

Advances in Chiral Resolution of Amino Acids by LC and LC/MS

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44th RMCAC Symposium, New Frontiers in Separation Science, Denver, CO, July 2002

Abstract

Proteomics and the analysis of amino acids for human health coupled with the utility of amino acids in biotechnology and other pharmaceuticals has made the chiral analysis of amino acids imperative. There is a long history in chiral separation of amino acids from the development of the first LC methods for separating various alpha, primary amines by the chiral crown ether design of Cram, to the work of Davenkov and ligand exchange chromatography. The application of macrocyclic glycopeptides since 1995 however, has opened the doors to an array of applications for all forms of amino acids including primary, secondary as well as beta and gamma amino acids. This technique has also allowed for the resolution of a wide variety of N-blocked amino acids. This presentation will cover the history of chiral stationary phase development in this area and focus primarily on developments with macrocyclic glycopeptides. Very large increases in selectivity have been obtained with the aglycone forms of at least one macrocyclic in particular, teicoplanin. Comparison of amino acid separations on different versions of these various CSP's will be presented and the utility of some for preparative application will be outlined. The use of these novel stationary phases has not only expanded the applications possible in this area but has tied the technology to a wide variety of methodologies like SFC and LC/MS.

Amino Acids - the Building Blocks of Peptides, Proteins and Pharmaceutical Targets

Chiral Purity is Essential

Presentation contents:

- *Historical Development of CSP's for Amino Acids Chiral Recognition

- *Applications:

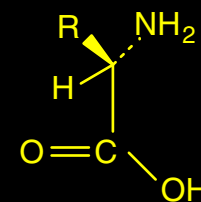
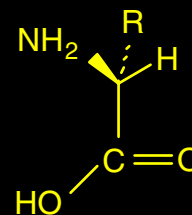
 - Free alpha, beta and gamma amino acids

 - Cyclic and unusual amino acids

 - N-blocked amino acids

 - LC/MS of amino acids

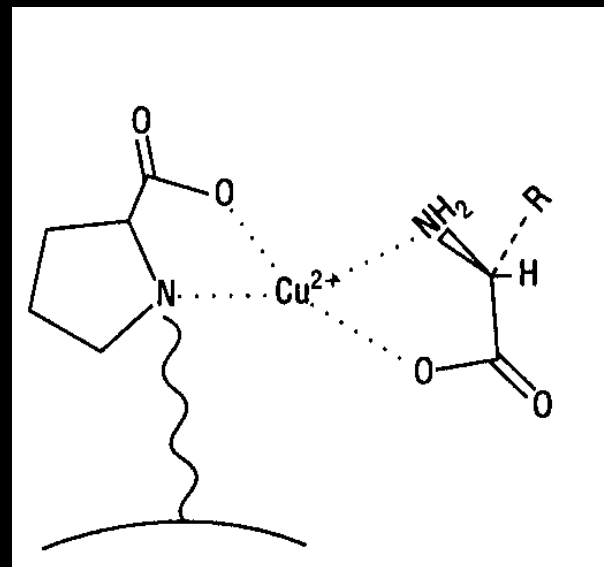
 - Clinical applications for genetic diseases, (LC/MS)



Ligand Exchange

Schematic of the three dimensional complex formed between the bonded amino acid proline, copper (II) and an amino acid.

Reference: Davenkov et al, J. Chromatogr., 60, 280 (1971).

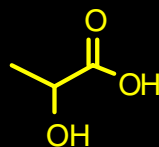


Key Points for Ligand Exchange

1. First practical approach to the separation of underivatized amino acids.
2. Requires a bidentate ligand. Examples of the type of chiral ligands employed include but are not limited to proline, hydroxyproline, histidine, penicillamine and phenylalanine amide.
3. Requires a transition metal with a +2 valence, copper being the most effective.
4. Copper complex formed with the free amino acid in solution can be visualized at 254 nm UV.
5. In addition to alpha amino acids, alpha hydroxy acids and amino alcohols have been resolved on these types of CSP's.
6. Various alcohols and pHs can be used to effect retention and resolution.
7. Elution order can be reversed by using different enantiomeric form of the bidentate ligand.

