# CHIROBIOTIC™

# HANDBOOK

## A GUIDE TO USING MACROCYCLIC GLYCOPEPTIDE BONDED PHASES FOR CHIRAL LC SEPARATIONS

5th EDITION

astec

Advanced Separation Technologies Inc.

# CHIROBIOTIC™

## Bonded Macrocyclic Glycopeptide Phases for Liquid Chromatography

The first part of this Handbook describes the CHIROBIOTIC phases and their manipulation in general terms. More specific detail for each of the CHIROBIOTIC phases is described under each type.

## Introduction

CHIROBIOTIC phases are manufactured by linking macrocyclic glycopeptides through five covalent bonds to a silica surface. To date, we have bonded vancomycin (CHIROBIOTIC V), teicoplanin (CHIROBIOTIC T), teicoplanin aglycone (CHIROBIOTIC TAG) and ristocetin A (CHIROBIOTIC R). Since their introduction by Dr. Daniel Armstrong as chiral stationary phases for HPLC in 1994, these products have established themselves as valuable tools for the separation of a wide variety of chiral molecules. In addition to their broad selectivity, these phases have demonstrated the ability to differentiate small changes in molecular structure and, therefore, have been ideal for drug stability and metabolism studies, monitoring biocatalysis reactions for bioconversions and following the course of a compound throughout its development. The stability of these phases have made them most useful for the above applications since the type of solvent, be it aqueous buffer (bioreactors) or halogenated solvent, has no material effect on the performance or stability of the stationary phase. The mobile phases used are compatible with the requirements for mass spectrometry which has further contributed to their success. Over 18 LC/MS publications have been published in the last two years.

The structures of the macrocyclic glycopeptides used in these phases have been well documented and can be reviewed in this Handbook. Over the years the CHIROBIOTIC line has extended from the original CHIROBIOTIC V and T, to the CHIROBIOTIC R and TAG. In 2003, two new versions were added, the CHIROBIOTIC V2 and T2. The development of the CHIROBOTIC V2 and T2 came about as a result of an intense study on the effect of the type of linkage used to bond these unique chiral structures to silica. The position of those linkages and the length of the spacer was also studied. It was noted that the response to these factors studied was also a function of the type of mobile phase used to evaluate them. Since the polar organic and polar ionic modes are the most desirable mobile phases from the standpoint of their utility in preparative and LC/MS applications, the study focused on increasing the performance of these two phases in those particular modes. Additionally, we found a significant increase in capacity for preparative applications by factors of 20-30 times. CHIROBIOTIC V2 and T2 are valuable when used in the optimization process in the polar organic and polar ionic modes only.

Each of these four chiral stationary phases has unique selectivity characteristics but, in addition, they have been found to offer complementary separations. The use of the term “complementary” describes the condition where an increase in selectivity is obtained in the exact same mobile phase conditions on a different CHIROBIOTIC phase. This complementary nature allows for improved resolution by simply substituting the CHIROBIOTIC V with the T or with the R. The CHIROBIOTIC TAG is complementary to the T. The reasons for this phenomenon have to do with the subtle differences in diastereomeric binding sites between the four phases.

## Separation Mechanism

CHIROBIOTIC stationary phases have demonstrated broad selectivity in reversed phase, normal phase and the new polar ionic and polar organic phase modes. The stationary phase is unaffected when switching between the mobile phase systems. Since these chiral stationary phases contain peptide, carbohydrate and other ionizable groups, it is not surprising that the enantioselectivity appears to be different in each of these modes. This allows for the potential to separate a greater variety of chiral analytes. The structure of these phases indicates that all of the typical interactions defined for protein phases and stationary phases based on modified cellulose or amylose are present. The potential interactions and their relative strength are listed below:

| | | |
|---|---|---|
| ■ | π-π complexation | strong |
| ■ | hydrogen bonding | strong |
| ■ | inclusion | weak* |
| ■ | dipole stacking | medium strong |
| ■ | steric interactions | weak |

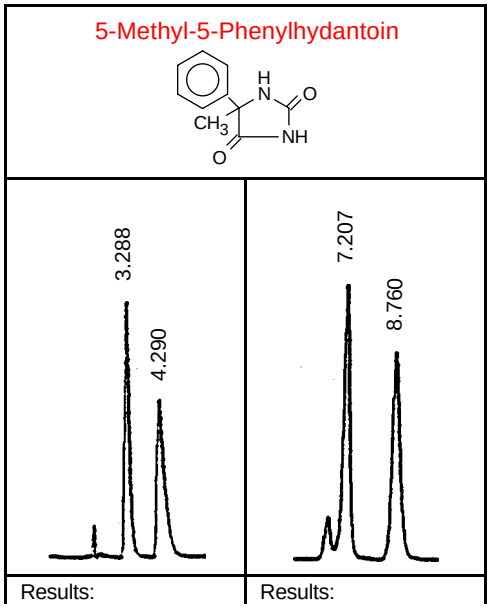
| | | |
|---|---|---|
| ■ | anionic or cationic binding | very strong |

**The shallow pockets for inclusion yield weaker binding energies when compared to cyclodextrins. This is beneficial as the kinetics of the inclusion process are much faster, leading to faster, more efficient separations.*

Obviously, the choice of mobile phase conditions enhances certain binding energies. For instance, reversed phase conditions favor inclusion and hydrogen bonding. Under these conditions, changes in pH can produce cationic or anionic interactions. The π-π complexation and dipole stacking are favored in normal phase solvents. The new polar ionic mode enhances the potential for all of the above interactions, especially ionic, and is, therefore, very broad in the spectrum of molecules that it can separate.

## Mobile Phase Design

Due to the nature of these complex macrolides and the number and type of ionizable groups, they function equally well in reversed or normal phase solvents and a modified polar organic mode. All three solvent modes generally show different selectivity with different analytes. Sometimes equivalent separations were demonstrated in both reversed and normal phase modes. A typical example of this is 5-methyl-5-phenylhydantoin on the CHIROBIOTIC V. This ability to operate in two different solvent modes was an advantage in determining the best preparative methodology where sample solubility is a key issue.




| | | | |
|---|---|---|---|
| to | 1.85 | to | 2.8 |
| $k_1$ | 0.78 | $k_1$ | 1.57 |
| $k_2$ | 1.32 | $k_2$ | 2.13 |
| α | 1.69 | α | 1.35 |
| R | 2.18 | R | 3.0 |
| 100% EtOH | | 10/90: THF/20mM $NH_4NO_3$, pH 5.5 | |

## Polar Organic Versus Polar Ionic Modes

The terminology used to describe the polar organic mode has been compromised by the application of this general term to describe the composition of this mobile phase as being only polar alcohols. The use of methanol, ethanol, alone or in combination and sometimes modified with acetonitrile are useful solvent combinations for chiral separations on a number of popular chiral stationary phases. When applied to the CHIROBIOTIC phases, only neutral molecules have demonstrated exceptional selectivity and speed in a mobile phase consisting of just methanol, ethanol, acetonitrile or combinations of these anhydrous solvents. See specific CHIROBIOTIC phases under these conditions for some typical examples. When, however, the compound has an ionizable group present the need to add small amounts of acid and base or a volatile salt is imperative. This is due to the presence of ionizable groups present within the macrocyclic glycopeptide itself. We refer to this latter mobile phase condition as the polar ionic mode to differentiate it from the polar organic mode. Both the polar organic and polar ionic modes are very useful systems for preparative applications while the latter is especially useful for the LC/MS platform.

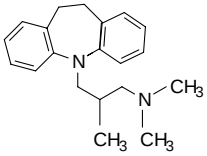
It has been further noted that in order for the polar ionic mode to obtain selectivity there must be at least two functional groups present in the compound, one of which must be ionizable. These functional groups can include alcohols, halogens (I,Br,Cl,F), nitrogen in any form (primary, secondary, tertiary), carbonyl, carboxyl, oxidized forms of sulfur or phosphorus. The composition of the polar ionic mode is generally 100 parts anhydrous methanol and a 1:1 ratio of acid to base generally in the concentration range of 0.01 to 1%, most typically 0.1%. It is possible to further enhance resolution by altering this ratio from 1:2 or 2:1. Ratios up to 5:1 have been observed.

Many acids and bases as well as volatile salts can be used on the CHIROBIOTIC phases. In particular, for LC/MS applications, ammonium acetate is typical for acidic molecules, and ammonium trifluoroacetate and

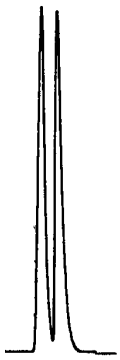
ammonium formate are used for basic molecules. In this case, the weighed salt is added directly to the methanol.

*Acid-Base Effects – Polar Ionic Mode*

Trimipramine

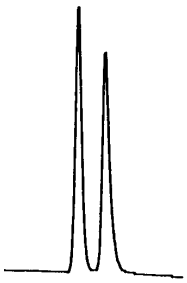



Peak 1 - 16.2 min.
Peak 2 - 17.3 min.



CHIROBIOTIC V
250 x 4.6mm
MeOH/HOAc/TEA:
100/0.03/0.02
1.0 mL/min.

Peak 1 - 20.7 min.
Peak 2 - 22.8 min.



CHIROBIOTIC V
250X4.6mm
MeOH/AFTA:
100/0.02 %v/w
1.0 mL/min.

If the analyte is eluting too fast, the concentration of acid/base or volatile salt is reduced. Conversely, if the analyte is too long retained, the acid/base or volatile salt concentration is increased. Above 1% the analyte is considered too polar and generally indicates a typical reversed phase system may be preferred. Below 0.001% indicates that the polar organic or a normal phase system should be tried. See New Polar Ionic Mode under each section for the appropriate CHIROBIOTIC phase.

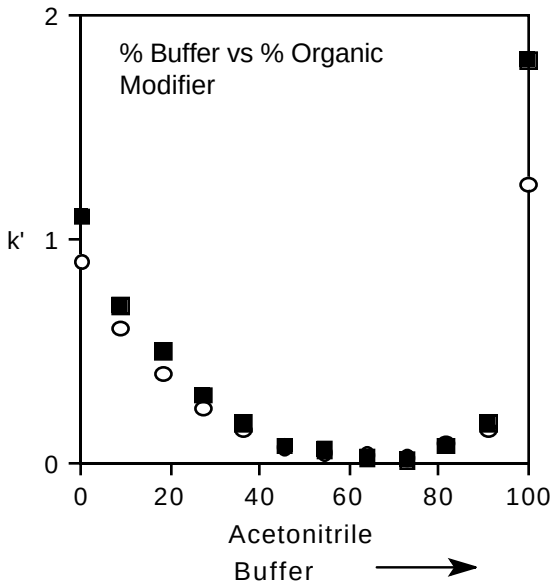
For analytes that have only one functional group or have low solubility in methanol, the typical normal phase solvents (Hexane/EtOH) or reversed phase solvents (THF, ACN or MeOH/Buffer) are employed. Dioxane and DMSO may be used with methanol to improve solubility.

## Reversed Phase Mode

Selectivity and optimization of reversed phase separations are accomplished by controlling the amount of organic modifier to adjust retention and type of organic modifier, type of buffer and pH to control selectivity. Efficiency and selectivity are also affected by the ionic strength, buffer type, flow rate and temperature.

A variety of organic modifiers have been tested for their selectivity. Acetonitrile, methanol, ethanol, isopropanol and tetrahydrofuran give good selectivities for various analytes. Screening of potential organic modifiers is recommended if no separation is observed with the first solvent choice.

*Reversed Phase Separation*
*Enantiomers of 5-Methyl-5-Phenylhydantoin*




Ref: Anal. Chem., Vol. 66(9), p1473-1484, May 1994.

*Note: Selectivity has been observed at both high aqueous and high organic composition indicating a potential for both reversed phase and normal phase operation.*

Plots for retention and resolution versus organic modifier/aqueous composition demonstrate separation at both high and low concentrations of organic modifier. This precludes the use of gradient systems but also indicates that mobile phases can be designed for the solubility of the analyte. In the reversed phase mode the amount of organic modifier can be very low usually in the order of 10-20%. Typical starting composition is 10/90: organic modifier/buffer, pH 3.5-7.0. Alcohols as modifiers

generally require higher starting concentrations, i.e., 20% for comparable retention to $CH_3CN$ or THF.

## Buffers in the Reversed Phase Mode

The efficiency and selectivity of CHIROBIOTIC separations have been substantially increased in some instances with the use of certain buffers. To obtain efficient and reproducible separations in the reversed phase mode, some buffer should always be present even with neutral compounds. Ammonium nitrate, ammonium acetate and triethylammonium acetate (TEAA) buffers have been used successfully. Other buffers can and have been used such as sodium citrate for the separation of profens.

## Preparation of TEAA

TEAA can be prepared by adjusting a 0.1% aqueous solution of triethylamine with acetic acid to the appropriate pH. Make sure the TEA used is anhydrous and fresh.

## Recommended Buffers in Order of Potential Use

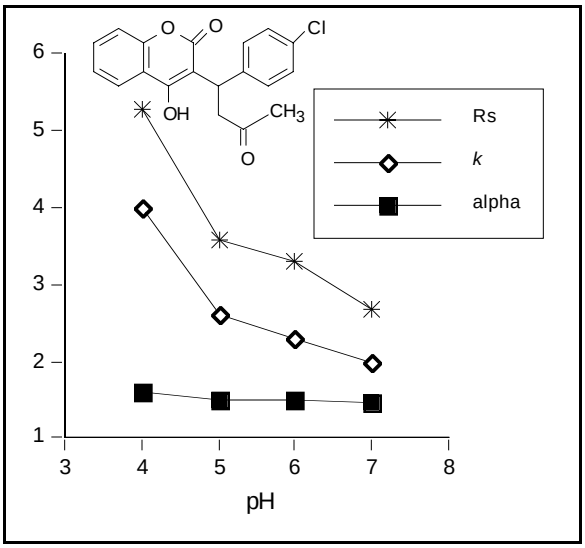
1. Triethylammonium Acetate
2. Ammonium Acetate
3. Ammonium Nitrate
4. Sodium Citrate

**NEVER STORE COLUMNS EVEN FOR SHORT PERIODS OF TIME IN BUFFER. WASH THE COLUMN FIRST WITH WATER, THEN WITH EITHER METHANOL OR ETHANOL.**

## pH in the Reversed Phase Mode

The pH of the buffer is the most important parameter in chiral selectivity in the reversed phase mode. CHIROBIOTIC phases have been used in the reversed phase mode with buffers in a pH range of 3.5 to 7.0. The stability of the chiral stationary phase/analyte complex is dependent on the charge of the analyte. In general, selectivity is enhanced by increasing the ionic complex, therefore, basic analytes prefer low pH, while acidic analytes prefer high pH. This assumes the ionizable group is on or near the chiral center. Ionizable groups far from the chiral center may require the reverse. Because of the complexities of these interactions, it is necessary to observe the retention and resolution as a function of pH, usually testing at low and high pH or 0.5 pH units above and below the pK value.

*Effect of pH on Resolution (Rs)*
*R,S-Coumachlor pH Profile*



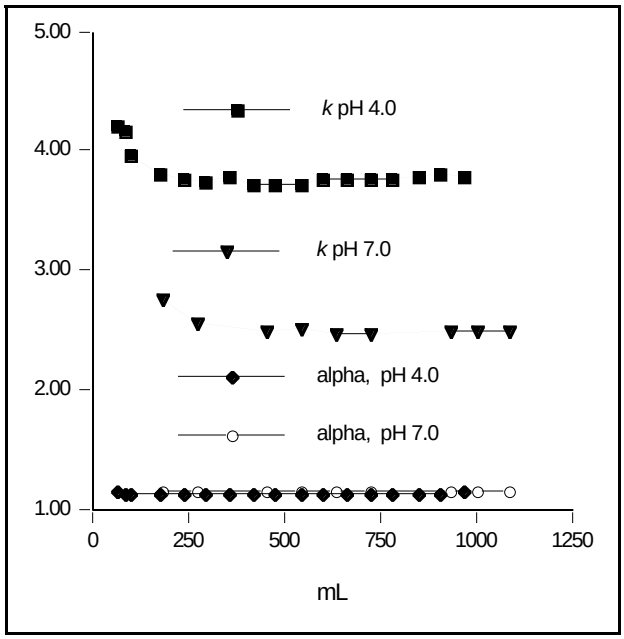

pH Stability

It has been determined that the safest and most stable pH ranges for the CHIROBIOTIC phases are as follows:

| CHIROBIOTIC Phase | pH Range |
|---|---|
| CHIROBIOTIC V and V2 | 3.5 - 7.0 |
| CHIROBIOTIC T and T2 | 3.8 - 6.8 |
| CHIROBIOTIC R | 3.5 - 6.8 |
| CHIROBIOTIC TAG | 3.0 - 6.8 |

CSP Stability Data

*Stability at pH 4.0 and pH 7.0 on CHIROBIOTIC V*




After testing for selectivity, a 250x4.6mm CHIROBIOTIC V column was run at 1.0 mL/min. with 10/90:$CH_3CN$/1% TEAA buffer at pH 7.0. A second column was similarly tested at pH 4.0. After 25 hours of continuous operation there was no observable

change in alpha. The k, after showing an initial drop of approximately 10%, was stable throughout the experiment. Application data from published literature indicate several thousand injections are possible.

A precolumn (before injector) of 40µm silica should always be used when operating silica based columns with aqueous mobile phases.

## LC/MS/MS Detection of Macrolides

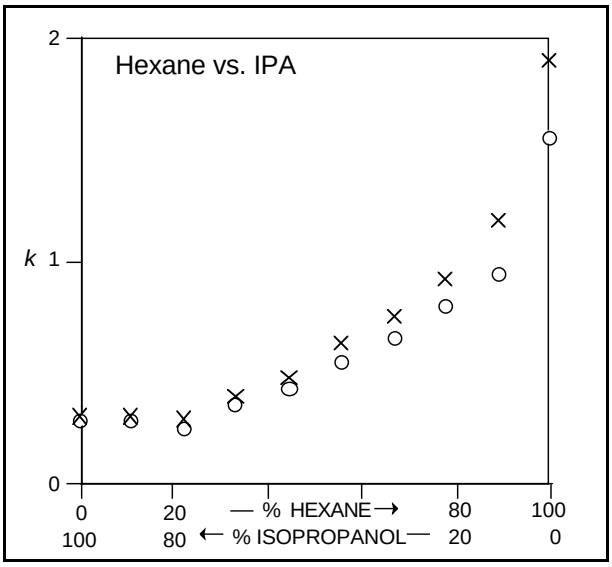
An LC/MS/MS method was established to determine the presence of vancomycin (characteristic ion 1450.3) in column eluates. To maximize sensitivity the eluates had to be acidified to reach a detection limit (LOD) of one picogram per milliliter. It was determined that the best washing system to remove unreacted vancomycin is 50/50: ACN/20mM $NH_4OAc$, pH 4.1. New columns showed different levels of vancomycin depending on the mobile phase used with reversed phase being the highest. Levels of free vancomycin decreased with increased washing with the above solvent to below detectable limits.

## Normal Phase Mode

CHIROBIOTIC phases can be used with all normal phase solvents with no detectable change in enantioselectivity of the stationary phase. Switching from reversed phase to normal phase and back again also has shown no irreversible change in performance. Flush the system with EtOH between changes. As is typical with normal phase conditions, retention is controlled by adjusting the ratio of nonpolar to polar organic solvents (the greater the polarity, the lower the retention).

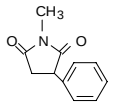
As a result of the linear response of solvent composition to resolution, gradients can be run in the normal phase mode to find the window of separation. Also, very high linear velocities can be run as flow rate has little effect on resolution.

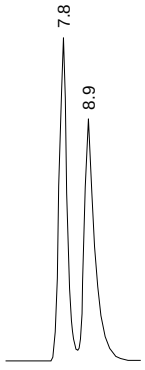
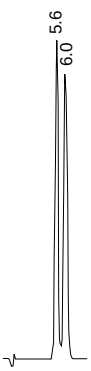
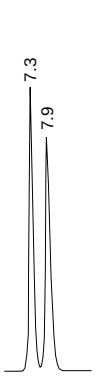
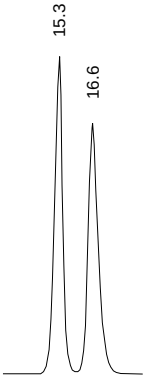
*Enantiomers of Mephenytoin*




For the CHIROBIOTIC phases, greater peak efficiency and resolution are obtained with ethanol as the polar constituent instead of the usual isopropanol, although there are a few cases where IPA proved to be a better modifier. It has been reported that halogenated solvents have also been successful on these stationary phases as well as solvents like dioxane, DMF and methyl *tert*-butyl ether (MtBE).

Phensuximide




| 50/50: Hex/IPA | 50/50: Hex/EtOH | 70/30: Hex/EtOH | |
|---|---|---|---|
|  |  |  |  |
| 1.0 mL/min. | 1.0 mL/min. | 1.0 mL/min. | 0.5 mL/min. |



## Temperature Effects

Temperature has a number of beneficial affects when applied to the separation of enantiomers. It can be a powerful tool in the hands of the chromatographer to

control selectivity, stability and retention for both analytical and preparative applications. The following are some critical areas that have been observed.

### *Temperature Can Increase Resolution*

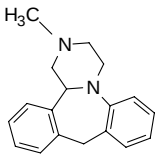
| N-Carbamyl-D,L-phenylalanine | | | |
|---|---|---|---|
| T°C | k | α | Rs |
| 0 | 0.51 | 1.39 | 1.5 |
| 5 | 0.39 | 1.34 | 1.3 |
| 15 | 0.38 | 1.23 | 1.0 |
| 22 | 0.31 | 1.20 | 0.8 |
| 35 | 0.27 | 1.11 | 0.7 |
| 45 | 0.22 | 1.00 | 0.0 |

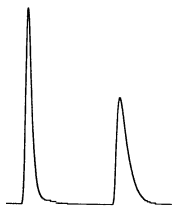
Mobile Phase: 10/90: $CH_3CN$/1% TEAA, pH 4.1.

### *Jetstream Plus Column Thermostat*

### *High Temperature Results in Higher Peak Efficiency and Shorter Analysis Time*

**Mianserin**

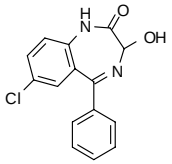


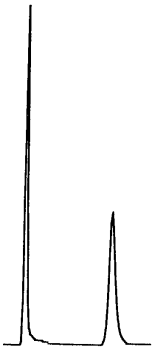
| 5°C | 35°C |
|---|---|
| $k_2' = 3.99$<br>$n_2 = 3627$ | $k_2' = 1.04$<br>$n_2 = 5942$ |
|  |  |

CHIROBIOTIC V, 250x4.6mm
100/0.1/0.1 (v/v/v): MeOH/HOAc/TEA
0.9 mL/min., UV@230nm

### *Temperature Controls on Racemization*

**Oxazepam**

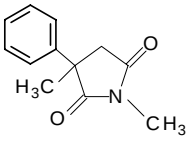


| 15°C | 35°C |
|---|---|
| $k_1' = 3.99$<br>$\alpha = 4.75$<br>Rs = 9.6 | $k_1' = 0.34$<br>$\alpha = 4.14$<br>Rs = 7.1 |
|  |  <br>**On-column racemization** |

CHIROBIOTIC T, 250x4.6mm
100% MeOH
0.9 mL/min., UV@230nm

### *Temperature Can Be Used to Reverse Elution Order*

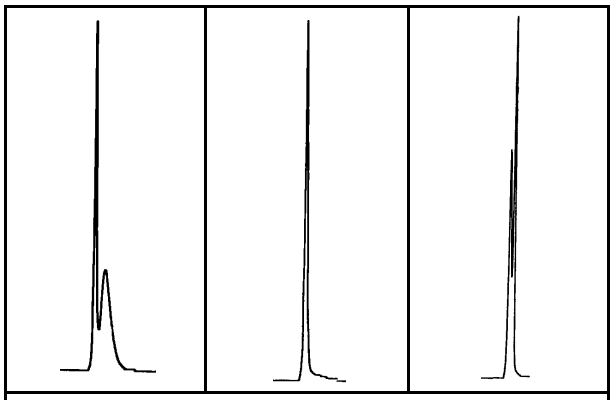
**Methsuximide**



| 5°C | 10°C | 15°C |
|---|---|---|
| 1 | 1,2 | 1 |

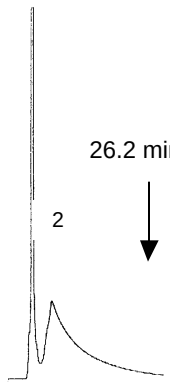
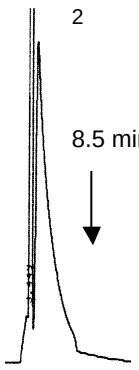
2

2



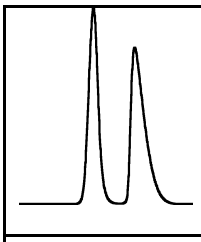
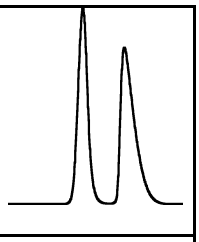
CHIROBOTIC T, 250x4.6mm
10/90: EtOH/Hex
0.9 mL/min., UV@ 230nm

*How Temperature Can Improve Preparative Throughputs*

| 23°C | 35°C |
|---|---|
| Peak 1 – 3.88 min.<br>Peak 2 – 7.34 min. | Peak 1 – 3.87 min.<br>Peak 2 – 4.79 min. |
|  |  |

Loading 15mg
CHIROBIOTIC T
250x4.6mm
100/0.1% (v/w): MeOH/$NH_4OAc$
2.0 mL/min.
UV@230nm

*Temperature Study*

| | | |
|---|---|---|
| *N*-Benzyl-α-methylbenzylamine<br>CHIROBIOTIC V, 250x4.6mm<br>Mobile Phase:100/0.02/0.02 v/v/v: MeOH/HOAc/TEA<br>Flow Rate: 1.0 mL/min.<br>Detector: UV@254nm<br>Temperature: 20°C | | |
|  | 70°C for 2 hours, then cooled to 20°C @ 1°C/min. |  |
| $t_1$ = 15.209<br>$t_2$ = 16.793 | | $t_1$ = 15.294<br>$t_2$ = 16.849 |

From a number of studies it was determined that the linkages between the silica and the macrolide allow the column to be operated at temperatures up to 70°C without any detrimental effect on column performance as long as the change does not exceed 2°C/minute. This is independent of mobile phase type.

