with acid/base or volatile salt < 0.1% wt.
 - Fast kinetics, largest group of separations
 - Best suited for LC/MS and Prep
- ✓ Reversed Phase
 - Methanol/Buffer
 - Second largest group of separations
- ✓ Polar Organic Mode
 - ACN, EtOH, MeOH or combos
 - Third largest group, neutral molecules only
- ✓ Normal Phase
 - Heptane/ethanol
 - Generally provides ca 10-15% of total methods

What is the Polar Ionic Mode[©]?

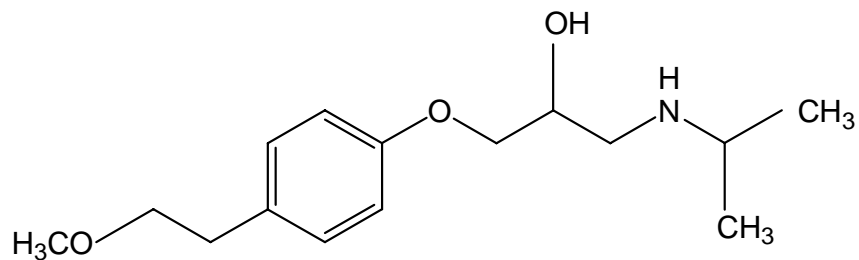
- The polar ionic mode is defined by the use of 100 % MeOH with added acid and base (typically 0.1%, each with a range of 1.0 to 0.001%), or equivalent volatile salts
- The mechanism of interaction is predominately ionic
- Method development and optimization is very simple and fast

What is the difference between Polar Ionic and Polar Organic Modes?

- The Polar Organic Mode terminology is now frequently applied to the Daicel phases, but in this case it means 100% organic only, usually MeOH, EtOH, ACN or combinations
- The mechanism (predominantly hydrogen bonding) for the POM on Daicel is *different* from that on the CHIROBIOTIC phases and the optimization process is *different*

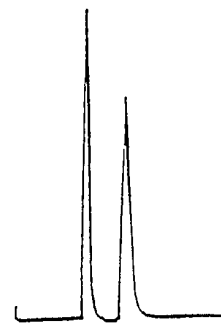
Normal Phase vs Polar Ionic Mode[©]

Metoprolol



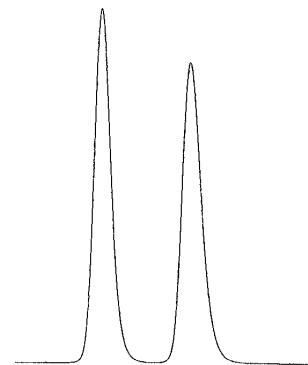
CHIRACEL OD[®]

Peak 1 – 11.9 min.
Peak 2 – 18.2 min.



CHIROBIOTIC T[®]

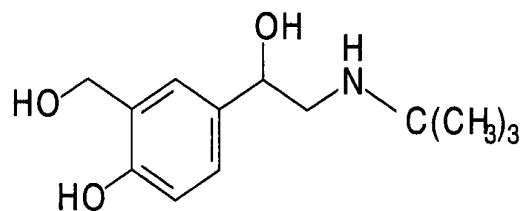
Peak 1 – 15.36 min
Peak 2 – 17.11 min



Chiracel is the trademark of Daicel

Polar Organic Mode vs Normal Phase Mode

Albuterol

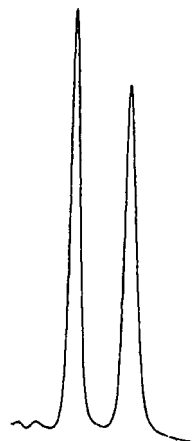


1 - 8.66 min.

2 - 9.74 min.

$\alpha = 1.19$

$R_s = 1.8$



CHIROBIOTIC T

250X4.6mm

100/0.2/0.1:

MeOH/HOAc/TEAA

1 - 16.52 min.

2 - 19.63 min.

$\alpha = 1.24$

$R_s = 1.8$



CHIROBIOTIC T

250x4.6mm

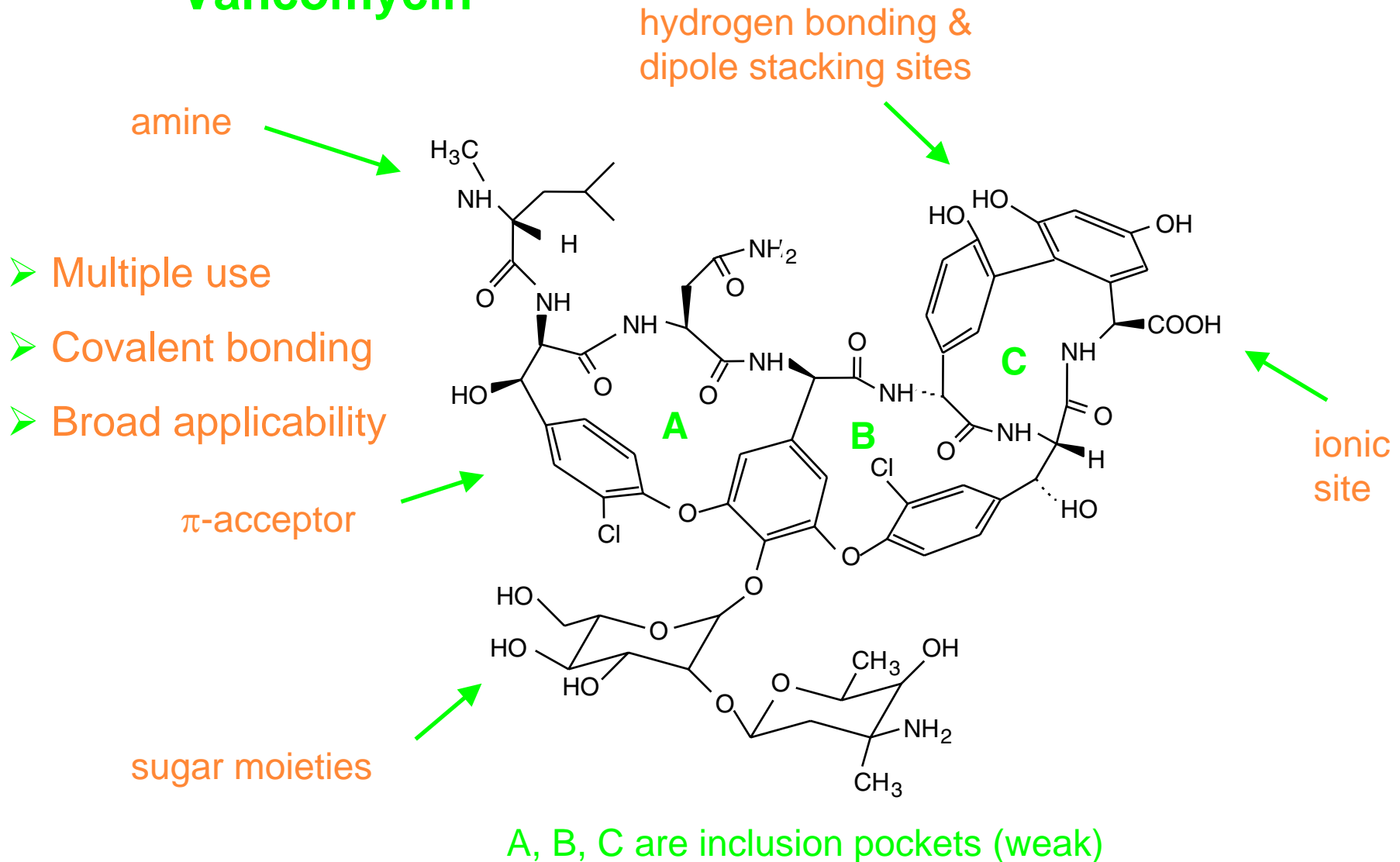
50/50/0.3/0.6:

EtOH/Hex/TFA/TEA

NOTE: R_t is 2x longer for same R_s

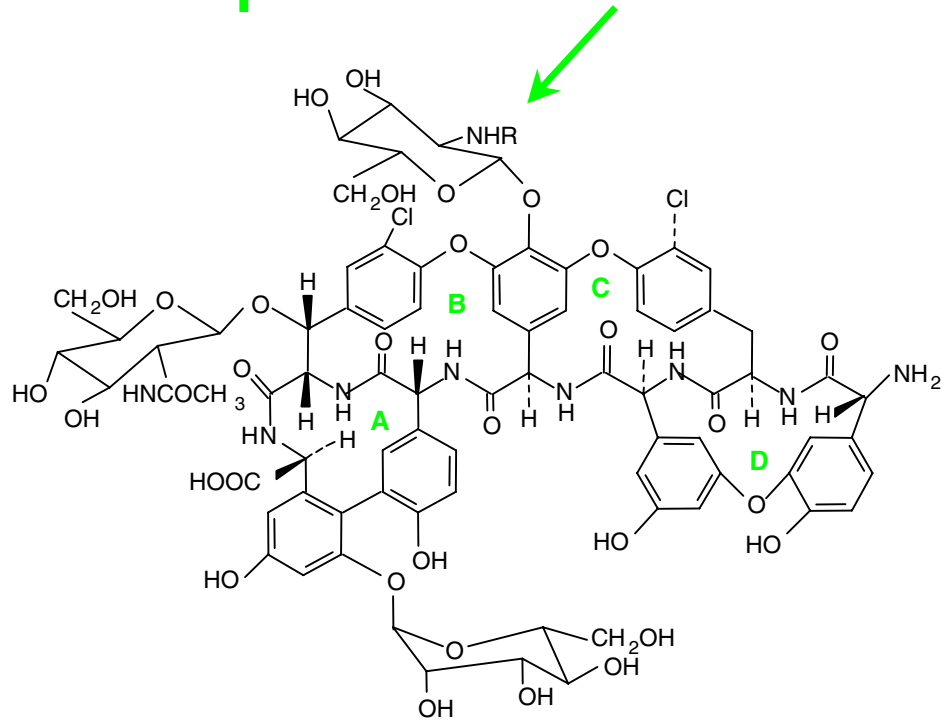
Proposed Structures of Glycopeptide CSPs