Separation of Alpha Hydroxy Acids

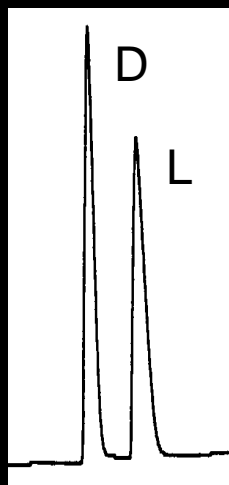
Lactic Acid



Astec CLC-L

Peak 1: 9.24 min.

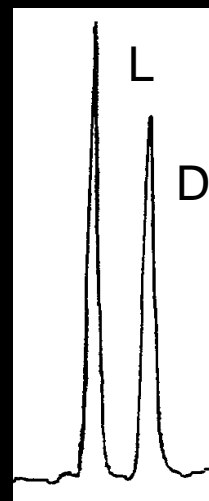
Peak 2: 12.58 min.



Astec CLC-D

Peak 1: 9.27 min.

Peak 2: 12.18 min.



Mobile Phase:
5mM CuSO₄

Separation D, L Proline Astec Ligand CLC-D

Peak 1: 4.25 min.

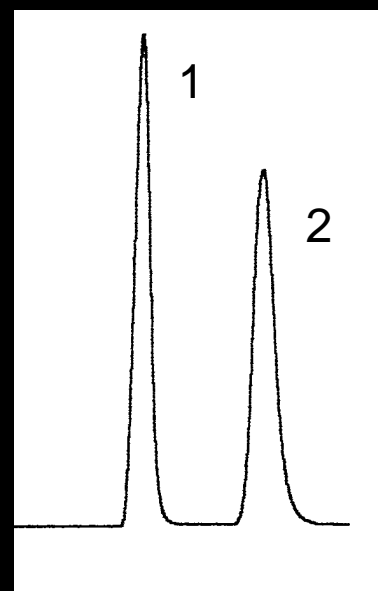
Peak 2: 7.41 min.

Mobile Phase: 3mM CuSO₄

Flow Rate: 1.5mL/min

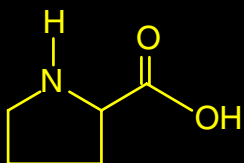
UV: $\lambda=254$ nm

Inj: 2 μ L (10mg/mL)



Trace Analyses on Astec CLC Columns

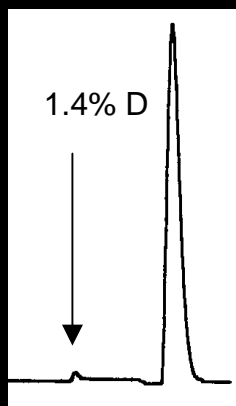
Proline



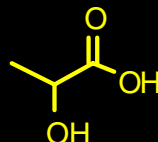
Astec CLC-L

Peak 1: 6.29 min. (D)

Peak 2: 11.87 min. (L)



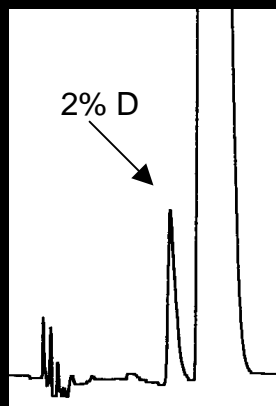
Lactic Acid



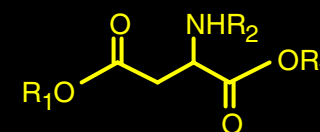
Astec CLC-L

Peak 1: 9.24 min. (D)

Peak 2: 12.58 min. (L)



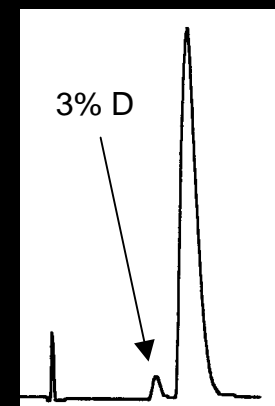
Aspartic Acid



Astec CLC-L

Peak 1: 9.17 min. (D)

