Chiral Method Development Screening Protocols

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Abstract

The increasing backlog of new compounds in search of chromatographic methods has grown exponentially. This is as true in the area of chiral molecules where the task is much more difficult. Small changes in structure can often greatly affect the ability to resolve a racemate for any given CSP. More and more effort, therefore, has been expended to come up with generic screening methods utilizing the best of the stationary phases that are commercially available.

Two approaches have been designed for both speed and high throughput. The first method is based on column coupling of the bonded glycopeptides, vancomycin, teicoplanin and ristocetin. For a broad range of molecule this method can yield positive selectivity for 60-70% of the compounds in one or more of three distinctly different mobile phases in under 120 minutes.

The second screen yields a statistical gain of 85-95% but requires an overnight run. It can, however, be given a priority sequence that will yield results in a shorter timeframe for many separations. This presentation will demonstrate both techniques and give examples relating to the pharmaceutical industry.

Features of Phases Used in the Screening Process

- Phases can be used effectively with more than one mobile phase type. (Multimodal)
- CHIROBIOTIC phases R, V & T can be coupled for fast screening
- Typical mobile phase types for chiral separations:
  - ✓ 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

Differentiating Polar Organic and Polar Ionic Modes

- Originally the term Polar Organic Mode, developed by Dr. D. W. Armstrong and Astec in 1985, was applied to cyclodextrin (CYCLOBOND) technology.

- CYCLOBOND composition : ACN/MeOH/HOAc/TEA;95/5/0.3/0.2,v/v/v/v

- CHIROBIOTIC composition: MeOH/HOAc/TEA; 100/0.1/0.1, range of acid to base 1.0 to 0.001% or equivalent salts, NH_4OAc, NH_4OCF_3, 0.2 to 0.01%wt.

- Method development: simple and fast by manipulation of acid, base ratio or salt concentration

Differentiating Polar Organic and Polar Ionic Modes

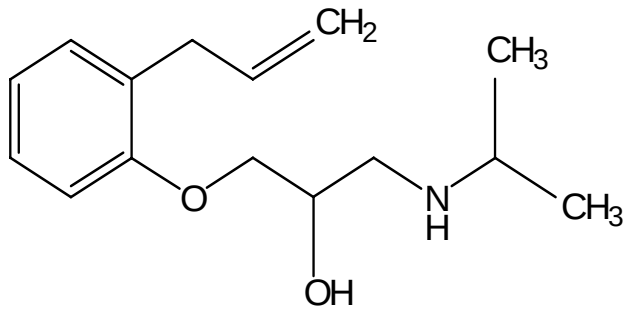
- The Polar Organic Mode terminology is now frequently applied to the Daicel phases, but in this case means 100% organic only, usually MeOH, EtOH, ACN or combinations.

- The mechanism (predominantly hydrogen bonding) for the POM on Daicel phases is *different* from that on the CHIROBIOTIC phases and the optimization process is *different.*

What is the Polar Ionic Mode?

The mechanism for chiral recognition on the CHIROBIOTIC phases is predominantly ionic, making it essential for acid and base to be added for ionizable compounds. Thus, the term Polar *Ionic* Mode©

Alprenolol



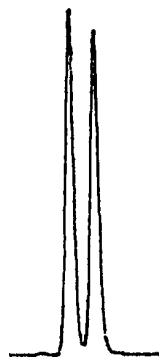
CHIRACEL OD®

Peak 1 - 12.4 min
Peak 2 - 16.4 min

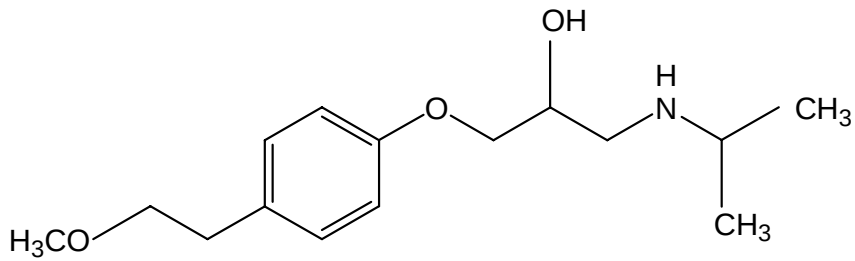
Normal Phase 20/80/0.1: IPA/Hex/TFA

CHIROBIOTIC V® Peak 1 – 7.69 min.
Peak 2 – 8.33 min.