Vancomycin

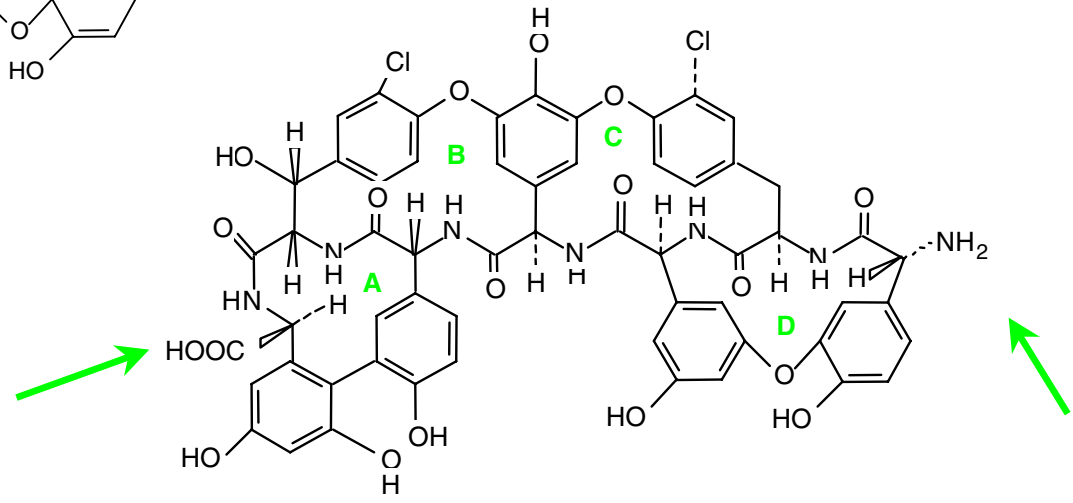


Proposed Structures of Glycopeptide CSPs

Teicoplanin



Teicoplanin Aglycone



→ Key sites

Applications by Compound Classes

● CHIROBIOTIC V and V2

Acids: Profens

Bases: Amino alcohols, Calcium channel blockers, tricyclic antidepressants, ergot alkaloids, cyclic imides, pyridones, carbonitriles and other amines.

Neutrals: Barbitals, coumarins, hydantoins, oxazolidinones, and sulfoxides.

● CHIROBIOTIC T, T2 and TAG

Acids: Amino acids, N-blocked amino acids, cyclic amino acids, hydroxy and O-blocked hydroxy acids, α -bromo-acids and phenoxy propionic acids.

Bases: Amino alcohols, ergot alkaloids, aryl dihydropyrimidine carboxylates

Neutrals: Oxazolidinones, Diazepines, hydantoins and sulfoxides

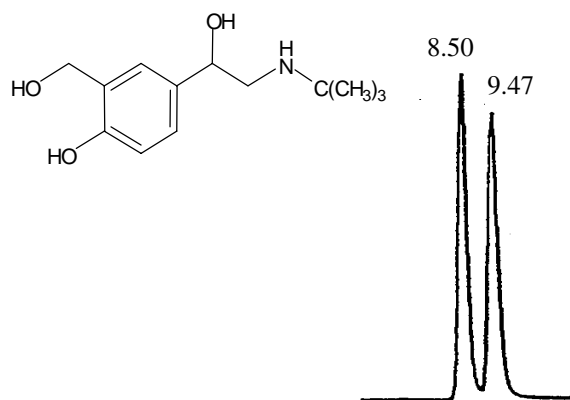
● CHIROBIOTIC R

Acids: Profens, N-blocked, cyclic and unusual amino acids, hydroxy, O-blocked and halogenated acids.

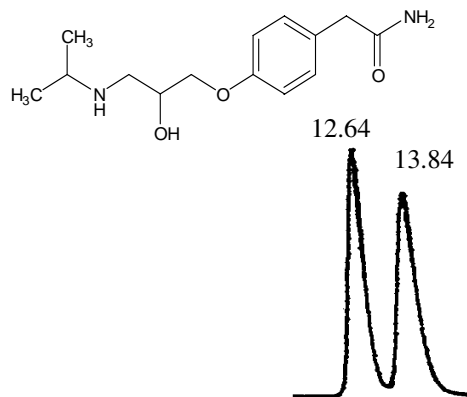
Neutrals: Oxazolidinones, hydantoins

Broad Selectivity Based on the Same Stereogenic Center: CHIROBIOTIC T: Amino Alcohols

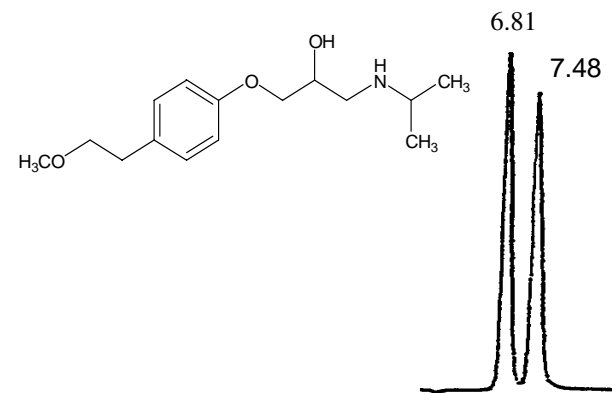
Albuterol



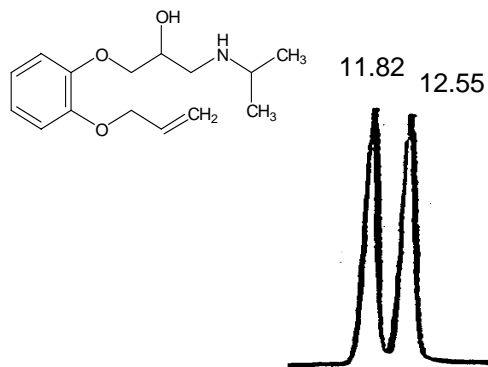
Atenolol



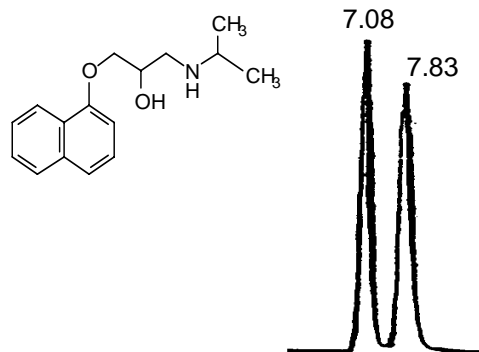
Metoprolol



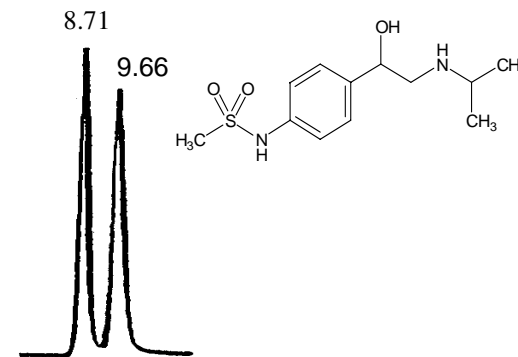
Oxprenolol



Propranolol

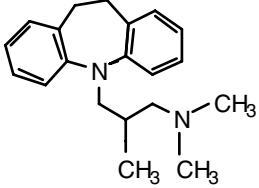
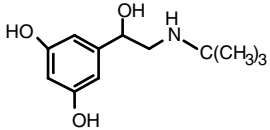
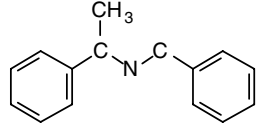
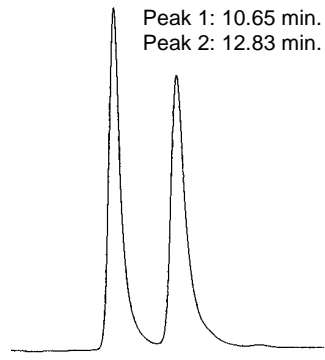
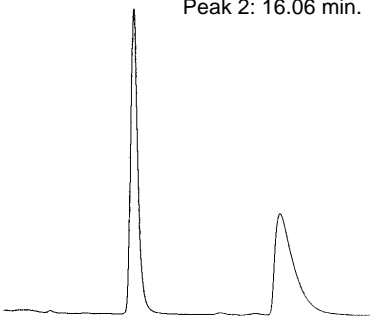
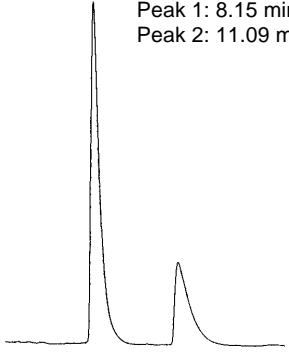


Sotalol



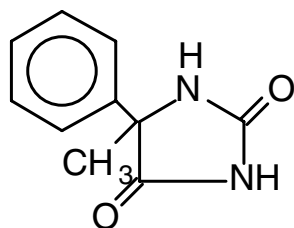
Mobile Phase: 100/0.1/0.1:MeOH/HOAc/TEA @ 2.0 mL/minute

Enhanced Chiral Selectivity with V2 and T2 in Polar Ionic Mode

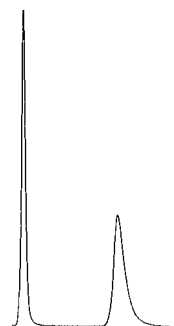
<p><i>Separation of Trimipramine</i></p>  <p><chem>CN(C)CC12C=CC=CC1C3=CC=CC=C23</chem></p>	<p><i>Separation of Terbutaline</i></p>  <p><chem>CC(C)(C)NCC(O)c1ccc(O)cc1</chem></p>	<p><i>Separation of N-Benzyl-α-methyl-benzylamine</i></p>  <p><chem>CC1=CC=CC=C1CN(C2=CC=CC=C2)CC3=CC=CC=C3</chem></p>
 <p>Peak 1: 10.65 min. Peak 2: 12.83 min.</p>	 <p>Peak 1: 9.70 min. Peak 2: 16.06 min.</p>	 <p>Peak 1: 8.15 min. Peak 2: 11.09 min.</p>
<p>CHIROBIOTIC V2, 5μM 250x4.6mm 100/0.1w%: MeOH/NH₄TFA 1.0 mL/min. Detection: 230nm</p>	<p>CHIROBIOTIC T2, 5μM 250x4.6mm 100/0.1w%: MeOH/NH₄TFA 1.0 mL/min. Detection: 230nm</p>	<p>CHIROBIOTIC V2, 5μM 250x4.6mm 100/0.1w%: MeOH/NH₄TFA 1.0 mL/min. Detection: 230nm</p>

Selectivity of Methanol for Neutral Molecules on CHIROBIOTIC TAG

5-Methyl-5-phenylhydantoin

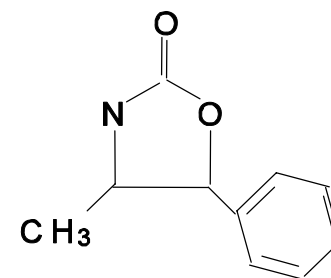


1. 5.08 min
2. 9.62 min

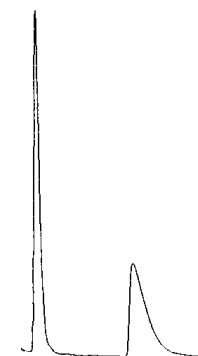


100% MeOH @ 0.8 mL/min

4-Methyl-5-phenyl-2-oxazolidinone



1. 5.35 min.
2. 8.21 min.