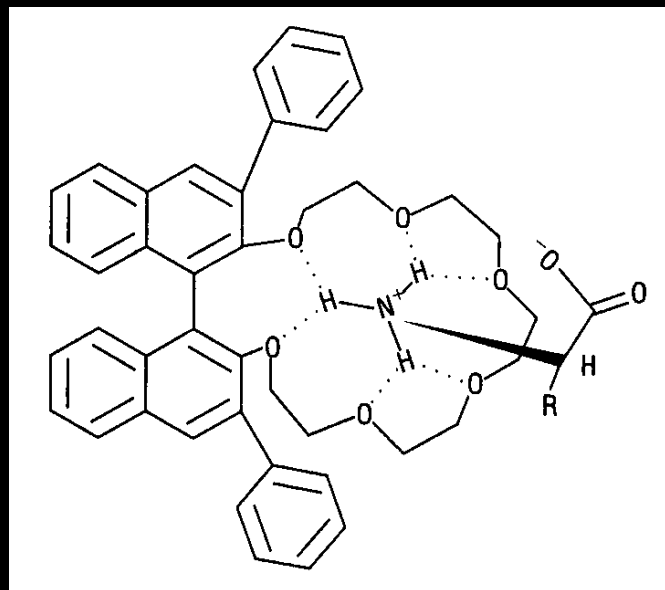
Peak 2: 11.14 min. (L)



Chiral Crown Ethers

References: Cram, et al.,

- 1.) J. Am. Chem. Soc. 96, 6762 (1974).
- 2.) J. Am. Chem. Soc. 101, 4941 (1979).

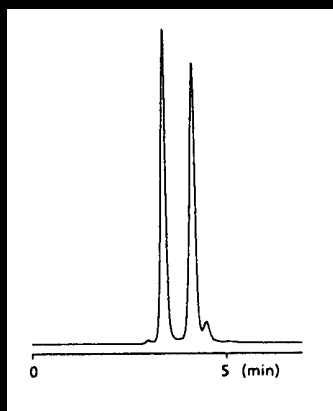


Key Points for Chiral Crown Ethers

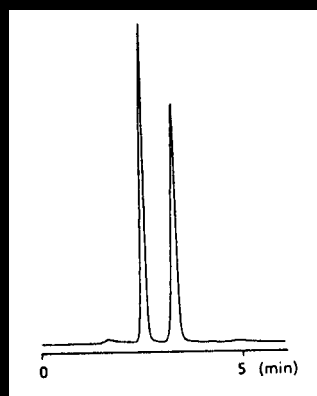
1. Expands the reach of chiral separations to a greater variety of primary amine containing analytes.
2. Crown-6-polyethers chirality is obtained with the attachment of steric bulk in the form of chiral planes that can be made (+) or (-).
3. Amine must be in the form of an ammonium ion to form the required inclusion complex.
4. Uses only perchloric acid as the mobile phase to promote and maintain the ammonium ion formation.
5. Secondary amines do not separate.
6. Temperature can be used as an operating parameter to effect retention and selectivity.