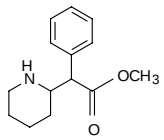
Column reproducibility can be established by maintaining a constant temperature condition within 1°C using 18°C as a base temperature. Astec offers the Jetstream Plus Column Thermostat from 0-80°C for solid temperature control which is ideal for chiral separation work.

## Flow Rate

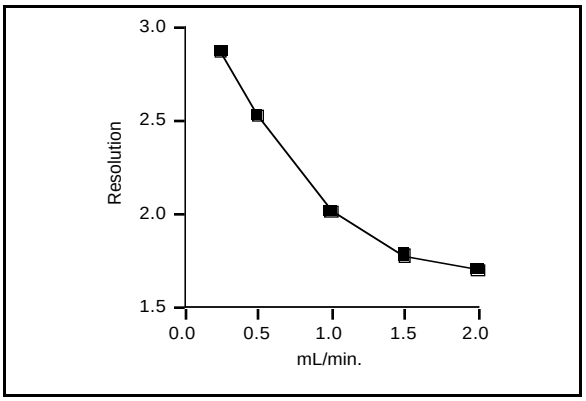
An increase in resolution with a decrease in flow rate is a general phenomenon observed with chiral stationary phases that have inclusion pockets. This change has mainly been observed in the reversed phase mode. This gives the chromatographer an additional operating parameter to improve resolution. Some changes have been observed in the polar organic and polar ionic modes while in typical normal phase solvents no effect on resolution has been observed. In the normal phase, a three-fold increase in flow rate showed no change in selectivity or resolution so analysis time can be significantly reduced in this solvent mode by increasing flow rate.

*Effect of Flow Rate on Resolution*

Methylphenidate



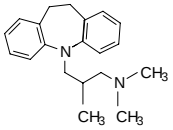

95/5: MeOH/1.0% TEAA, pH 4.1



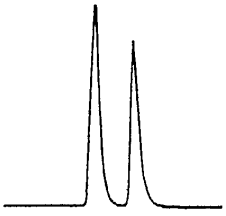

## Comparing Macrocyclic Glycopeptide Phases to Other Stationary Phases

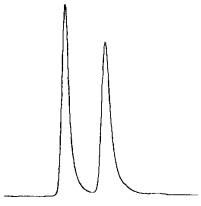
When comparing CHIROBIOTIC phases to other types of stationary phases, it is usually necessary to test the CHIROBIOTIC column in a different mobile phase system. Differences in chiral stationary phase construction require modification of the mobile phase type since the mobile phase composition is what drives the separation. The most flexible and successful comparison of the CHIROBIOTIC phases to others has been with the new polar ionic mode. Separation occurred in about 65% of the cases, the balance being in the reversed phase mode. Only a small percentage (<5%) have resulted from a direct normal phase comparison.

Trimipramine




| POPULAR CELLULOSIC CSP | ASTEC CHIROBIOTIC V2 |
|---|---|
| Peak 1 – 24.9 min. | Peak 1 – 10.65 min. |
| Peak 2 – 28.2 min. | Peak 2 – 12.83 min. |
| | LC/MS Compatible |



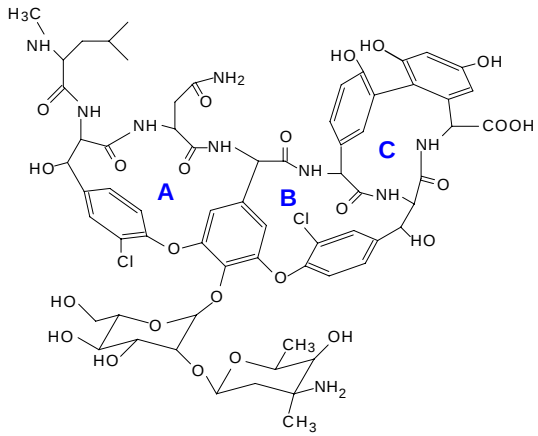


| 40/60: MeCN/1.0M NaClO4 | 100/0.1 w%:MeOH/NH4TFA |
|---|---|

# CHIROBIOTIC V and V2 (Vancomycin)

- ✓ Complex chiral environment
- ✓ π-π interactions
- ✓ Chiral hydrogen bonding sites
- ✓ Peptide binding site
- ✓ Carbohydrate binding site
- ✓ Inclusion complexation
- ✓ Multi-modal possibilities
- ✓ Advantages of protein phases with higher capacity and greater stability
- ✓ Advantages of cellulose and amylose phases with greater solvent versatility and higher throughputs
- ✓ Complementary to CHIROBIOTIC T and CHIROBIOTIC R

*Proposed Structure of the Macrocyclic Glycopeptide Vancomycin*




General Physical Data:
Molecular Weight - 1449
pK's - 2.9, 7.2, 8.6, 9.6, 10.4, 11.7
Isoelectric Point - 7.2
Chiral Centers - 18
Sugar Moieties - 2
Inclusion Cavities-A,B,C

# Types of Chiral Analytes CHIROBIOTIC V

Neutral molecules, amides, acids, esters and amines show considerable enantioselectivity on this phase. A wide variety of secondary and tertiary amines have been separated on the CHIROBIOTIC V in the polar ionic mode. The CHIROBIOTIC V has demonstrated many of the separation characteristics of protein based stationary phases with exceptional stability and much higher sample capacity. Some chiral analytes have been resolved that have not been reported separated on any other chiral stationary phase.

## Screening for Selectivity – CHIROBIOTIC V

There are four solvent modes that can be used for selectivity with this phase and the response is different for different structures in each of these mobile phase compositions. A screening mobile phase composition has been designed to give the broadest range of possibilities but each mobile phase type should be tested according to the following statistical responses that have been documented.

| Compound Type | Polar Organic Mode | Polar Ionic Mode | Reversed Phase Mode | Normal Phase Mode |
|---|---|---|---|---|
| Acids | | | √ | |
| Bases | | √ | √ | |
| Neutrals | √ | | √ | √ |

## Mobile Phase Types

### New Polar Ionic and Polar Organic Phase Separations on CHIROBIOTIC V

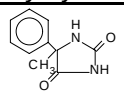
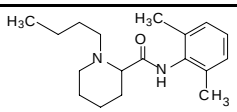
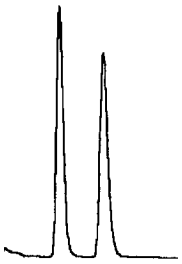
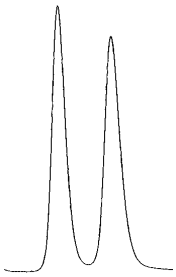
The polar ionic mode is applicable to all molecules with at least one ionizable group on or near the chiral center and one additional functional group anywhere in the structure. Usually, basic compounds demonstrate more selectivity in this mobile phase. This novel and very versatile mobile phase can and should be used whenever possible because of its high volatility and beneficial ionization effect for LC/MS.

Best Starting Polar Ionic Mode Composition
100/0.1/0.1 (v/v/v): MeOH/HOAc/TEA

The acetic used is glacial and the triethylamine should be anhydrous and fresh. These components have broad selectivity in this composition. Once selectivity is observed then volatile components can be evaluated as well as salts like ammonium trifluoroacetate, ammonium formate or ammonium acetate. The amount of these salts will vary widely depending on the compound.

Best Starting Polar Organic Mode Composition
100% Methanol

For neutral molecules only, the polar organic mode is very effective with the CHIROBIOTIC V. This mobile phase is typically a single solvent like methanol, ethanol, acetonitrile or sometimes combinations of these. No acid or base is required here.

| Polar Organic Mode | Polar Ionic Mode |
|---|---|
| 5-Methyl-5-Phenylhydantoin | Bupivacaine |
|  |  |
| Peak 1 – 3.70 min.<br>Peak 2 – 4.21 min. | Peak 1 – 5.59 min.<br>Peak 2 – 6.15 min. |
|  |  |
| CHIROBIOTIC V<br>250x4.6mm<br>100% MeOH<br>1.0 mL/min.<br>UV-220nm | CHIROBIOTIC V<br>250x4.6mm<br>100/0.1w%:<br>MeOH/NH4TFA<br>1.0 mL/min.<br>UV-230nm |

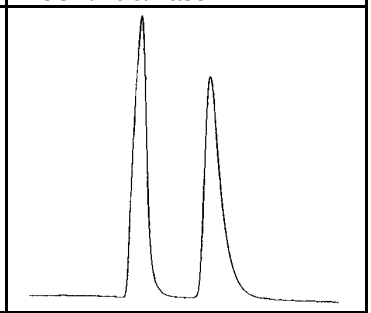
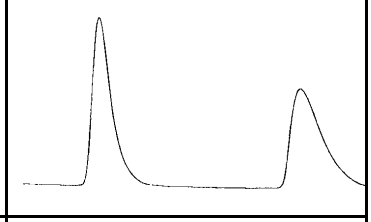
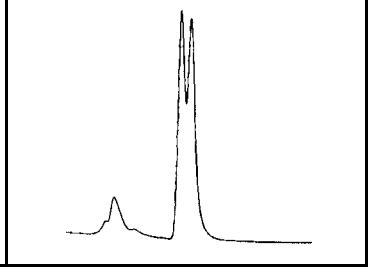
Optimization in the Polar Organic Mode

Test single solvent first: MeOH>EtOH>IPA>ACN. To reduce retention or enhance peak shape the more polar alcohols can be modified with small amounts of ACN.

Optimization in the Polar Ionic Mode

Step 1: Choose the proper acid and base or volatile salt components. To further enhance resolution it has been observed that the ratio of acid to base may need to be altered. Since basic analytes are favored in this mobile phase on the CHIROBIOTIC V, the acid component is higher in order to completely protonate the basic analyte. Increase the acetic acid to 2:1 and then 3:1 and so on to find the maximum ratio for the analyte being tested. It is necessary to adjust the ratio so as not to have more acid than is necessary for the protonation of the basic analyte or selectivity will begin to decrease. This is especially true when changing from the TEA base to an ammonia base or any acid and base change. The determination of the most useful ratio for other acids or bases can be made by substituting water for the methanol and measuring the apparent pH of the starting composition. Using other bases like ammonia, adjust pH to same value and record the amount used. This is now the new ratio for chromatographic use in the polar ionic mode with methanol as the carrier.

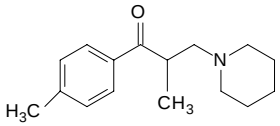
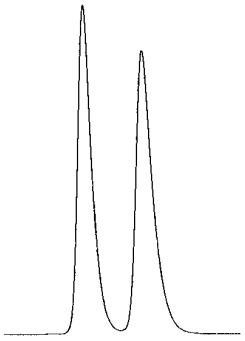
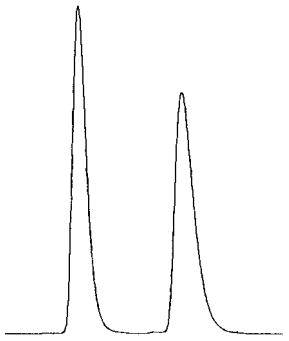
*Acid/Base Effect in the Polar Ionic Mode*

| Example | Mianserin |
|---|---|
| Mobile Phase | MeOH/Acid/Base |
| 100/0.1/0.1<br><br>Peak 1 – 6.21 min.<br>Peak 2 – 7.36 min.<br><br>Ratio: 1:1 |  |
| 100/0.15/0.05<br><br>Peak 1 – 10.44 min.<br>Peak 2 – 14.46 min.<br><br>Ratio: 3:1 |  |
| 100/0.05/0.15<br><br>Peak 1 – 3.43 min.<br>Peak 2 – 3.58 min.<br><br>Ratio: 1:3 |  |

Step 2: Change the flow rate. Lower flow rates often result in higher resolution in this particular mode. Flow rates down to 0.3 mL/min. for a 250x4.6mm column have been reported.

Step 3: 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.

Step 4: Evaluate CHIROBIOTIC V2. This is done under the exact same mobile phase conditions that have been established to date from Steps 1 to 3. Enhanced resolution has been observed for a number of compounds. There is also a great improvement in loadability so, if the project is going to prep, this is a worthwhile evaluation.

| CHIROBIOTIC V | CHIROBIOTIC V2 |
|---|---|
| Tolperisone | |
|  | |
| Peak 1 – 8.87 min.<br>Peak 2 – 9.90 min. | Peak 1 – 8.80 min.<br>Peak 2 – 10.69 min. |
|  |  |
| CHIROBIOTIC V<br>250x4.6mm<br>100/0.1w%:<br>MeOH/NH4TFA<br>1.0 mL/min.<br>UV-230nm | CHIROBIOTIC V2<br>250x4.6mm<br>100/0.1w%:<br>MeOH/NH4TFA<br>1.0 mL/min.<br>UV-230nm |

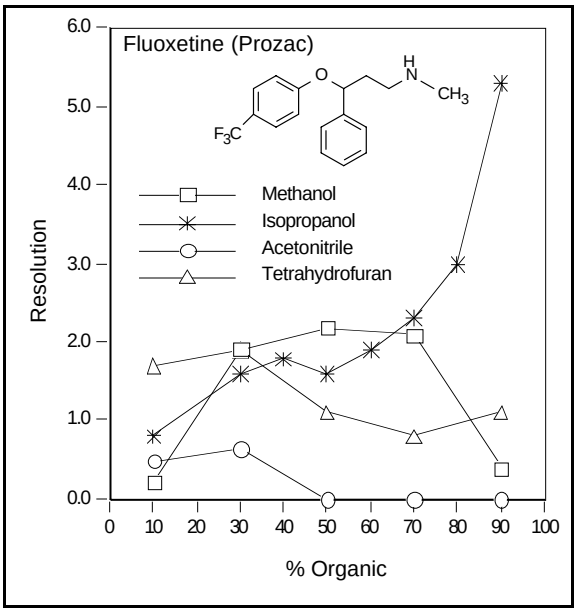
## Reversed Phase Separations CHIROBIOTIC V

The organic modifier, even at 10-20%, has a profound effect on resolution. For the CHIROBIOTIC V, a number of solvents have shown a broad selectivity response. As can be seen in the chart below, resolution can be increased with the proper solvent choice. This response differs with each analyte. Methanol is generally used as a first choice for screening purposes.

Best Starting Reversed Phase Composition

20/80: MeOH/20mM NH4OAc, pH 4.0

## *Effect of Organic Modifier on Resolution*




*Courtesy of Scott Sharpe, Eli Lilly & Co.*

Acidic and basic compounds alike have shown selectivity in the reversed phase mode on the CHIROBIOTIC V.

## Optimization in the Reversed Phase Mode

Step 1: Evaluate the optimum organic modifier: THF>MeOH>ACN.

Step 2: Evaluate the concentration of the organic modifier. Higher concentrations result in lower retention.

Step 3: Evaluate pH of aqueous buffer.

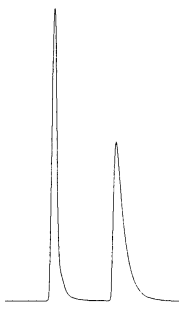
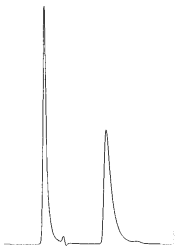
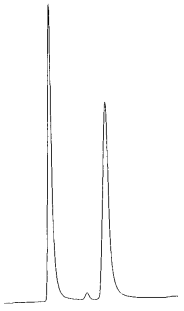
Step 4: Evaluate the best buffer: TEAA, $NH_4OAc$, $NH_4NO_3$ and Na citrate.

Step 5: Evaluate the concentration of the aqueous buffer. Range 0.05-1.0%.

Step 6: Evaluate flow rate. Lower flow rates typically lead to higher resolution when k′ is less than 3.

Step 7: Evaluate temperature: Lower temperatures often lead to higher resolutions.

*pH Effect on Reversed Phase*

| Example | Mandelic Acid | |
|---|---|---|
| Mobile Phase | 20/80: MeOH/0.1% TEAA | |
| **pH 4.1** | **pH 5.0** | **pH 6.5** |
| Peak 1 – 3.35 min.<br>Peak 2 – 5.08 min. | Peak 1 = 2.73 min.<br>Peak 2 – 4.37 min. | Peak 1 – 2.33 min.<br>Peak 2 – 3.70 min. |
|  |  |  |
| $t_o$ = 3.2 min. | | |

# Normal Phase Separations
# CHIROBIOTIC V

CHIROBIOTIC V phases can tolerate a wide variety of solvents including chlorinated types. The most common solvent combination has been hexane/ethanol.

Best starting Normal Phase Composition

80/20: Heptane/EtOH

Optimization in Normal Phase Mode

Step 1: Evaluate the polar solvent EtOH vs IPA. Both are useful for different analytes. The combination of ACN/MtBE also should be tried.

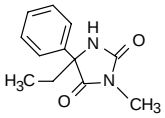
Step 2: Evaluate concentration of the polar solvent. Higher concentrations result in lower retention.

Step 3: Add small amounts of acid and base as modifiers to reduce tailing.

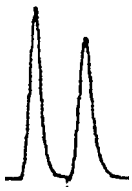
Step 4: Evaluate temperature. Lower temperature can increase resolution. Higher temperature can reverse elution order.

*Note: Low flow rates have no effect on resolution, therefore, higher flow rates can be used to speed assay results.*

Mephenytoin




Peak 1 - 11.26 min.
Peak 2 - 12.85 min.

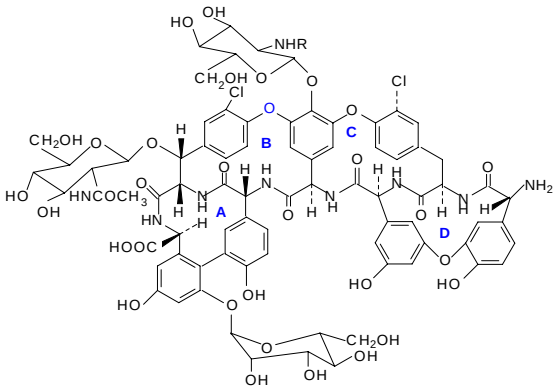


95/5: Hex/EtOH @ 1.5 mL/min.

# CHIROBIOTIC T and T2 (Teicoplanin)

- ✓ Complex chiral environment
- ✓ π-π interactions
- ✓ Chiral hydrogen bonding sites
- ✓ Peptide binding site
- ✓ Inclusion complexation
- ✓ Multi-modal possibilities
- ✓ Excellent alternative to crown ether and ligand exchange for amino acids and hydroxy acids
- ✓ Can resolve α, β, γ or cyclic amino acids and peptides
- ✓ Complementary to CHIROBIOTIC V and CHIROBIOTIC R

*Proposed Structure of the Macrocyclic Glycopeptide Teicoplanin*



General Physical Data:
Molecular Weight ~ 1885
Chiral Centers - 23

Sugar Moieties - 3
Inclusion Cavities - 4 (A,B,C,D)
R - $CH_3$-decanoic acid

# Types of Chiral Analytes CHIROBIOTIC T

The CHIROBIOTIC T has unique selectivity for a number of classes of molecules, specifically underivatized α, β, γ or cyclic amino acids, N-derivatized amino acids, i.e., FMOC, CBZ, t-BOC and alpha hydroxy-carboxylic acids, acidic compounds including carboxylic acids and phenols, small peptides, neutral aromatic analytes and cyclic aromatic and aliphatic amines. Separations normally obtained on a chiral crown ether or ligand exchange type phase are also possible on the CHIROBIOTIC T. In addition, all of the known beta-blockers (amino alcohols), and dihydrocoumarins have been resolved. One of the major features of CHIROBIOTIC T is its "complementary stereoselectivity" to the CHIROBIOTIC V and/or CHIROBIOTIC R column. If, after optimization, the CHIROBIOTIC V column does not resolve the analyte to baseline, using the CHIROBIOTIC T or CHIROBIOTIC R column in the same mobile phase can result in complete resolution. This phenomenon also works in reverse. For enhanced resolution of a number of these compound classes see CHIROBIOTIC TAG section.

| Compound Type | Polar Organic Mode | Polar Ionic Mode | Reversed Phase Mode | Normal Phase Mode |
|---|---|---|---|---|
| Acids | | √ | √ | |
| Bases | | √ | √ | |
| Neutrals | √ | | √ | √ |

# Mobile Phase Types

## New Polar Ionic and Polar Organic Mode Separations on CHIROBIOTIC T

The molecular structure requirements are the same as those presented for the CHIROBIOTIC V, i.e., at least two functional groups must be present in the analyte, one on or near the stereogenic enter, the second one anywhere else in the molecule. One of the functional groups must be ionizable to work in the polar ionic mode. If not, the molecule is considered neutral and the polar organic mode is suitable. On occasion, if the two polar groups are alpha to one another, strong internal hydrogen bonding requires a higher acid/base concentration or a formulation incorporating $CH_3CN$/$CH_3OH$ as the organic modifiers. For the majority of cases, the conversion to the 100% methanol composition is effective and highly efficient. Any β-blocker (amino alcohol) can be resolved with this mobile phase.

Best Starting Polar Ionic Mode Composition

100/0.1/0.1:$CH_3OH$/HOAc/TEA

In addition to HOAc/TEA, HOAc/$NH_4OH$ and TFA/$NH_4OH$ have been used. When TFA is employed, a lower concentration is usually required. Also, salts such as ammonium acetate, ammonium formate or ammonium trifluoroacetate can be used.

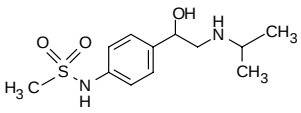
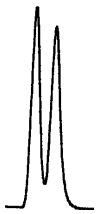
Optimization in the Polar Ionic Mode

Step 1: With a 1:1 ratio of acid:base some selectivity is typically observed, and then different ratios of 1:2 and 2:1 are run to note the change in resolution indicating the trend. This ratio is determined after any dilution of the acid/base concentration for weakly polar molecules.

If the analyte is eluting too fast, the concentration of acid/base is reduced. Conversely, if the analyte is too long retained, the acid/base concentration is increased. The parameters for concentration are between 1% and .001%. Above 1% the analyte is too

polar and indicates a typical reversed phase system should be used.

*New Polar Ionic Mode Separations on CHIROBIOTIC T - Optimization Procedure*

| Sotalol | |
|---|---|
|  | |
| Peak 1 - 17.3 min.<br>Peak 2 - 18.6 min. | Peak 1 - 12.5 min.<br>Peak 2 - 13.7 min. |
|  |  |
| 100/0.1/0.1:<br>MeOH/HOAc/TEA | 100/0.2/0.1:<br>MeOH/HOAc/TEA |

Step 2: Change the flow rate. Lower flow rates often result in higher resolution in this particular mode. Flow rates down to 0.3 mL/min. for a 250x4.6mm column have been reported.

Step 3: 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.

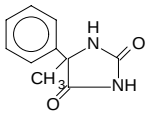
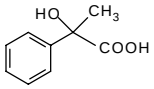
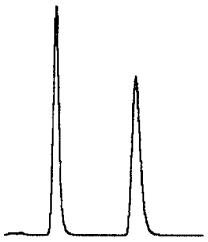
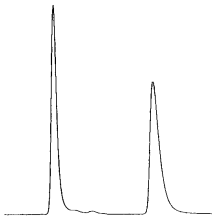
Step 4: Evaluate CHIROBIOTIC T2. This is done under the exact same mobile phase conditions that have been established to date from Steps 1 to 3. Enhanced resolution has been observed for a number of compounds. There is also a great improvement in loadability so, if the project is going to prep, this is a worthwhile evaluation.

Best Starting Polar Organic Mode Composition

100% MeOH

Optimization in the Polar Organic Mode

Test single solvents MeOH>EtOH>IPA>ACN. To reduce retention or enhance peak shape the more polar alcohols can be modified with small amounts of ACN.

| Polar Organic Mode | Polar Ionic Mode |
|---|---|
| 5-Methyl-5-phenyl-hydrantoin | Atrolactic Acid |
|  |  |
| Peak 1 – 3.83 min.<br>Peak 2 – 5.05 min. | Peak 1 – 2.90 min.<br>Peak 2 – 4.24 min. |
|  |  |
| 100% MeOH | 100/0.1w%:<br>MeOH/ $NH_4OAc$ |

## Reversed Phase Separations CHIROBIOTIC T

As in most reversed phase systems, retention and selectivity are controlled by the concentration and nature of the organic modifier, pH and, to a lesser degree, concentration and nature of the buffer. Temperature and flow rate are two additional parameters that can effectively influence resolution in this mode.

