



# Enhanced Selectivity on the Teicoplanin Aglycone Chiral Stationary Phase in HPLC

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Chirality-2001  
Orlando, Florida USA  
July 2001



# Abstract



Macrocyclic glycopeptide-based chiral stationary phases (CSPs) are becoming more popular due to their broad selectivity, multi-modal capability and ruggedness. Among them, Teicoplanin has proven to be the best choice for the chiral separation of amino acids. Recently, it has been reported that the removal of three sugar moieties on the Teicoplanin, resulting in the aglycone structure, gives different selectivity compared to its counterpart. Specifically, Teicoplanin aglycone CSP is showing more pronounced selectivity with acids (amino acids) and some classes of cyclic and neutral compounds. The enhancement could be 2-5 times both in selectivity and resolution. Due to the stronger polarity of this chiral stationary phase, pure organic solvent systems like alcohols and acetonitrile can be applied for separating neutral molecules.

Detailed information on the separation power of this unique phase will be presented. Examples and comparisons will be given under normal, reversed and polar organic phase<sup>2</sup> systems to show the versatility of this aglycone stationary phase.

<sup>1</sup> A. Berthod, X. Chen., J.P. Kullman, D.W. Armstrong, F. Gasparrini, I. D'Acquarica, C. Villani, A. Carotti, *Anal. Chem.* 72, 1767-1780 (2000).

<sup>2</sup> T. Beesley, A.X.Wang, Eastern Analytical Symposium, October 30-November 3, 2000, Atlantic City, NJ, Paper 153.





# Introduction

Studies demonstrating enhanced selectivity when the sugars are removed from the Teicoplanin CSP was work conceived and published by Dr. Francesco Gasparrini of the Universits Degli Studi Di Roma, Italy.<sup>1</sup>

The enhanced selectivity was observed for most amino acids but recent studies indicated this CSP also demonstrates excellent selectivity for sulfoxides, some acids containing either a phenoxy or hydrogen donating group alpha or beta to the carboxyl and some neutral molecules. As with the Teicoplanin CSP, methanol has been shown to be the best modifier. With a number of neutral molecules just 100% methanol, ethanol or acetonitrile has been shown to be the best mobile phase.

In all cases studied, the Teicoplanin CSP has shown some selectivity when compared to the aglycone form, therefore, in the column coupling screening method<sup>2</sup> the CHIROBIOTIC T (Teicoplanin) is always used as a primary screening tool and the CHIROBIOTIC TAG (Teicoplanin aglycone) is tried in the optimization step.

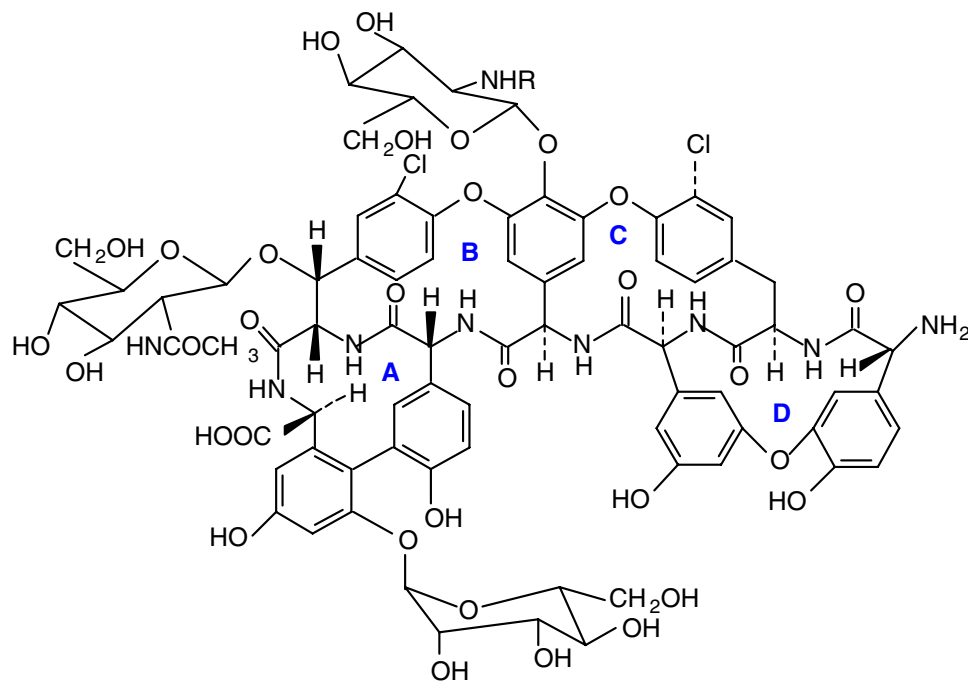
<sup>1</sup> A. Berthod, X. Chen., J.P. Kullman, D.W. Armstrong, F. Gasparrini, I. D'Acquarica, C. Villani, A. Carotti, Anal. Chem. 72, 1767-1780 (2000).

<sup>2</sup> A.X. Wang, J.T. Lee, T.E. Beesley, LG.GC, 18(6), 626-639 (2000).