Polar Ionic Mode 100/0.01/0.01: MeOH/HOAc/TEA



Metoprolol

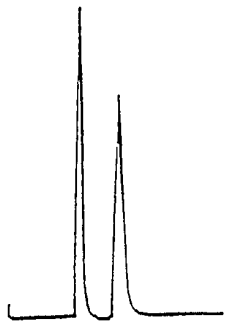


CHIRACEL OD®

Peak 1 – 11.9 min.
Peak 2 – 18.2 min.

Normal Phase

20/80/0.1: IPA/Hex/DEA

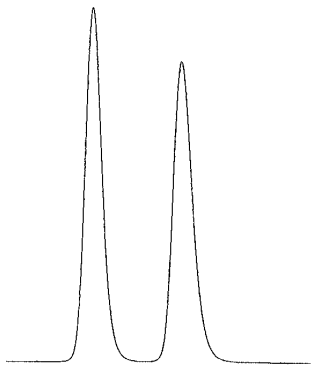


CHIROBIOTIC T®

Peak 1 – 15.36 min
Peak 2 – 17.11 min

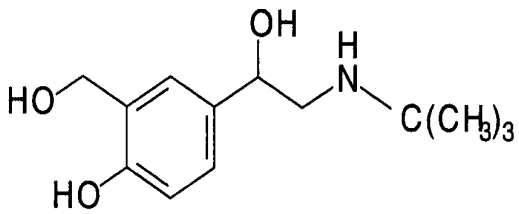
Polar Ionic Mode

MeOH/0.1% ATFA

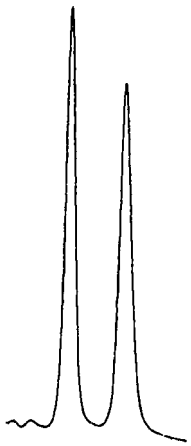


Polar Organic Mode vs Normal Phase Mode

Albuterol

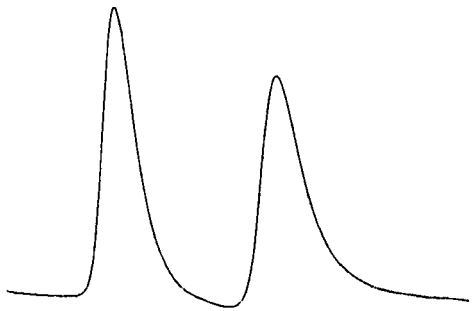


1 - 8.66 min.
2 - 9.74 min.
α = 1.19
Rs = 1.8



CHIROBIOTIC T
250X4.6mm
100/0.2/0.1:
MeOH/HOAc/TEAA

1 - 16.52 min.
2 - 19.63 min.
α = 1.24
Rs = 1.8



CHIROBIOTIC T
250x4.6mm
50/50/0.3/0.6:
EtOH/Hex/TFA/TEA

***NOTE:* Rt is 2x longer for same Rs**

Advantages of Polar Ionic Mode© on CHIROBIOTIC V, T, R

- No problem with salt containing compounds
- Compatible with column switching from C_{18} column to chiral column
- Scale-up is easy due to the volatility of mobile phase components
- Complementary to hexane/ethanol separations on polysaccharide CSPs
- For LC/MS, can use ammonium trifluoroacetate, ammonium acetate or formate (0.001 to 0.5% w/v in methanol)
- Applicable to the use of gradients for method development

Method Development Techniques Using Coupled Columns

- 3 solvents systems, plus 3 different CHIROBIOTIC columns (close coupled kit) provides 9 screens in 120 minutes

Screening mobile phases:

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

Method Development Techniques Using Column Coupling

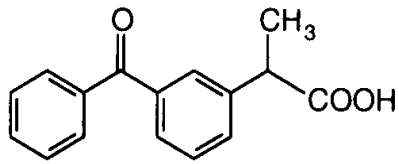
- ✓ Generic screening methods for fast chiral method development
- ✓ Column coupling utilized to enable simultaneous multi-column screening
- ✓ Some selectivity lost due to reversal of elution order between coupled columns. Failure rate about 5 to 10%.