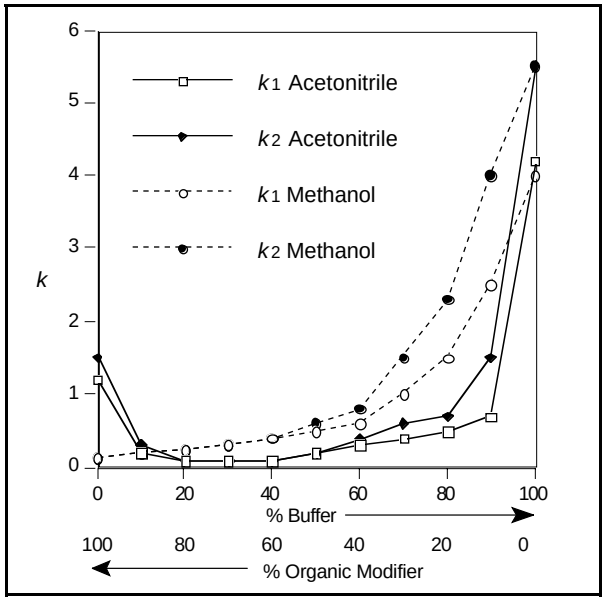
Best Starting Reversed Phase Composition

20/80: MeOH/20mM $NH_4OAc$, pH 4.0

Effect of Organic Modifiers in the Reversed Phase Mode

The CHIROBIOTIC T has shown high selectivity as a function of the organic modifier. Organic modifier/aqueous buffer plots obtained for retention and resolution show separation at both high and low concentrations of organic modifier. Typical compositions are 20/80: alcohol/buffer and 10/90: $CH_3CN$ or THF/buffer. Overall, the CHIROBIOTIC T separations appear to favor the alcohol type mobile phase by a large margin and it is, therefore, suggested as the starting mobile phase. The order of priority is $CH_3OH$ > $C_2H_5OH$ > THF > $CH_3CN$ > IPA. The exceptions are amino acids where ethanol demonstrated higher selectivity. It must be emphasized that there are occasions when only one of the solvents has shown selectivity for a separation.

## *Effect of Organic Modifier on Retention (k)*




Optimization in the Reversed Phase Mode

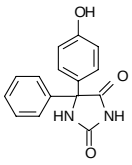
Step 1: Evaluate the organic modifier: MeOH>ACN>THF

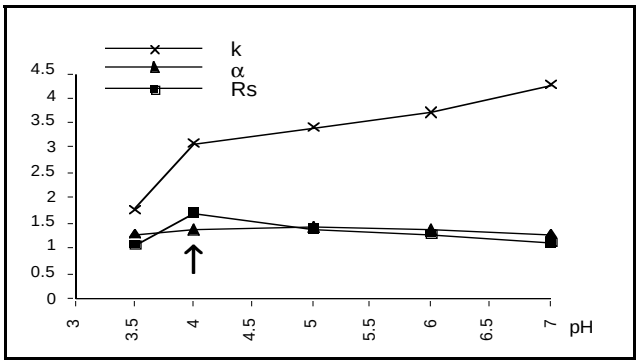
Step 2: Evaluate the concentration of the organic modifier. Higher concentrations result in lower retention.

Step 3: Effect of pH in the reversed phase mode.

## *Effect of pH on Retention (k), Selectivity (α) and Resolution (Rs)*

5-(4-hydroxyphenyl)-5-phenylhydantoin
20/80: MeOH/1% TEAA




Step 4: Evaluate the best buffer: TEAA, $NH_4OAc$, $NH_4NO_3$.

Step 5: Evaluate the concentration of the aqueous buffer. Range 0.05 – 1.0%.

Step 6: Evaluate flow rate. Lower flow rates in the reversed phase mode can have a dramatic effect on resolution.

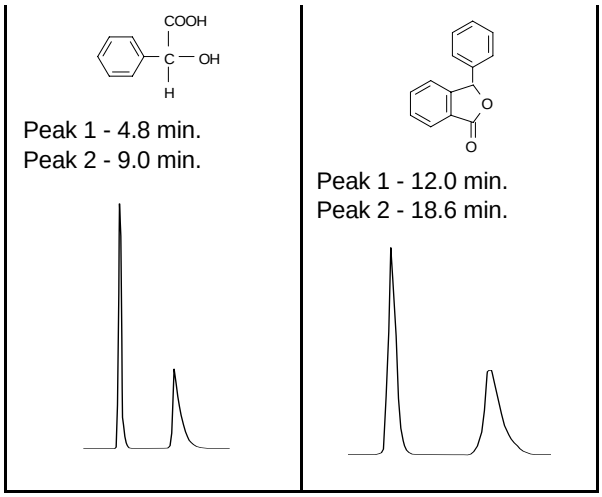
Step 7: Evaluate temperature. Lower temperatures can increase resolution. Higher temperatures can be used to increase efficiency leading to high resolution. Care should be exercised here because higher temperatures have been reported to reverse elution order.

It has been determined that the safest and most stable pH range for the CHIROBIOTIC T is 3.8 to 6.8. Decreasing pH to 3.8 produces a significant increase in retention for analytes with free carboxyl groups. In all cases, both selectivity and resolution vary with pH. Nonionizable analytes typically show less variation or a decrease in retention with a decrease in pH.

## *Reversed Phase Separation of Hydroxy Carboxylic Acids and Anhydrides*

Acids and anhydrides are generally run in 20/80: $CH_3OH$/1% TEAA, pH 4.0.

| Mandelic Acid | Phenylphthalide |
|---|---|

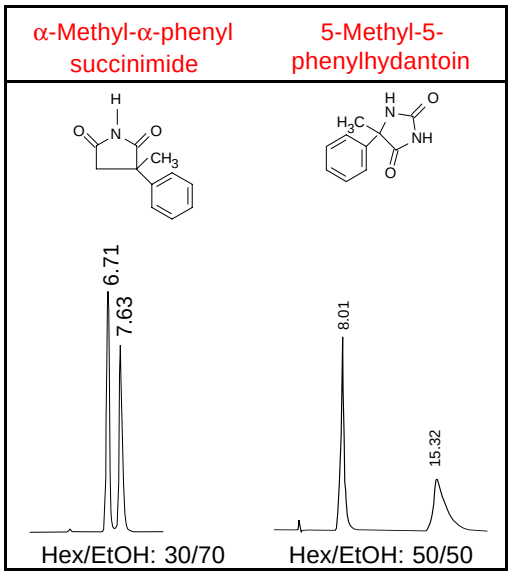



## Normal Phase Separations CHIROBIOTIC T

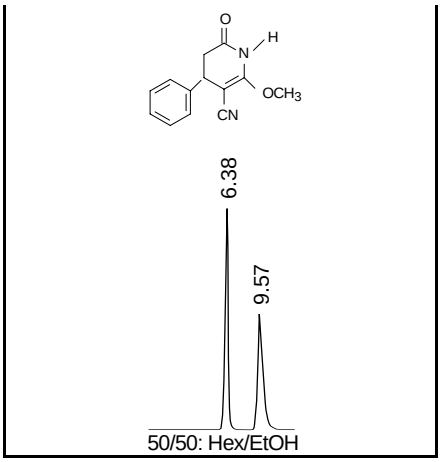
CHIROBIOTIC T can be used with all normal phase solvents. There is no limitation on the stability of this stationary phase in any chlorinated or lipophilic solvent. As is typical with normal phase conditions, retention is controlled by adjusting the ratio of nonpolar to polar constituent. We have noticed a particular preference for ethanol as the polar constituent. There are a few cases where the standard IPA works best.

Best Start Normal Phase Composition

80/20: Hex/EtOH




4-Phenyl-2-methoxy-6-oxo-1,4,5,6-tetrahydropyridine-3-carbonitrile




"A Covalently Bonded Teicoplanin Chiral Stationary Phase for HPLC Enantioseparations", D.W. Armstrong, Y. Liu, K.H. Ekborg-Ott, *Chirality*, **Vol. 7, No. 6** (1995).

"Enantioresolution of Substituted 2-Methoxy-6-oxo-1,4,5,6-Tetrahydro-pyridine-3 Carbonitriles on Macrocyclic Antibiotic and Cyclodextrin Stationary Phases", S. Chen, Y. Liu, D.W. Armstrong, P. Victory, B. Martinez-Teipel, *J. Liq. Chrom.*, **Vol. 18 (8)**, 1495-1507 (1995).

Optimization in Normal Phase Mode

Step 1: Evaluate the polar solvent EtOH vs IPA. Both are useful for different analytes.

Step 2: Evaluate concentration of the polar solvent. Higher concentrations result in lower retention.

Step 3: Add small amounts of acid and base as modifiers to reduce tailing.

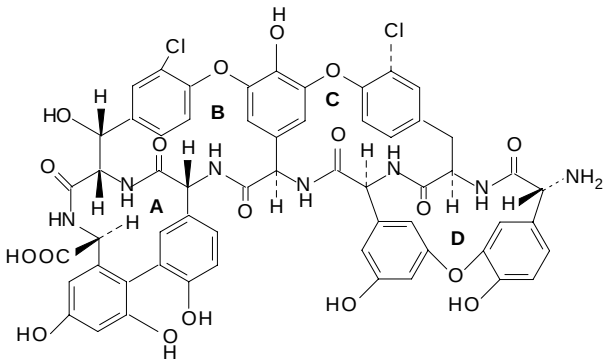
Step 4: Evaluate temperature. Lower temperature can increase resolution. Higher temperature can reverse elution order.

Step 5: Low flow rates have no effect on resolution, therefore, higher flow rates can be used to speed assay results.

# CHIROBIOTIC TAG (Teicoplanin Aglycone)

- ✓ Complex chiral environment
- ✓ π-π interactions
- ✓ Chiral hydrogen bonding sites
- ✓ Inclusion complexation
- ✓ Multi-modal possibilities
- ✓ Complementary to CHIROBIOTIC T
- ✓ High selectivity: amino acids; N-blocked amino acids, peptides
- ✓ High selectivity: neutral molecules, diazepines, hydantoins, oxazolidinones, sulfoxides

*Proposed Structure of the Macrocyclic Glycopeptide Teicoplanin Aglycone*




General physical data:
Molecular Weight ~ 1197
Chiral Centers - 8
Inclusion Cavities - 4 (A,B,C,D)

## Types of Chiral Analytes CHIROBIOTIC TAG

The CHIROBIOTIC TAG has shown excellent complementary selectivity to the CHIROBIOTIC T. The removal of the three carbohydrates has enhanced resolution for many of the amino acids, alpha, beta, gamma and cyclic. It has shown remarkable selectivity especially towards sulfur containing molecules (sulfoxides) including amino acids, methionine, histidine and cysteine. Resolution of the classes of molecules like amino alcohols which were excellent on the CHIROBIOTIC T have shown reduced resolution on the TAG with the exception of propranolol. A number of neutral molecules like the oxazolidinones, hydantoins and diazepines have shown enhanced resolution and, more remarkably, in the single solvents like methanol, ethanol and acetonitrile. Some acidic molecules have also shown increased selectivity.

*The preparation and applications of the aglycone form of CHIROBIOTIC T was a concept conceived and published by Dr. Francesco Gasparrini, Universita Degli Studi Di Roma, Italy. It was under his guidance that we have produced this product.*

## Screening for Selectivity – CHIROBIOTIC TAG

There are four solvent modes that can be used for selectivity with this phase and the response is different for different structures in each of these mobile phase compositions. A screening mobile phase composition has been designed to give the broadest range of possibilities but each mobile phase type should be tested according to the following statistical responses that have been documented.

| Compound Type | Polar Organic Mode | Polar Ionic Mode | Reversed Phase Mode | Normal Phase Mode |
|---|---|---|---|---|
| Acids | | √ | √ | |
| Bases | | √ | √ | |
| Neutrals | √ | | √ | √ |

## Mobile Phase Types

### New Polar Ionic and Polar Organic Phase Separations on CHIROBIOTIC TAG

The polar ionic mode is applicable to all molecules with at least one ionizable group on or near the chiral center and one additional functional group anywhere in the structure. A broad variety of compounds demonstrate selectivity in this mobile phase. This novel and very versatile mobile phase can and should be used whenever possible because of its high volatility and beneficial ionization effect for LC/MS.

Best Starting Polar Ionic Mode Composition
100/0.1/0.1 (v/v/v): MeOH/HOAc/TEA

The acetic used is glacial and the triethylamine should be anhydrous and fresh. These components have broad selectivity in this composition. Once selectivity is observed then volatile components can be evaluated as well as salts like ammonium trifluoroacetate, ammonium formate or ammonium acetate. The amount of these salts will vary widely depending on the compound.

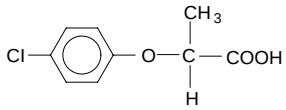
Best Starting Polar Organic Mode Composition
100% Methanol

For neutral molecules only, the polar organic mode is very effective with the CHIROBIOTIC TAG. This

mobile phase is typically a single solvent like methanol, ethanol, acetonitrile or sometimes combinations of these. No acid or base is required here. Of all the CHIROBIOTIC phases, CHIROBIOTIC TAG has the greatest selectivity in this mobile phase.

*New Polar Ionic Mode Separation on CHIROBIOTIC TAG*

2-(4-Chlorophenoxy) propionic acid

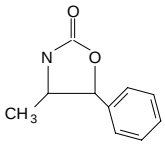



Peak 1 - 3.43 min.
Peak 2 - 4.74 mIn.

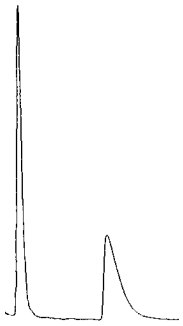
CHIROBIOTIC TAG
250 x 4.6mm
100/0.2/0.1:MeOH/HOAc/TEA
1.0 mL/min.

*Polar Organic Mode Separation on CHIROBIOTIC TAG*

4-Methyl-5-phenyl-2-oxazolidinone



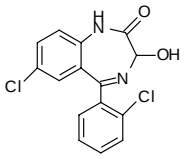

Peak 1 - 5.35 min.
Peak 2 - 8.21 min.

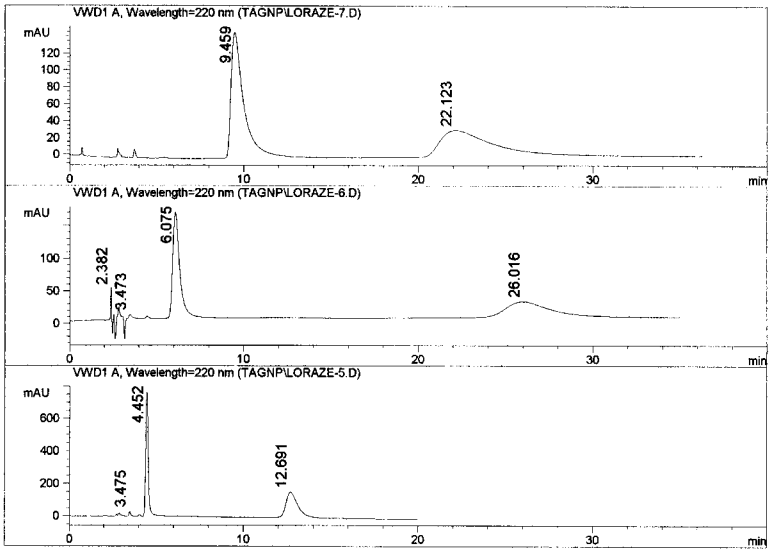


CHIROBIOTIC TAG
250x4.6mm
100% MeOH
0.8 mL/min.

*Effect of Single Solvents on Selectivity for Neutral Molecules in the Polar Organic Mode*

*Lorazepam*





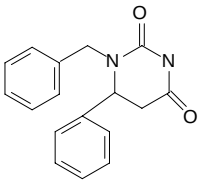

$CH_3CN$
1.0 mL/min.
$\alpha$=2.9

EtOH
1.0 mL/min.
$\alpha$=7.0

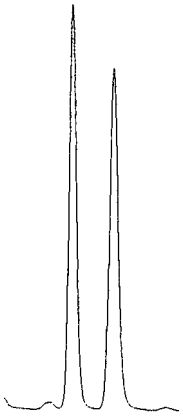
MeOH
1.0 mL/min.
$\alpha$=6.0

## *Polar Organic Mode Separations on CHIROBIOTIC TAG*

1-Benzyl-6-phenyl-5,6-dihydrouracil

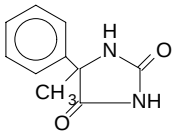


Peak 1 – 4.91 min.
Peak 2 – 5.58 min.

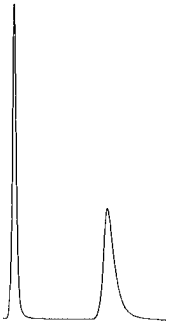


CHIROBIOTIC TAG, 250x4.6mm
100% MeOH @ 0.8 mL/min.

5-Methyl-5-phenylhydantoin



Peak 1 - 5.08 min.
Peak 2 - 9.62 min.



100% MeOH @ 0.8 mL/min.

Optimization in the Polar Organic Mode

Test single solvent first: MeOH>EtOH>IPA>ACN. To reduce retention or enhance peak shape the more polar alcohols can be modified with small amounts of ACN.

Optimization in the Polar Ionic Mode

Step 1: Choose the proper acid and base or volatile salt components. To further enhance resolution it has been observed that the ratio of acid to base may need to be altered. Both acidic and basic analytes are favored in this mobile phase on the CHIROBIOTIC TAG, the acid component is higher in order to completely protonate the basic analyte. Increase the acetic acid to 2:1 and then 3:1 and so on to find the

maximum ratio for the analyte being tested. For acidic molecules the reverse will be true. It is necessary to adjust the ratio so as not to have more acid than is necessary for the protonation of the basic analyte or selectivity will begin to decrease. This is especially true when changing from the TEA base to an ammonia base or any acid and base change. The determination of the most useful ratio for other acids or bases can be made by substituting water for the methanol and measuring the apparent pH of the starting composition. Using other bases like ammonia, adjust pH to same value and record the amount used. This is now the new ratio for chromatographic use in the polar ionic mode with methanol as the carrier.

Step 2: Change the flow rate. Lower flow rates often result in higher resolution in this particular mode. Flow rates down to 0.3 mL/min. for a 250x4.6mm column have been reported.

Step 3: 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.

## Reversed Phase Separations CHIROBIOTIC TAG

No significant change has been observed for use of this phase in the reversed phase mode. All of the points made under the CHIROBIOTIC T are applicable here, including the selectivity of methanol as the primary organic component.

Best Starting Reversed Phase Composition
20/80: MeOH/20mM $NH_4OAc$, pH 6.0

Optimization in the Reversed Phase Mode

Step 1: Evaluate the organic modifier: MeOH>ACN>THF.

Step 2: Evaluate the concentration of the organic modifier. Higher concentrations result in lower retention.

Step 3: Effect of pH in the reversed phase mode.

Step 4: Evaluate the best buffer: TEAA, $NH_4OAc$, $NH_4NO_3$.

Step 5: Evaluate the concentration of the aqueous buffer. Range 0.05 – 1.0%.

Step 6: Evaluate flow rate. Lower flow rates in the reversed phase mode can have a dramatic effect on resolution.

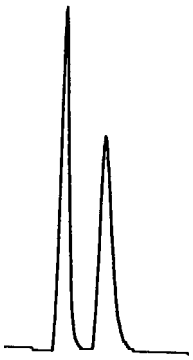
Step 7: Evaluate temperature. Lower temperatures can increase resolution. Higher temperatures can be used to increase efficiency leading to high resolution. Care should be exercised here because higher temperatures have been reported to reverse elution order.

It has been determined that the safest and most stable pH range for the CHIROBIOTIC TAG is 3.0 to 6.8. Decreasing pH to 3.0 produces a significant increase in retention for analytes with free carboxyl groups. In all cases, both selectivity and resolution vary with pH. Nonionizable analytes typically show less variation or a decrease in retention with a decrease in pH.

Carnitine

$(CH_3)_3NCH_2CH(OH)CH_2COOH$

Peak 1 – 12.19 min.
Peak 2 – 14.89 mIn.



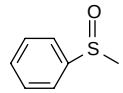
CHIROBIOTIC TAG
250 x 4.6mm
85/15:MeOH/25mM $NH_4OAc$, pH 6.0
1.0 mL/min.
Detector: ELSD
T=80°C
Nitrogen Flow: 1.0 SLPM

## Normal Phase Separations CHIROBIOTIC TAG

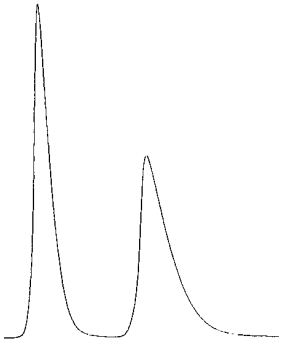
Best Starting Normal Phase Composition
20/80: EtOH/Heptane

As with other CHIROBIOTIC phases, all typical normal phase solvents can be used. There is no limit on the stability of this stationary phase in any chlorinated or lipophilic solvent. Many sulfoxide type molecules have been separated in the typical hexane/ethanol type mobile phase.

Methyl-phenyl sulfoxide



Peak 1 - 10.86 min.
Peak 2 - 14.49 min.



CHIROBIOTIC TAG
250 x 4.6mm
40/60:EtOH/Hex @1.5 mL/min.

## Optimization in Normal Phase Mode

Step 1: Evaluate the polar solvent EtOH vs IPA. Both are useful for different analytes.

Step 2: Evaluate concentration of the polar solvent. Higher concentrations result in lower retention.

Step 3: Add small amounts of acid and base as modifiers to reduce tailing.

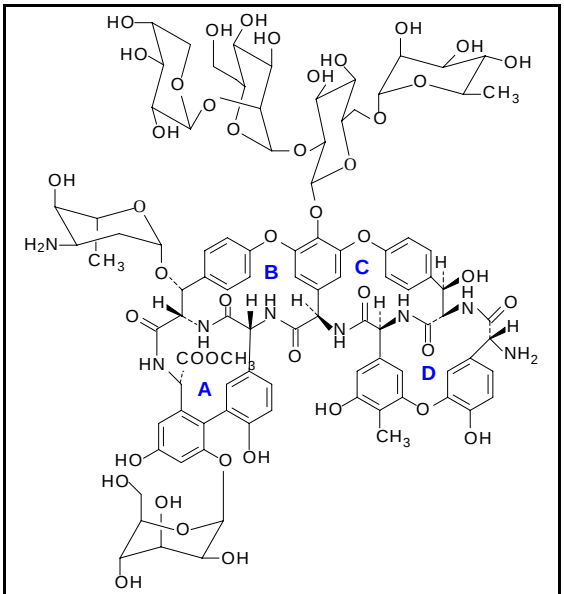
Step 4: Evaluate temperature. Lower temperature can increase resolution. Higher temperature can reverse elution order.

Step 5: Low flow rates have no effect on resolution, therefore, higher flow rates can be used to speed assay results.

# CHIROBIOTIC R (Ristocetin A)

- ✓ Covalently bonded glycopeptide - Ristocetin A
- ✓ Multi-modal - operates in normal, reversed, polar organic and polar ionic modes
- ✓ Targets anionic chiral molecules
- ✓ Complementary to CHIROBIOTIC V and CHIROBIOTIC T

*Proposed Structure of the Macrocyclic Glycopeptide Ristocetin A*




General physical data:
Molecular weight - 2066
Chiral centers - 38
Inclusion cavities-A,B,C,D
Sugar moieties - 6

## Types of Chiral Analytes CHIROBIOTIC R

| New Polar Ionic Mode |
|---|
| α-hydroxy/halogenated acids<br>profens<br>N-blocked amino acids |
| **Normal Phase Mode** |
| imides<br>hydantoins<br>N-blocked amino acids |
| **Reversed Phase Mode** |
| α-hydroxy/halogenated acids<br>substituted aliphatic acids<br>profens<br>N-blocked amino acids<br>hydantoins |
| peptides |

## Screening for Selectivity – CHIROBIOTIC R

There are four solvent modes that can be used for selectivity with this phase and the response is different for different structures in each of these mobile phase compositions. A screening mobile phase composition has been designed to give the broadest range of possibilities but each mobile phase type should be tested according to the following statistical responses that have been documented.

| Compound Type | Polar Organic Mode | Polar Ionic Mode | Reversed Phase Mode | Normal Phase Mode |
|---|---|---|---|---|
| Acids | | √ | √ | |
| Bases | | | | |
| Neutrals | √ | | √ | √ |

## Mobile Phase Types

### New Polar Ionic and Polar Organic Phase Separations on CHIROBIOTIC R

The polar ionic mode is applicable to all molecules with at least one ionizable group on or near the chiral center and one additional functional group anywhere in the structure. This stationary phase favors acidic molecules. This novel and very versatile mobile phase can and should be used whenever possible because of its high volatility and beneficial ionization effect for LC/MS.

Best Starting Polar Ionic Mode Composition
100/0.1/0.1 (v/v/v): MeOH/HOAc/TEA

The acetic used is glacial and the triethylamine should be anhydrous and fresh. These components have broad selectivity in this composition. Once selectivity is observed then volatile components can be evaluated as well as salts like ammonium trifluoroacetate, ammonium formate or ammonium acetate. The amount of these salts will vary widely depending on the compound.