Crown Ether Separations

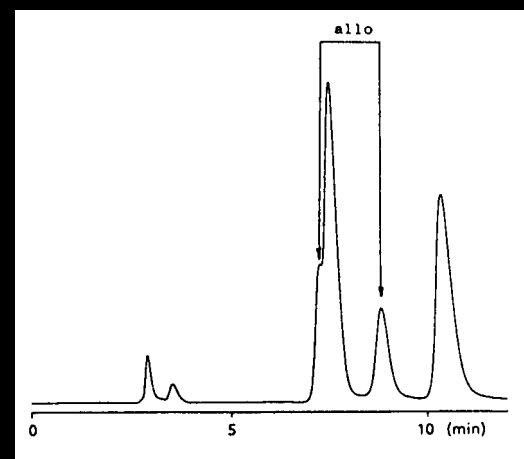
Glutamine



Methionine



Isoleucine

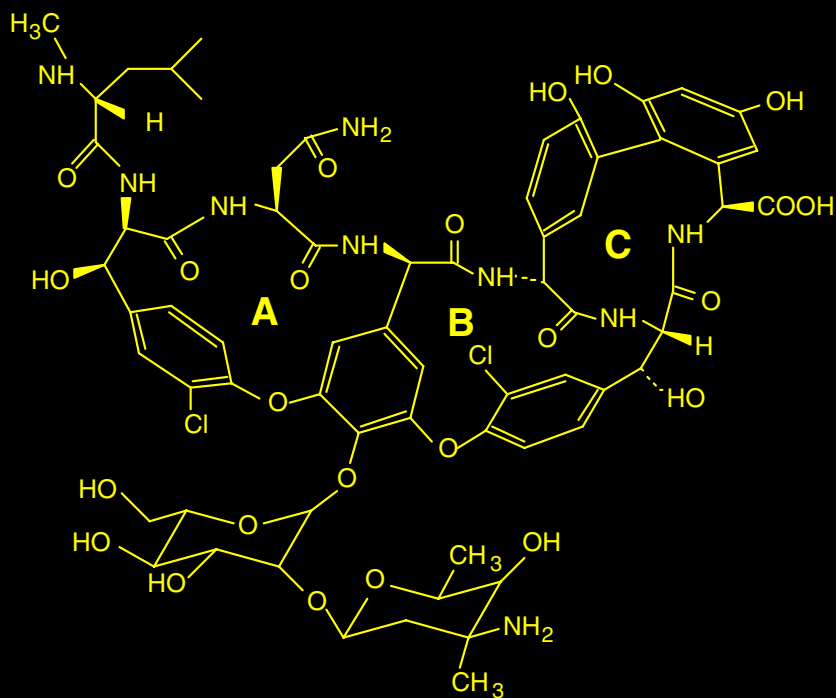


Load: 5 mM, 2 μ L
Eluent: Aq. HClO₄ pH 2.0
Flow rate: 0.4 mL/min
Temperature: 25°C
Detection: UV 200 nm

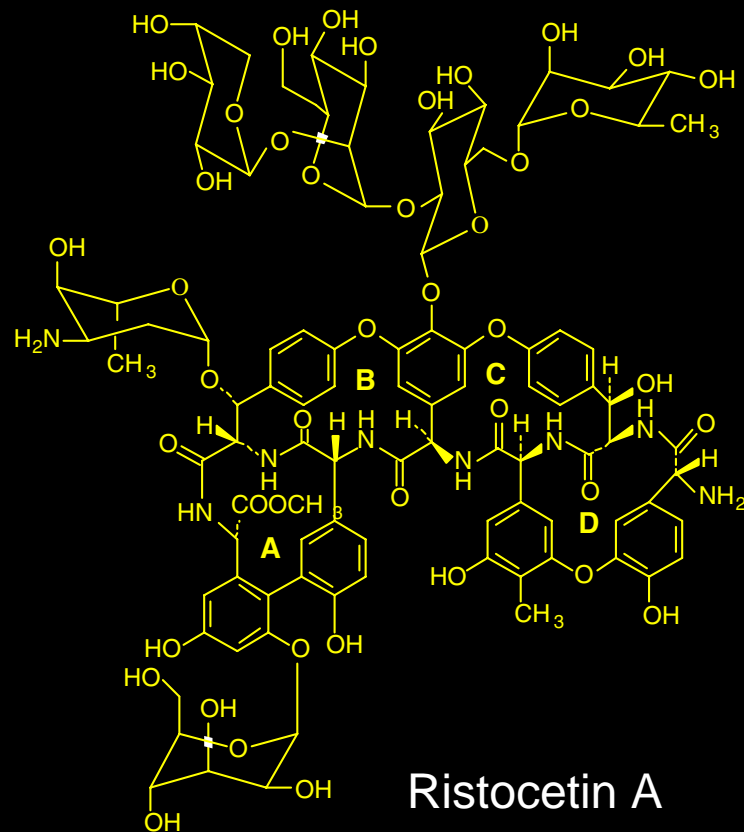
Load: 5 mM, 2 μ L
Eluent: Aq. HClO₄ pH 2.0
Flow rate: 0.8 mL/min
Temperature: 25°C
Detection: UV 200 nm

Load: 5 mM, 10 μ L
Eluent: Aq. HClO₄ pH 2.0
Flow rate: 0.4 mL/min
Temperature: 0°C
Detection: UV 200 nm