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, NH_4OH or salt NH_4Ac, $HCOONH_4$, NH_4TFA).
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.

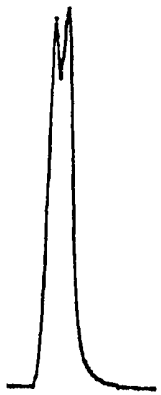
Ketoprofen



1- 3.27 min.

2 - 3.37 min

Screen



R+V+T (10cm)

100/0.02/0.01

2 mL/min.

1 – 7.84 min.

2 – 8.81 min.

Optimized

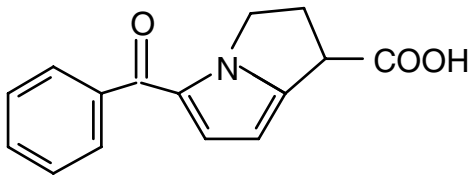
R (25cm)

100/0.02/0.01

0.8 mL/min.

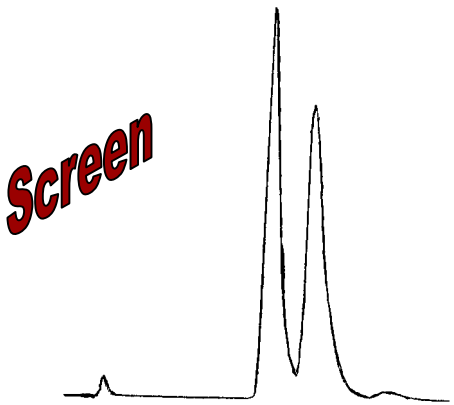
Coupled Column Screen and Optimization

Ketorolac



1 - 9.11 min

2 - 10.43 min



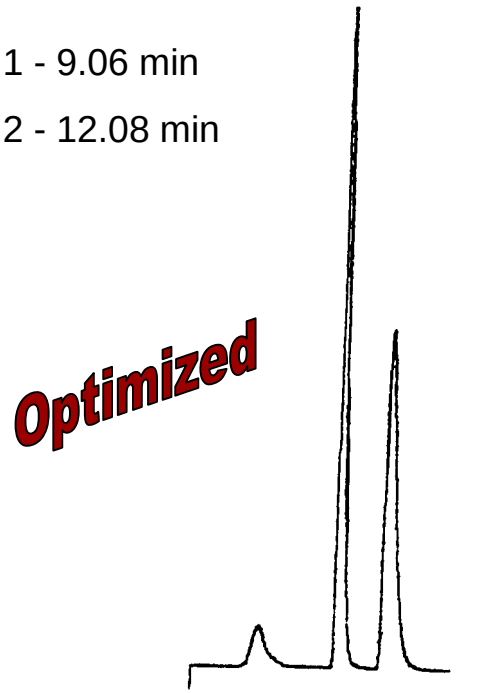
R+V+T (10cm)

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

1.0mL/min

1 - 9.06 min

2 - 12.08 min



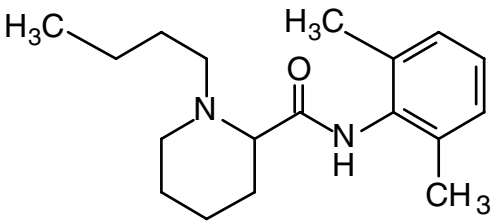
T (25 cm)

40/60;MeOH/20mM NH_4OAc pH 5.5

0.9mL/min

Coupled Column Screen and Optimization

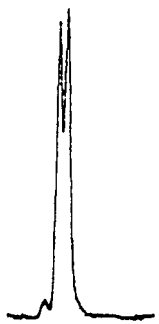
Bupivacaine



Screen

1 – 4.70 min.

2 – 4.88 min.