Best Starting Polar Organic Mode Composition

100% Methanol

For neutral molecules only, the polar organic mode is very effective with the CHIROBIOTIC R. This mobile phase is typically a single solvent like methanol, ethanol, acetonitrile or sometimes combinations of these. No acid or base is required here.

Optimization in the Polar Organic Mode

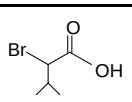
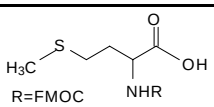
Test single solvent first: MeOH>EtOH>IPA>ACN. To reduce retention or enhance peak shape the more polar alcohols can be modified with small amounts of ACN.

Optimization in the Polar Ionic Mode

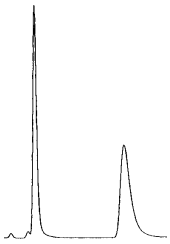
Step 1: Choose the proper acid and base or volatile salt components. To further enhance resolution it has been observed that the ratio of acid to base may need to be altered. Acidic analytes are favored in this mobile phase on the CHIROBIOTIC R. Test the acid to base ratio from 2:1 to 1:2 and so on to find the maximum ratio for the analyte being tested. It is necessary to adjust the ratio so as not to have more acid or base than is necessary. This is especially true when changing from the TEA base to an ammonia base or any acid and base change. The determination of the most useful ratio for other acids or bases can be made by substituting water for the methanol and measuring the apparent pH of the starting composition. Using other bases like ammonia, adjust pH to same value and record the amount used. This is now the new ratio for chromatographic use in the polar ionic mode with methanol as the carrier.

Step 2: Change the flow rate. Lower flow rates often result in higher resolution in this particular mode. Flow rates down to 0.3 mL/min. for a 250x4.6mm column have been reported.

Step 3: 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.

| 2-Bromo-3-methylbutyric acid | FMOC-Methionine |
|---|---|
|  |  |



| | |
|---|---|
| Peak 1 - 6.23 min.<br>Peak 2 - 8.41 min.<br>  <br>CHIROBIOTIC R<br>250x4.6mm<br>100/0.2/0.1:MeOH/HOAc/TEA<br>0.8mL/min. | Peak 1 –5.44 min.<br>Peak 2 – 9.90 min.<br>  <br>CHIROBIOTIC R<br>250x4.6mm<br>100/0.1%w: MeOH/ATFA<br>1.0mL/min. |

## Reversed Phase Separations CHIROBIOTIC R

As in most reversed phase systems, retention and selectivity are controlled by the concentration and nature of the organic modifier, pH and, to a lesser degree, concentration and nature of the buffer. Temperature and flow rate have a significant effect on resolution.

Best Starting Reversed Phase Composition

20/80: MeOH/20mM $NH_4OAc$, pH 6.0

Optimization in the Reversed Phase Mode

Step 1: Evaluate the organic modifier: MeOH>ACN>THF

Organic Modifiers in the Reversed Phase Mode

The CHIROBIOTIC R has shown high selectivity as a function of the organic modifier. Typical U-shaped plots are obtained for retention and resolution with organic modifier/aqueous buffer systems. Typical compositions are 10/90: $CH_3CN$ or THF/buffer and 20/80: alcohol/buffer. Overall, the CHIROBIOTIC R separations appear to favor the alcohol type mobile phase by a large margin and it is, therefore, suggested as the starting mobile phase. The order of priority is $CH_3OH$ > $C_2H_5OH$ > THF > $CH_3CN$ > IPA but changes in the organic modifier, even when the composition is typically <20%, can have a dramatic impact on resolution.

Step 2: Evaluate the concentration of the organic modifier. Higher concentrations result in lower retention.

Step 3: Effect of pH in the reversed phase mode. Working 0.5 pH units above and below pk of the molecule will give the overall impact of pH. If that pH

exceeds the range of the stationary phase, then use the highest possible pH.

It has been determined that the safest and most stable pH range for the CHIROBIOTIC R is 3.5 to 6.8. Decreasing pH to 3.5 produces a significant increase in retention for analytes with free carboxyl groups. In all cases, both selectivity and resolution vary with pH. Nonionizable analytes typically show less variation or a decrease in retention with a decrease in pH.

Step 4: Evaluate the best buffer: TEAA, $NH_4OAc$, $NH_4NO_3$.

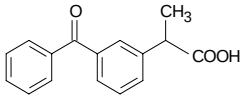
Step 5: Evaluate the concentration of the aqueous buffer. Range 0.05 – 1.0%.

Step 6: Evaluate flow rate. Lower flow rates in the reversed phase mode can have a dramatic effect on resolution. Flow rates down to 0.3 mL/minute can double or triple resolution.

Step 7: Evaluate temperature. Lower temperatures can increase resolution. Higher temperatures can be used to increase efficiency leading to high resolution. Care should be exercised here because higher temperatures have been reported to reverse elution order.

*Optimized Reversed Phase Separation*

Ketoprofen




Peak 1 - 7.46 min.
Peak 2 - 9.55 min.

CHIROBIOTIC R, 250x4.6mm
50/50:MeOH/0.1% TEAA, pH 6.8
0.8 mL/min.

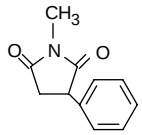
## Normal Phase Separations CHIROBIOTIC R

The CHIROBIOTIC R can be used with all normal phase solvents. There is no limitation on the stability of this stationary phase in any chlorinated or lipophilic solvent. Retention and resolution are controlled by the amount of polar solvent used. Ethanol is recommended more often since it results in more efficient peaks than isopropanol.

Best Starting Normal Phase Composition
20/80: EtOH/Heptane

As with other CHIROBIOTIC phases, all typical normal phase solvents can be used. There is no limit on the stability of this stationary phase in any chlorinated or lipophilic solvent. Many sulfoxide type molecules have been separated in the typical hexane/ethanol type mobile phase.

Phensuximide




Peak 1 - 5.27 min.
Peak 2 - 5.99 min.

CHIROBIOTIC R
40/60: Hex:EtOH
1.5mL/min.

For some N-blocked amino acids that are enantioresolved in hexane/ethanol, small amounts of glacial acetic acid (0.01-0.04) have proven beneficial in improving efficiency and reducing retention (see Ref. 8).

Optimization in Normal Phase Mode

Step 1: Evaluate the polar solvent EtOH vs IPA. Both are useful for different analytes.

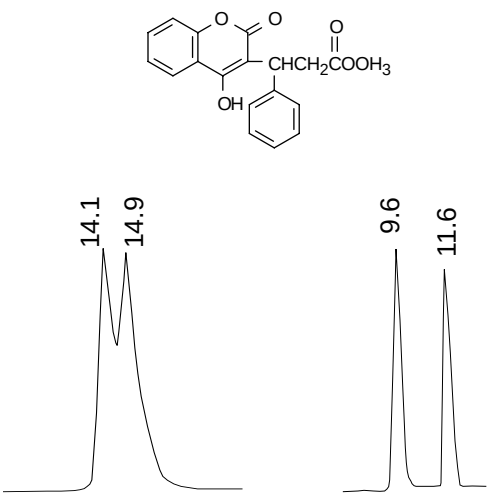
Step 2: Evaluate concentration of the polar solvent. Higher concentrations result in lower retention.

Step 3: Add small amounts of acid and base as modifiers to reduce tailing.

## COMPLEMENTARY SEPARATIONS

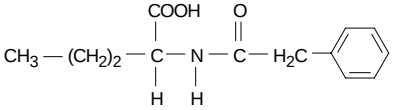
A unique property of the CHIROBIOTIC V, T, TAG and R is their complementary selectivity. Many examples emerged. This characteristic has related primarily to the new polar ionic and reversed phase separations. If after attempts to optimize a separation fail to obtain complete resolution on the CHIROBIOTIC T, then the CHIROBIOTIC R or CHIROBIOTIC TAG is switched into the exact same mobile phase. In some instances the CHIROBIOTIC V is preferred. The reason for this characteristic is the subtle differences in binding sites between these phases.

CHIROBIOTIC T vs. CHIROBIOTIC V
Warfarin



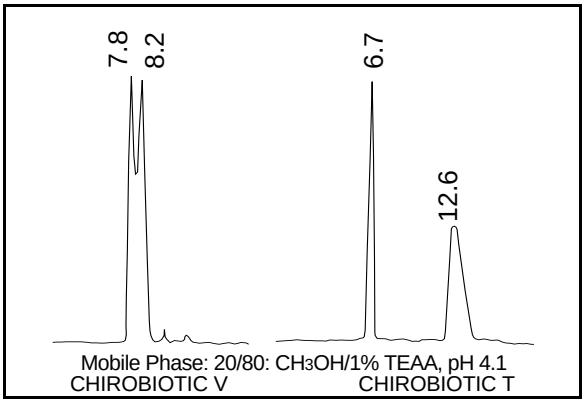

Mobile Phase: 10/90: $CH_3CN$/1% TEAA, pH 4.1
CHIROBIOTIC T CHIROBIOTIC V

CHIROBIOTIC V vs. CHIROBIOTIC T
N-CBZ-Norvaline



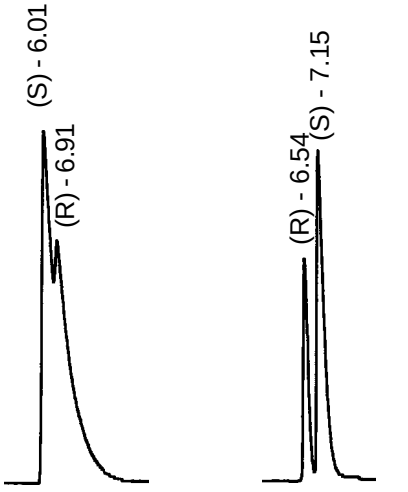
Step 4: Evaluate temperature. Lower temperature can increase resolution. Higher temperature can reverse elution order.

Step 5: Low flow rates have no effect on resolution, therefore, higher flow rates can be used to speed assay results.



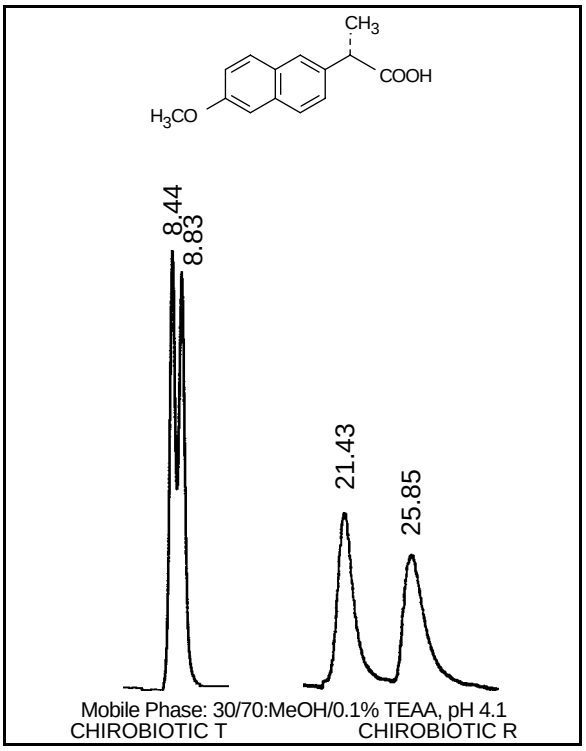

Mobile Phase: 20/80: $CH_3OH$/1% TEAA, pH 4.1
CHIROBIOTIC V CHIROBIOTIC T

CHIROBIOTIC R vs. CHIROBIOTIC V
4-Benzyl-2-oxazolidinone




Mobile Phase: 50/50: Hex/EtOH
CHIROBIOTIC R CHIROBIOTIC V

CHIROBIOTIC T vs. CHIROBIOTIC R
Naproxen




# SCREENING FOR CHIRAL SELECTIVITY

All of the defined mechanisms for chiral recognition are contained within CHIROBIOTIC phases, including ionic interaction, hydrogen bonding, steric and π-π interactions as well as inclusion complexation. This allows for a broad range of chiral separations in all known mobile phase types. The proper mobile phase can be chosen based on the solubility of the analyte. Each of these four CHIROBIOTIC phases has its own unique selectivity characteristics and, in addition, offers "complementary" separations in many cases. The term "complementary" describes the mobile phase condition where an increase in selectivity is obtained when switching from one CHIROBIOTIC phase to another in the same mobile phase. The fact that four basic mobile phase systems are used together with four 10cm CHIROBIOTIC columns allows for very broad chiral selectivity. It has further been observed that if a Rs of 0.6 or greater is obtained in the column screen a Rs >1.5 can be optimized on a 25cm column for the selected stationary phases.

## Kit Description

The Chiral Selectivity Screening Kit offers the chromatographer a broad range of separation capabilities using a simplified method to screen for chiral selectivity.

The kit contains:
1 - CHIROBIOTIC V HPLC Column, 100x4.6mm
1 - CHIROBIOTIC T HPLC Column, 100x4.6mm
1 - CHIROBIOTIC TAG HPLC Column, 100x4.6mm
1 - CHIROBIOTIC R HPLC Column, 100x4.6mm
2 - Column Couplers
1 - CHIROBIOTIC Handbook

## Solvent Storage

The stationary phases are stored in MeOH which is both useful for long term storage and the ability to efficiently proceed in either reversed phase, polar organic or polar ionic modes. It is suggested that after finishing any experimentation the columns be washed with a mobile phase of 50/50: ACN/20mM $NH_4OAc$ for at least 20 column volumes. When using for normal phase screening, use EtOH or IPA as an intermediate solvent.

## Stationary Phases versus Stereochemical Structures

**New polar ionic mode:** Requires compounds with two or more functional groups and should be tried first. It gives short run time with high efficiency. Functional groups include alcohols, halogens (I,Br, Cl, F), amines, (1°,2°,3°), carbonyl, carboxyl, oxidized forms of sulfur and phosphorus.

CHIROBIOTIC V: Amino alcohols and (cyclic) amines.
CHIROBIOTIC T: Amino alcohols, acids and N-blocked amino acids.
CHIROBIOTIC TAG: Amino alcohols and all types of acids
CHIROBIOTIC R: α-Hydroxy/halogenated acids, profens and N-blocked amino acids

**Reversed phase mode:** statistically separates the most compounds.

CHIROBIOTIC V: Amines, imides, coumarins profens
CHIROBIOTIC T: α-Hydroxy acids, oxazolidinones, underivatized and N-blocked amino acids and peptides
CHIROBIOTIC TAG: N-blocked amino acids and α-hydroxy/halogenated acids
CHIROBIOTIC R: α-Hydroxy acids, substituted aliphatic acids, profens, N-blocked amino acids, hydantoins and peptides

**Polar organic mode:** Separates neutral molecules

CHIROBIOTIC V: Cyclic amides, uracils, hydantoins.
CHIROBIOTIC T: Cyclic amides, uracils, hydantoins, benzodiazepines
CHIROBIOTIC TAG: Cyclic amides, uracils, hydantoins, oxazolidinones, benzodiazepines
CHIROBIOTIC R: Cyclic amides

**Normal phase mode:** Typical alcohol/hexane combinations.

CHIROBIOTIC V: Hydantoins, barbiturates, imides and oxazolidinones.
CHIROBIOTIC T: Hydantoins and imides.
CHIROBIOTIC TAG: Sulfoxides, hydroxy coumarins, hydantoins, oxazolidinones
CHIROBIOTIC R: Imides, hydantoins and N-blocked amino acids.

## Suggested Blind Screening Sequence 100x4.6mm Columns

| Column | CHIROBIOTIC V and T |
|---|---|
| Reversed Phase | 20/80: MeOH/20mM $NH_4OAc$, pH 4.0 |
| Polar Ionic Mode | 100/0.02/0.01: MeOH/HOAc/TEA |
| Normal Phase | 20/80: EtOH/Heptane |
| Polar Organic Mode | EtOH |

| Column | CHIROBIOTIC R and TAG |
|---|---|
| Reversed Phase | 20/80: MeOH/20mM $NH_4OAc$, pH 6.0 |
| Polar Ionic Mode | 100/0.02/0.01: MeOH/HOAc/TEA |
| Normal Phase | 20/80: EtOH/Heptane |
| Polar Organic Mode | EtOH |

*Note: TEAA is triethylammonium acetate. It is prepared by diluting HPLC grade anhydrous triethylamine to 0.1% (v/v) with HPLC grade water and adjusting the pH with 5% acetic acid to pH 4.0 or 6.0.*

*Note: Ethanol is also used to rinse column between reversed phase and polar ionic or normal phase modes.*

Run Conditions:

| | |
|---|---|
| Flow rate | 1.0 mL/min. |
| Equilibration time | 15 minutes |
| Run time | 25 minutes |
| Temperature | Ambient |
| Detector | UV |
| Sample | 1 mg/mL |

## General Operating Instructions for Screening

### Injection Volumes and Concentrations

Typical samples of 1-5µL of a 1 mg/mL concentration are required for good sensitivity. In other cases, the load volume and concentration may be 5 to 10 times higher without affecting resolution. Therefore, begin the separation study at the lowest volumes and concentrations until a proper determination can be made of its effect. All Astec columns may be operated from either direction without loss of performance due to the uniform packing system that produces uniform packing density

### Pressure

Operating pressure for the CHIROBIOTIC phases (100x4.6mm) is generally in the range of 300-500 psi for the new polar ionic mode, 600-800 psi for the polar organic mode, 800-1200 psi for the reversed phase and about 500-700 psi for the normal phase mode. Care should always be exercised in prefiltering and degassing the buffer and solvent when used with these columns. In general, pressure should not exceed 3,500 psi.

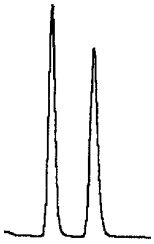
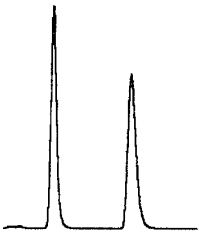
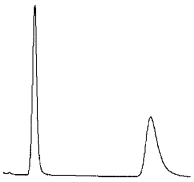
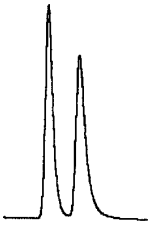
### Storage

Subsequent to the actual quality control test, the column is conditioned with methanol for storage and shipment. When analysis is complete, the column should be returned to this solvent to ensure long life. If in reversed phase conditions, wash the column with water first removing buffer before storage in methanol.

### Quality Control Test Procedures

Quality control tests are recommended to be used routinely to assure consistent performance.
Conditions for all columns:

Sample: 5-Methyl-5-Phenylhydantoin (Aldrich 18,082-3)
Column size: 250x4.6mm
Mobile phase: 100% MeOH
Flow rate: 1.0 mL/min.
Detection: UV220nm

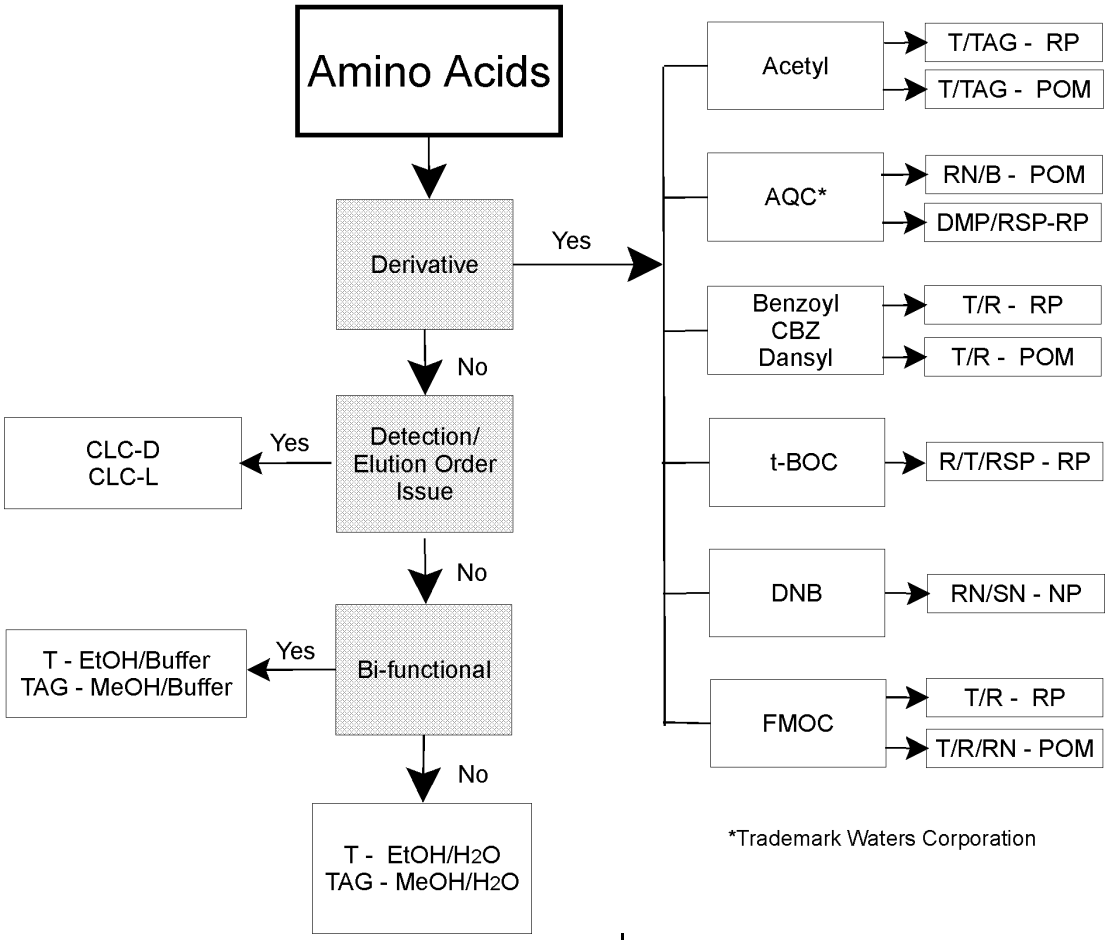
| CHIROBIOTIC V | CHIROBIOTIC T | CHIROBIOTIC TAG | CHIROBIOTIC R |
|---|---|---|---|
| Peak 1 – 3.70 min.<br>Peak 2 – 4.21 min. | Peak 1 – 3.83 min.<br>Peak 2 – 5.05 min. | Peak 1 – 3.98 min.<br>Peak 2 – 7.22 min. | Peak 1 – 4.15 min.<br>Peak 2 – 4.63 min. |
|  |  |  |  |

*Note: For 100x4.6mm columns, set flow rate to 0.5 mL/min.*

# ENANTIOMERIC SEPARATION OF AMINO ACIDS AND N-BLOCKED AMINO ACIDS

As a result of the explosive field of proteomics and its essential value to human health, a wide variety of single amino acid enantiomers and derivatives have increased interest to the pharmaceutical and biotechnology industries. The analytical method development and large scale preparation of these analytes often require the separation of enantiomers or diastereomers by HPLC. A number of CHIROBIOTIC phases have been identified as excellent chiral stationary phases for the separation of this class of molecules. In order to identify the best possible column and conditions for each of the numerous possibilities we have created a decision tree. Information on the CYCLOBOND phases can be found in the CYCLOBOND HANDBOOK, a guide to using cyclodextrin bonded phase for chiral LC separations. The CHIROBIOTIC T and CHIROBIOTIC TAG have been found to be the most useful CSPs for the separation of D,L-amino acids and many of the N-blocked amino acids. For complex matrices, i.e., food products where detection is an issue, the Astec CLC columns with copper coordination complex first proposed by Davenkov, have been used.

# Decision Tree for the Separation of D,L-amino acids and N-blocked Amino Acids




*Trademark Waters Corporation

## Legend for Decision Tree Column Designations

Column Designations

| | |
|---|---|
| CLC-D | Astec CLC, D configuration |
| CLC-L | Astec CLC, L configuration |
| B | CYCLOBOND I *2000* |
| DMP | CYCLOBOND I *2000* DMP |
| RSP | CYCLOBOND I *2000* RSP |
| RN | CYCLOBOND I *2000* RN |
| SN | CYCLOBOND I *2000* SN |
| V | CHIROBIOTIC V |
| T | CHIROBIOTIC T |
| TAG | CHIROBIOTIC TAG |
| R | CHIROBIOTIC R |

Mobile Phase Designations

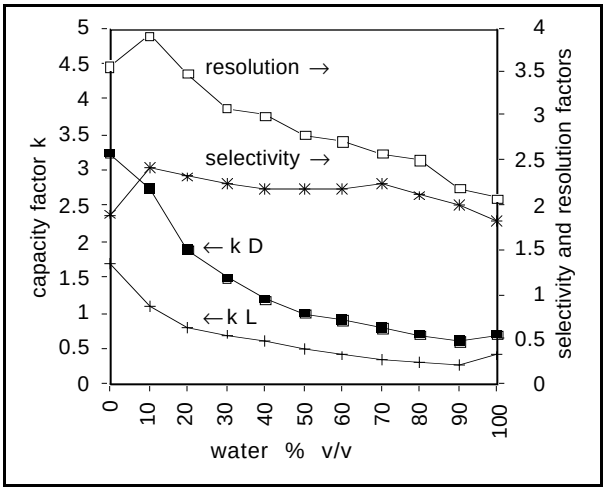
POM Polar Organic Mode
For CYCLOBOND phases: 95/5/0.3/0.2:
$CH_3CN$/MeOH/HOAc/TEA
For CHIROBIOTIC phases: 100/0.1/0.1:
MeOH/HOAc/TEA
RP Reversed Phase
NP Normal Phase

*Note : A complete description of the CLC and CYCLOBOND chiral stationary phases can be found in the Astec Chromatography Product Guide.*

# Underivatized D,L-Amino Acids

Underivatized D,L-amino acids can be separated in simple ethanol or methanol and water mobile phases. This type of mobile phase allows for low UV detection or the use of light scattering detectors. Note in the following chart the effect of organic composition on resolution. An increase in the alcohol content of the mobile phase typically results in a dramatic increase in resolution while selectivity is largely unaffected.