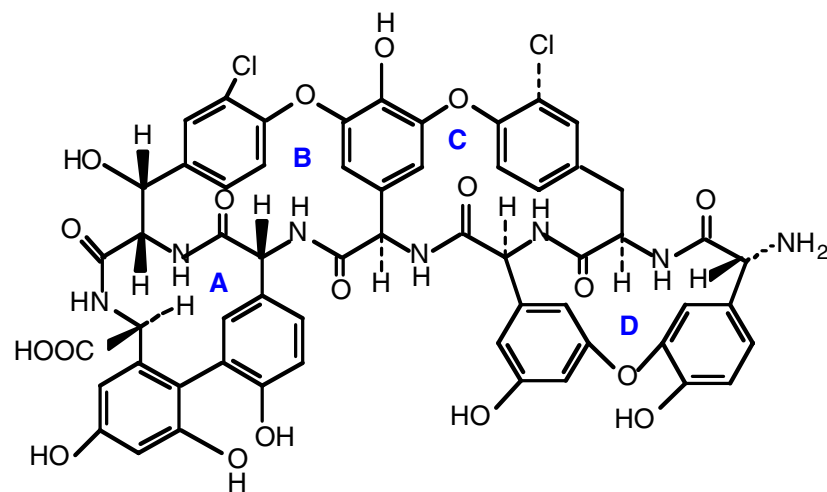


# Proposed Structures of Glycopeptide CSPs

Teicoplanin



Teicoplanin Aglycone



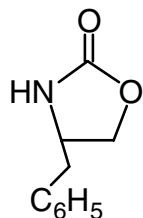
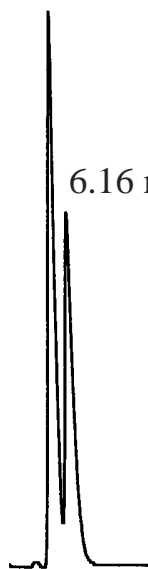
# Selectivity Enhancement Teicoplanin vs. Teicoplanin Aglycone

Separation of the Neutral Molecule 4-Benzyl-2-oxazolidinone

CHIROBIOTIC T

5.57 min.

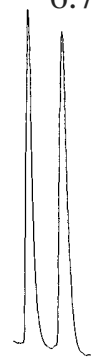
6.16 min.



CHIROBIOTIC TAG

5.60 min.

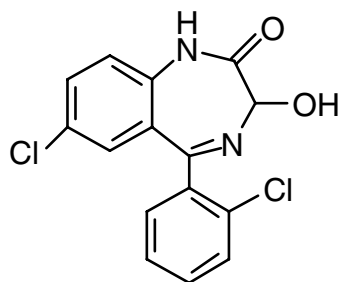
6.72 min.



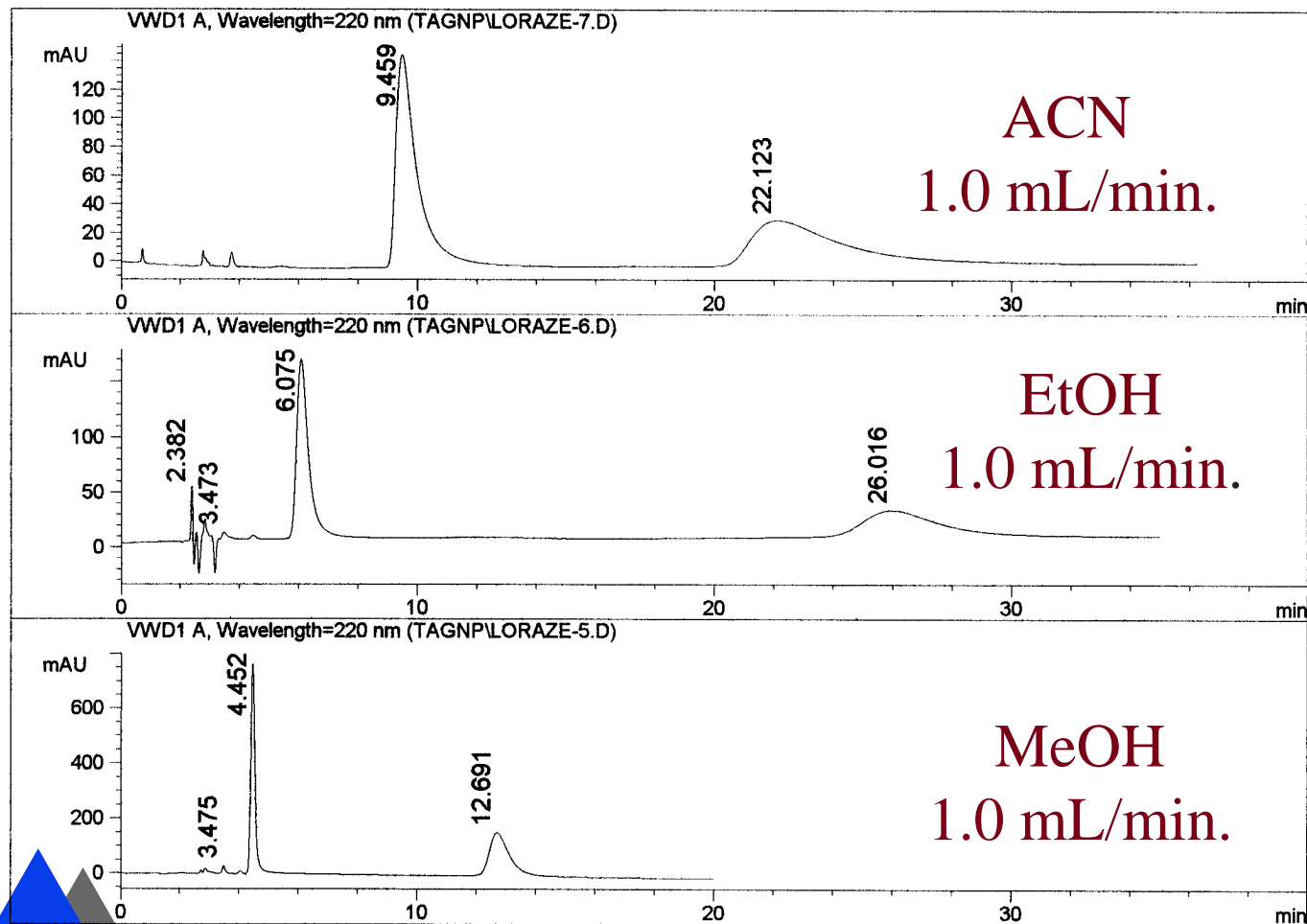
100% MeOH @ 1.0 mL/min.