R+V+T (10cm)

MeOH/HOAc/TEA:

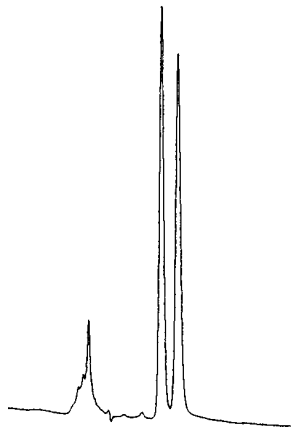
100/0.02/0.01

2mL/min

Optimized

1 - 5.59 min.

2 – 6.15 min



V (25cm)

MeOH/NH_4TFA

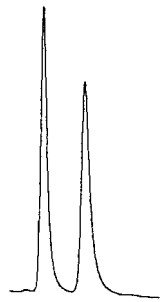
100/0.1% wt.

1mL/min

Optimized

1 – 6.15 min.

2 – 7.63 min.



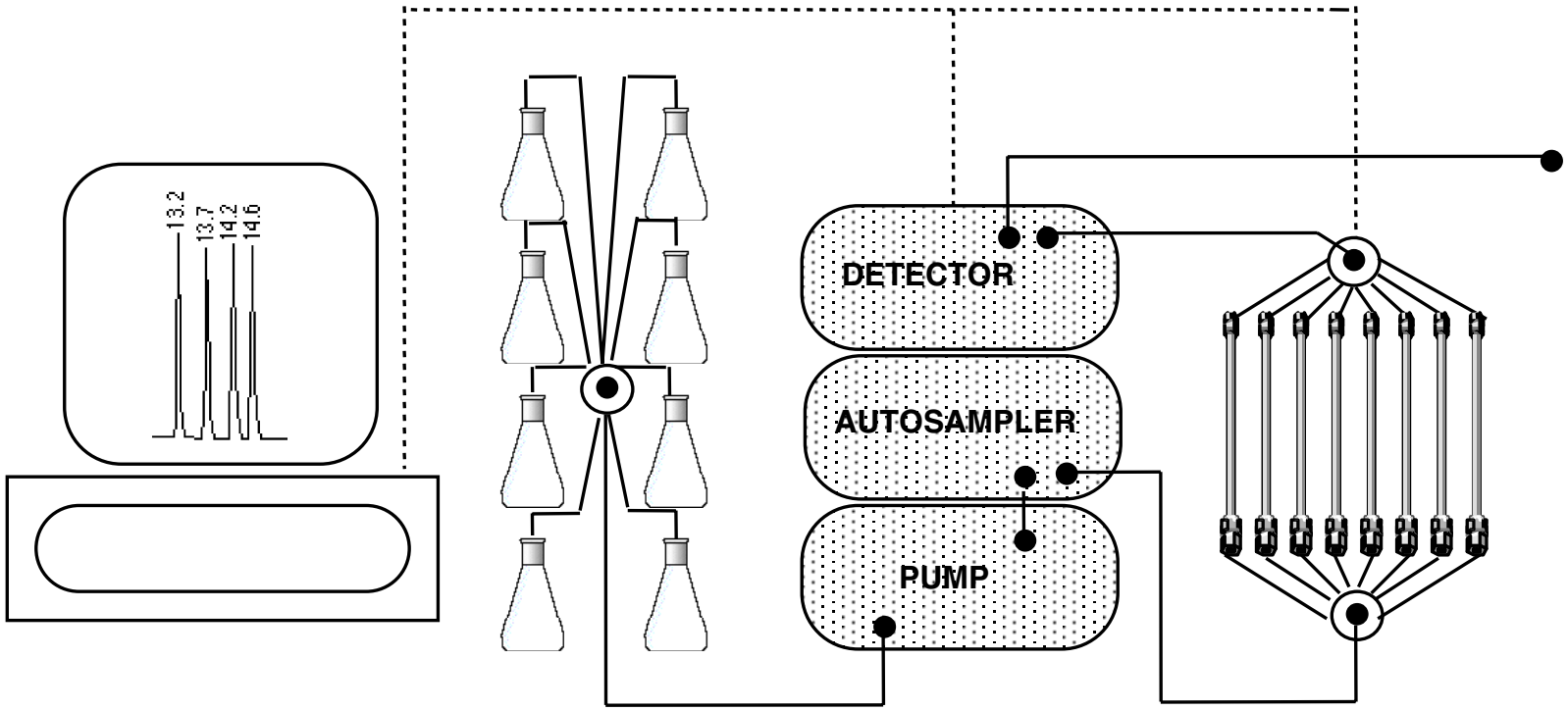
V2 (25cm)

MeOH/NH_4TFA

100/0.1% wt.