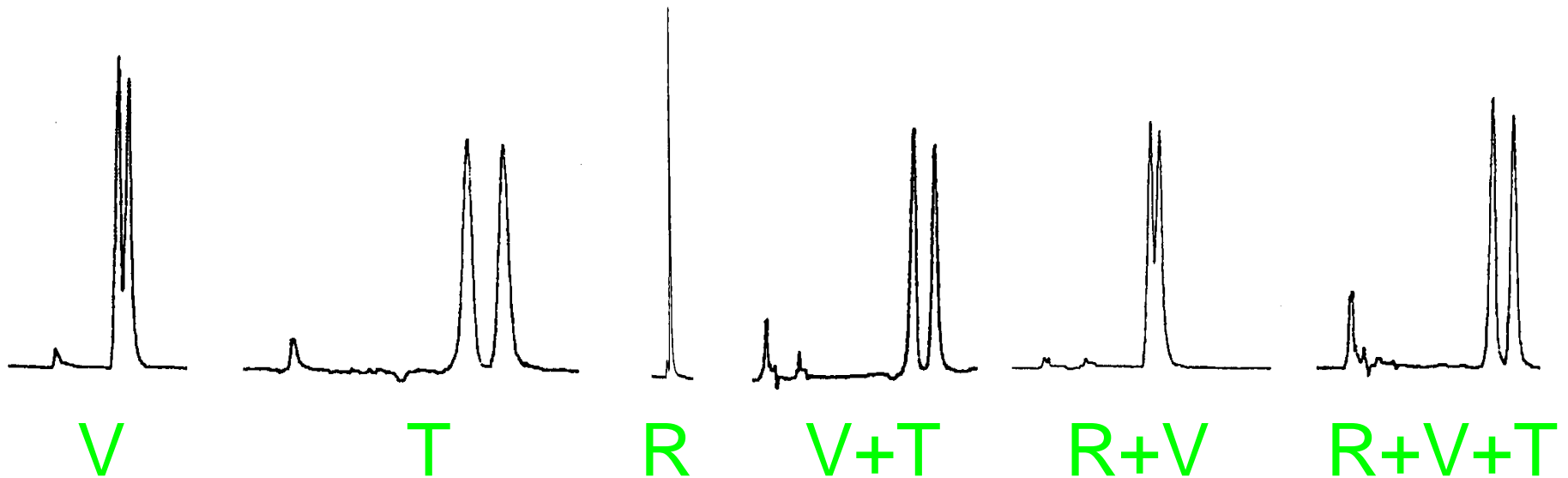


100% MeOH @ 0.8 mL/min

Column Coupling

Albuterol

Moblie Phase: 100/0.02/0.01
MeOH/HOAc/TEA
Flow Rate: 2.0 mL/min.
Detection: UV@254nm
Temperature: 23°C



Initial studies indicated no loss in Rs with coupling but column order had to be R+V+T.

Method Development Techniques Using Coupled Columns

SCREENING MOBILE PHASES:

- Polar ionic mode©
MeOH/AcOH/TEA, 100/0.02/0.01, 2.0 mL/min
- Reversed phase mode
MeOH/TEAA (0.1%, pH 6.0), 25/75, 1.0 mL/min
- Normal phase mode
Hex/EtOH, 40/60, 1.5 mL/min

Coupled Column Screen and Optimization

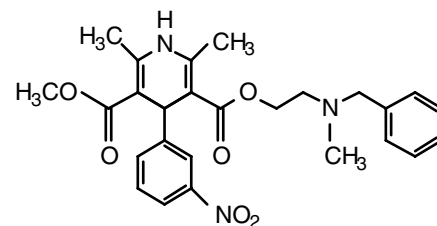
Optimization in the Polar Ionic Mode

1. Use single analytical column to determine the most selective phase (10 or 25cm R, V or T).
2. Choose proper acid/base (HOAc, TEA, TFA, NH₄OH or salt NH₄Ac, HCOONH₄, NH₄TFA).
3. Adjust acid/base ratio (4/1 to 1/4) or salt concentration 0.01 to 1%.
4. Change the concentration of acid and base (0.001% to 1%). Higher concentration of acid and base results in lower retention.
5. Change flow rate. Lower flow rate often results in higher resolution.
6. Decreased temperature can increase resolution.
7. Evaluate V₂ and T₂ under best set of conditions.

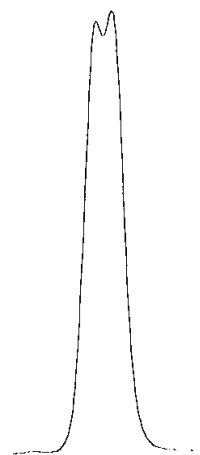
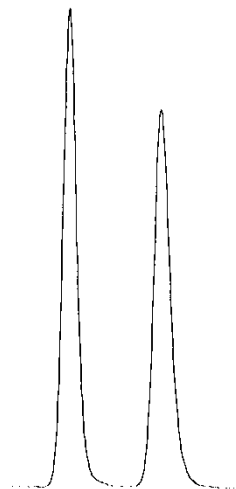
Optimization Study

Column: CHIROBIOTIC V

Sample: Nicardipine

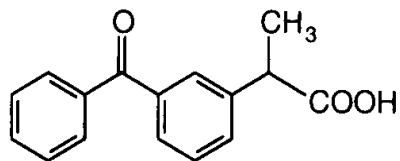


100/0.1w%, MeOH/NH₄TFA 100/0.1w%, MeOH/NH₄OAc

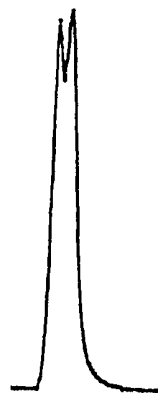


Coupled Column Screen and Optimization

Ketoprofen

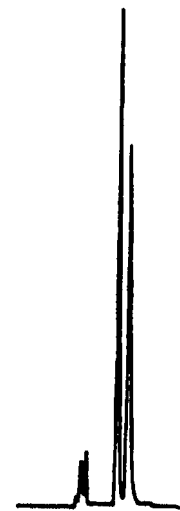


Screen



R+V+T (10cm)
100/0.02/0.01
2 mL/min.

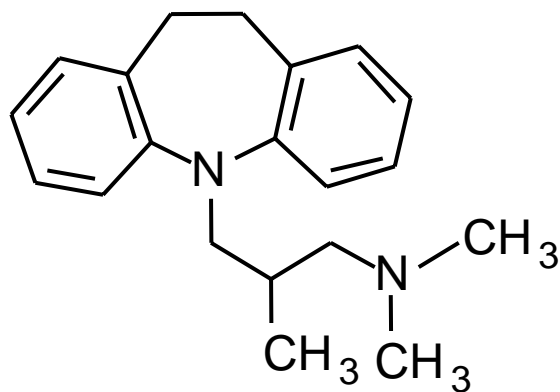
Optimized



R (25cm)
100/0.02/0.01
0.8 mL/min.

Coupled Column Screen and Optimization

Trimipramine

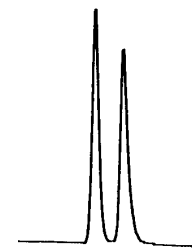


Screen



R+V+T (10cm)
MeOH/HOAc/TEA:
100/0.02/0.01
2 mL/min.

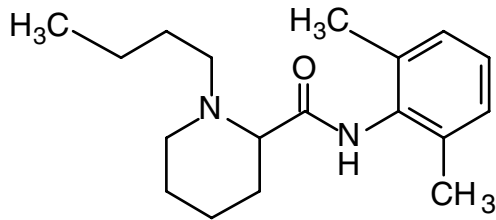
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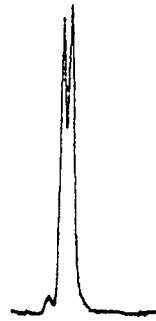
V (25cm)
100/0.02 v/w:
MeOH/AFTA
0.8 mL/min.

Coupled Column Screen and Optimization

Bupivacaine

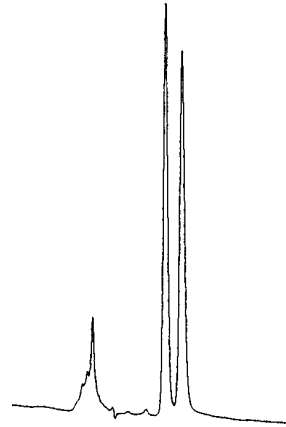


Screen



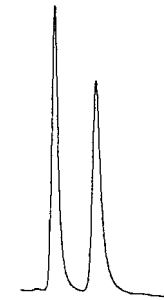
R+V+T (10cm)
MeOH/HOAc/TEA:
100/0.02/0.01
2mL/min

Optimized



V (25cm)
MeOH/NH₄TFA
100/0.1% wt.
1mL/min

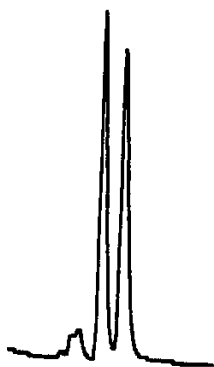
Optimized



V2 (25cm)
MeOH/NH₄TFA
100/0.1% wt.
1mL/min

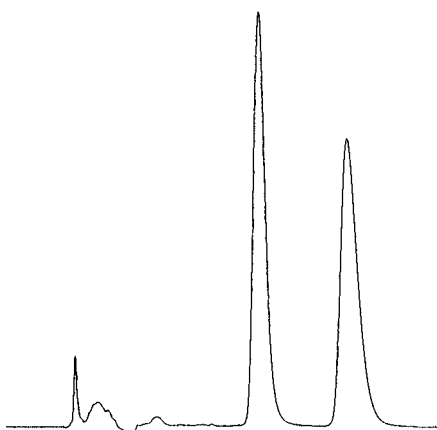
Example of Improved Resolution and Capacity for a Basic Compound