# Effect of Single Solvent on Selectivity for Neutral Molecules

Lorazepam

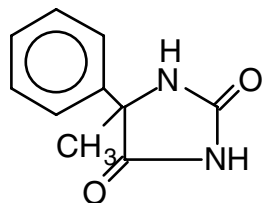


CHIROBIOTIC  
TAG

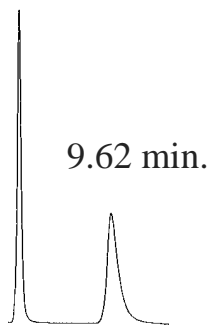


# Selectivity of Methanol for Neutral Molecules on Bonded Teicoplanin Aglycone

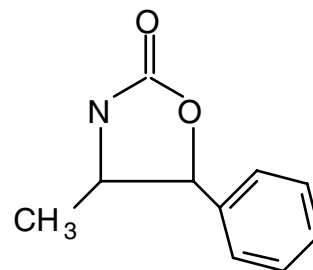
5-Methyl-4-phenylhydantoin



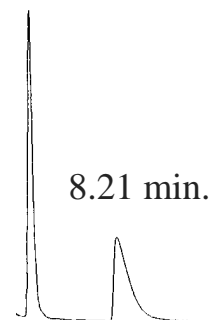
5.08 min.



4-Methyl-5-phenyl-2-oxazolidinone



5.35 min.