1mL/min

Configuration of MDS with Isocratic System



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_4OAc, pH 4.0	20/80
2	MeOH/20mM NH_4OAc, pH 6.0	25/75
3	ACN/20mM NH_4OAc, pH 4.0	20/80
4	ACN/20mM NH_4OAc, 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 ($NH_4O_2CCF_3$ for bases, NH_4OAc 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
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	
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	

4. 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

1. 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.
2. 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.
3. 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.
4. If a separation occurs in the polar ionic mode©, for a neutral molecule, change to 100% organic solvent (i.e. MeOH, EtOH or ACN).
5. 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)	● 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	● Eliminate MeOH ● Test alternative acid/base ratios
Reversed phase mode	● Test smaller pH changes ● Change organic to THF, ACN, MeOH ● Change buffer type and buffer concentration ● Change temperature
Normal phase mode	● Change EtOH concentration

6. OPTIMIZING FOR MS DETECTION

1. Use salts, as in Step 5, when using the polar ionic or polar organic modes.
2. 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.

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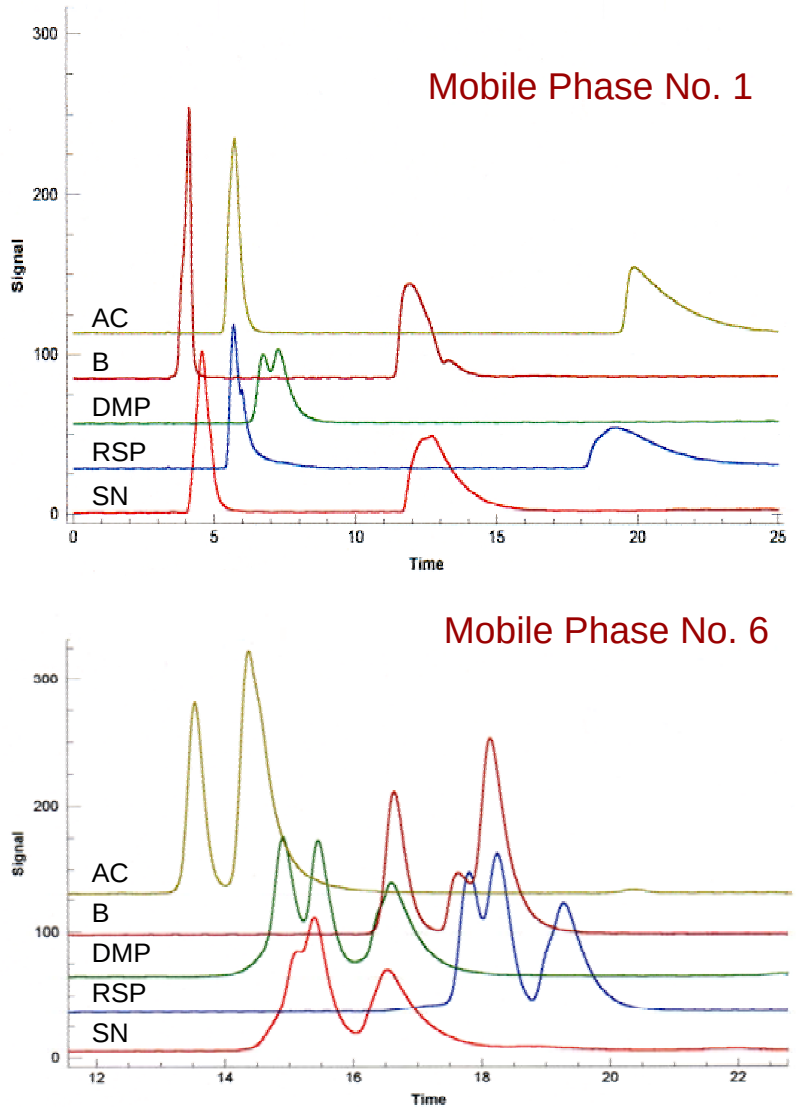
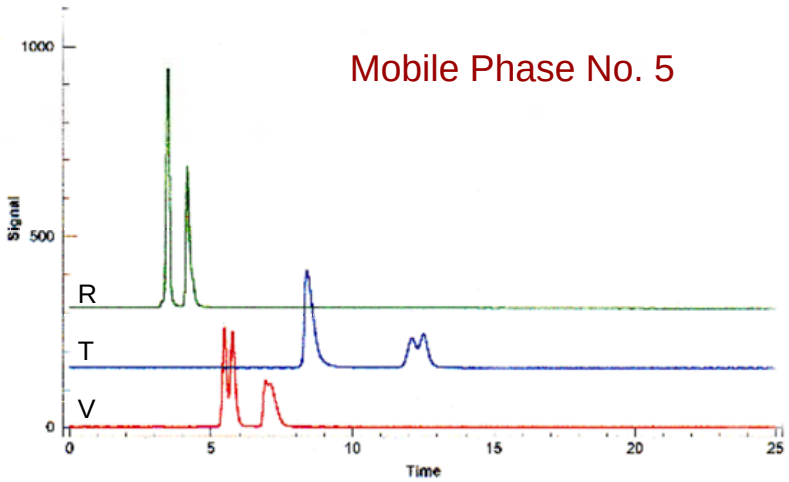
Positive Screening Results