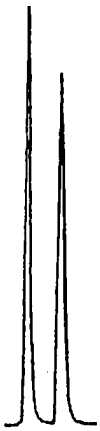
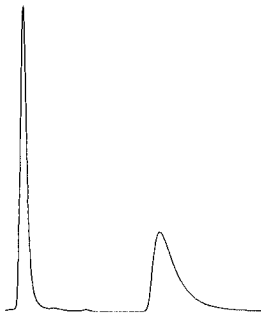
## *Effect of Alcohol Modifier on Retention, Selectivity and Resolution*
## *Sample: Methionine*



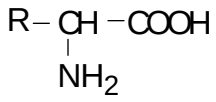

*Note: To detect amino acids at 195-210nm UV the best quality water and methanol must be used.*

The table on page 28 lists the common, essential, α-amino acids separated on the three most useful CHIROBIOTIC phases. Beta, gamma and cyclic amino acids have been separated as well. Separation data for these latter types of amino acids can be found in the references listed below the chart. It should also be noted that the bi-functional amino acids require control of the pH with the addition of an appropriate buffer. While triethylamine acetate has been reported here, the use of phosphate and ammonium acetate buffers have also been successfully employed.

*Comparison of the CHIROBIOTIC T and CHIROBIOTIC TAG*
*Enantiomeric Separation of D,L-Methionine*

| CHIROBIOTIC T | CHIROBIOTIC TAG |
|---|---|
| $t_1$ = 4.4<br>$t_2$ = 5.4 | $t_1$ = 4.7<br>$t_2$ = 8.0 |
|  |  |
| 10/90: EtOH/$H_2O$ @ 1.0 mL/lmin.<br>Detection: 210nm | 30/70: MeOH/$H_2O$ @ 1.0 mL/min.<br>Detection: 210nm |

*Enantioresolution of Underivatized α-Amino Acids*

|  | | CHIROBIOTIC T[C1] | | CHIROBIOTIC TAG[C2] | | CHIROBIOTIC R[C3] | |
|---|---|---|---|---|---|---|---|
| α-Amino Acid | R-Moiety | k′ | Rs | k′ | Rs | k′ | Rs |
| Alanine | -$CH_3$ | 0.56 | 2.9 | 0.16 | 4.0 | 0.30 | 1.7 |
| Arginine | -$(CH_2)_3$-NH-CNH-$NH_2$ | 1.17 | 2.1 | 2.17 | 3.0 | N/A | N/A |
| Aspartic | -$CH_2$-COOH | 1.49 | 1.9 | 0.95 | 2.0 | N/A | N/A |
| Asparagine | -$CH_2$-CO-$NH_2$ | 0.58 | 2.1 | 0.29 | 3.7 | 1.45 | 1.56 |
| Cysteine | -$CH_2$-SH | 0.45 | 1.6 | 0.20 | 1.8 | 1.78 | 1.50 |
| Glutamic | -$CH_2$-$CH_2$-COOH | 1.15 | 2.2 | 0.64 | 2.5 | N/A | N/A |
| Glutamine | -$(CH_2)_2$-$CONH_2$ | 1.13 | 1.6 | 0.82 | 3.5 | N/A | N/A |
| Histidine | | 3.10 | 1.5 | 3.96 | 1.5 | 1.13 | 1.45 |
| Isoleucine | -CH($CH_3$)-$CH_2$-$CH_3$ | 0.40 | 2.5 | 0.18 | 3.0 | 1.03 | 2.9 |
| Leucine | -$CH_2$-CH-$(CH_3)_2$ | 0.47 | 3.5 | 0.60 | 5.5 | 0.27 | 2.2 |
| Lysine | -$(CH_2)_4$-$NH_2$ | 0.81 | 2.2 | 1.21 | 2.5 | 1.27 | 1.97 |
| Methionine | -$CH_2$-$CH_2$-S-$CH_3$ | 0.55 | 3.3 | 0.47 | 3.5 | 1.23 | 2.5 |
| Phenylalanine | | 0.87 | 2.0 | 0.98 | 7.2 | 0.64 | 2.5 |
| Proline | | 0.58 | 2.5 | 0.43 | 6.2 | 2.00 | 3.24 |
| Serine | -$CH_2$OH | 0.69 | 1.5 | 0.11 | 1.9 | 1.13 | 0.8 |
| Threonine | -CHOH-$CH_3$ | 0.75 | 1.4 | 0.46 | 4.0 | 0.19 | 1.0 |
| Tyrosine | | 0.60 | 1.9 | 0.76 | 2.9 | 0.52 | 1.0 |
| Tryptophan | | 1.01 | 2.0 | 2.05 | 3.5 | 1.12 | 2.0 |
| Valine | -CH($CH_3$)$_2$ | 0.56 | 1.9 | 2.48 | 4.5 | 1.22 | 2.0 |

N/A - not available.

A. Typical mobile phases for amino acids:

1. Neutral
   - T: 60/40: EtOH/$H_2O$
   - TAG: 30/70: MeOH/$H_2O$ or 60/40: MeOH/H2O
   - R: 50/50: MeOH/$H_2O$ or 50/50: ACN/H2O
2. Acidic
   - T: 60/40: EtOH/$H_2O$, pH 3.8 with HOAc
   - TAG: 80/20: MeOH/$H_2O$, pH 3.8 with HOAc
3. Basic:
   - T: 50/50: EtOH/100mM $NaH_2PO4$
   - TAG: 50/50: MeOH/100mM $NaH_2PO4$

B. L-form eluted first for all cases.

C. For more information, please consult the following articles:


(1) Berthod, Liu, Bagwell and Armstrong, J. Chromatog. A., 731, 123-137 (1996)

(2) Berthod, Gasparrini and Carotti, Anal. Chem. Vol. 72, 1767-1780 (2000).

(3) Ekborg-Ott, Liu and Armstrong, Chirality 10, 434-483 (1998).


Removal of the carbohydrates from the CHIROBIOTIC T created the CHIROBIOTIC TAG which demonstrates very high selectivity for amino acids, especially useful for preparative applications.

## Amino Acids Derivatives

To enhance selectivity or resolution and to enhance detection, different derivatizing methods; FMOC,t-BOC, AQC, Acetyl, Dansyl and others have been employed. We supply below information on some of the more widely used derivatives. Please note that it is essential that the analyte have a ″free carboxyl group″ for these chiral stationary phases to demonstrate selectivity. A comprehensive handbook has been prepared to more thoroughly cover this matter. For more extensive information, request a copy of the Handbook of Amino Acid Separations.

### 1. N-FMOC (9-Fluorenylmethyl chloroformate) D,L-Amino Acids

The two best stationary phases for the separation of FMOC-D,L essential amino acids are the CHIROBIOTIC T and CHIROBIOTIC R. The chart below lists the columns, mobile phase conditions and resolution data. Other FMOC D,L-amino acids have been separated. For more extensive coverage of the separation of this class of molecule see the Handbook of Amino Acid Separations.

*Chiral Separation of N-FMOC Amino Acids*

| Compound | Mobile Phase | Column | $k_1$ | $\alpha$ | Rs |
|---|---|---|---|---|---|
| Alanine | 50/50, MeOH/20mM $NH_4OAc$ | R | 0.38 | 3.89 | 3.5 |
| | 40/60, MeOH/1% TEAA, pH 4.1 | T | 1.26 | 2.27 | 5.5 |
| | 100/0.02w%, MeOH/$NH_4OAc$ | R | 0.57 | 2.37 | 2.2 |
| Arginine | 20/80, MeOH/0.1%TEAA, pH 6.8 | R | 3.28 | 1.46 | 1.6 |
| | 100/0.1w%, MeOH/$NH_4TFA$ | R | 1.69 | 2.95 | 4.6 |
| Asparagine | 100/1/1, MeOH/HOAc/TEA | T | | | 1.7 |
| | 40/60, MeOH/1% TEAA, pH 4.1 | T | 0.63 | 1.81 | 3.0 |
| | 100/0.1w%, MeOH/$NH_4TFA$ | R | 4.41 | 1.22 | 1.3 |
| | 30/70, MeOH/20mM $NH_4OAc$ | R | 1.55 | 1.49 | 1.8 |
| Aspartic acid | 20/80, MeOH//0.1%TEAA, pH 6.8 | R | | | 2.0 |
| | 40/60, MeOH/0.1% TEAA, pH 4.1 | T | 0.46 | 1.68 | 1.8 |
| | 100/0.1w%, MeOH/$NH_4TFA$ | R | 2.59 | 1.23 | 1.3 |
| Citrulline | 40/60,MeOH/0.1% TEAA, pH 4.1 | T | 1.07 | 2.50 | 4.0 |
| | 100/1/1, MeOH/HOAc/TEA | T | | | 3.0 |
| | 30/70, MeOH/20mM $NH_4OAc$ | R | 1.34 | 2.05 | 2.6 |
| Cysteine | 65/35, EtOH/Hexane | R | | | 1.7 |
| Glutamic acid | 20/80, MeOH/0.1%TEAA, pH 6.8 | R | | | 1.6 |
| | 100/1/1, MeOH/HOAc/TEA | T | | | 1.3 |
| | 40/60, MeOH/0.1% TEAA, pH 4.1 | T | 1.07 | 1.60 | 3.8 |
| Glutamine | 40/60, MeOH/0.1% TEAA, pH 4.1 | T | 0.61 | 2.85 | 5.0 |
| | 100/0.1w%, MeOH/$NH_4OAc$ | R | 1.90 | 2.04 | 3.8 |
| | 30/70, MeOH/20mM $NH_4OAc$ | R | 0.93 | 2.46 | 3.6 |
| Histidine | 20/80, MeOH/0.1%TEAA, pH 4.1 | R | | | 1.0 |
| Isoleucine | 40/60, MeOH/0.1% TEAA, pH 4.1 | T | 1.08 | 1.78 | 2.2 |
| | 100/0.1w%, MeOH/$NH_4OAc$ | R | 0.45 | 1.87 | 2.3 |
| | 30/70, MeOH/20mM $NH_4OAc$ | R | 2.32 | 1.85 | 1.6 |
| Isoleucine,allo | 100/0.1w%, MeOH/$NH_4OAc$ | R | 0.53 | 1.57 | 2.0 |
| Isoserine | 65/35, EtOH/Hexane | R | | | 1.5 |
| | 50/50, MeOH/1% TEAA, pH 5.5 | T | | | 4.5 |
| Leucine | 40/60, MeOH/0.1% TEAA, pH 4.1 | T | 1.03 | 2.45 | 5.0 |
| | 100/0.1w%, MeOH/$NH_4TFA$ | R | 0.46 | 2.41 | 3.5 |
| Lysine | 50/50, MeOH/1% TEAA, pH 5.5 | T | | | 1.4 |
| | 100/0.1w%, MeOH/$NH_4TFA$ | R | 0.79 | 2.12 | 3.4 |
| Methionine | 40/60, MeOH/0.1% TEAA, pH 4.1 | T | 0.96 | 3.43 | 6.0 |
| | 100/1/1, MeOH/HOAc/TEA | T | | | 3.0 |
| | 100/0.1w%, MeOH/$NH_4TFA$ | R | 0.94 | 2.70 | 4.5 |
| | 50/50, MeOH/20mM $NH_4OAc$ | R | 0.27 | 5.77 | 5.4 |
| Norleucine | 40/60, MeOH/0.1%TEAA, pH 4.1 | T | 1.20 | 2.87 | 6.5 |
| | 100/0.1w%,MeOH/$NH_4TFA$ | R | 0.50 | 2.0 | 3.0 |
| | 30/70, MeOH,20mM $NH_4OAc$ | R | 2.92 | 2.15 | 3.0 |
| Norvaline | 100/0.1w%, MeOH/$NH_4TFA$ | R | 0.61 | 2.56 | 3.5 |
| | 30/70, MeOH/20mM $NH_4OAc$ | R | 2.12 | 3.56 | 6.5 |
| | 40/60, MeOH/0.1% TEAA, pH 4.1 | T | 0.99 | 3.19 | 5.5 |
| Ornithine | 50/50, MeOH/1% TEAA, pH 5.5 | T | | | 1.4 |
| | 100/0.1w%, MeOH/$NH_4TFA$ | R | 1.22 | 1.72 | 3.0 |
| Phenylalanine | 100/1/1, MeOH/HOAc/TEA | T | | | 3.0 |
| | 40/60, MeOH/0.1% TEAA, pH 4.1 | T | 1.54 | 2.82 | 6.0 |
| | 100/0.02w%, MeOH/$NH_4OAc$ | R | 0.45 | 6.65 | 5.0 |
| | 50/50, MeOH/20mM $NH_4OAc$ | R | 0.12 | 8.53 | 6.0 |
| Proline | 100/0.02/0.01, MeOH/HOAc/TEA | R | | | 1.0 |
| | 95/5/0.3/0.2, ACN/MeOH/AA/TEA | CBII | | | 1.4 |
| | 100/0.4/0.6, ACN/HOAc/TEA | RN | | | 4.1 |
| Serine | 20/80, MeOH/0.1%TEAA, pH 6.8 | R | | | 1.6 |
| | 100/1/1.5, MeOH/HOAc/TEA | T | | | 1.4 |

| | | | | | |
|---|---|---|---|---|---|
| | 20/80, MeOH/0.1% TEAA, pH 4.1 | T | 2.28 | 1.61 | 3.3 |
| | 30/70, MeOH/20mM $NH_4OAc$ | R | 1.07 | 1.80 | 2.5 |
| Threonine | 30/70, MeOH/0.1%TEAA, pH 4.1 | T | 1.56 | 1.35 | 1.7 |
| | 30/70, MeOH/20mM $NH_4OAc$ | R | 0.93 | 1.44 | 1.8 |
| Tryptophan | 65/35, EtOH/Hexane | R | | | 1.1 |
| | 40/60, MeOH/0.1% TEAA, pH 4.1 | T | 2.04 | 1.88 | 3.7 |
| | 100/0.1w%, MeOH/$NH_4$TFA | R | 1.21 | 6.16 | 5.8 |
| | 100/0.02w%,MeOH/$NH_4OAc$ | R | 0.34 | 9.30 | 5.3 |
| p-Tyrosine | 100/0.02w%,MeOH/$NH_4OAc$ | R | 0.44 | 6.76 | 5.0 |
| Valine | 40/60, MeOH/0.1% TEAA, pH 4.1 | T | 0.90 | 1.90 | 4.0 |
| | 30/70, MeOH/20mM $NH_4$Oac | R | 2.01 | 2.68 | 4.0 |
| | 100/0.1w%, MeOH/$NH_4$TFA | R | 0.70 | 1.76 | 2.5 |

Legend: TEAA = Triethylammonium acetate
T = CHIROBIOTIC T
R = CHIROBIOTIC R
RN = CYCLOBOND I *2000* RN (R-naphtylethyl carbamate)
CBII = CYCLOBOND II (gamma-cyclodextrin) (See CYCLOBOND HANDBOOK).

## 2. N-tert-Butoxycarbonyl (t-BOC) Amino Acids

The cyclodextrin phase CYCLOBOND I *2000* RSP has been the most widely used stationary phase for the separation of this class of derivatized amino acids. It has been found, however, that the CHIROBIOTIC T and CHIROBIOTIC R complement this phase quite well. The chart below can be used as a guide for the separation of this class of analytes.

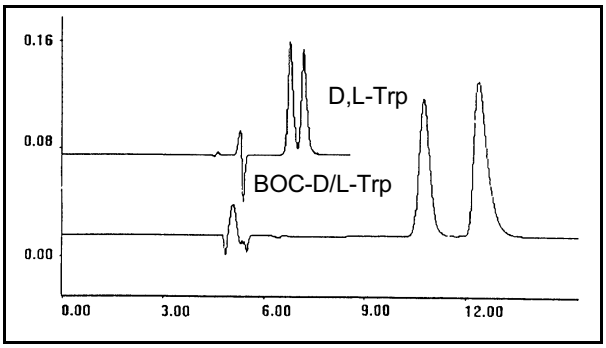
### *Chiral Separation of N-t-BOC Amino Acids*

| Compound | Mobile Phase | Column | $k_1$ | $\alpha$ | Rs |
|---|---|---|---|---|---|
| Alanine | 20/80, MeOH/0.1%TEAA, pH 4.1 | R | 1.08 | 1.77 | 3.3 |
| | 10/90, MeOH/0.1%TEAA, pH 4.1 | T | 0.45 | 1.55 | 2.4 |
| Asparagine | 20/80, MeOH/0.1%TEAA, pH 4.1 | T | 1.30 | 1.36 | 2.0 |
| Glutamine | 20/80, MeOH/0.1%TEAA, pH 4.1 | R | 1.10 | 1.38 | 2.0 |
| | 10/90, MeOH/0.1%TEAA, pH 4.1 | T | 0.41 | 1.37 | 1.4 |
| Histidine | 20/80, MeOH/0.1%TEAA, pH 6.0 | R | 0.81 | 1.37 | 1.8 |
| | 20/80, MeOH/0.1%TEAA, pH 4.1 | T | 1.53 | 1.66 | 2.0 |
| Isoleucine | 20/80, MeOH/0.1%TEAA, pH 4.1 | R | 1.67 | 1.25 | 1.6 |
| | 10/90, ACN/20mM NH4OAc | RSP | 2.00 | 1.54 | 1.6 |
| Methionine | 20/80, MeOH/0.1%TEAA, pH 6.0 | R | 0.34 | 20.3 | 12.0 |
| | 20/80, MeOH/0.1%TEAA, pH 4.1 | T | 0.48 | 3.90 | 5.5 |
| | 10/90, ACN/20mM $NH_4OAc$ | RSP | 1.20 | 4.09 | 10.0 |
| Phenylalanine | 20/80, MeOH/0.1%TEAA, pH 6.0 | R | 1.02 | 3.30 | 4.8 |
| | 10/90, MeOH/0.1%TEAA, pH 6.0 | T* | 0.44 | 1.55 | 1.6 |
| | 10/90, ACN/20mM $NH_4OAc$ | RSP | 1.90 | 1.16 | 1.2 |
| Phenylglycine | 20/80, MeOH/0.1%TEAA, pH 6.0 | R | 0.15 | 9.24 | 5.0 |
| | 20/80, MeOH/0.1%TEAA, pH 4.1 | T | 0.52 | 4.65 | 3.5 |
| | 10/90, ACN/20mM $NH_4OAc$ | RSP | 3.59 | 1.29 | 1.5 |
| Serine | 20/80, MeOH/0.1%TEAA, pH 4.1 | R | 0.88 | 1.30 | 2.4 |
| Tryptophan | 20/80, MeOH/0.1%TEAA, pH 6.0 | R | 0.61 (D) | 3.89 | 5.4 |
| | 20/80, MeOH/0.1%TEAA, pH 4.1 | T | 0.73 (D) | 2.17 | 2.2 |
| | 10/90, ACN/20mM $NH_4OAc$ | RSP | 1.46 (D) | 2.96 | 5.7 |
| p-Tyrosine | 20/80, MeOH/0.1%TEAA, pH 6.0 | R | 0.79 | 4.31 | 5.5 |
| | 10/90, MeOH/0.1%TEAA, pH 6.0 | T* | 0.24 | 1.77 | 1.4 |
| | 10/90, ACN/20mM $NH_4OAc$ | RSP | 1.05 | 1.16 | 1.3 |
| Valine | 20/80, MeOH/0.1%TEAA, pH 4.1 | R | 1.44 | 1.26 | 2.0 |
| | 10/90, ACN/20mM $NH_4OAc$ | RSP | 1.64 | 1.45 | 1.6 |

Legend: TEAA = Triethylammonium acetate
T = CHIROBIOTIC T
R = CHIROBIOTIC R
RSP = CYCLOBOND I *2000* RSP

Flow rate = 1mL/min; *0.5mL/min
UV = 220 nm
For all compounds tested, L-form eluted first except for tryptophan.

*Enantioseparation of D,L-Try and N-t-BOC-Tryptophan on CHIROBIOTIC T*




20/80: Acetonitrile/1% Triethylamine Acetate, pH 4.1

Ref. E. Tesarova, Z. Bosakova, V. Pacakova, J. Chromatogr. A, 838, 121-129 (1999).

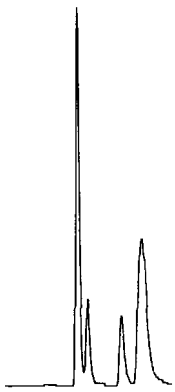
## 3. Amino Acid Diastereomers

A number of amino acids i.e., isoleucine and threonine, have allo forms. The CHIROBIOTIC R and CHIROBIOTIC TAG have proven useful for the separation of these pairs of enantiomers in simple alcohol/water mixtures.

*Preparative Purification of Isoleucine on CHIROBIOTIC TAG*

### Isoleucine

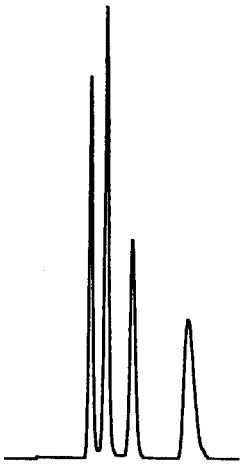
Peak 1 - L-Isoleucine
Peak 2 - L-Allo-Isoleucine
Peak 3 - D-Allo-Isoleucine
Peak 4 - D -Isoleucine



CHIROBIOTIC R, 250x4.6mm
80/20: MeOH/$H_2O$ @ 0.6 mL/min.
Detector: ELSD
T=80°C, Nitrogen Flow: 0.8 SLPM

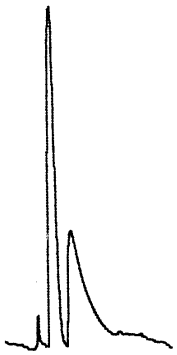
### Threonine

Peak 1,3- DL-Threonine
Peak 2,4 - DL-Allo-Threonine



CHIROBIOTIC TAG, 250x4.6mm
60/40: MeOH/$H_2O$ @ 1.0 mL/min.
Detector: ELSD
T=85°C, Nitrogen Flow: 0.9 SLPM

### Isoleucine



CHIROBIOTIC TAG
250x22.1mm
30/70: MeOH/$H_2O$
20 mL/min.
Inj. - 170 mg in 10 mL mobile phase

*Note: This preparative separation is typical of the capacity of the CHIROBIOTIC TAG for amino acid purification.*

# PREPARATIVE SEPARATIONS

CHIROBIOTIC phases offer unique opportunities for preparative purifications. Key factors are:

1. No solvent limitations. Halogenated solvents as well as very polar solvents are well tolerated on these CSPs. This solvent tolerance is especially useful when optimizing for sample solubility.
2. Can be run in four distinctly different mobile phase types. Use of acid/base on any one of these CSPs does not preclude their use in other mobile phases. Mobile phases listed here in the order of success:
   a) Polar ionic mode©:
      MeOH/Acid/Base or
      MeOH/$NH_4$ salt
   b) Reversed phase mode:
      ACN/Buffer

*Please note efficient workup procedures available for the reversed phase mode. Method outlined for analytical C18 column. See workup following Example 3: Warfarin.*

   c) Polar orgoanic mode:
      MeOH, EtOH, ACN or combination
   d) Normal phase mode:
      Heptane/EtOH

*Note: The chiral recognition mechanism is different in each of these mobile phase types.*

3. Very long term stability with these CSPs due, in part, to the multiple linkages used in anchoring the CSP and, secondly, to the mild conditions typically required.

4. Range of capacities is compound dependent. Significantly overlaps cellulose and amylase phases based on throughput primarily because separations on these CSPs are usually very fast. Capacities on CHIROBIOTIC V2/T2 phases have been 2.5 mg/gm with an alpha of 1.5. Maximum capacity achieved was 300mg “on column” using a 250x21.2mm column with an alpha of 2.0.

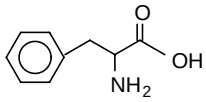
5. Excellent economics especially with the polar organic and polar ionic modes. Ionic interactions play a significant role in the chiral recognition mechanism on these phases. Solvents here are anhydrous, more volatile and less toxic than the typical normal phase solvents.

## Preparative Method Development Procedure

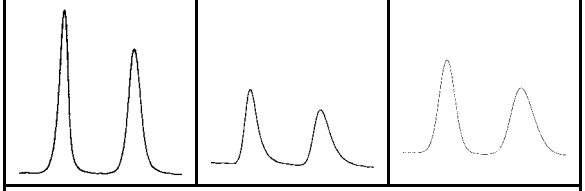
1. Test sample solubility in various solvent systems.
2. Screen multiple columns in mobile phase for maximum analyte solubility and maximum capacity.
3. Detune detector sensitivity.
4. Optimize conditions on the best CSP.
5. Correlation between analytical (5µm) and preparative (10µm or 16µm) packing materials.
6. Overload an analytical column packed with 10 or 16µm material. Target 85% + resolution.
7. Determine purity and yield from analytical run.
8. Determine options for any interfering impurities.
9. Evaluate systems and features available as maximum flow rate versus operating pressure, recycling and shaving, variable wavelength and minimized data point collection, all to determine final column dimensions.