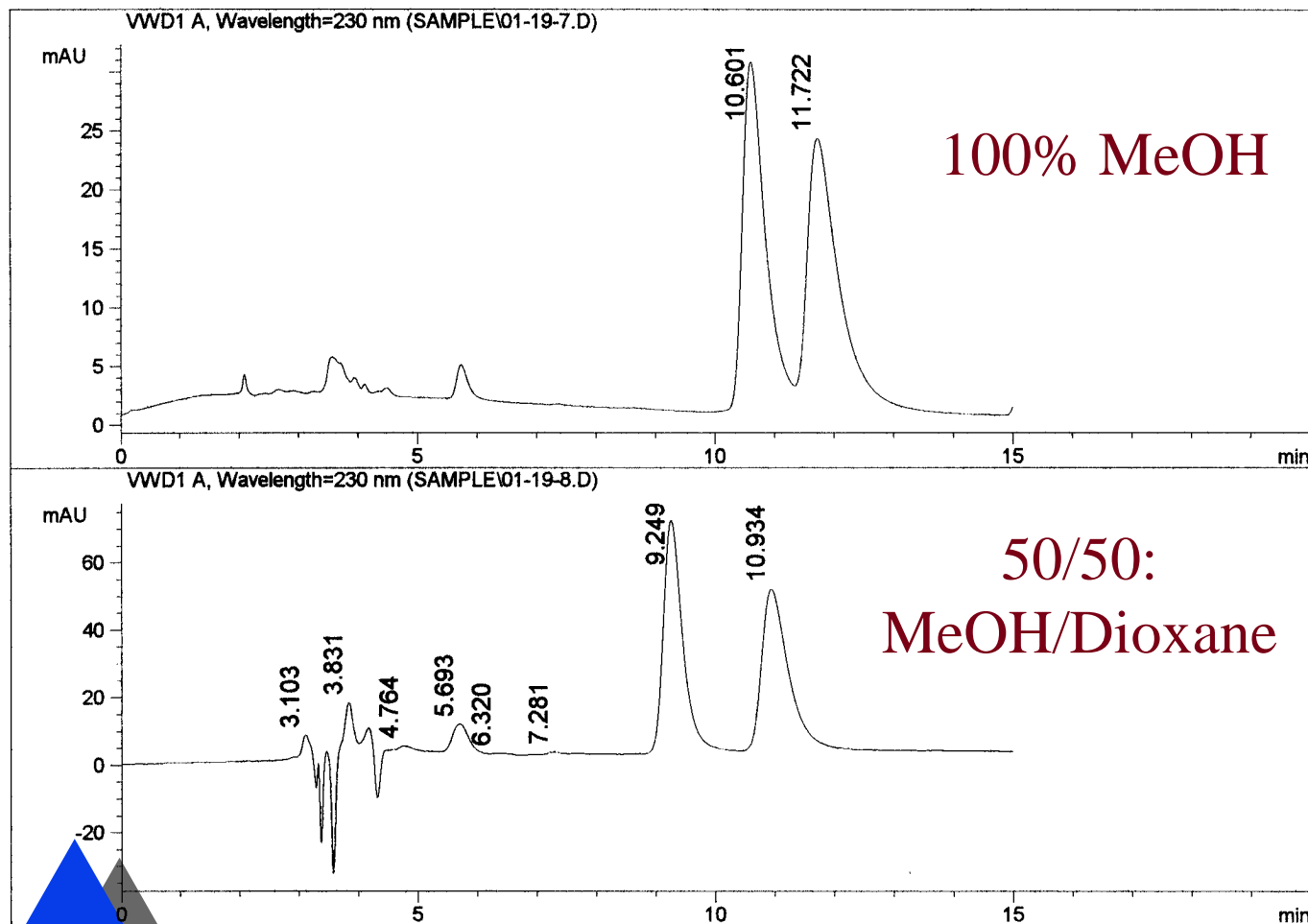


100% MeOH @ 0.8 mL/min.

# Effect of Dioxane on Selectivity and Resolution for Some Neutral Racemates

CHIROBIOTIC  
TAG

0.8 mL/min.

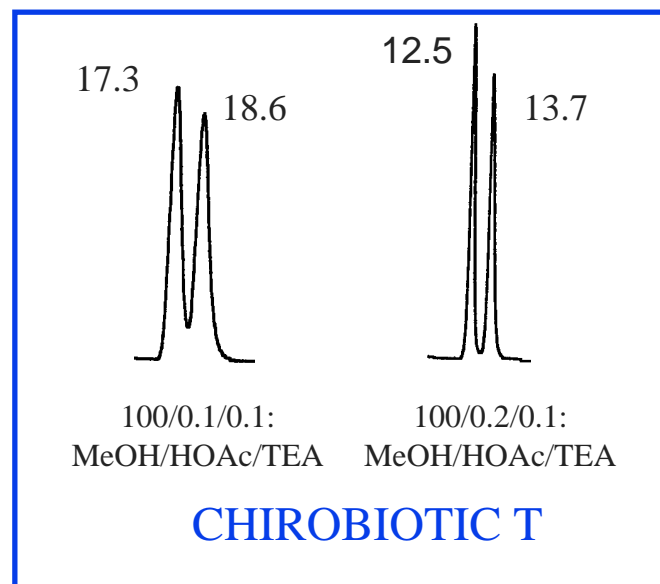
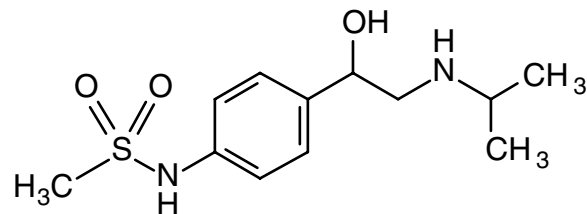




# Optimization in the New Polar Organic Mode

1. Use a single analytical column (25cm T or TAG).
2. Choose proper acid, base or salt (HOAc, TEA, TFA,  $\text{NH}_4\text{OCOCF}_3$ , etc.).
3. Adjust acid/base ratio (4/1 to 1/4).
4. Change the concentration of acid and base (0.001% to 1%). Note: Higher concentration of acid and base results in lower retention.
5. Change flow rate. Lower flow rate often results in higher resolution.

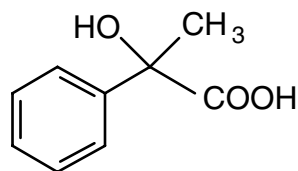
## Sotalol



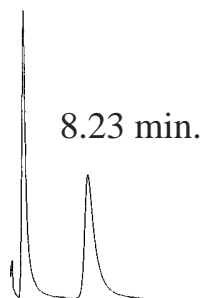
# Bonded Teicoplanin

## Aglycone Separation of Acids in the Polar Organic Mode

Atrolactic acid

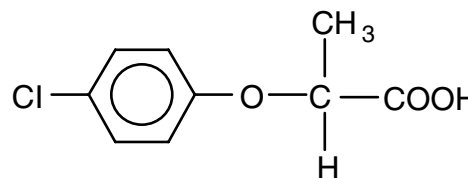


3.93 min.