Analytical Separation on
CHIROBIOTIC V, 5 μ M
250x4.6mm



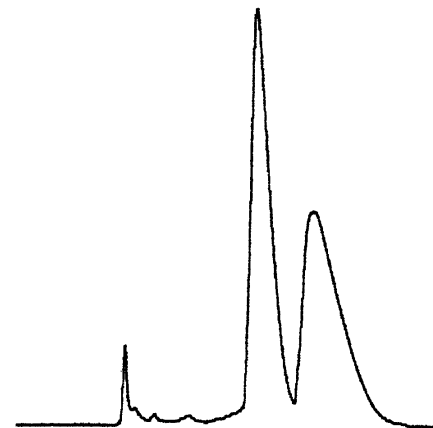
100/0.2/0.1:MeOH/HOAc/TEA
0.9 mL/min.
Inj. 2.0 μ L (5mg/mL)

Analytical Separation on
CHIROBIOTIC V2, 5 μ M
250x4.6mm



100/0.5/0.5:MeOH/HOAc/TEA
1.0 mL/min.
Inj. 10.0 μ L (5mg/mL)

Preparative Separation on
CHIROBIOTIC V2, 5 μ M
250x21.2mm



100/0.2/0.1:MeOH/HOAc/TEA
15.0 mL/min.
Inj. 140mg (in 2mL MeOH)

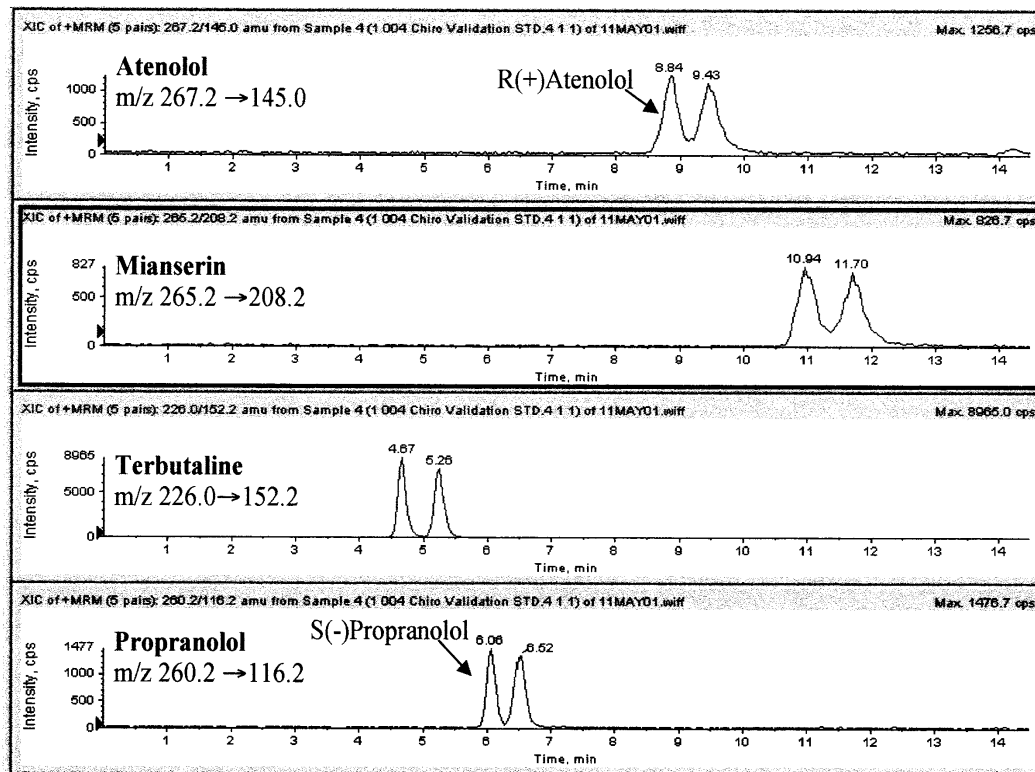
LC/MS Method Development Techniques

Use of On-line Dual Extraction in Conjunction with Chiral Liquid Chromatography Tandem Mass Spectrometry for Determination of Terbutaline Enantiomers in Human Plasma, Yuan-Qing Xia, David Q. Liu, and Ray Bakhtiar, Department of Drug Metabolism, Merck Research Laboratories, Rahway, New Jersey. *Chirality* 14:742-747 (2002).

Analytes:	Terbutaline in human plasma
Sample Prep:	On-line SPE
Column:	CHIROBIOTIC T, 100x4.6mm or R+V+T
Mobile Phase:	MeOH/0.05% ammonium trifluoroacetate salt, v/wt
Flow Rate:	1.2 mL/min
Analysis Time:	5.5 min
Detection Limits:	1 ng/mL

Note: Good comparison of single column to coupled column CHIROBIOTIC phases.

Using the CHIROBIOTIC R,V,T Kit for Drug Metabolism: LC/APCI/MRM Chromatograms



Neat solution at 10 ng/mL of: Atenolol (m/z 267.2 → 145.0), Mianserin (m/z 265.2 → 208.2), Terbutaline (m/z 226.0 → 152.2), Propranolol (m/z 260.2 → 116.2). [Ref: R Bakhtiar, in press]

LC/MS Clinical Applications

Determination of the Enantiomers of Salbutamol and its 4-O-sulfate Metabolites in Biological Matrices by Chiral Liquid Chromatography/Tandem Mass Spectrometry, Joyce, K.B., Jones, A.E., Scott, R.J., Biddlecombe, R.A., Pleasance, S., Rapid Commun. Mass Spectrom. 12, 1899-1910 (1998).

Analytes:	Salbutamol and 4-O-sulfate metabolite
Sample Prep:	Robotic SPE, Processed 4000 human plasma samples
Column:	CHIROBIOTIC T, 250x4.6mm
Mobile Phase:	Methanol/acetic acid/ammonium hydroxide; 100/0.5/0.1
Flow Rate:	2.0 mL/min
Analysis Time:	2.0 min (metabolite), 3.2,3.7 min enantiomers
Detection:	LLQ 100 pg/mL and 5ng/mL, respectively

LC/MS Clinical Applications

Liquid Chromatography/Atmospheric Pressure Chemical Ionization Tandem Mass Spectrometry Enantiomeric Separation of d,l-threo-Methylphenidate Using a Macrocyclic Antibiotic as the Chiral Selector. Ramos, L., Bakhtairk, R. Majumdar, T., Hayes, M., Tse, F., Rapid Commun. Mass Spectrom., 13, 2054-2062 (1999).

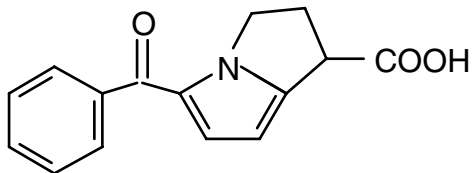
Analytes:	Methylphenidate (Ritalin®)
Sample Prep:	Liquid-liquid extraction
Column:	CHIROBIOTIC V, 150x4.6mm Processed 2500 human plasma samples
Mobile Phase:	100 methanol/0.05% (wt) ammonium trifluoroacetate
Flow Rate:	1.0 mL/min
Analysis Time:	6.1, 7.2 min
Detection:	LLQ 87 pg/mL

Optimization in Reversed Phase Mode

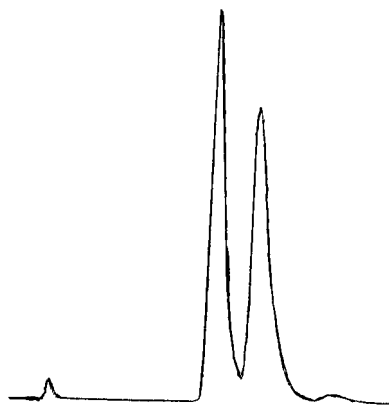
- Evaluate single analytical column (R,V and T).
- Choose best organic modifier (THF for V. MeOH for R, T or TAG).
- Change the concentration of organic modifier; higher concentration results in lower retention.
- Choose the best buffer (TEAA, NH₄Ac).
- Change the concentration of aqueous buffer. Range: 0.05% to 1%.
- Change pH of aqueous buffer.
- Change flow rate. Lower flow rate results in higher resolution.
- Increase column length to 25cm.
- Change temperature. Lower temperature leads to higher resolution.

Coupled Column Screen and Optimization

Ketorolac



Screen

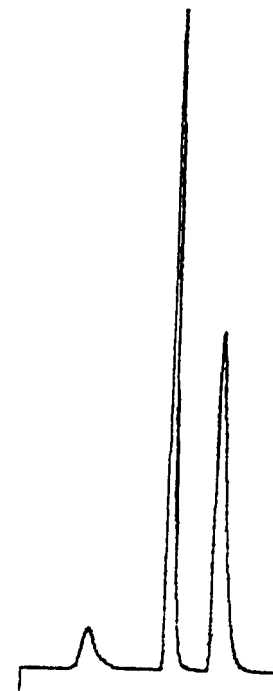


R+V+T (10cm)

25/75;MeOH/0.1% TEAA, pH 6

1.0mL/min

Optimized



T (25 cm)

40/60;MeOH/20mM NH₄OAc pH 5.5

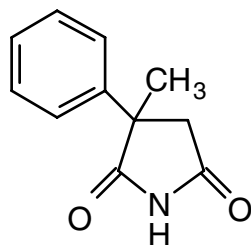
0.9mL/min

Optimization in Normal Phase Mode

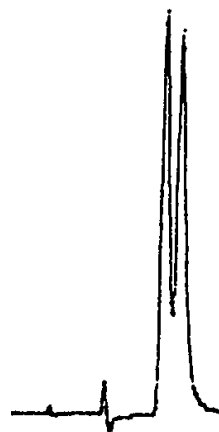
- Evaluate single analytical column (R, V and T).
- Evaluate polar solvent (EtOH, IPA, etc).
- Change the concentration of polar solvent: higher concentration results in lower retention.
- Add small amounts of acid or acid + base as modifiers.
- Increase column length to 25cm.
- Change temperature. Selectivity and elution order may change with temperature. Lower temperature increases R_s , higher temperature may lead to co-elution and finally reversal of elution order.
- Higher flow rates are possible to speed assay times – little effect on resolution.