Proposed Structures of Glycopeptide CSP's

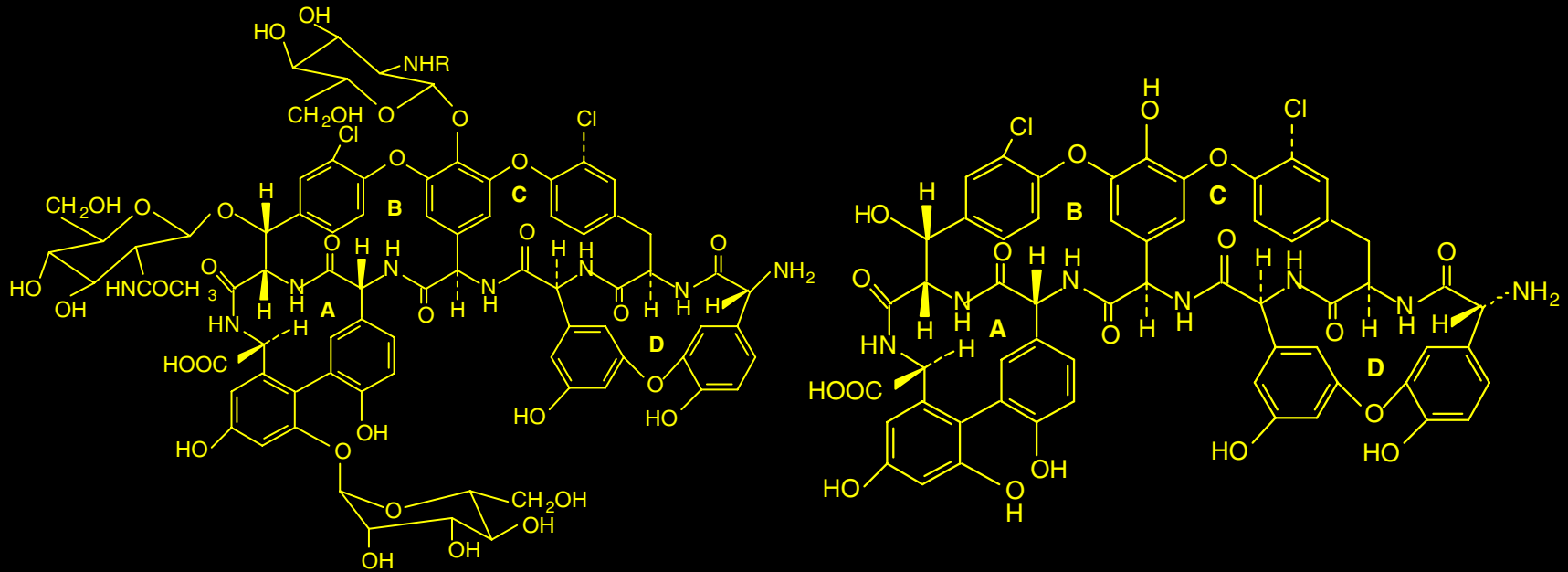


Vancomycin



Ristocetin A

Proposed Structures of Glycopeptide CSP's



Teicoplanin

Teicoplanin Aglycone

Possible Mechanism for the Separation of Amino Acids on CHIROBIOTIC CSP's

Work of Berthod and Armstrong:

J. Chromatogr. A. 731(1996) 123-137

Indicated that the primary amine on the CHIROBIOTIC T was largely responsible for the separation of racemic amino acids but that several other mechanisms were also at work.

Work of Jandera:

J. Chromatogr. A. 917 (2001) 123-133

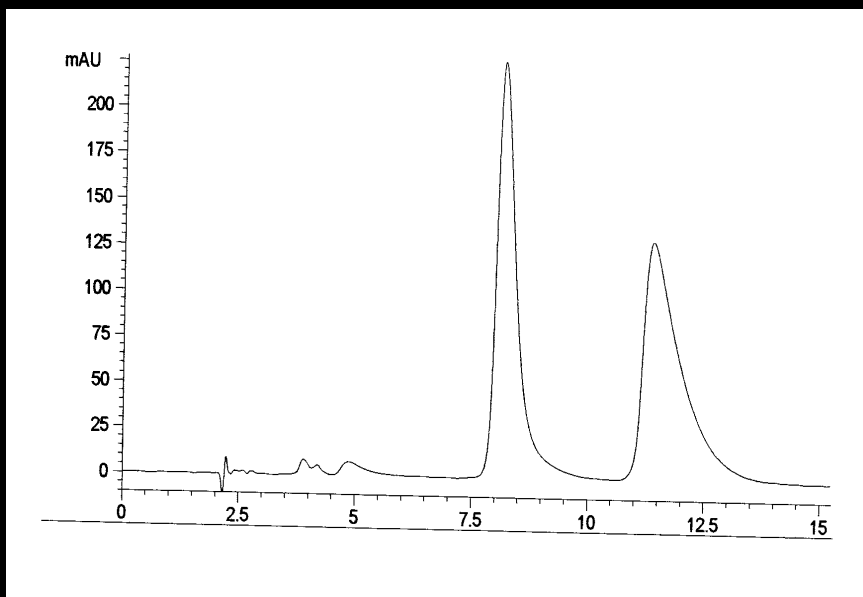
J. Chromatogr. A. 919 (2001) 67-77

Indicated that at least two selective sites and one non-selective site take part in chiral recognition on the CHIROBIOTIC T.

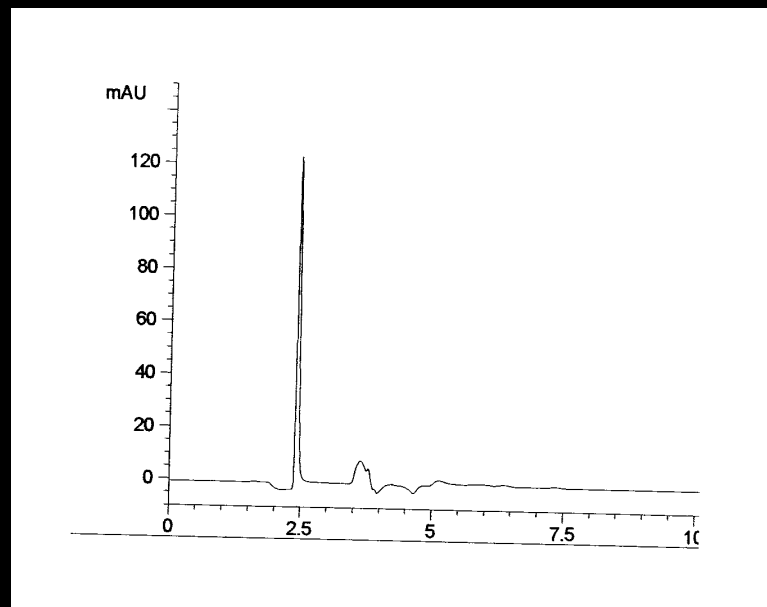
Key Functional Group for CSP Interaction

Column: CHIROBIOTIC T (250x4.6mm)
Mobile Phase: 50/50, EtOH/H₂O
Flow rate: 0.8mL/min.
UV: 254nm