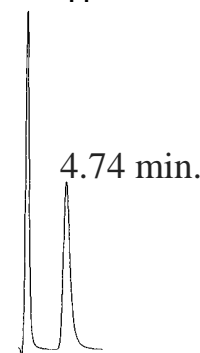


100/0.1/0.1: MeOH/HOAc/TEA  
1.0 mL/min.

2-(4-Chlorophenoxy) propionic acid



3.43 min.



100/0.2/0.1: MeOH/HOAc/TEA  
1.0 mL/min.



# Optimization in the Reversed Phase Mode

## Parameters That Affect Selectivity and Resolution

### Organic Modifier with broadest selectivity

CH<sub>3</sub>OH

Other organic modifiers include CH<sub>3</sub>CN, IPA and EtOH

### Solvent/Buffer

Retention is adjusted by the amount of organic modifier. Selectivity affected at both high and low amounts of organic modifier.

### pH Effect

Large effects since both anionic and cationic functionalities exist in these CSPs.

Safe operating pH range for T and TAG - 3.8 - 6.5

### Buffer Type and Strength

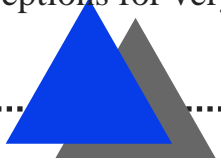
Smaller impact on selectivity. Ionic strength is a function of the stationary phase T < TAG.

### Flow Rate

Strong response in the reversed phase mode, moderate in the polar organic mode and no effect in the normal phase mode for all phases.

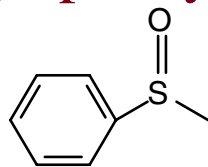
### Temperature

Response similar to flow rate parameter for all phases. Decrease temperature generally increases Rs. Exceptions for very polar molecules.



# Selectivity of Bonded Teicoplanin Aglycone for Sulfur Containing Racemates

Methyl-phenyl sulfoxide

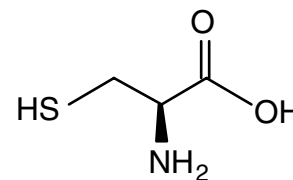


10.86 min.

14.49 min.

40/60: EtOH/Hexane  
1.5 mL/min.

Cysteine



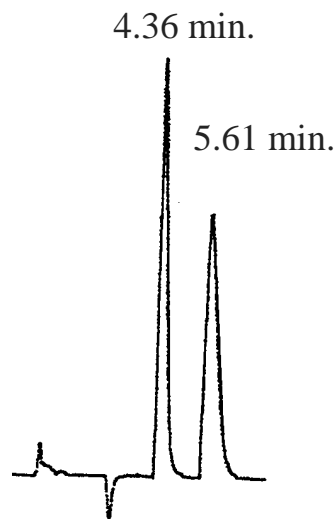
3.60 min.

5.20 min.

60/40: MeOH/H<sub>2</sub>O  
1.0 mL/min.

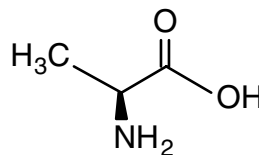
# Selectivity Comparison T vs TAG for Aliphatic $\alpha$ -Amino Acids

## CHIROBIOTIC T

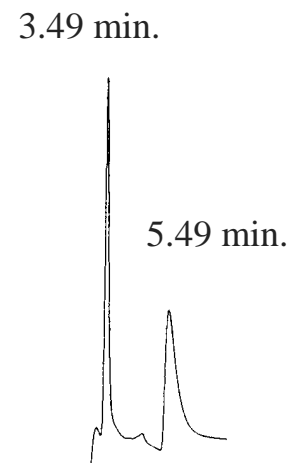


50/50: EtOH/H<sub>2</sub>O  
1.0 mL/min.  
 $\alpha = 1.80$

## Alanine



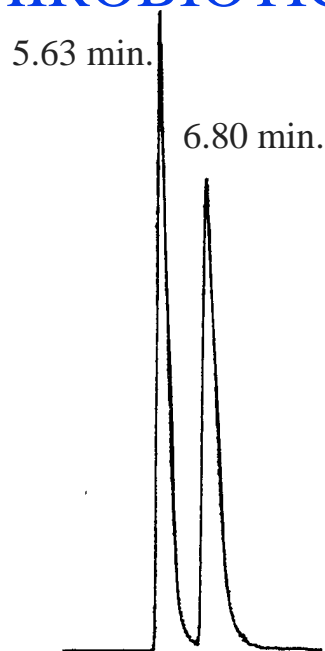
## CHIROBIOTIC TAG



30/70: MeOH/H<sub>2</sub>O  
1.0 mL/min.  
 $\alpha = 5.19$

# Selectivity Comparison T vs TAG for Aromatic $\alpha$ -Amino Acids

CHIROBIOTIC T

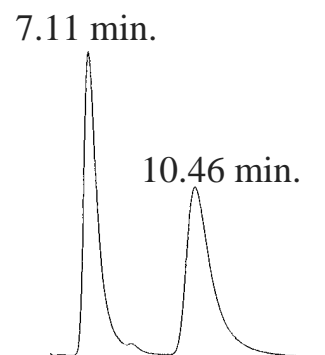


30/70: EtOH/H<sub>2</sub>O

1.0 mL/min.