Results of Normal Phase Screen

α -Methyl- α -phenylsuccinimide



Screen



CHIROBIOTIC

R+V+T (10cm)

60/40: EtOH/Hexane

1.5 mL/min

Optimized



CHIROBIOTIC

T (25cm)

20/80: EtOH/Hexane

3.0 mL/min

Generic Screening Method

✓ 9 Columns

✓ 9 Mobile Phases

astec

CHIRAL METHOD DEVELOPMENT SCREEN IN DRUG DISCOVERY

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	25/75
3	ACN/20mM NH ₄ OAc, pH 4.0	20/80
4	ACN/20mM NH ₄ OAc, pH 6.0	25/75
POLAR IONIC MODE®:		
5	MeOH/HOAc/TEA*	100/0.2/0.1
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)**	20/80
8	Washing cycle	100% EtOH
9	Column storage	100% IPA or MeOH**

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

** If stored in MeOH, wash with EtOH before proceeding to normal phase.

3. COLUMN CHOICE AND RUN TABLE

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

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

RUN CONDITIONS

Flow Rate: 1.0 mL/min.
 Equilibration Time: 25 minutes
 Run Time: 25 minutes
 Temperature: Ambient
 Detector: UV
 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 10 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 1.0/0.5. 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) ● Change the acid/base to a salt (ammonium trifluoroacetate, formate or acetate at a concentration of 0.1%)
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

- Use salts, as in Step 5, when using the polar ionic or polar organic modes.
- Use ammonium acetate or formate when using 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

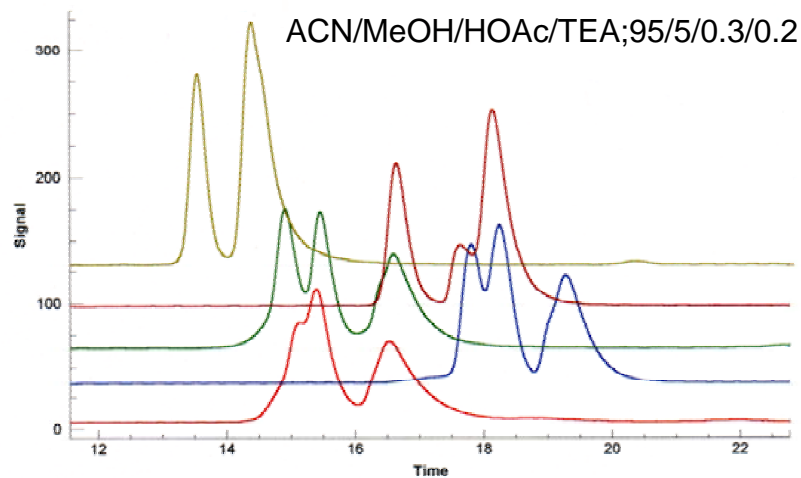
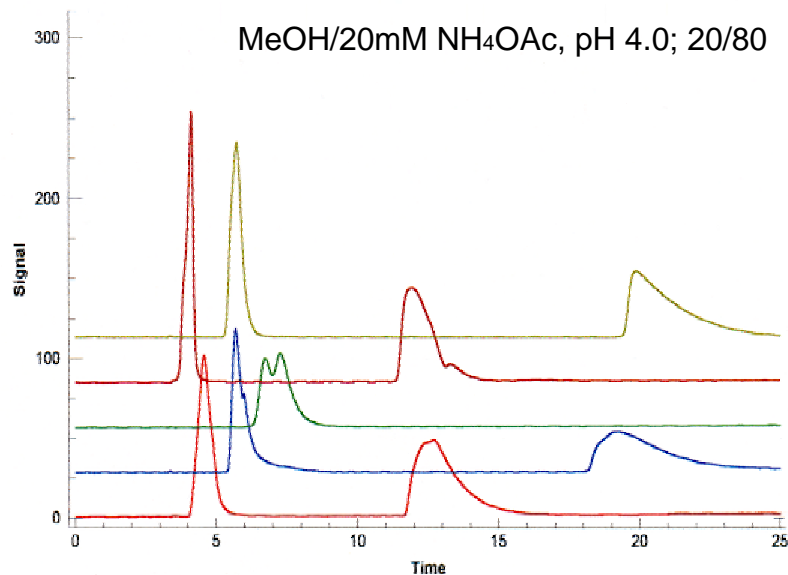
World Headquarters: 37 Leslie Court, Post Office Box 297, Whippany, NJ 07981 USA Tel: (973) 428-9080 Fax: (973) 428-0152
 E-mail: astecusa@aol.com www.astecusa.com

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@astecuro.com www.astecuro.com

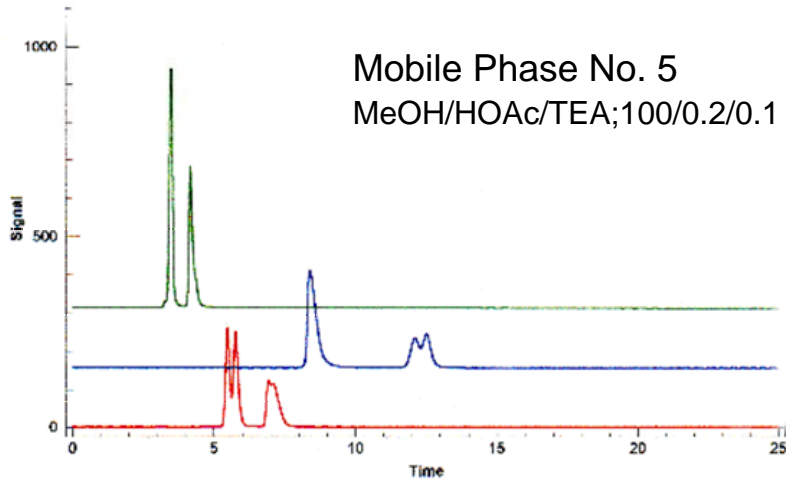
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Positive Screening Results

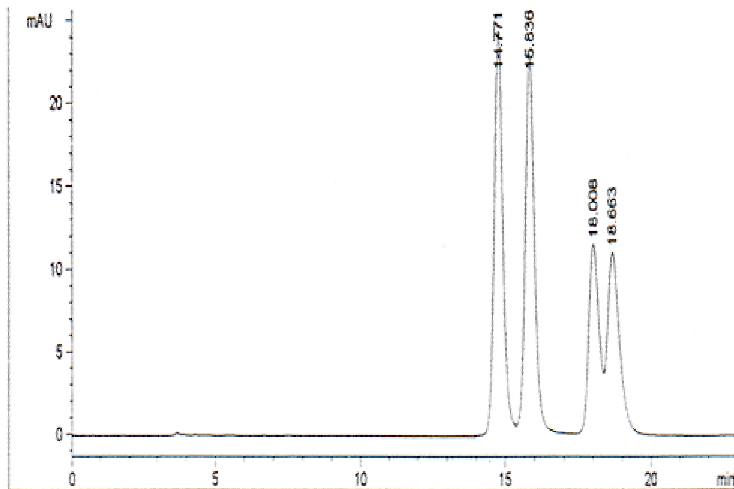
Sample: unknown structure - 2 chiral centers



Best Positive Screening Results



Optimized CHIROBIOTIC V



MeOH/NH₄TFA; 100/0.02 WT%

Chiralyzer® Optical Rotation

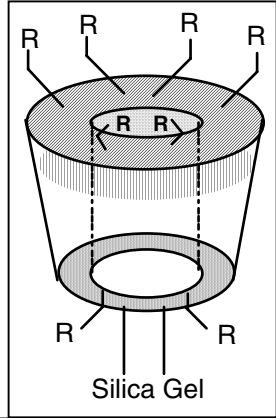


Bonded Derivatized Cyclodextrins

CYCLOBOND I 2000 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 RP mode with the organic component in the range of 5-40%.

For preparative separations a C₁₈ recovery system is ideal with these typical mobile phases.



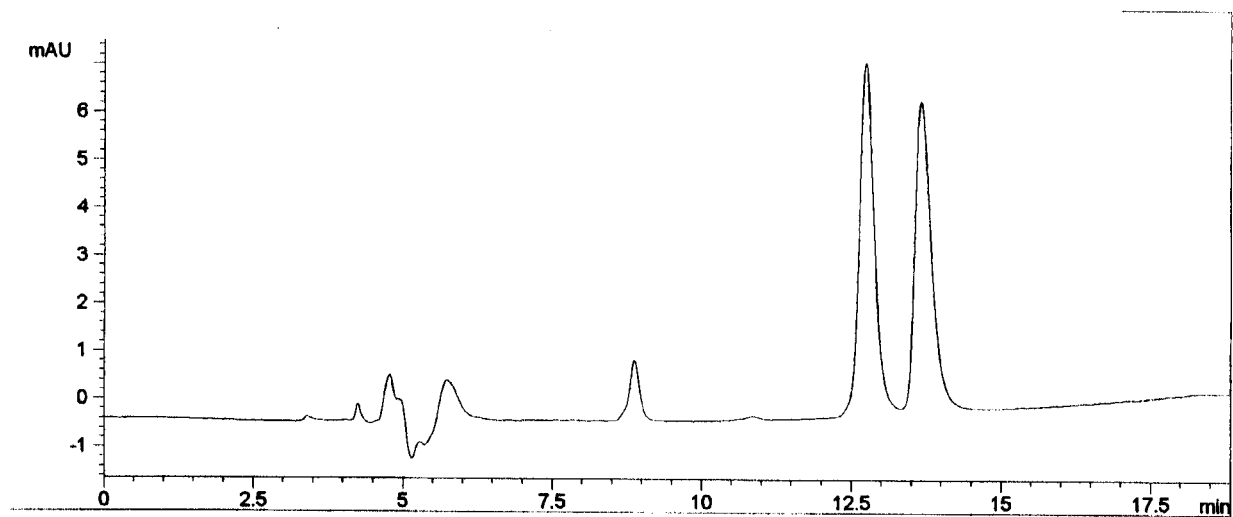
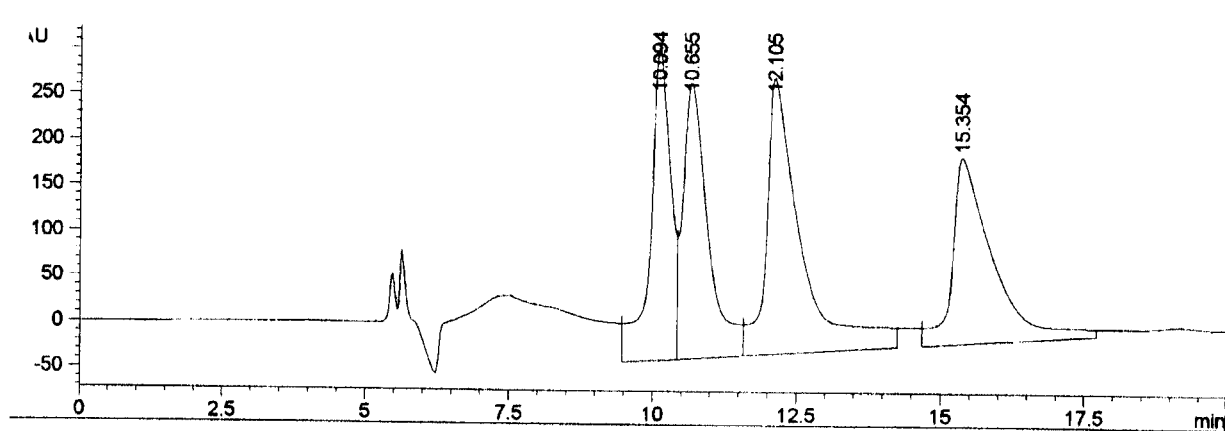
R=	CYCLOBOND I 2000 SUFFIX
$-\text{COCH}_3$	AC** (acetylated)
$-\text{CH}_2\underset{\text{*}}{\overset{\text{OH}}{\text{C}}}\text{CH}_3$	SP or RSP (hydroxypropyl ether)
$-\text{CONH}\underset{\text{*}}{\overset{\text{CH}_3}{\text{C}}}-\text{C}_6\text{H}_4-\text{C}_6\text{H}_4$	RN or SN (naphthylethyl carbamate)
$-\text{CONH}-\text{C}_6\text{H}_3(\text{CH}_3)_2$	DMP (3,5-dimethylphenyl carbamate)

* Stereogenic Center.