Sample: unknown structure - 2 chiral centers

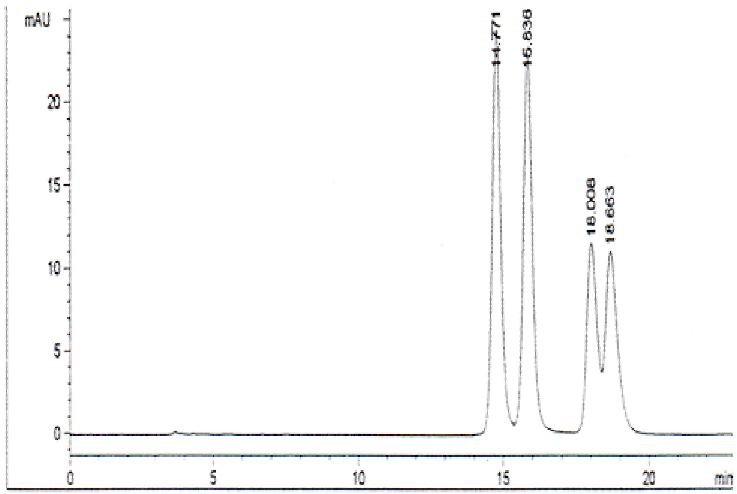


Best Positive Screening Results

Mobile Phase No. 5

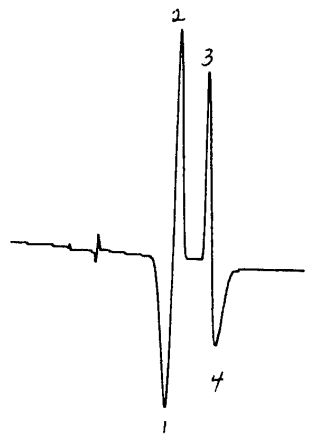


Optimized CHIROBIOTIC V



MeOH/NH4TFA; 100/0.02 WT%

Chiralyzer® Optical Rotation



Conclusions

- ✓ Chiral Stationary Phases used in these screening methods are multimodal with selectivity possible in more than one mobile phase type.
- ✓ Polar Ionic Mode© is a very effective mobile phase for the chiral separation of ionizable racemates on CHIROBIOTIC phases. Especially CHIROBIOTIC V2 and T2.
- ✓ Column coupling of 10 cm CHIROBIOTIC phases R + V+ T is a fast technique for selectivity screening: three mobile phase types in 150 minutes.
- ✓ The Chiral Method Development Chart offers the most effective method for selectivity screening overnight.
- ✓ The Chiral Method Development Screen utilizes a variety of mobile phase types compatible with LC/MS and preparative purification methods.
- ✓ The Wall Chart handles compounds with multiple chiral centers and is very selective for achiral components.

"Don't leave home without it".