$\alpha = 1.42$

CHIROBIOTIC TAG

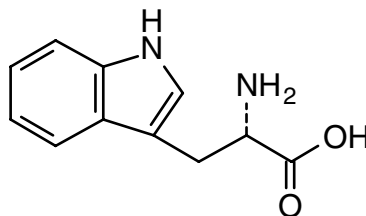


60/40: MeOH/H<sub>2</sub>O

1.0 mL/min.

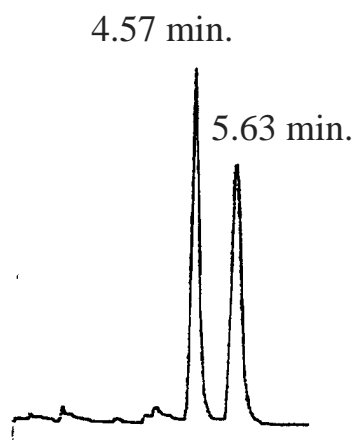
$\alpha = 1.82$

Tryptophan



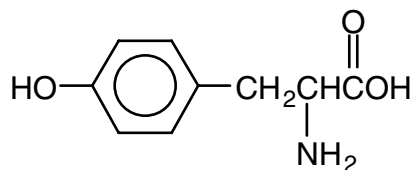
# Selectivity Comparison T vs TAG for Aromatic $\alpha$ -Amino Acids

CHIROBIOTIC T

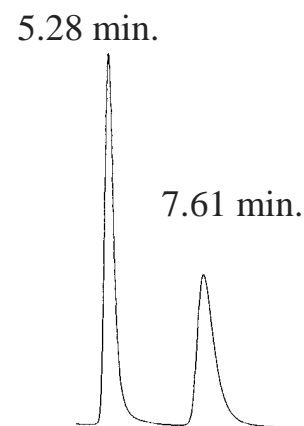


50/50: EtOH/H<sub>2</sub>O  
1.0 mL/min.  
 $\alpha$  - 1.60

p-Tyrosine



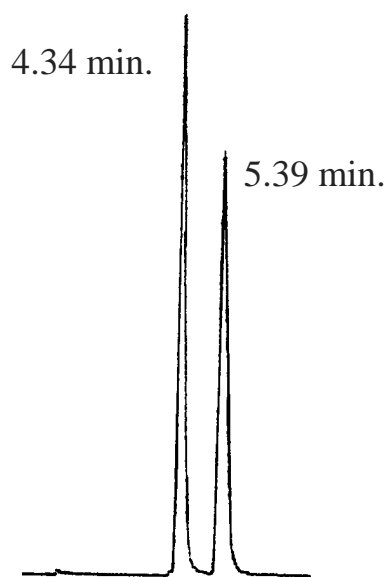
CHIROBIOTIC TAG



30/70: MeOH/H<sub>2</sub>O  
1.0 mL/min.  
 $\alpha$  = 2.02

# Selectivity Comparison T vs TAG for Sulfur Containing $\alpha$ -Amino Acids

CHIROBIOTIC T

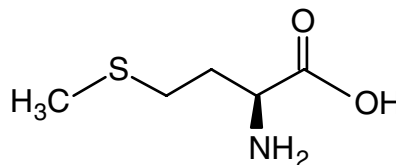


20/80: EtOH/H<sub>2</sub>O

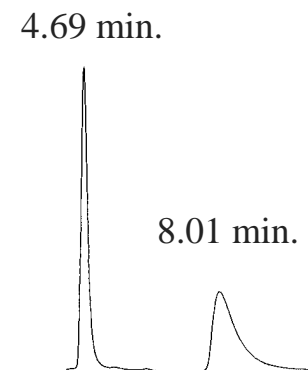
1.0 mL/min.

$\alpha = 1.32$

Methionine



CHIROBIOTIC TAG



30/70: MeOH/H<sub>2</sub>O

1.0 mL/min.

$\alpha = 2.98$

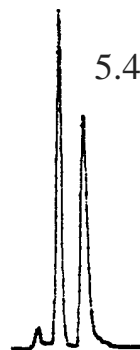


# Selectivity Comparison T vs TAG for Basic $\alpha$ -Amino Acids

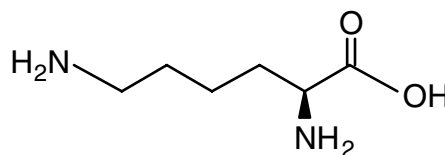
CHIROBIOTIC T

4.81 min.

5.44 min.



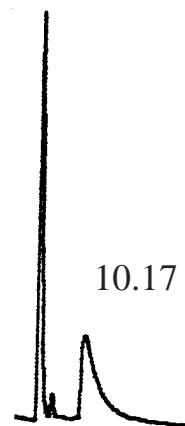
Lysine



CHIROBIOTIC TAG

7.07 min.

10.17 min.



20/80: EtOH/100mM NaH<sub>2</sub>PO<sub>4</sub>, pH 4.6

1.0 mL/min.

$\alpha = 1.32$

20/80: MeOH/100mM NaH<sub>2</sub>PO<sub>4</sub>

1.0 mL/min.