*Comparison of 5µm, 10µm and 16µm CHIROBIOTIC T Separations*

Phenylalanine

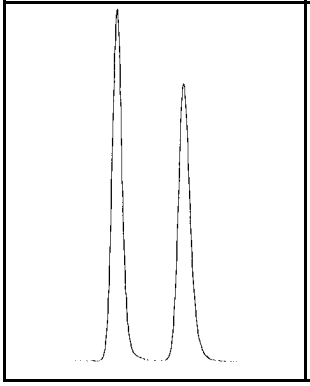
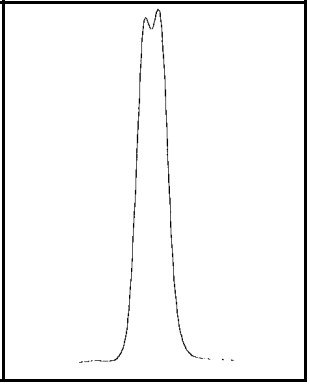



| 5µm | 10µm | 16µm |
|---|---|---|
| Peak 1 – 5.75<br>Peak 2 – 7.17 | Peak 1 – 5.86<br>Peak 2 – 7.13 | Peak 1 – 5.84<br>Peak 2 – 7.57 |



CHIROBIOTIC T, 250x4.6mm
50/50: EtOH/$H_2O$
0.9 mL/min.

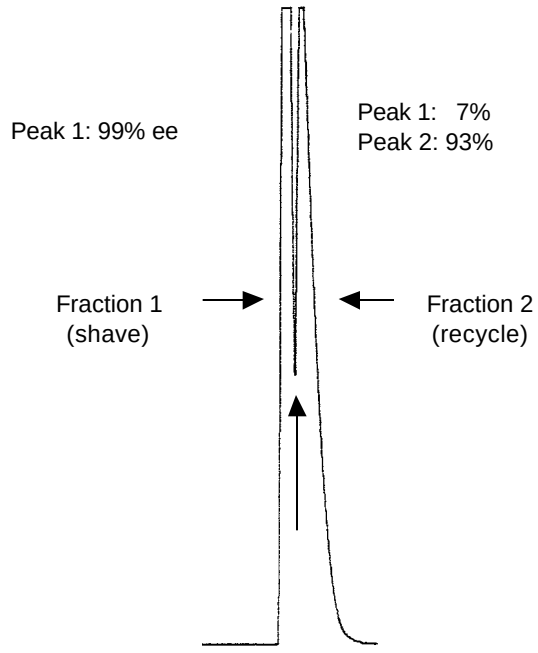
*Case Study: Nicardipine*
*Evaluation of Volatile Buffers*

| 100/0.1w%: MeOH/$NH_4TFA$ | 100/0.1w%: MeOH/$NH_4OAc$ |
|---|---|
| Peak 1 – 4.07 min.<br>Peak 2 – 4.69 min.<br>α - 1.50 | Peak 1 – 3.41 min.<br>Peak 2 – 3.47 min.<br>α - 1.09 |
|  |  |

*Recycle and Shave Purification Method Using the New Polar Ionic Mode*

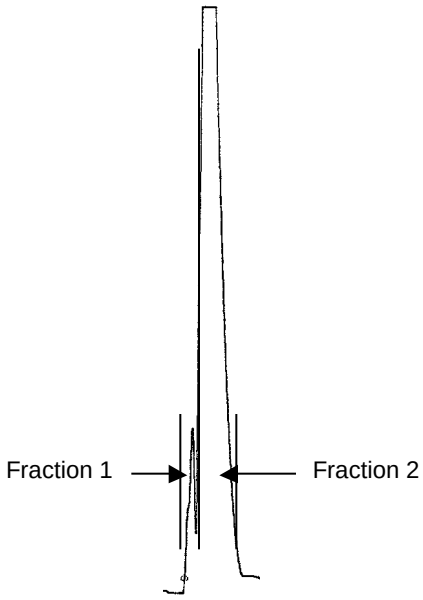
Nicardipine

Peak 1 – 4.95 min.
Peak 2 – 5.24 min.




CHIROBIOTIC V
250x22.1mm
100/0.005/0.002: MeOH/TFA/$NH_4OH$
18 mL/min.
Inj. 25 mg
UV-254nm

Recycle Peak 2 - Nicardipine




CHIROBIOTIC V
250x22.1mm
100/0.005/0.002: MeOH/TFA/$NH_4OH$
18 mL/min.
Inj. 20 mg
UV-254nm

| Final Results | Peak 1 | Peak 2 |
|---|---|---|
| ee | 99% | 99% |
| Yield | 95% | 90% |

## Method Development Protocol for Preparative Reversed Phase

When sample solubility is a concern, compromise between selectivity and solubility by varying the concentration of organic modifier/buffer.
Target 85% + resolution.

Example: Warfarin (not soluble in $H_2O$)

| EtOH/0.1% TEAA, 4.1 | k1 | Solubility (mg/mL) | Selectivity |
|---|---|---|---|
| 20/80 | 4.94 | 0.1 | 1.50 |

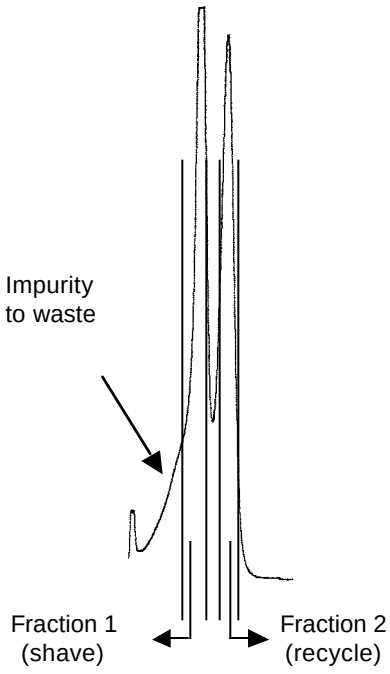
| | | | |
|---|---|---|---|
| 30/70 | 2.31 | 0.3 | 1.60 |
| 40/60* | 2.19 | 0.6 | 1.47 |
| 50/50 | 0.29 | 3.0 | 1.45 |

*Prep scale composition chosen as a compromise.

*Preparative Reversed Phase*

Warfarin

Peak 1 – 7.24 min.
Peak 2 – 8.67 min.




CHIROBIOTIC V
250x22.1mm
40/60: EtOH/0.1% TEAA, pH 4.1 @15 mL/min.
Inj. 20 mg
UV-220nm

## General Procedure: $C_{18}$ Recovery of Enantiomers from Aqueous Mobile Phases

The degree of hydrophobicity of the analyte is the main criterion in applying this technique. The following procedure can be used to ascertain the capacity of the $C_{18}$ column and the size column required for recovery of separated analytes from a full scale prep run.

1. Equilibrate an analytical $C_{18}$ column with HPLC grade water.

2. Pump the recovered chiral column eluant* containing the enantiomer through the column until the compound breaks through.

3. Wash column with water to remove any buffer present and eluate with an appropriate organic solvent. Methanol is often the best choice but ethanol or acetonitrile can be used as well. Measure the volume of collected solvent and assay for recovered analyte.

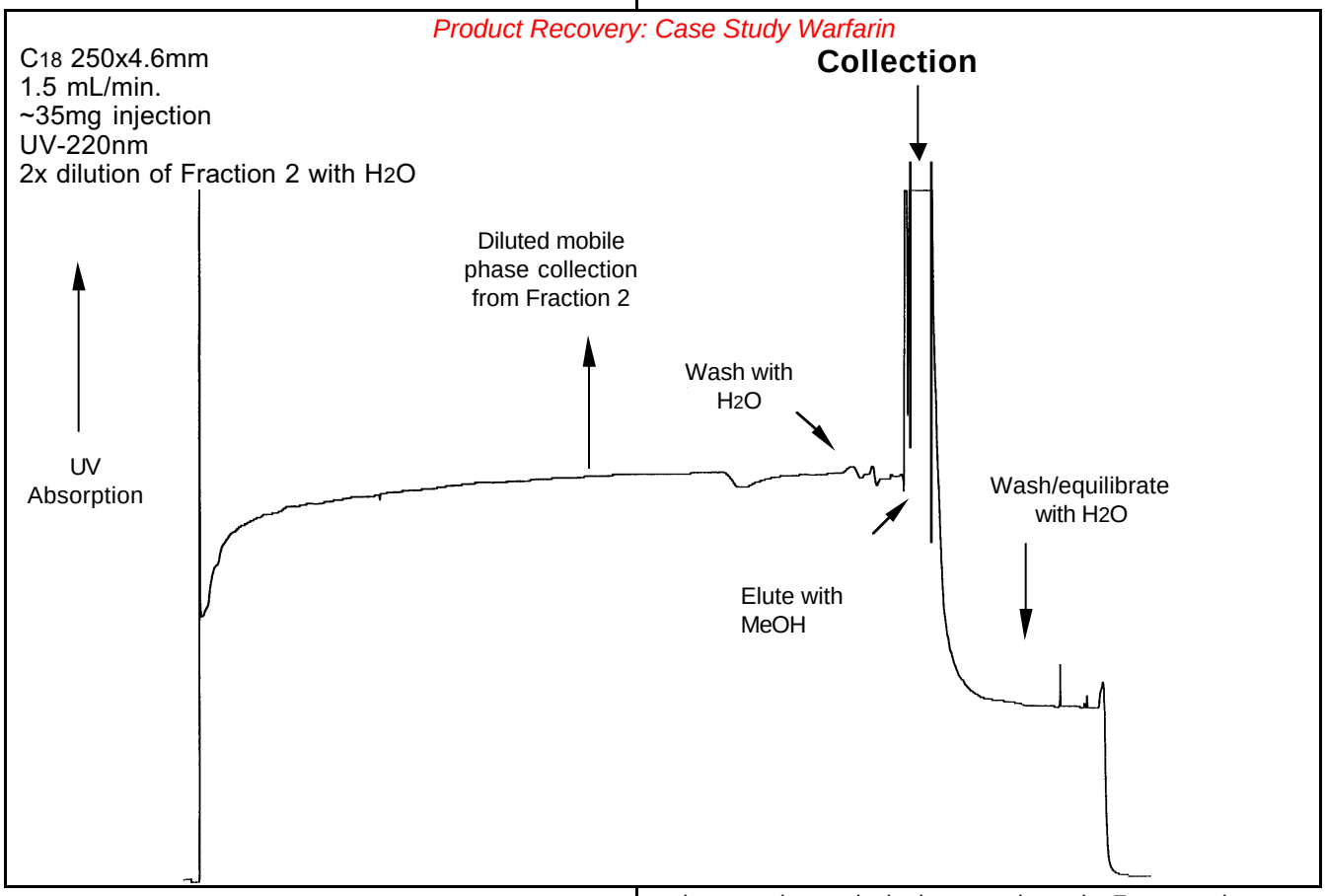
4. If recovered amount falls below anticipated capacity it is always possible to further dilute the eluant that is being charged with water. In addition, larger size $C_{18}$ columns can be used.

5. After elution of the compound of interest the column is equilibrated with water for the next addition.

6. From analytical runs it is possible to calculate the size of the column required for a larger scale run. Typically, doubling the column diameter is equivalent to four times the volume.

**Note: Adjust pH to increase hydrophobicity and convert all cations and anions to neutral salt. Principle is to suppress the ionization of the analyte.*

*Product Recovery: Case Study Warfarin*




## Warfarin Reversed Phase Mode

$C_{18}$ recovery method makes for efficient use of the reversed phase mode with CHIROBIOTIC phases. Also note speed of recovery of the separated enantiomers from a $C_{18}$ is faster than evaporation of an equivalent volume of heptane.

Results:

| Final Results | Peak 1 | Peak 2 |
|---|---|---|
| ee | 98.5% | 99% |
| Yield | 90% | 85% |

Optimization Studies

1. Prime consideration in dealing with preparative applications is the balance between selectivity and sample solubility. See Example 3.

2. Check the CHIROBIOTIC V2 or T2 if the polar organic or polar ionic modes have been chosen. These two CSPs may offer increased resolution and increased capacity in these modes only. For neutral molecules, the polar organic mode will work best eliminating the need for acid/base or volatile salt.

3. When operating in the polar ionic mode check carefully the choice of volatile salt as it may effect selectivity dramatically. Ammonium trifluoroacetate usually favors bases while ammonium acetate favors acids.

## Issued on Mass Overload

1. Study injection volume and concentration effects:
   Compromise between injection volume and analyte concentration is typically required. For neutral molecules a smaller volume can be used but for separations in the polar ionic mode samles have to be more dilute and larger volumes injected.

2. Salts of chiral drugs:
   a. Increased buffer strength. When a large amount of sample is injected onto the column, consideration has to be given to the

quick disassociation of the sample. Usually increasing the buffer strength by a factor of 2 will accomplish the task.

b. Solubility. If the salt reduces the solubility of the sample, water may be added for all modes but especially in the polar ionic mode.

3. Overload studies:
Minimize detector overload by using a less sensitive wavelength. It is necessary to evaluate column overload not detector overload. The analytical column is injected with increasing amounts of sample until resolution decreases to about 85%. This number can then be used for direct scale up. From a 250x4.6mm column to a 21.2mm ID is a factor of 20x. From the analytical to a 50mm ID column is a factor of 120x.

4. Non linear adsorption isotherms are observed when overloading, i.e, retention decreases with increasing load and peak splitting can result at some critical point.

5. Connecting tubing. To minimize band spread keep tubing connections to 0.01″ (0.25mm) or 0.02″ (0.5mm) especially if recycle and shave methodologies are used.

## Specific Examples of CSP Capacity

| | Compound | Loading Capacity* | Column |
|---|---|---|---|
| 1 | Chlorokynurenine | 2.0 | CHIROBIOTIC T |
| 2 | Dehydroproline | 10.0 | CHIROBIOTIC T |
| 3 | Thalidomide | 12.5 | CHIROBIOTIC V |
| 4 | Succinimide | 2.8 | CHIROBIOTIC T |
| 5 | Phenylhydantoin | 1.8 | CHIROBIOTIC V |
| 6 | Phenylalanine | 10.0 | CHIROBIOTIC T |
| 7 | Isoleucine | 24.0 | CHIROBIOTIC TAG |

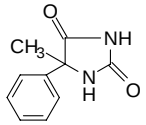
**mg racemate per gram CSP/hour*

The examples on the right were chosen as simple demonstrations of some of the points covered above. They do not represent the best or the worst examples of what is currently a broad base of experience.

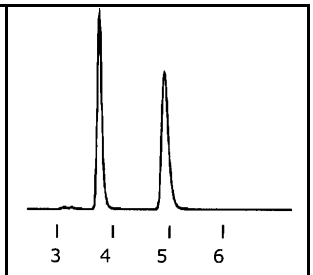
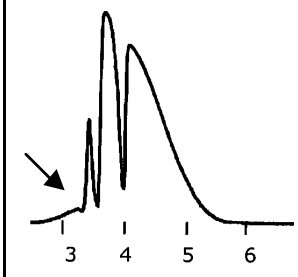
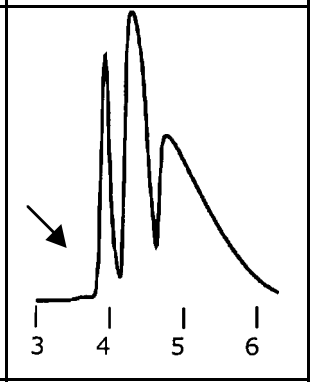
## Conclusions

1. The 10µm and 16µm CHIROBIOTIC phases show excellent scalability directly from analytical to 2" (5cm) ID column.
2. The retention times and resolution data were nearly identical to published application notes.
3. The CHIROBIOTIC phases can be used in normal, reversed phase, polar ionic and polar organic modes with no alteration of the stationary phase.
4. CHIROBIOTIC columns up to 2″ ID have stable beds at linear velocities up to 400 mL/min.
5. The use of recycle and shave methodology showed significant advantages in increased throughput and final purity.
6. The use of recycle method reduced solvent consumption by 25-50%.
7. CHIROBIOTIC phases have demonstrated no solvent limitations.
8. CHIROBIOTIC phases have demonstrated capacities from 2 to 24 grams/hour/kilo CSP.

### *Separation in the Polar Organic Mode*
### *Example 1: 5-Methyl-5-phenyl hydantoin*




Solubility: >80 mg/mL in MeOH
Column: CHIROBIOTIC T
Mobile Phase: 100% MeOH
Selectivity: 2.1

| | |
|---|---|
| Analytical: 250x4.6mm, 5µM<br>Load: 2mg/mL x 5µL (10µg)<br>Flow rate: 1 mL/min.<br>UV: 220nm<br><br>Peak 1 – 3.83 min.<br>Peak 2 – 5.05 min. |  |
| Analytical: 250x4.6mm, 5µM<br>Load: 80mg/mL x 0.1mL (8mg)<br>Flow rate: 1 mL/min.<br>UV: 270nm |  |
| Prep: 250x21.2mm, 5µM<br>Load: 80mg/mL x 1.5mL (120mg)<br>Flow rate: 18 mL/min.<br>UV: 270nm<br>Throughput: 20mg/g CSP/hr |  |

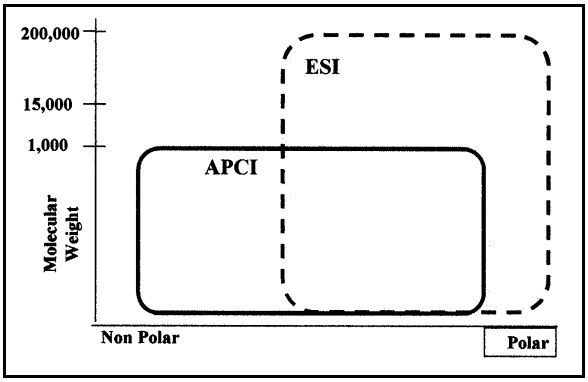
# CHIROBIOTIC PHASES FOR LC/MS

Since 1996, the LC/MS platform has gained increasing status as an analytical and developmental tool especially within the pharmaceutical industry. To date, of all the publications relating to chiral separations utilizing this technique, six papers have appeared applying Chiral Technologies products, one applying our CYCLOBOND and 15 citing our CHIROBIOTIC phases. Please refer to CHIROBIOTIC bibliography references 30 and 57 for method development techniques.

One of the reasons that the CHIROBIOTIC phases have gained so much interest has to do with the success of the polar ionic mode as a simple, effective mobile phase that results in an easy to validate method. CHIROBIOTIC phases avoid the use of inorganic buffers and rarely require the use of normal phase solvents like hexane. Instead, a typical mobile phase would be methanol with low concentrations (0.1– 0.001%, v/wt) of volatile salts like ammonium trifluoroacetate, ammonium formate or ammonium acetate, enhancing MS detection. Speed of analysis is another very favorable factor, especially when using the polar ionic mode. Further, CHIROBIOTIC technology allows for the use of any solvent compatible with MS even halogenated solvents, which is not possible with competitive products.

Since CHIROBIOTIC phases operate in both the polar ionic and reversed phase modes, they are useful in APCI and ESI methods. Their applications can be summarized in the figure below.

*Analyte Compatibility*




## Adapting LC Methods to LC-API-MS

### Solvents

All known solvents compatible with MS are compatible with the CHIROBIOTIC phases including methanol, ethanol, acetonitrile, water, IPA, dichloromethane, chloroform, hexane and THF.

### Acids

Formic and acetic are recommended. Trifluoroacetic acid sometimes causes ion suppression in both positive and negative modes.

### Bases

Ammonium hydroxide and ammonia solutions are recommended. Triethylamine and trimethylamine may enhance deprotonation for negative ion formation.

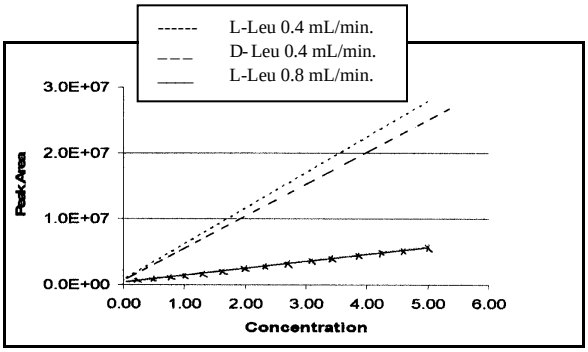
### Buffers

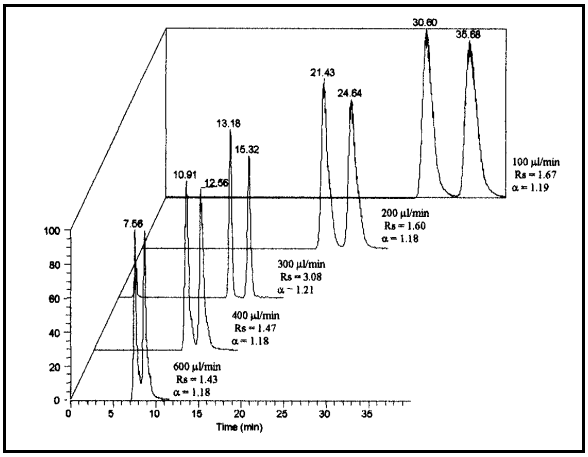
Ammonium formate, ammonium trifluoroacetate and ammonium acetate have been widely used.

### Flow Rate

Flow rate is an important parameter in the optimization of any chiral or non-chiral LC/ESI-MS method. The best sensitivity is often achieved with moderate to low flow rates.

*Flow Rate Dependence on Senstivity for ESI*






*Effect of flow rate on the separation of clenbuterol enantiomers. An optimum flow rate of 300 µL/min provided for the best resolution and enantioselectivity. The separation conditions are: 250x2.1mm (ID) CHIROBOTIC T column, mobile phase: 100/0.1w%: MeOH/NH4TFA.*

Following is selected data abstracted from the LC/MS publications that can be found in the CHIROBIOTIC bibliography. They are categorized under appropriate application headings.

## Method Development Techniques

Reference 28: 8 drugs, HT screening, CHIROBIOTIC V and T, polar ionic mode
Reference 30: 6 drugs, HT screening, CHIRIOBIOTIC V+R+T, polar ionic mode

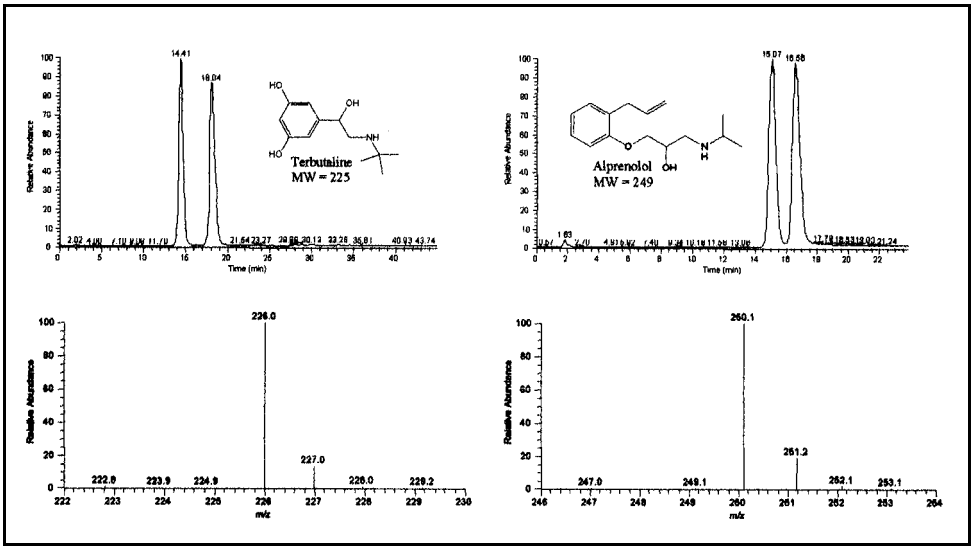
## Nutraceuticals

Reference 36: Selenomethonine and selenthionine, CHIROBIOTIC T, reversed phase mode

## Clinical Applications

Reference 12: Salbutamol and 4-sulfate metabolite, CHIROBIOTIC T, polar ionic mode
Reference 26: Methylphenidate, CHIROBIOTIC V, polar ionic mode
Reference 38: 2-Hydroxyglutaric acid, CHIROBIOTIC R, reversed phase mode
Reference 40: 15 Underivatized protein amino acids, CHIROBIOTIC T, reversed phase mode
Reference 46: Fluoxetine (Prozac™), CHIROBIOTIC V, polar ionic mode
Reference 53: L-Pipecolic acid, CHIROBIOTIC T, reversed phase mode
Reference 54: Glyceric acid, CHIROBIOTIC T, reversed phase mode
Reference 57: Terbutaline, CHIROBIOTIC T, polar ionic mode
Reference 83: R,S-Propranolol, CHIROBIOTIC T, polar ionic mode
Reference 84: R,S-Methylphenidate, CHIROBIOTIC V, polar ionic mode
Reference 87: 2S,2S,2R,2S-Captopril, CHIROBIOTIC T, reversed phase mode
Reference 89: R,S-Albuterol, CHIROBIOTIC T, polar ionic mode

*HPLC-ESI-MS of Polar Compounds Separated on CHIROBIOTIC T*
*Mobile Phase: 100/0.1% MeOH/ATFA*



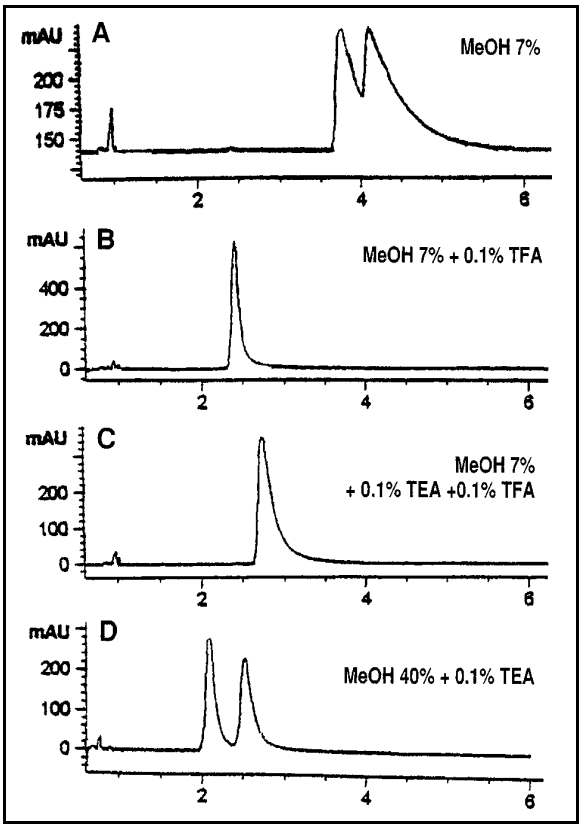

## Conclusions

1. PIM and POM modes are ideal for ESI.
2. APCI is usually best for RP separations but require more optimization.
3. Additives effect both separation selectivity and detection sensitivity
4. Native amino acids work best with APCI and alcohol/water mixtures.
5. Lower flow rates tend to enhance detection sensitivity and overall performance.