**Note: Acetylated versions also available in gamma (CYCLOBOND II AC) and alpha (CYCLOBOND III AC).

Unknowns from Screen

CYCLOBOND I 2000 RSP



Mobile Phase 1

20/80; MeOH/20mM NH₄OAc, pH 4.0

Method Development Screen

System 1 CHIROBIOTIC Series

Columns CHIROBIOTIC V and T

CHIROBIOTIC TAG and R

Notes: This method uses 100x4.6mm columns, the V/T and R purchased as a Methods Development Kit (Product Guide, pg 17, 23) giving the option to run as coupled or single columns depending on the number of samples to be handled. The TAG is brought separately and offers unique selectivity for certain classes of compounds like neutrals (oxazolidinones & hydantoins), chiral sulfoxides, amino acids and N-blocked amino acids and certain amines. The columns are operated at 1.0 mL/min with an equilibration time of 15 minutes. The columns and mobile phases are sequenced in a statistical priority.

Method Development Screen

System 2 CYCLOBOND Series

Columns CYCLOBOND I 2000 RSP and AC

CYCLOBOND I 2000 and SN

The CYCLOBOND columns have to be 250x4.6mm columns operated at 1.0 mL/min and require a 25 minute equilibration time and a 25 minute run time. In going from the polar organic mode to the normal phase mode it is necessary to flush the column first with EtOH.

**Experimental Protocols for
Preparative Purifications
Using Macrocyclic
Glycopeptide Chiral
Stationary Phases**

CHIROBIOTIC phases offer unique opportunities for preparative purifications:

Optimization Studies

CHIROBIOTIC T vs CHIROBIOTIC TAG

Column Conditions:

Sample:

5-Methyl-5-Phenylhydantoin

Column Size:

250x4.6mm

Mobile Phase:

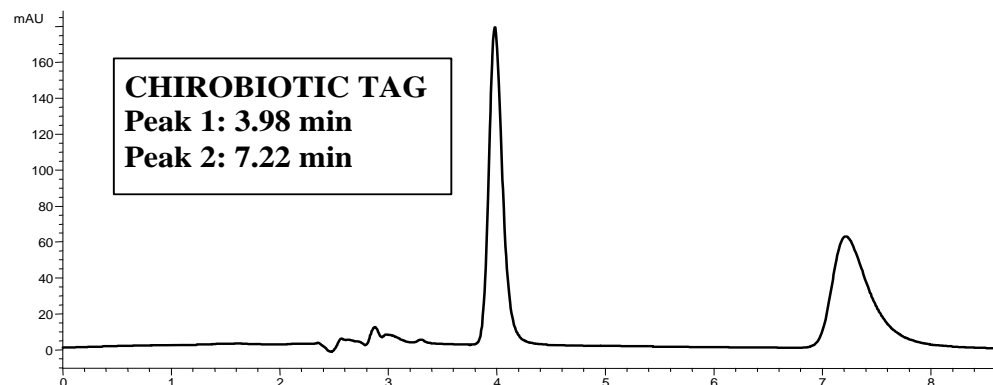
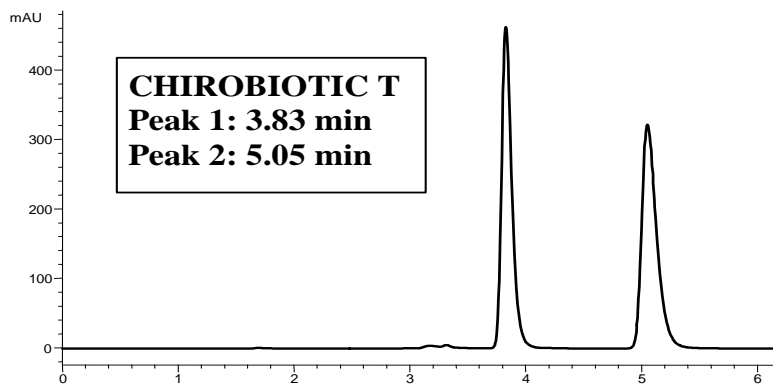
100% MeOH

Flow Rate:

1 mL/min

UV:

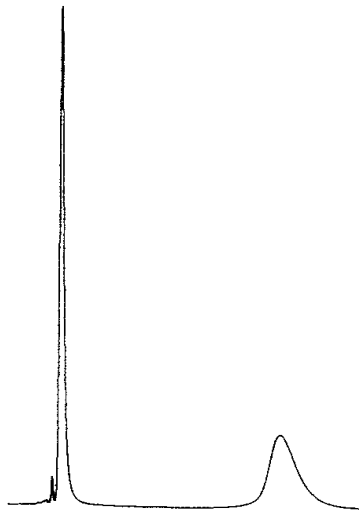
220nm



Case Study: N-Acetyl Typtophan

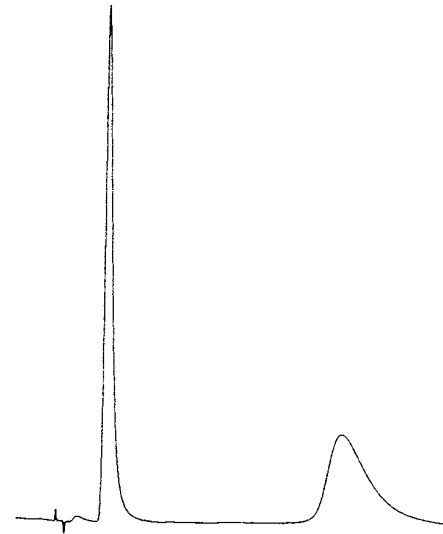
Column: CHIROBIOTIC TAG (250x4.6mm, 5 μ m)
UV: 254 nm
Flow rate: 1 mL/min

Mobile phase:
100/0.1w%, MeOH/NH₄OAc



$k_1 = 0.36, \alpha = 12.7$

Mobile phase:
40/60 MeOH/0.1% TEAA, pH4.1



$k_1 = 1.09, \alpha = 5.87$

Case Study : N-Acetyl Tryptophan

Column: CHIROBIOTIC TAG
(250x21.2mm, 5 μ m)

Load: 200mg in 6mL

Mobile phase: 100/0.1w%, MeOH/NH₄OAc

UV: 300 nm

Flow rate: 35 mL/min

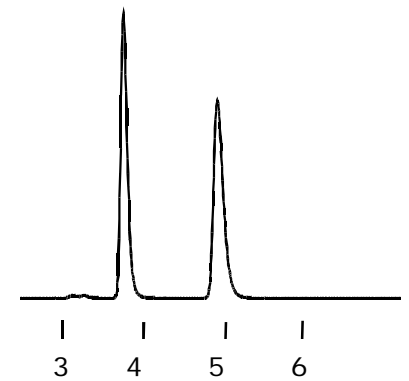
Throughput: 20 mg/g CSP/hr



Load Study

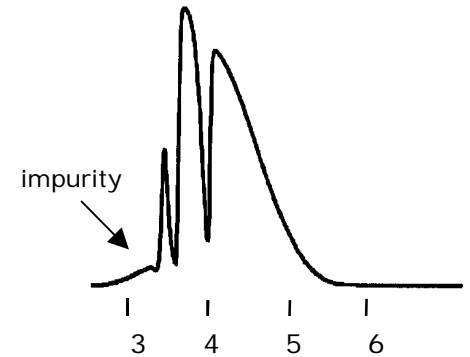
Analytical: 250x4.6mm, 5 μ M
Load: 2 mg/mL x 5 μ L (10 μ g)
Flow Rate: 1 mL/min
UV: 220nm

Peak 1: 3.83 min
Peak 2: 5.05 min

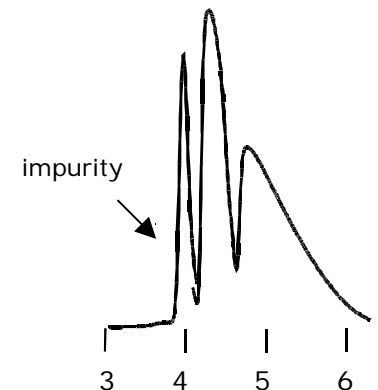


Example 1: 5-Methyl-5-phenyl hydantoin

Analytical: 250x4.6mm, 5 μ M
Load: 80mg/mL x 0.1mL (8 mg)
Flow Rate: 1mL/min
UV: 270 nm



Prep: 250x21.2mm, 5 μ M
Load: 80mg/mL x 1.5mL (120 mg)
Flow Rate: 18 mL/min
UV: 270 nm
Throughput: 20 mg/g CSP/hr



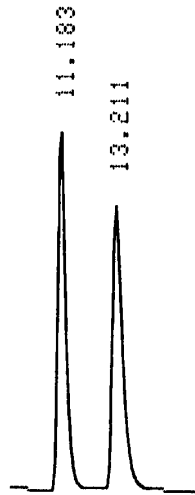
Free Amine Acid Mobile Phase

Column: CHIROBIOTIC T (250X4.6mm)