$\alpha = 1.80$

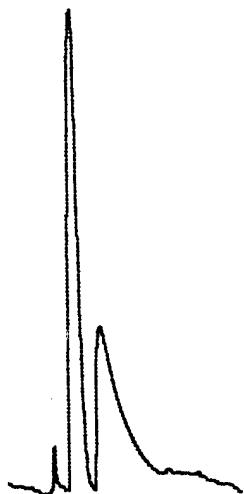
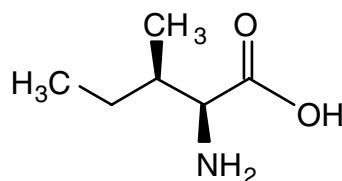
# Selectivity: Underivatized $\alpha$ -Amino Acids

$\alpha$ -Amino Acid	CHIROBIOTIC T			CHIROBIOTIC TAG		
	k'	$\alpha$	Mobile Phase	k'	$\alpha$	Mobile Phase
Alanine	0.56	1.80	50/50: EtOH/H <sub>2</sub> O	0.16	5.19	30/70: MeOH/H <sub>2</sub> O
Arginine	1.17	1.34	20/80 :EtOH/100mM NaH <sub>2</sub> PO <sub>4</sub>	2.49	1.88	20/80: MeOH/100mM NaH <sub>2</sub> PO <sub>4</sub>
Asparagine	0.58	1.61	50/50:EtOH/H <sub>2</sub> O	0.29	2.79	30/70: MeOH/H <sub>2</sub> O
Aspartic	1.49	1.58	70/20/10 :EtOH/MeOH/H <sub>2</sub> O,pH 4.1*	0.87	1.98	60/40: MeOH/H <sub>2</sub> O, pH 3.2*
Cysteine	0.45	1.60	60/40: EtOH/H <sub>2</sub> O	0.20	3.67	60/40:MeOH/H <sub>2</sub> O
Glutamic	1.15	1.59	80/20: EtOH/0.1% TEAA, pH 4.2*	0.72	2.68	60/40: MeOH/H <sub>2</sub> O, pH 3.2*
Glutamine	1.13	1.38	50/50: EtOH/H <sub>2</sub> O	0.82	2.07	60/40:MeOH/H <sub>2</sub> O
Histidine	3.10	1.19	60/40: EtOH/160mM NaH <sub>2</sub> PO <sub>4</sub>	2.70	1.19	30/70: MeOH/100mM NaH <sub>2</sub> PO <sub>4</sub>
Isoleucine	0.40	2.00	60/40: EtOH/H <sub>2</sub> O	0.25	5.87	30/70: MeOH/H <sub>2</sub> O
Leucine	0.47	1.92	30/70: EtOH/H <sub>2</sub> O	0.46	3.33	30/70: MeOH/H <sub>2</sub> O
Lysine	0.72	1.32	20/80: EtOH/100mM NaH <sub>2</sub> PO <sub>4</sub>	1.21	1.80	20/80: MeOH/100mM NaH <sub>2</sub> PO <sub>4</sub>
Methionine	0.55	1.68	20/80: EtOH/H <sub>2</sub> O	0.56	2.98	30/70: MeOH/H <sub>2</sub> O
Phenylalanine	0.87	1.53	50/50: EtOH/H <sub>2</sub> O	0.90	2.08	60/40:MeOH/H <sub>2</sub> O
Proline	1.90	1.55	100% H <sub>2</sub> O	2.20	1.97	100% H <sub>2</sub> O
Serine	0.89	1.36	70/30: EtOH/H <sub>2</sub> O	0.11	2.45	30/70:MeOH/H <sub>2</sub> O
Threonine	0.75	1.39	70/30: EtOH/H <sub>2</sub> O	0.13	4.46	60/40: MeOH/H <sub>2</sub> O
Tryptophan	1.01	1.42	30/70: EtOH/H <sub>2</sub> O	1.37	1.82	60/40: MeOH/H <sub>2</sub> O
Tyrosine	0.63	1.60	50/50: EtOH/H <sub>2</sub> O	0.76	2.02	30/70: MeOH/H <sub>2</sub> O
Valine	0.56	1.58	50/50: EtOH/H <sub>2</sub> O	2.48	4.06	60/40: MeOH/H <sub>2</sub> O

\*pH of aqueous portion of the mobile phase.