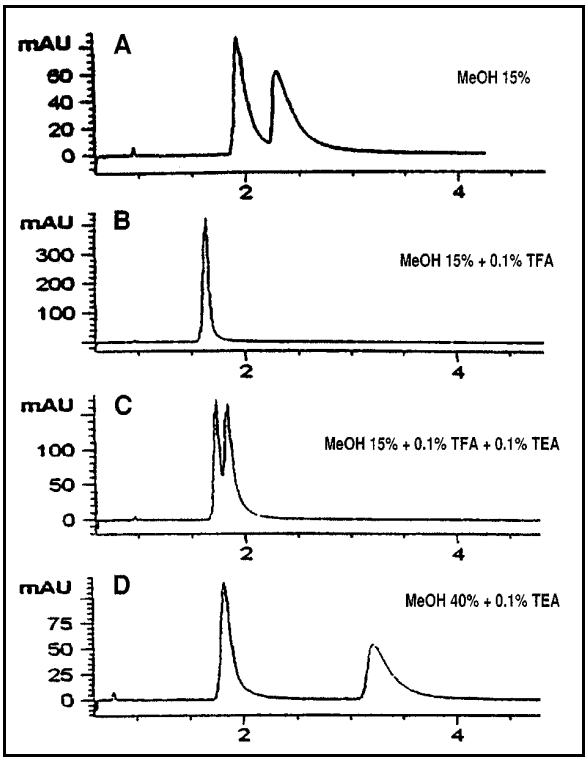
# SFC SUPER/SUB CRITICAL FLUID ENANTIOMERIC SEPARATION ON CHIROBIOTIC PHASES

All packed CHIROBIOTIC phases are stable to sub critical and super critical fluid methodologies and have been tested under a variety of operating conditions. Several recent publications attest to the effectiveness of these stationary phases in this methodology. Because of the polarity of these phases and the presence of ionic functionality, additives such as methanol in the range of 10-25% had to be added to the carbon dioxide. For some ionizable compounds additional additives like TFA and TEA had to be added in the range of 0.05-0.5% v/v.

*Effect of Additives*
*2(4-Chlorophenoxy) propionic acid*
*CHIROBIOTIC T, 250x4.6mm*
*SFC Mobile Phase @4 mL/min.*

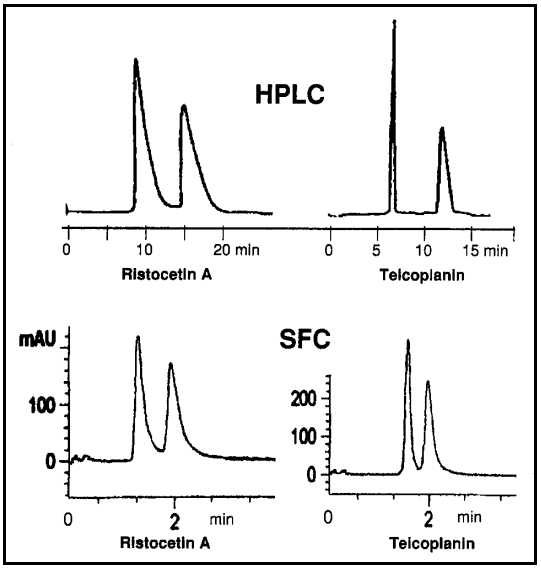



*Effect of Additives*
*DNPyr-leucine*
*CHIROBIOTIC T, 250x4.6mm*
*SFC Mobile Phase @4 mL/min.*




Armstrong has published the first super critical separation of amino acids (Reference 70) and the separation of 24 chiral dihyrofuorcoumarins in a recent paper, Chromatographia 58, 775-780 (2003).

*Comparison HPLC/SFC*
*CBZ-Norvaline*
*HPLC: 20/80: MeOH/TEAA, pH 4*
*SFC:60/39.96/0.4:$CO_2$/MeOH/TEA*




# GENERAL OPERATING CONDITIONS

## Conditioning New CHIROBIOTIC Columns and First-Step Regeneration

A new column should always be washed first with 50/50: $CH_3CN$/50mM $NH_4OAc$ for 20 column volumes. Then rinse the column with pure HPLC water for 10 column volumes, followed by a wash with pure organic solvent, i.e., $CH_3CN$ or $CH_3OH$.

## Injection Volumes and Concentrations

Typical samples of 1-5 µL of a 1 mg/mL concentration are required for good resolution. In other cases, the load volume and concentration may be 5 to 10 times higher without affecting resolution. Therefore, begin the separation study at the lowest volumes and concentrations until a proper determination can be made of its effect.

A precolumn (before injector) of 40µm silica should always be used when operating silica based columns with aqueous mobile phases.

Astec columns may be operated from either direction without loss of performance due to the uniform packing system that produces uniform packing density.

## Changing Mobile Phases

Care should always be exercised in switching from one mobile phase type to another. Always wash the column connected to the system in order to purge all connecting lines and detector cell. In switching from reversed phase to normal phase first wash with water, then methanol. In changing from the polar ionic mode, wash with methanol first to remove any acid and base especially if TFA was used.

## Pressure

Operating pressure for CHIROBIOTIC columns is generally in the range of 800 (54 bars) to 1000 (68 bars) psi (150x4.6mm) and 1100 (75 bars) to 1200 (82 bars) psi (250x4.6mm) at 1.0 mL/minute for 10/90: THF/20mM $NH_4NO_3$. As with standard reversed phase columns, the higher the water/methanol ratio, the higher the back pressure, with a maximum at 50/50: MeOH/$H_2O$. Care should always be exercised in prefiltering and degassing the water and solvent used with these columns. In general, pressure should not exceed 3500 psi (238 bars). With 50/50: EtOH/$H_2O$ at 1.0 mL/min. the pressure will be 3200-3500 psi.

## Stability Data

Typical production columns have been run at 1.0 mL/min. for extended periods with acetonitrile/ buffer:10/90 at the limits of pH stability (in our test 4.0 and 7.0). After 1000 hours of continuous operation there was no observable change in alpha for the selectivity standard. Reversed phase separations are the first to show signs of column deterioration. See quality control test on the following page.

## Regeneration

Columns showing decreased resolution can sometimes be regenerated by passing several column volumes of 50/50: $CH_3CN$/50mM $NH_4OAc$ pure HPLC grade water, pure ethanol and then acetonitrile or methanol through the column at 0.5 mL/min. Ethanol is more efficient for displacing substances from these phases than methanol. If a column has been operating at a particular pH for any length of time, it is sometimes helpful to wash the column with a buffer at the opposite range of the pH to elute opposing anions or cations. Remember these stationary phases have both anionic and cationic characteristics. Acetonitrile may be used for final displacement and storage. Long term storage (>24 hours) is best done in methanol.

## Storage

**NEVER STORE COLUMNS EVEN FOR SHORT PERIODS OF TIME IN BUFFER. WASH THE COLUMN FIRST WITH WATER, THEN WITH EITHER METHANOL OR ETHANOL.**

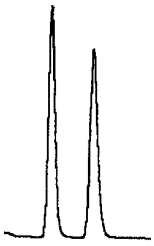
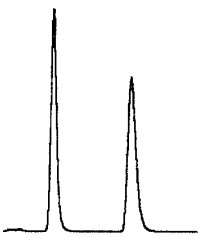
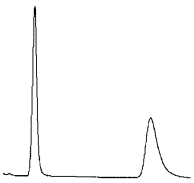
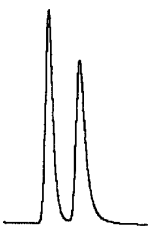
The column is best stored in methanol since it easily allows testing and conversion to reversed phase or polar organic and polar ionic modes. Subsequent to storing, the selectivity test should be performed.

Preferred test samples for selectivity control tests are:

## Quality Control Test Procedures

Quality control tests are recommended to be used routinely to assure consistent performance.
Conditions for all columns:

Sample: 5-Methyl-5-Phenylhydantoin (Aldrich 18,082-3)
Column size: 250x4.6mm
Mobile phase: 100% MeOH
Flow rate: 1.0 mL/min.
Detection: UV220nm

| CHIROBIOTIC V | CHIROBIOTIC T | CHIROBIOTIC TAG | CHIROBIOTIC R |
|---|---|---|---|
| Peak 1 – 3.70 min.<br>Peak 2 – 4.21 min. | Peak 1 – 3.83 min.<br>Peak 2 – 5.05 min. | Peak 1 – 3.98 min.<br>Peak 2 – 7.22 min. | Peak 1 – 4.15 min.<br>Peak 2 – 4.63 min. |
|  |  |  |  |

*Note: For 100x4.6mm columns, set flow rate to 0.5 mL/min.*

# BIBLIOGRAPHY


1. Macrocyclic Antibiotics as a New Class of Chiral Selectors for Liquid Chromatography, D.W. Armstrong, Y. Tang, S. Chen, Y. Zhou, C. Bagwill, J.R. Chen, *Anal. Chem.*, **Vol. 66, No. 9**, 1473-1484 (1994).

2. A Covalently Bonded Teicoplanin Chiral Stationary Phase for HPLC Enantioseparations, D.W. Armstrong, Y. Liu, K.H. Ekborg-Ott, *Chirality*, **Vol. 7, No. 6**, 474-497 (1995).

3. Enantioresolution of Substituted 2-Methoxy-6-oxo-1,4,5,6-Tetrahydropyridine-3 Carbonitriles on Macrocyclic Antibiotic and Cyclodextrin Stationary Phases, S. Chen, Y. Liu, D.W. Armstrong, P. Victory, B. Martinez-Teipel, *J. Liq. Chrom.*, **Vol. 18 (8)**, 1495-1507 (1995).

4. Facile RPLC Enantioresolution of Native Amino Acids and Peptides Using a Teicoplanin Chiral Stationary Phase, A. Berthod., Y.Liu, C. Bagwill, D.W. Armstrong, *J. of Chromatogr. A*, **731**, 123-137 (1996).

5. Separation of Enantiomers of 4-Aryldihydropyrimidines by Direct Enantioselective HPLC. A Critical Comparison of Chiral Stationary Phases, O.P. Kleidernigg, C.O. Kappe, *Tetrahedron:Asymmetry*, **Vol. 8**, 2057-2067 (1997).

6. High-performance Liquid Chromatographic Separation of Enantiomers of Unusual Amino Acids on a Teicoplanin Chiral Stationary Phase, A. Peter, G. Torok, D.W. Armstrong, *J.of Chromatogr. A.*, **793**, 283-296 (1998).

7. Stereochemical Analyses of Food Components, K. H. Ekborg-Ott, D.W. Armstrong, Chiral Separations: Application and Technology, Ch. 9, 201-270 (1997), American Chemical Society, Washington, DC.

8. Highly Enantioselective HPLC Separations Using the Covalently Bonded Macrocyclic Antibiotic, Ristocetin A, Chiral Stationary Phase, K. H. Ekborg-Ott, Y. Liu, D. W. Armstrong, *Chirality*, **10**, 434-483 (1998).

9. Enantiomeric Impurities in Chiral Catalysts, Auxiliaries and Synthons Used in Enantioselective Synthesis, D. W. Armstrong, J. T. Lee, L. W. Chang, *Tetrahedron: Asymmetry* **9**, 2043-2064 (1998).

10. Enantiomeric Impurities in Chiral Catalysts, Auxiliaries, Synthons and Resolving Agents. Part 2, D. W. Armstrong, L. He, T. Yu, J. T. Lee, Y. Liu, *Tetrahedron: Asymmetry* **10**, 37-60 (1999).

11. The Evolution of Chiral Stationary Phases for Liquid Chromatography, D. W. Armstrong, *Journal of the Chinese Chemical Society*, **45**, 581-590 (1998).

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

13. Determination of the Enantiomers of Albuterol in Human and Canine Plasma by Enantioselective High-Performance Liquid Chromatography on a Teicoplanin-Based Chiral Stationary Phase, K. M. Fried, P. Koch, I. W. Wainer, *Chirality* **10**, 484-491 (1998).

14. Enantiomeric Separation of Several Cyclic Imides on a Macrocyclic Antibiotic (Vancomycin) Chiral Stationary Phase Under Normal and Reversed Phase Conditions, H. Y. Aboul-Enein, V. Serignese,*Chirality* **10**, 358-361 (1998).

15. Plant and Soil Enantioselective Biodegradation of Racemic Phenoxyalkanoic Herbicides, J. M. Schneiderheinze, D. W. Armstrong, A. Berthod, *Chirality* **11**, 330-337 (1999).

16. Comparison of Enantioselective Separation of *N-tert.*-butyloxycarbonyl Amino Acids and Their Non-blocked Analogues on Teicoplanin-based Chiral Stationary Phase, E. Tesarova, A. Bosakova, V. Pacakova, *J. of Chrom. A*, **838**, 121-129 (1999).

17. Chiral Separations Performed by Enhanced Fluidity Liquid Chromatography on a Macrocyclic Antibiotic Chiral Stationary Phase, Q. Sun, S.V. Olesik, *Anal. Chem.* **71**, 2139-2145 (1999).

18. Chiral Separation of Enantiomers of Amino Acid Derivatives by HPLC on Vancomycin and Teicoplanin Chiral Stationary Phases, J. Lehotay, K. Hrobonová, J. Krupcík, J. Cizmárik, *Pharmazie* **53**, 863-865 (1998)12.

19. Effect of Selector Coverage and Mobile Phase Composition on Enantiomeric Separations with Ristocetin A Chiral Stationary Phases, K. H. Ekborg-Ott, X. Wang, D. W. Armstrong, *Microchemical Journal* **62**, 26-49 (1999).

20. Optimum Operating Conditions for Chiral Separations in Liquid Chromatography, R.P.W. Scott, T. E. Beesley, *Analyst*, **124**, 713-719 (1999).

21. Enantioseparation of Semisynthetic Ergo Alkaloids on Vancomycin and Teicoplanin Stationary Phases, E. Tesarova, K. Zaruba, M. Flieger, *J. of Chrom. A*, **844**, 137-147 (1999).

22. Quantitative Determination of Clenbuterol Enantiomers in Human Plasma by High-performance Liquid Chromatography Using the Macrocyclic Antibiotic Chiral Stationary Phase Teicoplanin, H. Y. Aboul-Enein and V. Serignese, *Biomed. Chromatogr.,* **13**, 520-524 (1999).

23. Role of the Carbohydrate Moities in Chiral Recognition on Teicoplanin-Based LC Stationary Phases, Berthod, A., Chen, X., Kullman, J.P., Armstrong, D.W., Gasparrini, F., D'Acquarica, I., Villani, C., Carotti, A., *Anal. Chem.,* **72**, 1767-1780 (2000).

24. Chiral Separations of Polar Compounds by Hydrophilic Interaction Chromatography with Evaporative Light Scattering Detection, Risley, D.S, Strege, M.A., *Anal. Chem*., **72**, 1736-1739 (2000).

25. Synthesis and Structure of Biologically Active Ferrocenylalkyl Polyfluoro Benzimidazoles, Snegur, L.V., Boev, V.I., Nekrasov, Y.S., Ilyin, M.M., Davankov, V.A., Starikova, Z.A., Yanovsky, A.I., Kolomiets, A.F., Babin, V.N., *J. of Organometallic Chem.,* **580**, 26-35 (1999).

26. Liquid Chromatographic/Atmospheric Pressure Chemical Ionization Tandem Mass Spectrometry Enantiomeric Separation of *dl-threo*-Methylphenidate, (Ritalin®) Using a Macrocyclic Antibiotic as the Chiral Selector, Ramos, L., Bakhtiar, R., Majumdar, T., Hayes, M., Tse, F., *Rapid Commun. Mass Spectrom*., **13**, 2054-2062 (1999).

27. Coupling Chiral Stationary Phases as a Fast Screening Approach for HPLC Method Development, Wang, A. X., Lee, J. T., Beesley, T.E., *LC•GC*, **18(6)**, 626-639 (2000).

28. High-Throughput Chiral Liquid Chromatography/Tandem Mass Spectrometry, Bakhtiar, R., Tse, F.L.S., *Rapid Commun. Mass Spectrom*., **14**, 1128-1135 (2000).

29. Macrocyclic Antibiotics as Effective Chiral Selectors for Enantiomeric Resolution by Liquid Chromatography and Capillary Electrophoresis, Aboul-Enein, H. Y., Ali, I., *Chromatographia,* Vol. 52, No. 11/12, (December 2000).

30. Use of Atmospheric Pressure Ionization Mass Spectrometry in Enantioselective Liquid Chrom atography, Bakhtiar, R., Ramos, L., Tse, F. L. S., *Chirality* 13, 63-74 (2001).

31. Enantiomeric High-Performance Liquid Chromatographic Separation of β-Substituted Tryptophan Analogues, Török, G., Peter, A., Vékes, E, Sápi, J., Laronze, M. Laronze, J.-Y., Armstrong, D. W., *Chromatographia Supplement*, Vol. 51 ,S165-S174 (2000).

32. Chiral Separation by Simultaneous Use of Vancomycin as Stationary Phase Chiral Selector and Chiral Mobile Phase Additive, Sun, Q., Olesik, S. V., *J. of Chrom*. B, 745, 159-166 (2000).

33. Simultaneous Analysis of Enantiomeric Composition of Amino Acids and N-Acetyl-Amino Acids by Enantioselective Chromatography, Yu, Y-P., Wu, W-H., *Chirality* 13:231-235 (2001).

34. Modification of the Chiral Bonding Properties of Teicoplanin Chiral Stationary Phase by Organic Additives. HPLC Separation of Enantiomers of Alkoxysubstituted Esters of Phenylcarbamic Acid, J. Lehoytay, K. Hrobonová, J. Cizmárik, M. Renèová, D. W. Armstrong, *J. Liq. Chrom. & Rel. Technol*., 23(5), 609-624 (2001).

35. Validation of a Chiral Capillary Electrochromatographic Method for Metoprolol on a Teicoplanin Stationary Phase, Carlsson, E., Wikström, H., Owens, P.K., *Chromatographia* 53, April No. 7/8, 419-424 (2001).

36. Chiral Speciation and Determination of Selenomethionine Enantiomers in Selenized Yeast by HPLC-ICP-MS Using a Teicoplanin-based Chiral Stationary Phase, S. Pérez Méndez, E. Blanco González, A. Sanz Medel, J. Anal. At. *Spectrom.* 15, 1109-1114 (2000).

37. Dansyl Amino Acid Enantiomer Separation on a Teicoplanin Chiral Stationary Phase: Effect of Eluent pH, Peyrin, E., Ravelet, C., Nicolle, E., Villet, A., Grosset, C., Ravel, A., Alary, J., *J. of Chrom. A*, 923, 37-43 (2001).

38. Chiral Liquid Chromatography Tandem Mass Spectrometry in the Determination of the Configuration of 2-Hydroxyglutaric Acid in Urine, Rashed, M.S., AlAmoudi, M., Aboul-Enein, H.Y., *Biomedical Chromatogr*. 14, 317-320 (2000).

39. Interactions Between D,L Dansyl Amino Acids and Immobilized Teicoplanin: Study of the Dual Effect of Sodium Citrate on Chiral Recognition, Peyrin, E., Ravel, A., Grosset, C., Villet, A., Ravelet, C., Nicolle, E., Alary, *Chromatographia*, 53, 645-650 (2001).

40. Simultaneous Analysis of Underivatized Chiral Amino Acids by Liquid Chromatography – Ionspray Tandem Mass Spectrometry Using a Teicoplanin Chiral Stationary Phase, Petritis, K., Valleix, A., Elfakir, C., Dreux, M., *J. of Chrom*. A, 913, 331-340 (2001).

41. Retention and Selectivity of Teicoplanin Stationary Phases After Copper Complexation And Isotopic Exchange, Berthod, A., Valleix, A., Tizon, V., Leonce, E., Caussignac, C., Armstrong, D. W., *Anal. Chem*. 73, 5499-5508 (2001).

42. Direct Chiral Separation of Unnatural Amino Acids by High Performance Liquid Chromatography on a Ristocetin A-Bonded Stationary Phase, Török, G., Péter, A., Armstrong, D.W., Tourwé, D., Tóth, G., Sápi, J., *Chirality*, 13, 648- 656 (2001).

43. High-performance Liquid Chromatographic Enantioseparation of β-amino Acids, Péter, A., Lázár, L., Fülöp, F., Armstrong, D.W., *J. of Chrom. A*., 926, 229-238 (2001).

44. Quantification of Methylphenidate (Ritalin®) in Rabbit Fetal Tissue Using a Chiral Liquid Chromatography/ Tandem Mass Spectrometry Assay, Bakhtiar, R., Ramos, L., Tse, F.L.S., *Rapid. Commun. Mass. Spectrom.,* 16, 81-83 (2002).

45. Analysis of the Band Profiles of the Enantiomers of Phenylglycine in Liquid Chromatography, Jander, P., Backovská, V., Felinger, A., *J. of Chrom. A.,* 919, 67-77 (2001).

46. Enantiomeric Separation and Quantification of Fluoxetine (Prozac®) in Human Plasma by Liquid Chromatography/Tandem Mass Spectrometry Using Liquid-liquid Extraction in 96-well Plate Format, Shen, Z., Wang, S., Bakhtiar, R., *Rapid Commun. Mass Spectrom.,* 16, 332-338 (2002).

47. Enantiomeric Separation of Some Cyclic Ketones and Dioxalene Derivatives by Chiral SFC, Toribio, L., David, F., Sandra, P., *Química Analytíca*, 18, 269-273 (1999).

48. Sulphostin, A Potent Inhibitor for Dipeptidyl Peptidase IV from *Streptomyces* sp. MK251-43F3, Akiyama, T., Abe, M., Harada, S., Kojima, F., Sawa, R., Takahashi, Y., Naganawa, H., Homma, Y., Hamada, M., Yamaguchi, A., Aoyagi, T., Muraoka, Y. Takeuchi, T., *The Journal of Antibiotics*, 54(9), 744-746 (2001).

49. Chiral Discrimination of Phenoxypropionic Acid Herbicides on Teicoplanin Phase: Effect of Mobile Phase Modifier, Guillaume, Y.C., Truong, T.T., Millet, J., Nicod, L., Guinchard, C., Robert, J.F., Thomassin, M., Chromatographia, 55, No. 3/4, 143-148 (2002).

50. Chiral Resolution of Flurbiprofen and Ketoprofen Enantiomers by HPLC on a Glycopeptide-Type Column Chiral Stationary Phase, Péhourcq, F., Jarry, C., Bannwarth, B., Biomed. Chromatogr. 15, 217-222 (2001).

51. HPLC Enantioselective Separation of Aromatic Amino and Hydrazino Acids on a Teicoplanin Stationary Phase and the Enantiomeric Purity Determination of L-Isomers Used as Drugs, Chirality 11, 394-403 (1999).

52. Effect of the Mobile Phase on the Retention Behavior of Optical Isomers of Carboxylic Acids and Amino Acids in Liquid Chromatography on Bonded Teicoplanin Columns, Jandera, P., Skavrada, M., Klemmova, K., Backovska, V., Guiochon, G., J. of Chrom. A., 917, 123-133 (2001).

53. Determination of L-Pipecolic Acid in Plasma Using Chiral Liquid Chromatography-Electrospray Tandem Mass Spectrometry, Rashed, M.S., Al-Ahaidib, L.Y., Aboul-Enein, H.Y., Al-Amoudi, M. Jacob, M., Clinical Chemistry 47:12, 2124-2130 (2001).