Phenylalanine



Phenylalanine methyl ester

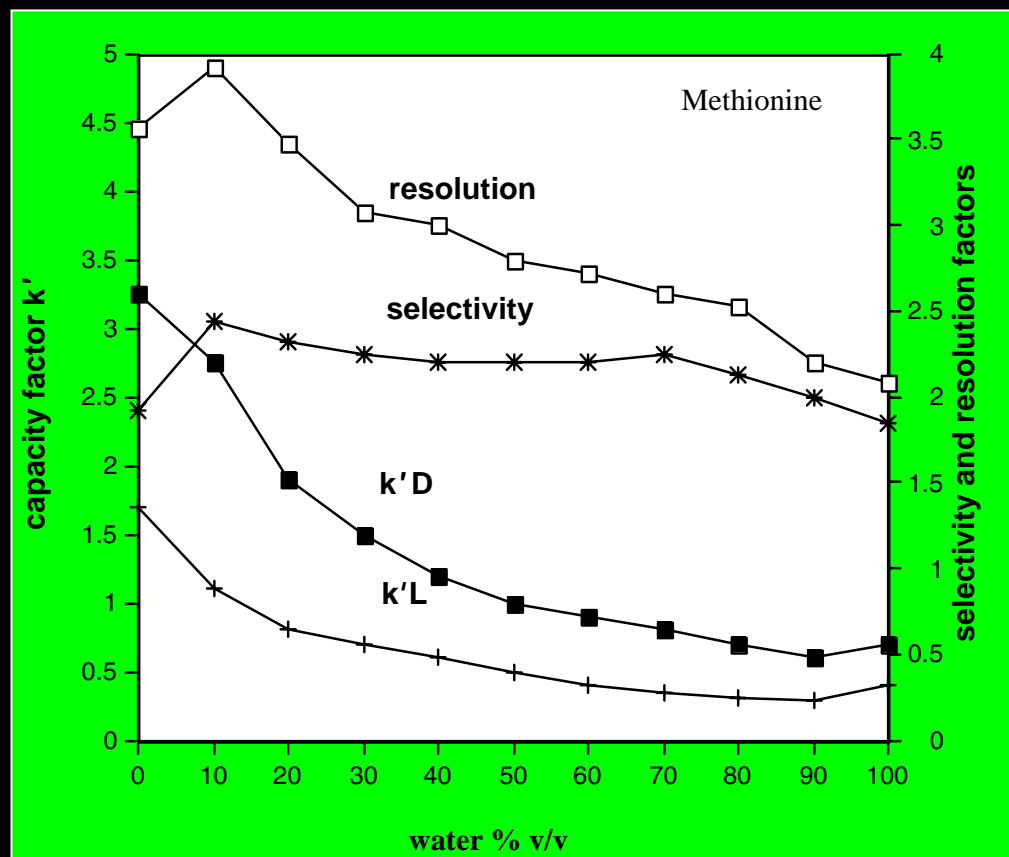


Underivatized D, L-Amino Acids

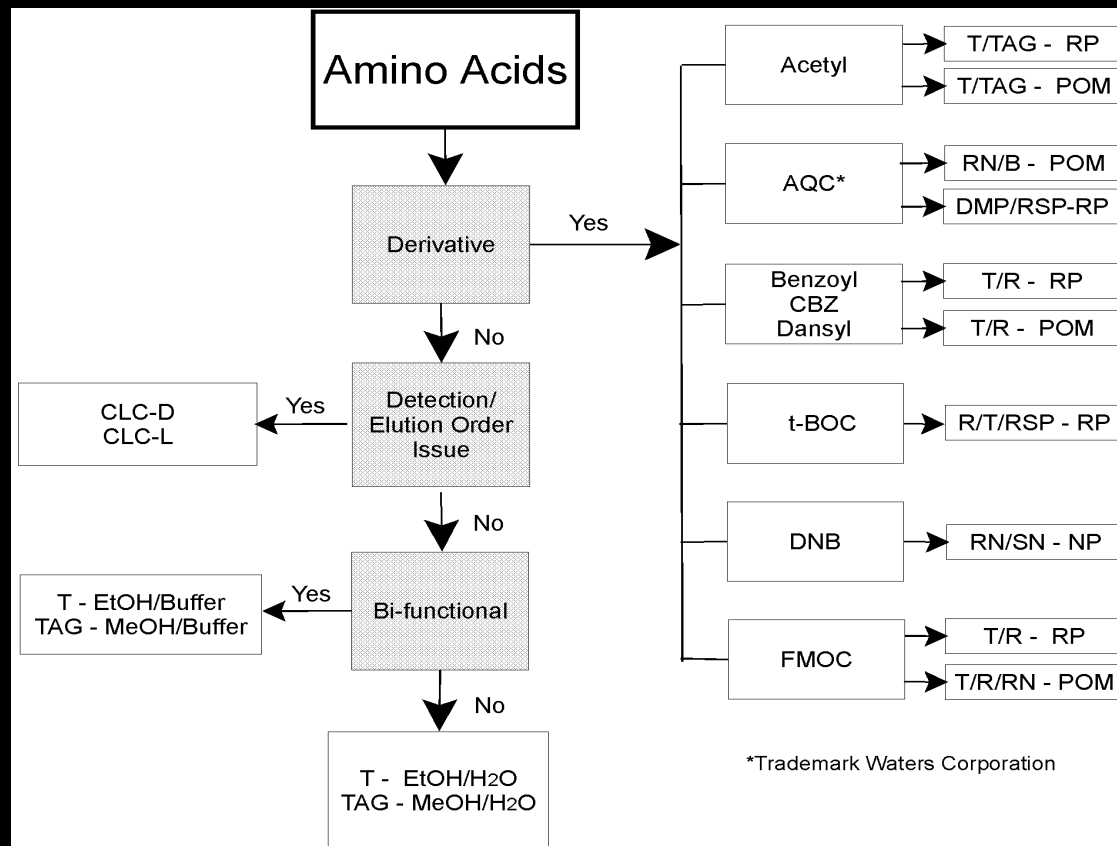
Effect of Alcohol Modifier on Retention, Selectivity and Resolution

Sample: Methionine

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.