# Preparative Purification of Isoleucine on Teicoplanin Aglycone

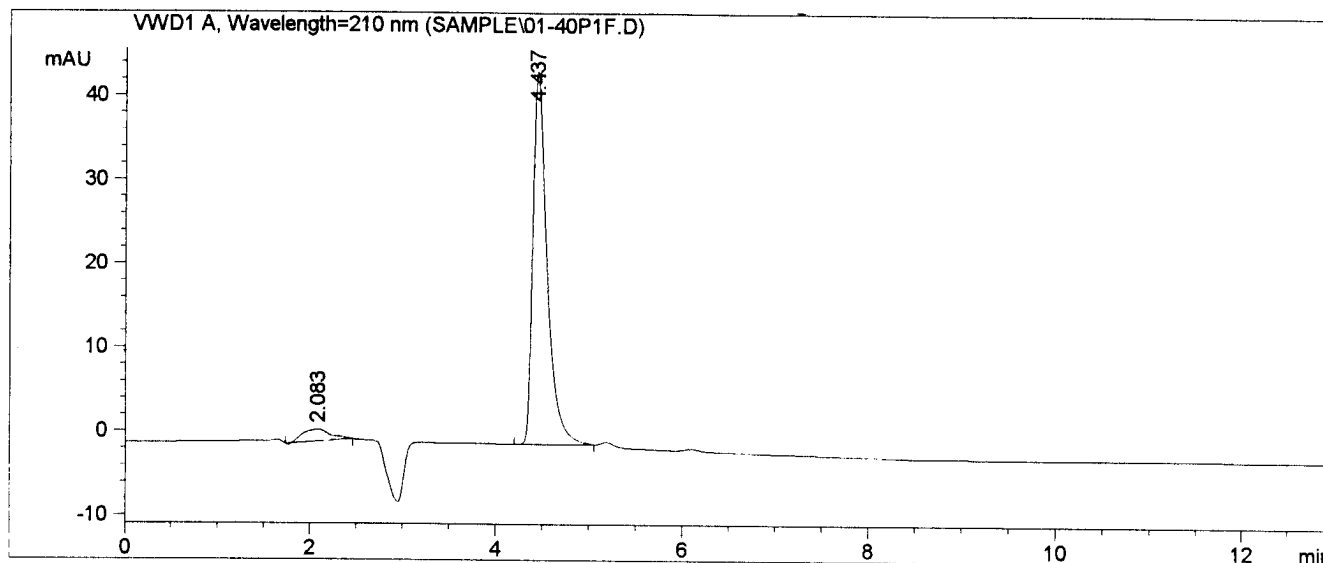
Isoleucine



Column:	CHIROBIOTIC TAG
Size:	250 x 22.1mm
Mobile Phase:	30/70: MeOH/H <sub>2</sub> O
Flow Rate:	20 mL/min.
Injection:	170 mg in 10 mL

# Recovered Isoleucine Enantiomers from 1.5 Grams of Process Racemate - Peak 1

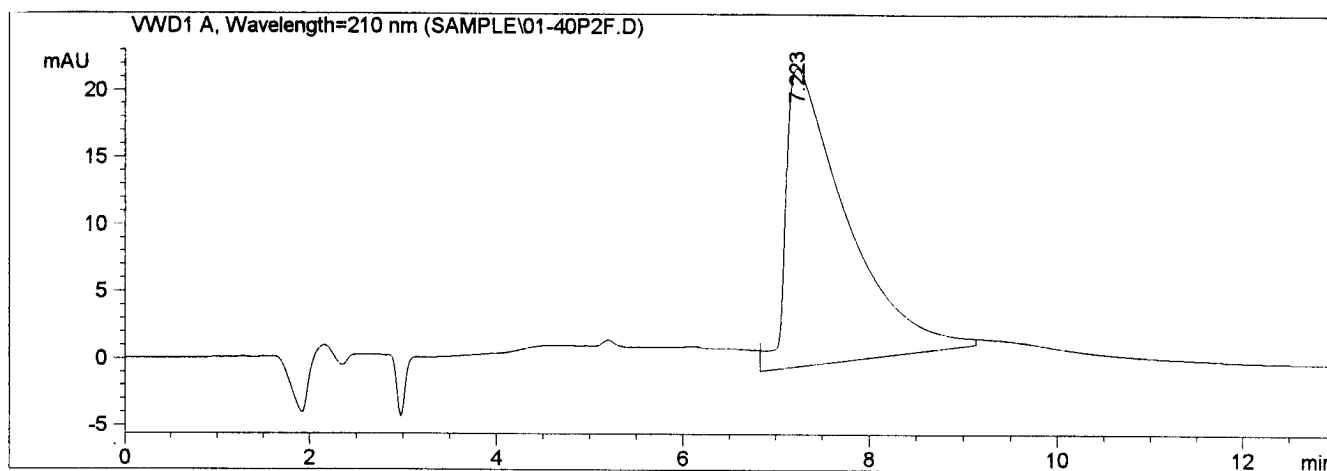
4.437 min: 100% ee



Throughput: 24mg/gram CSP/hour

# Recovered Isoleucine Enantiomers from 1.5 Grams of Process Racemate - Peak 2

7.223 min: 100% ee



Throughput: 24mg/gram CSP/hour

# Conclusions

- u Removal of the sugars from the bonded teicoplanin CSP to form the teicoplanin aglycone, enhances selectivity for alpha amino acids, a number of aromatic and phenoxy acids, sulfoxides and some neutral racemates.
- u On the bonded teicoplanin aglycone it is possible to use single solvents like methanol, which is the preferred, as well as ethanol or acetonitrile for the separation of neutral racemates. The addition of dioxane to the methanol could be used to improve peak shape and resolution for some neutral racemates.
- u Both the polar organic and reversed phase mode yielded excellent results for aromatic and phenoxy acids.
- u Overall, increased load capacity results from the substantially higher selectivity of the aglycone for amino acids. Increased stability to lower pH was also observed.