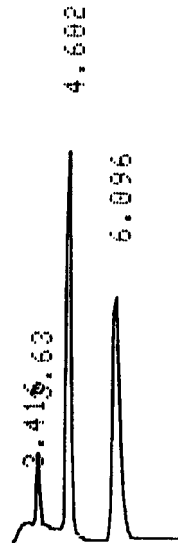
Temperature: 25°C

Mobile Phase: CH₃CN/H₂O 75/25

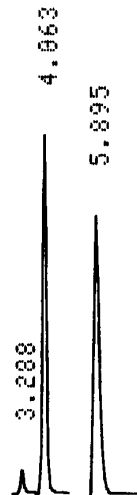
Flow Rate: 1.0 mL/min



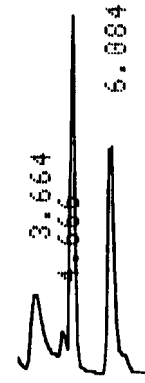
Alanine



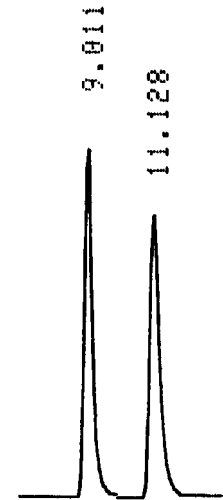
Proline



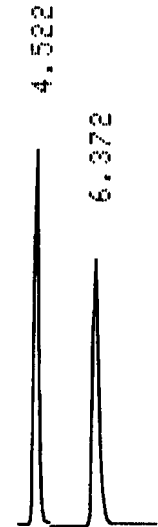
Phenylglycine



Pipecolic acid

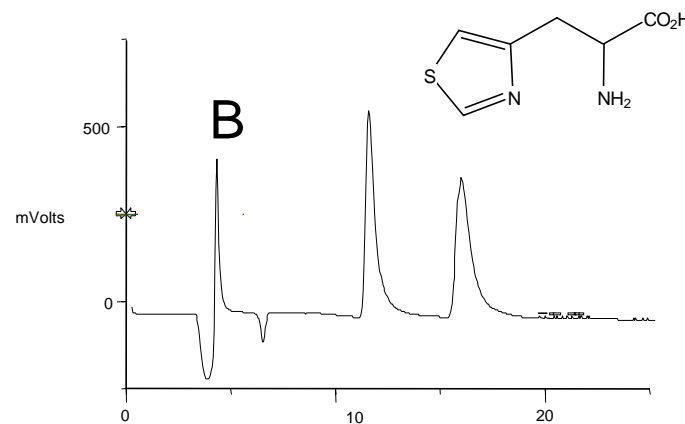
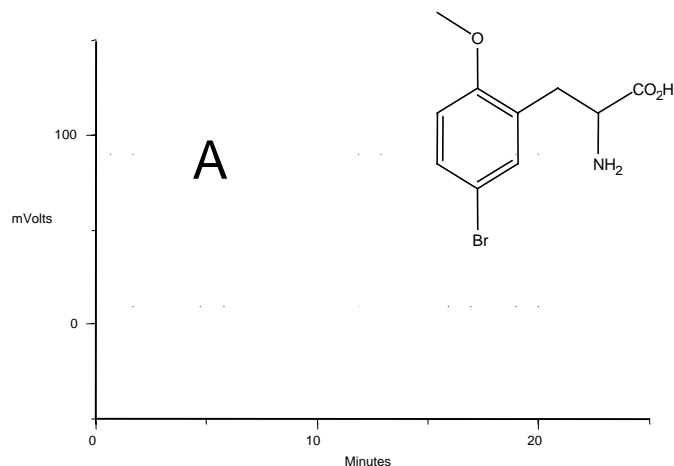


Methionine



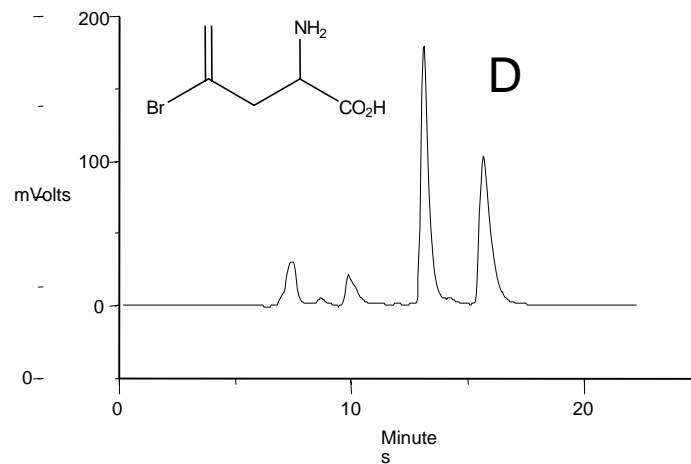
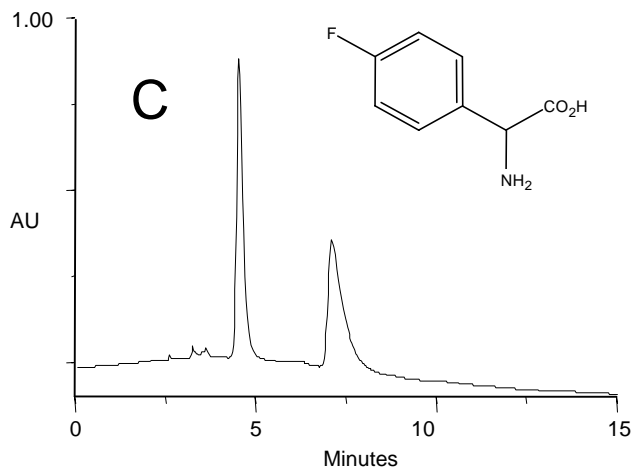
Dehydroproline

Unnatural Amino Acids as Chiral Synthesis Intermediates



Column:	CHIROBIOTIC T column (250 x 4.6mm)
Temperature:	Ambient
Detection:	UV at 210nm
Mobile phase:	A – 70% methanol, 30% water, 0.5 mL/min B – 50% methanol, 50% water, 0.5 mL/min

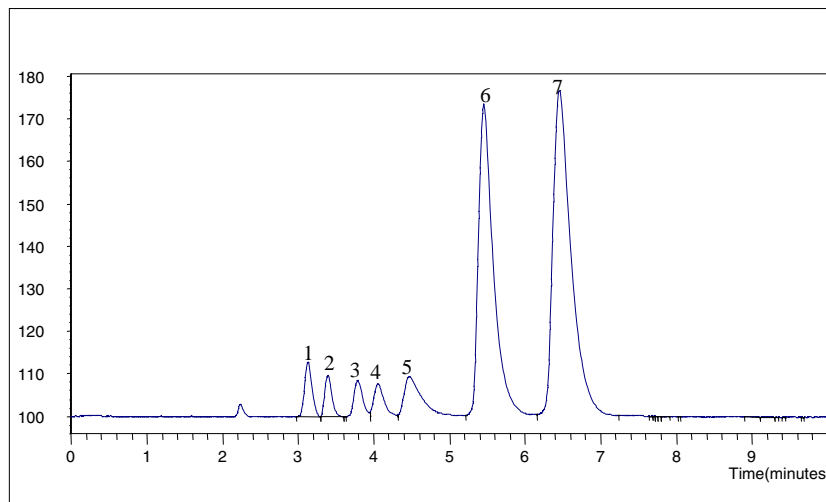
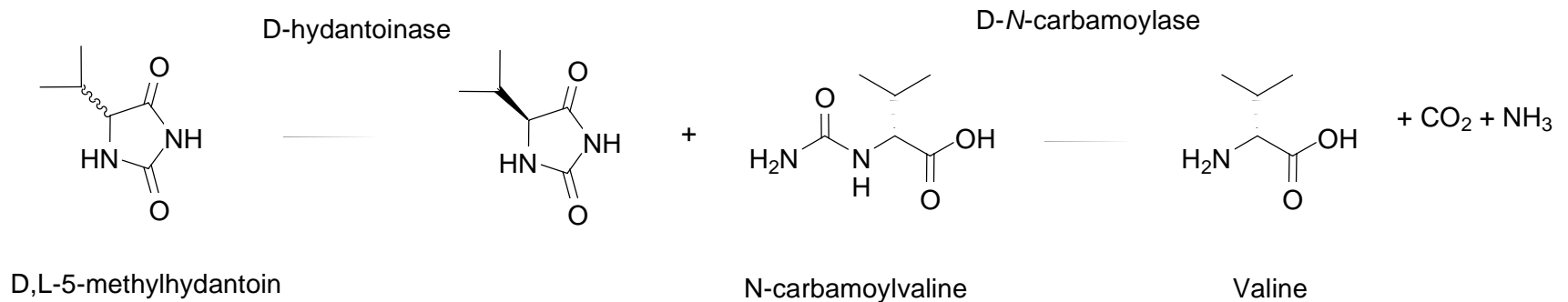
Unnatural Amino Acids as Chiral Synthesis Intermediates



- Column: CHIROBIOTIC T column (250 x 4.6mm)
- Temperature: Ambient
- Detection: UV at 210nm
- Mobile phase: C – 15% methanol, 85% water, 1.0 mL/min
D – 50% methanol, 50% water, 0.5 mL/min

Biocatalysis

Determination of the conversion and enantiomeric excess of substrate / reaction products in a D-hydantoinase / D-N-carbamoylase reaction



Column: CHIROBIOTIC T (250x4.6 mm, 5 μ m)