54. Chiral Liquid Chromatography Tandem Mass Spectrometry in the Determination of the Configurations of Glyceric Acid in Urine of Patients with D-glyceric and L-glyceric Acidurias, Rashed, M.S., Aboul-Enein, H.Y., AlAmoudi-M., Jakob, M., Al-Ahaideb, L.Y., Abbad, A., Shabib, S., Al-Jishi, E., Biomed. Chromatogr. 16, 191-198 (2002).

55. Enantiomeric Separation of Metoprolol and α-Hydroxymetoprolol by Liquid Chromatography and Fluorescence Detection Using a Chiral Stationary Phase, Mistry, B., Leslie, J. L., Eddington, N.D., *J. of Chromatogr. B*, 758, 153-161 (2001).

56. Enantioseparation by HPLC of Imino Acids on Macrocyclic Glycopeptide Stationary Phases and as their (*S*)-N-(4-Nitrophenoxycarbonyl)-phenylalanine Methoxyethyl Ester Derivatives, Péter, A., Armstrong, D. W.,, Tourwé, D., Chromatographia, 56, Suppl. S-41-S47 (2002).

57. Use of Online-Dual-Column Extraction in Conjunction with Chiral Liquid Chromatography Tandem Mass Spectrometry for Determination of Terbutaline Enantiomers in Human Plasma, Xia, Y-Q, Liu, D.Q., Bakhtiar, R., Chirality 14: 742-749 (2002).

58. Separation of Chiral Sulfoxides by Liquid Chromatography Using Macrocyclic Glycopeptide Chiral Stationary Phases, Berthod, A., Xiao, T. L., Liu, Y., Jenks, W.S., Armstrong, D.W., *J. of Chromatogr. A*, 955, 53-69 (2002).

59. High-performance Liquid Chromatographic and Capillary Electrophoretic Enantioseparation of Plant Growth Regulators and Related Indole Compounds Using Macrocyclic Antibiotics as Chiral Selectors, Hui, F., Ekborg-Ott, K.H., Armstrong, D.W., *J. of Chromatogr. A*, 906, 91-103 (2001).

60. Composition and Chirality of Amino Acids in Aerosol/Dust from Laboratory and Residential Enclosures, Armstrong, D.W., Kullman, J.P., Chen, X., Rowe, M., *Chirality* 13: 153-158 (2001).

61. Study of the Mechanism of Enantioseparation. I. Chiral Analysis of Alkylamino Derivatives of Aryloxypropanols by HPLC Using Macrocyclic Antibiotics as Chiral Selectors, Hrobonova, K., Lehotay, J., Cizmarikova, R., Armstrong, D.W., *J. Liq. Chrom. & Rel. Technol*., 25(15), 2225-2237 (2001).

62. Study of Local Anaesthetics. CLVIII. Chromatographic Separation of Some Derivatives of Substituted Phenylcarbamic Acid on a Vancomycin-Based Stationary Phase, Iungelova, J., Lehotay, J., Hrobonova, K., Cimarik, J., Armstrong, D.W., J. Liq. Chrom. & Rel. Technol., 25(2), 299-312 (2002).

63. Chiral Resolution of Clenbuterol, Cimaterol, and Mabuterol on CHIROBIOTIC V, T and TAG Columns, Aboul-Enein, H. Y., Imran, A., *J. Sep. Sci.* 25, 851-855 (2002).

64. Vancomycin Dimerization and Chiral Recognition Studied by High-Performance Liquid Chromatography, Slama, I., Dufresne, C., Jourdan, E., Fahrat, F., Villet, A., Ravel, A., Grosset, C. and Peyrin, E., *Anal. Chem*.74, 5205-5211 (2002).

65. Study of Mechanism of Enantioseparation. III. The Influence of Carbohydrate Moieties of Teicoplanin-bonded Chiral Stationary Phase on the Separation of Some Derivates of Phenylcarbamic Acid, Rojkovicova, T., Lehotay, J., Dungelova, J., Cizmarik, J., Armstrong, D. W., J. of Liq. Chrom. & Rel. Tech., Vol. 25, No. 18, 2723-2738 (2002).

66. Chiral Resolution of Cromakalim by HPLC on Teicoplanin Aglycon Chiral Stationary Phases, Aboul-Enein, H.Y., Ali, I., J. of Liq. Chrom. & Related Tech., Vol. 25, Nos. 13-15, 2337-2344 (2002).

67. Enantiomeric Separation of an AMPA Antagonist Using a CHIROBIOTIC T Column with HPLC and Evaporative Light-Scattering Detection, Guisbert, A. L., Sharp, V.S., Peterson, J.A., Risley, D.S., J. Liq. Chrom. & Rel. Technol., 23(7), 1019-1028 (2000).

68. Design, Synthesis, and Pharmacological Characterization of (+)-2-Aminobicyclo[3.1.0]hexane-2,6-dicarboxylic Acid (LY354740): A Potent, Selective, and Orally Active Group 2 Metabotropic Glutamate Receptor Agonist Possessing Anticonvulsant and Anxiolytic Properties, Monn, J.A., Valli, M. J., Massey, S.M., Wright, R.A., Salhoff, C.R., Johnson, B.G., Howe, T., Alt, C. A., Rhodes, G.A., Robey, R.L., Griffey, K.R., Tizzano, J.P., Kallman, M.J., Helton, D.R., Schoepp, D.D., J. Med. Chem., 40, 528-537 (1997).

69. In Vitro Study of Enzymatic Hydrolysis of Diperodon Enantiomers in Blood Serum by Two-dimensional C, Hrobonova, K., Lehotay, J., Cizmarik, J., Armstrong, D.W., Journal of Pharmaceutical and Biomedical Analysis, 30, 875-880 (2002).

70. Super/Subcritical Fluid Chromatography Chiral Separations with Macrocyclic Glycopeptide Stationary Phases, Liu, Y., Berthod, A., Mitchell, C.R., Xiao, T.L., Zhang, B., Armstrong, D.W., J. of Chrom. A 978, 185-204 (2002).

71. A Comparison of the Direct and Indirect LC Methods for Separating Enantiomers of Unusual Glycine and Alanine Amino Acid Analogues, Peter, A., Vekes, E., Gera, L., Stewart, J.M., Armstrong, D.W., Chromatographia Supple. 56, S-79-S89 (2002).

72. Column Selection and Method Development for the Determination of the Enantiomeric Purity of Investigational Non-Nucleoside Reverse Transcriptase Inhibitors, Aubry, A-F, Sebastian, D.S., Williams, R.C. Boucher, R.J. Chirality 13, 193-198 (2001).

73. Enantioselective Analysis of (R)- and (S)-Atenolol in Urine Samples by a High-Performance Liquid Chromatography Column-Switching Setup, Lamprecht, G., Kraushofer, T., Stoschitzky, K., Lindner, W., J. of Chrom. B, 740, 219-226 (2000).

74. Separation of Enantiomers of Some 1,4-Piperazine Derivatives of Aryloxy-Aminopropanols on a Vancomycin Chiral Stationary Phase, Lehotay, J., Hrobonova, K., Cizmarik, J., Celkova, H., Pharmazie 54, 743-745 (1999).

75. Comparison of Two Different Approaches of Sample Pretreatment for Stereoselective Determination of *(R,S)*-Propranolol in Human Plasma, Misl'anova, C., Stefancova, A., J. Trace and Microprobe Techniques, 19(1), 173-170 (2001).

76. Determination of (+) and (-)-Bromoisovalerylurea in Sera of Overdosed Subjects, Nishikawa, T., Kamijo, Y., Kondo, R., Sugie, H., Kurihara, K., Okuda, T., Matsumoto, N., Okada, Y., Ohtani, H., J. of Analytical Toxicology, Vol. 24, 691-695, Nov./Dec. (2000).

77. Direct High-Performance Liquid Chromatographic Determination of the Enantiomeric Purity of Levodopa and Methyldopa: Comparison with Pharmacopoeial Polarimetric Methods, Dolezalova, M., Tkaczykova, M., J. of Pharmaceutical and Biomedical Analysis, 19, 555-567 (1999).

78. High-Performance Liquid Chromatographic Separation of the Enantiomers of Unusual α-Amino Acid Analogues, Peter, A., Olajos, E., Casimir, R., Tourwe, D., Broxterman, Q.B., Kaptein, B., Armstrong, D.W., J. of Chrom. A., 871, 105-113 (2000).

79. Hybridation of Different Chiral Separation Techniques with ICP-MS Detection for the Separation and Determination of Selenomethionine Enantiomers: Chiral Speciation of Selenized Yeast, Mendez, S.P., Gonzalez, E.B., Sanz-Medel, A., Biomed. Chromatogr. 15, 181-188 (2001).

80. Resolution of Chiral Thiol Compounds Derivatized with N-(1-Pyrenyl)-Maleimide and Thioglo™3, Kullman, J.P., Yu, T., Chen, X., Neal, R., Ercal, N., Armstrong, D.W., J. Liq. Chrom. & Rel. Technol., 23(13), 1941-1952 (2000).

81. First Asymmetric Synthesis of Ch iral β-Iodo Baylis-Hillman Esters Via Tandem 1,4-Conjugate Addition/Carbonyl Coupling Reactions, Xu, X., Chen, D., Wei, H-X., Li, G., Xiao, T.L., Armstrong, D.W., Chirality, 15, 139-142 (2003).

82. Stereoselective HPLC-Assay for Citalopram and Its Metabolites, Zheng, Z., Jamour, M. Klotz, U., Therapeutic Drug Monitoring, 22, 219-224 (2000).

83. Automated Online Dual-column Extraction Coupled with Teiocoplanin Stationary Phase for Simultaneous Determination f *(R)* - and *(S)*-propranolol in Rat Plasma Using Liquid Chromatography – Tandem Mass Spectrometry, Xia, Y-Q, Bakhtiar, R., Franklin, R.B., J. of Chromatogr. B, 788, 317-329 (2003).

84. Quantification of Methylphenidate in Rat, Rabbit and Dog Plasma Using a Chiral Liquid-Chromatography/Tandem Mass Spectrometry Method, Application to Toxicokinetic Studies, Bakhtiar, R., Ramos, L., Tse, F.L.S., Analytica Chimica Acta 469, 261-272 (2002).

85. Effect of Temperature on Retention of Enantiomers of β-methyl Amino Acids on a Teicoplanin Chiral Stationary Phase, Péter, A., Török, G., Armstrong, D.W., Tóth, G., Tourwé, D., J. of Chromatogr. A., 828, 177-190 (1998).

86. Enantiomeric and Diastereomeric High-performance Liquid Chromatographic Separation of Cyclic β-substituted α-amino Acids on a Teicoplanin Chiral Stationary Phase, Schlauch, M., Frahm, A.W., J. of Chromatogr. A, 868, 197-207 (2000).

87. Direct Separation of Captopril Diastereoisomers Including their Rotational Isomers by RP-LC Using a Teicoplanin Column, Owens, P.K., Svensson, L.A., Vessman, J., J. Pharm. Biomed. Anal. 25, 453-464 (2001).

88. Effects of Temperature on Retention of Chiral Compounds on a Ristocetin A Chiral Stationary Phase, Péter, A., Vékes, E., Armstrong, D.W., J. of Chromatogr. A, 958, 89-107 (2002).

89. LC-MS Method for the Determination of Albuterol Enantiomers in Human Plasma Using Manual Solid-Phase Extraction and a Non-Deuterated Internal Standard, Jacobson, G.A., Chong, F.V., Davies, N.W., J. of Pharm. and Biomed. Analysis, 31, 1237-1243 (2003).

90. Comparison of Enantioseparation of Selected Benzodiazepine and Phenothiazine Derivatives on Chiral Stationary Phases Based on β-cyclodextrin and Macrocyclic Antibiotics, Tesarova, E., Bosakova, Z., J. Sep. Sci., 26, 661-668 (2003).

91. Separation of Enantiomers of Substituted Dihydrofurocoumarins by HPLC Using Macrocyclic Glycopeptide Chiral Stationary Phases, Xiao, T.L., Rozhkov, R.V., Larock. R.C., Armstrong, D.W., Anal. Bioanal. Chem. 377, 639-654 (2003).

92. HPLC Enantiomeric Resolution of Phenyl Isothiocyanated Amino Acids on Teicoplainin- Bonded Phase Using an Acetonitrile-Based Mobile Phase: A Structural Consideration, Chen, S., J. of Liq. Chrom. & Related Tech. Vol. 26, No. 20, 3475-3495 (2003).

93. Enantioselective Determination of Arotinolol in Human Plasma by HPLC Using Teicoplanin Chiral Stationary Phase, Biomed. Chromatogr. 17, 453-457 (2003).

94. Assignment of Absolute Configuration of a Chiral Phenyl-Substituted Dihydrofuroangelicin, Pescitelli, G., Berova, N., Xiao, T.L., Rozhkov, R.V., Larock, R.C., Armstrong, D.W., Org. Biomol. Chem., 1, 1-6 (2003).

95. Reanalysis of Chiral Discrimination of Phenoxypropionic Acid Herbicides on a Teicoplanin Phase Using a Bi-Langmuir Approach, Andre, C., Guillaume, Y.C., Chromatographia, 58, August (No. 3/4) 201-206 (2003).

96. Absolute Stereochemistry of Dihydrofuroangelicins Bearing C-8 Substituted Double Bonds: A Combined Chemical/Exciton Chirality Protocol, Tanaka, K., Pescitelli, G., Di Bari, L., Xiao, T.L., Nakanishi, K., Armstrong, D.W., Berova, N., Org. Biomol. Chem., 2, 48-58 (2004).

97. Validation of a Chiral HPLC Assay for (*R*)-Salbutamol Sulfate, Halabi, A., Ferrayoli, C., Palacio, M., Dabbene, V., Palacios, S., J. of Pharm. & Biomed. Analysis, 34, 45-51 (2004).

98. Evaluation of Generic Chiral Liquid Chromatography Screens for Pharmaceutical Analysis, Andersson, M.E., Aslan, D., Clark, A., Roeraade, J., Hagman, G., J. of Chromatogr. A, 1005, 83-101 (2003).

# ORDERING INFORMATION

## Where to Order

Advanced Separation Technologies Inc.
37 Leslie Court, Post Office Box 297
Whippany, NJ 07981 USA
Tel: (973) 428-9080, Fax: (973) 428-0152
E-mail: sales@astecusa.com

In the United Kingdom and Ireland Only:
Advanced Separation Technologies Ltd.
1 Blake Street
Congleton, Cheshire CW12 4DS UK
Tel: 01260 276276
Fax: 01260 290067
E-mail: sales@asteceuro.com

## When Ordering

Please specify shipping address, billing address, catalog number, product description, quantity and price. A purchase order number is required.

## Terms

All prices are f.o.b. Whippany, NJ USA.
Payment terms are net 30 days.
Prices are subject to change without notice.

## Shipping Instructions

All items are shipped via United Parcel Service (UPS) unless otherwise specified.

## Returns

Product returns are accepted with prior authorization only. Please contact our Customer Service Department for instructions. A 20% restocking charge is applied for products returned due to customer error.

## Liability

The products contained in this catalog are for laboratory or manufacturing use only. They are not intended for medicinal or food use. Advanced Separation Technologies Inc. assumes no responsibility if these products are used for medicinal or food purposes. Data and suggested applications are correct and reliable to the best of our knowledge and belief but cannot be guaranteed, and no responsibility is assumed with respect thereto.

## Warranty

All products manufactured by Advanced Separation Technologies are warranted for freedom from defects in material and workmanship. We will replace or repair without cost any materials which carry such defects. Maximum warranty period is limited to 90 days on LC columns unless previously agreed upon. Warranty is limited to the conditions set forth on the Column Assessment Parameter included with each column.

# AVAILABILITY

## CHIROBIOTIC V™ HPLC Columns - 5µm

| CAT # | DESCRIPTION | SIZE |
|---|---|---|
| 11018 | CHIROBIOTIC V | 100x2.1mm |
| 11019 | CHIROBIOTIC V | 150x2.1mm |
| 11020 | CHIROBIOTIC V | 250x2.1mm |
| 11021 | CHIROBIOTIC V | 50x4.6mm |
| 11022 | CHIROBIOTIC V | 100x4.6mm |
| 11023 | CHIROBIOTIC V | 150x4.6mm |
| 11024 | CHIROBIOTIC V | 250x4.6mm |
| 11026 | CHIROBIOTIC V | 500x4.6mm |
| 11034 | CHIROBIOTIC V | 250x10.0mm |
| 11036 | CHIROBIOTIC V | 500x10.0mm |
| 11044 | CHIROBIOTIC V | 250x21.2mm |
| 11046 | CHIROBIOTIC V | 500x21.2mm |
| 11100 | Guard Column | 2cmx4.0mm |
| 11101 | Microbore Guard Column | 2cmx1.0mm |
| 21150 | Guard Column Holder for 2cmx4.0mm Guard Columns | |

## CHIROBIOTIC V2™ HPLC Columns - 5µm

| CAT # | DESCRIPTION | SIZE |
|---|---|---|
| 15018 | CHIROBIOTIC V2 | 100x2.1mm |
| 15019 | CHIROBIOTIC V2 | 150x2.1mm |
| 15020 | CHIROBIOTIC V2 | 250x2.1mm |
| 15021 | CHIROBIOTIC V2 | 50x4.6mm |
| 15022 | CHIROBIOTIC V2 | 100x4.6mm |
| 15023 | CHIROBIOTIC V2 | 150x4.6mm |
| 15024 | CHIROBIOTIC V2 | 250x4.6mm |
| 15026 | CHIROBIOTIC V2 | 500x4.6mm |
| 15034 | CHIROBIOTIC V2 | 250x10.0mm |
| 15036 | CHIROBIOTIC V2 | 500x10.0mm |
| 15044 | CHIROBIOTIC V2 | 250x21.2mm |
| 15046 | CHIROBIOTIC V2 | 500x21.2mm |
| 15100 | Guard Column | 2cmx4.0mm |
| 15101 | Microbore Guard Column | 2cmx1.0mm |
| 21150 | Guard Column Holder for 2cmx4.0mm Guard Columns | |

## CHIROBIOTIC T™ HPLC Columns - 5µm

| CAT. # | DESCRIPTION | SIZE |
|---|---|---|
| 12018 | CHIROBIOTIC T | 100x2.1mm |
| 12019 | CHIROBIOTIC T | 150x2.1mm |
| 12020 | CHIROBIOTIC T | 250x2.1mm |
| 12021 | CHIROBIOTIC T | 50x4.6mm |
| 12022 | CHIROBIOTIC T | 100x4.6mm |
| 12023 | CHIROBIOTIC T | 150x4.6mm |
| 12024 | CHIROBIOTIC T | 250x4.6mm |
| 12026 | CHIROBIOTIC T | 500x4.6mm |
| 12034 | CHIROBIOTIC T | 250x10.0mm |
| 12036 | CHIROBIOTIC T | 500x10.0mm |
| 12044 | CHIROBIOTIC T | 250x21.2mm |
| 12046 | CHIROBIOTIC T | 500x21.2mm |
| 12100 | Guard Column | 2cmx4.0mm |
| 12101 | Microbore Guard Column | 2cmx1.0mm |
| 21150 | Guard Column Holder for 2cmx4.0mm Guard Columns | |

## CHIROBIOTIC T2™ HPLC Columns - 5µm

| CAT. # | DESCRIPTION | SIZE |
|---|---|---|
| 16018 | CHIROBIOTIC T2 | 100x2.1mm |
| 16019 | CHIROBIOTIC T2 | 150x2.1mm |
| 16020 | CHIROBIOTIC T2 | 250x2.1mm |
| 16021 | CHIROBIOTIC T 2 | 50x4.6mm |
| 16022 | CHIROBIOTIC T2 | 100x4.6mm |
| 16023 | CHIROBIOTIC T2 | 150x4.6mm |
| 16024 | CHIROBIOTIC T2 | 250x4.6mm |
| 16026 | CHIROBIOTIC T2 | 500x4.6mm |
| 16034 | CHIROBIOTIC T2 | 250x10.0mm |
| 16036 | CHIROBIOTIC T2 | 500x10.0mm |
| 16044 | CHIROBIOTIC T2 | 250x21.2mm |

| | | |
|---|---|---|
| 16046 | CHIROBIOTIC T2 | 500x21.2mm |
| 16100 | Guard Column | 2cmx4.0mm |
| 16101 | Microbore Guard Column | 2cmx1.0mm |
| 21150 | Guard Column Holder for 2cmx4.0mm Guard Columns | |

## CHIROBIOTIC TAG™ HPLC Columns - 5µm

| CAT. # | DESCRIPTION | SIZE |
|---|---|---|
| 14018 | CHIROBIOTIC TAG | 100x2.1 mm |
| 14019 | CHIROBIOTIC TAG | 150x2.1mm |
| 14020 | CHIROBIOTIC TAG | 250x2.1mm |
| 14021 | CHIROBIOTIC TAG | 50x4.6mm |
| 14022 | CHIROBIOTIC TAG | 100x4.6mm |
| 14023 | CHIROBIOTIC TAG | 150x4.6mm |
| 14024 | CHIROBIOTIC TAG | 250x4.6mm |
| 14026 | CHIROBIOTIC TAG | 500x4.6mm |
| 14034 | CHIROBIOTIC TAG | 250x10.0mm |
| 14036 | CHIROBIOTIC TAG | 500x10.0mm |
| 14044 | CHIROBIOTIC TAG | 250x21.2mm |
| 14046 | CHIROBIOTIC TAG | 500x21.2mm |
| 14100 | Guard Column | 2cmx4.0mm |
| 14101 | Microbore Guard Column | 2cmx1.0mm |
| 21150 | Guard Column Holder for 2cmx4.0mm Guard Columns | |

## CHIROBIOTIC R™ HPLC Columns - 5µm

| CAT # | DESCRIPTION | SIZE |
|---|---|---|
| 13018 | CHIROBIOTIC R | 100x2.1mm |
| 13019 | CHIROBIOTIC R | 150x2.1mm |
| 13020 | CHIROBIOTIC R | 250x2.1mm |
| 13021 | CHIROBIOTIC R | 50x4.6mm |
| 13022 | CHIROBIOTIC R | 100x4.6mm |
| 13023 | CHIROBIOTIC R | 150x4.6mm |
| 13024 | CHIROBIOTIC R | 250x4.6mm |
| 13026 | CHIROBIOTIC R | 500x4.6mm |
| 13034 | CHIROBIOTIC R | 250x10.0mm |
| 13036 | CHIROBIOTIC R | 500x10.0mm |
| 13044 | CHIROBIOTIC R | 250x21.2 mm |
| 13046 | CHIROBIOTIC R | 500x21.2mm |
| 13100 | Guard Column | 2cmx4.0mm |
| 13101 | Microbore Guard Column | 2cmx1.0mm |
| 21150 | Guard Column Holder for 2cmx4.0mm Guard Columns | |

## Availability - CHIROBIOTIC V ,T, TAG and R™ HPLC Columns - 10µm "Scout Columns" for Loading Studies for Preparative Separations

| CAT. # | DESCRIPTION | SIZE |
|---|---|---|
| 11124 | CHIROBIOTIC V (10µm) | 250x4.6mm |
| 15124 | CHIROBIOTIC V2 (10µm) | 250x4.6mm |
| 12124 | CHIROBIOTIC T (10µm) | 250x4.6mm |
| 16124 | CHIROBIOTIC T2 (10µm) | 250x4.6mm |
| 14124 | CHIROBIOTIC TAG (10µm) | 250x4.6mm |
| 13124 | CHIROBIOTIC R (10µm) | 250x4.6mm |

## Chiral Selectivity Screening Kit

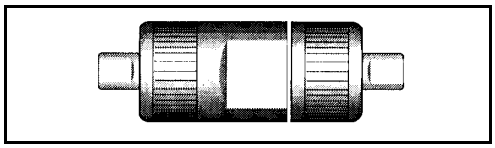
The Chiral Method Development Kit offers the chromatographer a broad range of separation capabilities using a simplified method to screen for chiral selectivity.

Catalog No. 10200
The kit contains:
1 - CHIROBIOTIC V HPLC Column, 100x4.6mm
1 - CHIROBIOTIC T HPLC Column, 100x4.6mm
1 - CHIROBIOTIC TAG HPLC Column, 100x4.6mm
1 - CHIROBIOTIC R HPLC Column, 100x4.6mm
2 - Column Couplers
1 - CHIROBIOTIC Handbook

## Guard Column System

The Astec 2cmx4.0mm guard column system is cartridge type and precision manufactured from 316 stainless steel. The inlet and outlet of holder connections are made using standard capillary tubing and fittings. Cartridges are packed with 5µm materials.

**Guard Cartridge Holder**



**Guard Cartridge Assembly**

## Preparative Columns and Media

The CHIROBIOTIC V, V2, T, T2, TAG and R are also available in 2" and 4" prepacked columns and bulk 10µm and 16µm media for preparative separations. We also offer contract services for the preparation of purified enantiomers in quantities up to 1 kilogram. Please contact our Sales Department for specific information.

## Astec Method Development Service

Astec provides a number of technical services based on the use of our chiral separation products. These include:

Program 1: LC screening for selectivity – 36 methods

Program 2: GC screening for selectivity – 16 methods

Program 3: Optimization, loading study and Purification

Program 4: Purification of gram quantities

For a detailed list of services and pricing, please contact our Sales Department.

5th Edition