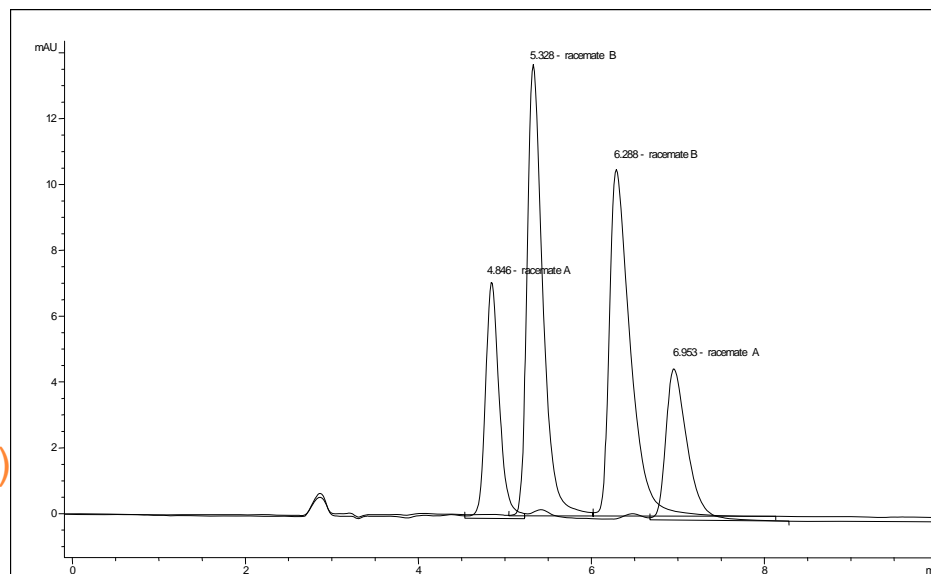
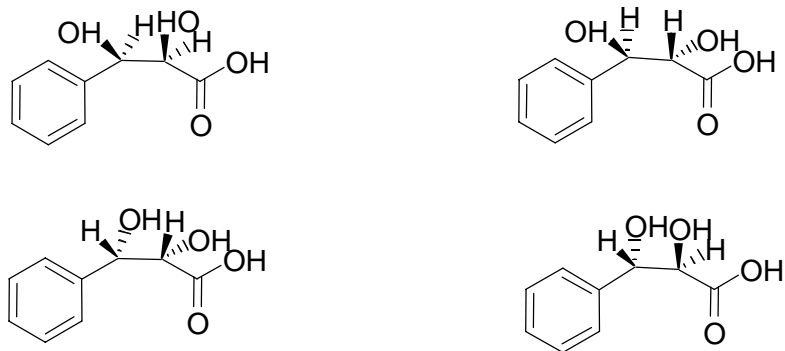
Eluent: 80/20; 15mM NH₄Ac pH 4.1/MeOH

Flow: 1.0 mL/min

Courtesy of DSM Fine
Chemicals

Biocatalysis

Determination of the enantiomeric excess of *cis*- and *trans*-diol reaction products from an epoxyhydrolase reaction



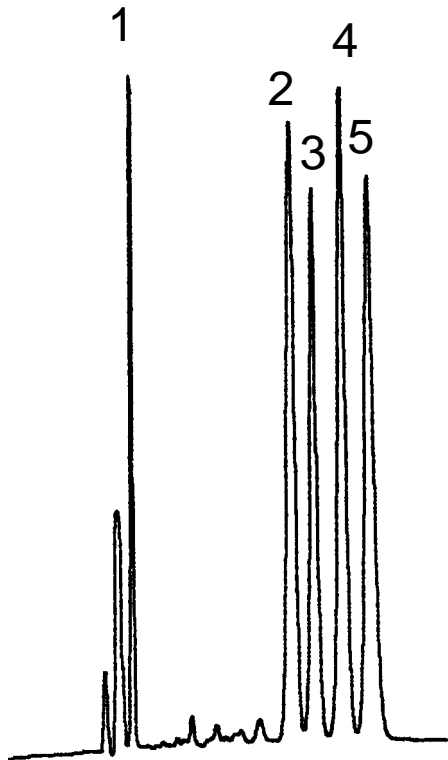
Column: CHIROBIOTIC R (250x4.6 mm, 5 μ m)

Eluent: 0.1% ammonia, pH 4.1 with formic acid / MeOH (50/50 %v/v)

Flow: 1.0 mL/min

Achiral Separation Basic Analytes

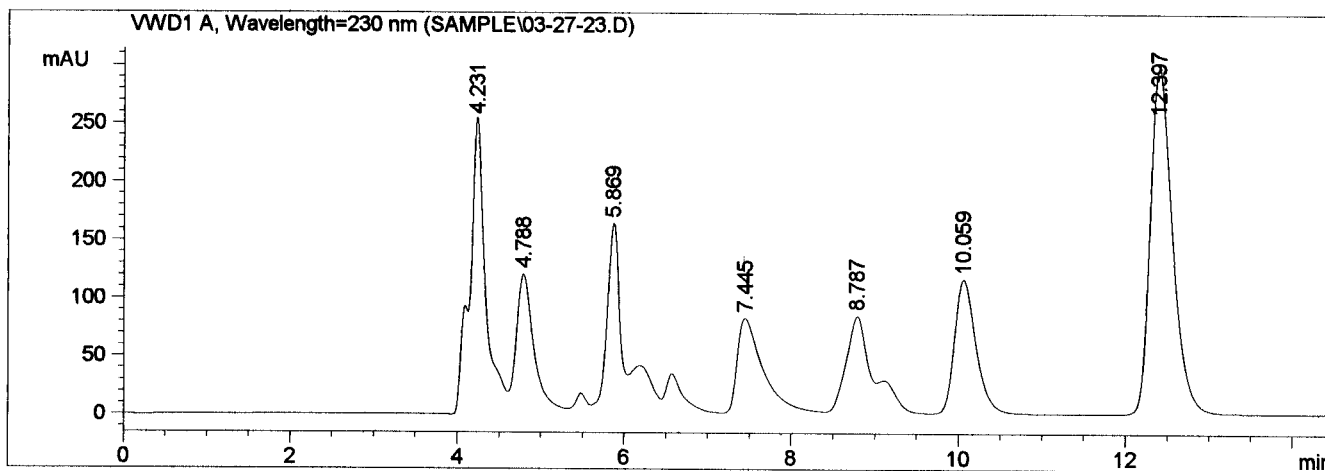
Column: CHIROBIOTIC V 250x4.6mm
Mobile Phase: 35/65; ACN/20mM NH₄OAc, pH4.0
Flow Rate: 0.8 mL/min



1. Uracil
2. Trimipramine
3. Imipramine
4. Clomipramine
5. Amitriptyline

Achiral Separation Aromatic Acids

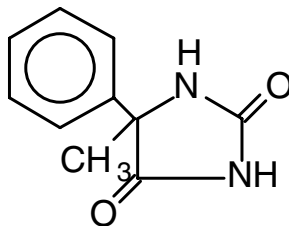
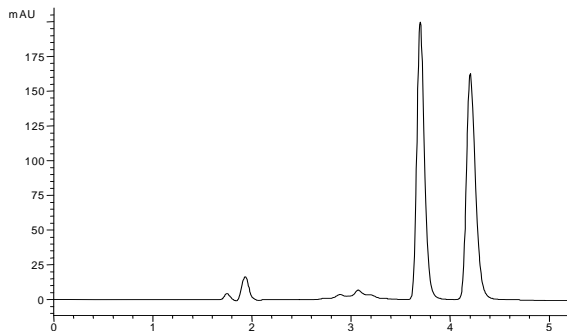
Column: CHIROBIOTIC T, 250x4.6mm
Mobile Phase: 10/90; MeOH/20mM NH₄OAc, pH 3.7
Flow Rate: 0.6 mL/min
Analytes: Sulfo-di-tri aromatic carboxylic acids



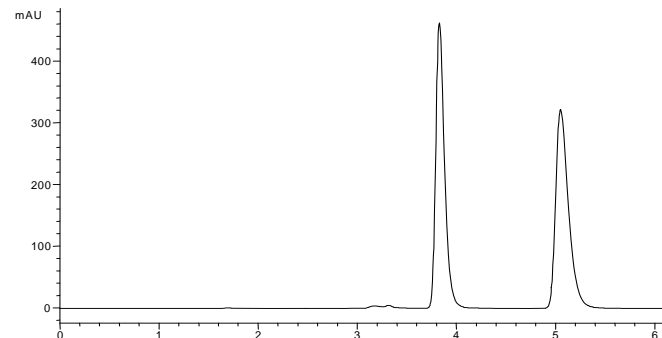
QC Testing of CHIROBIOTIC Columns

To ensure the selectivity and performance of all CHIROBIOTIC LC columns, periodically test your columns. This can now be accomplished with a single compound for all the CHIROBIOTIC phases in a very simple mobile phase of 100% MeOH.

CHIROBIOTIC V



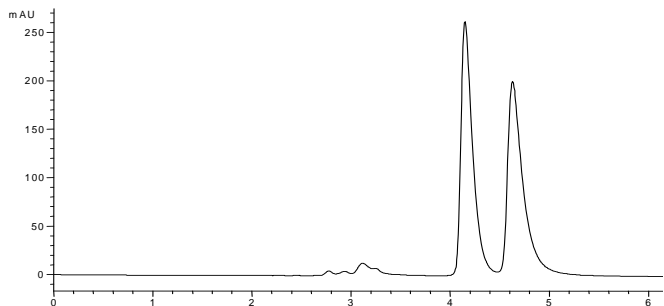
CHIROBIOTIC T



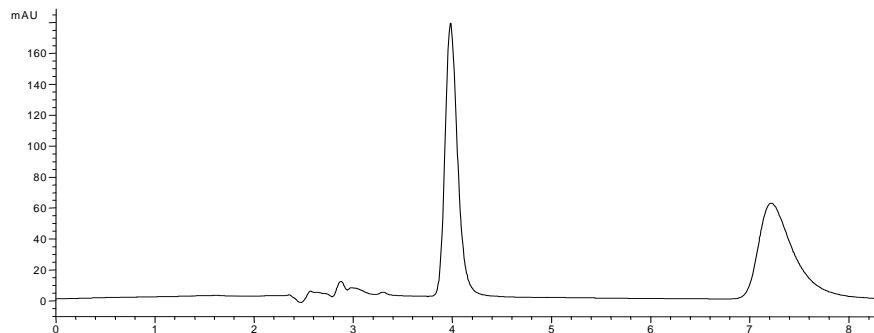
Conditions for all columns:

Sample: 5-Methyl-5-Phenylhydantoin (Aldrich 18,082-3)
Column size: 250x4.6mm
Mobile phase: 100% MeOH
Flow rate: 1 ML/min
UV: 220nm

CHIROBIOTIC R



CHIROBIOTIC TAG



Conclusions

1. CHIROBIOTIC phases are versatile, stable and complementary CSPs for chiral separations by HPLC
2. Polar ionic mode is the primary choice for LC/MS and preparative applications, especially V2 and T2
3. CHIROBIOTIC phases have unique ionic and hydrogen bonding capabilities for wide variety of structural differences.
4. Column coupling of 10 cm CHIROBIOTIC phases R + V+ T is a fast technique for selectivity screening: three mobile phase types in 150 minutes.

Conclusions

5. The Chiral Method Development Chart offers the most effective method for selectivity screening overnight.
6. The Chiral Method Development Screen utilizes a variety of mobile phase types compatible with LC/MS and preparative purification methods.
7. CHIROBIOTIC phases are useful for achiral separations of polar compounds and drug metabolism studies biocatalysis and drug discovery.