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



Enantioresolution of Underivatized α -Amino Acids

$\begin{array}{c} \text{R}-\text{CH}-\text{COOH} \\ \\ \text{NH}_2 \end{array}$		CHIROBIOTIC T ⁽¹⁾		CHIROBIOTIC TAG ⁽²⁾		CHIROBIOTIC R ⁽³⁾	
α -Amino Acid	R-Moiety	k'	Rs	k'	Rs	k'	Rs
Alanine	-CH ₃	0.56	2.9	0.09	4.0	1.35(L)	1.45
Arginine	-(CH ₂) ₃ -NH-C(NH ₂) ₂	1.17	2.1	2.17	3.0	N/A	N/A
Aspartic	-CH ₂ -COOH	1.49	1.9	0.34	3.4	N/A	N/A
Asparagine	-CH ₂ -CO-NH ₂	0.58	2.1	0.21	3.7	1.45(L)	1.56
Cysteine	-CH ₂ -SH	0.45	1.6	0.20	1.8	1.78(L)	1.50
Glutamic	-CH ₂ -CH ₂ -COOH	1.15	2.2	0.64	2.5	N/A	N/A
Glutamine	-(CH ₂) ₂ -CO-NH ₂	1.13	1.6	0.82	3.5	N/A	N/A
Histidine		3.10	0.8	8.45	2.3	1.13(L)	1.45
Isoleucine	-CH(CH ₃)-CH-CH ₃	0.40	2.5	0.18	3.0	1.25(L)	1.54
Leucine	-CH ₂ -CH-(CH ₃)-CH ₃	0.47	3.5	0.60	6.5	1.48(L)	1.45
Lysine	-(CH ₂) ₄ -NH ₃	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	1.52(L)	1.52

Enantioresolution of Underivatized α -Amino Acids (continued)

Phenylalanine		0.87	2.0	0.98	5.2	2.04(L)	1.63
Proline	-CH ₂ -CH ₂ -CH ₂	2.4	2.5	0.43	6.2	2.00(L)	3.24
Serine	-CH ₂ OH	0.69	1.5	0.04	1.9	1.13(L)	0.8
Threonine	-CHOH-CH ₃	0.75	1.4	0.46	4.0	N/A	N/A
Tyrosine		0.60	1.9	0.76	2.9	1.73(L)	1.52
Tryptophan		1.01	2.0	2.05	6.5	2.36(L)	1.55
Valine	-CH ₂ (CH ₃)-CH ₃	0.56	1.9	0.48	5.5	1.42(L)	1.55

N/A - not available.

Typical mobile phases:

T = 60/40: EtOH/H₂O and EtOH/H₂O @ pH 3.8

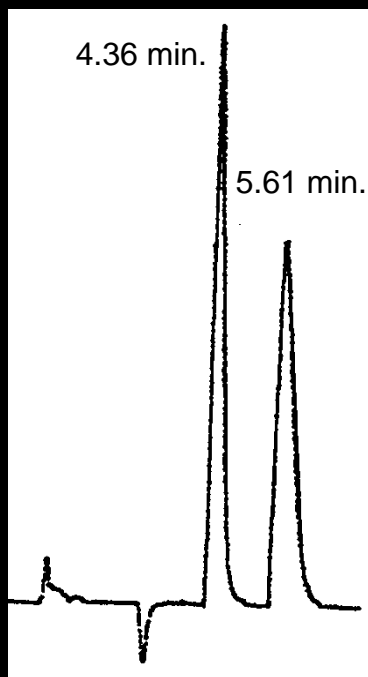
TAG = 20/80: MeOH/H₂O/pH 3.8 and/or 0.1% TEAA, pH 4.1

R = MeOH/H₂O: 50/50 and MeOH/0.1% TEAA, pH4 and pH7

- (1) Berthod, Liu, Bagwell and Armstrong, J. Chromatog. A., 731, 123-137 (1996).
- (2) Berthod, Gasparrini and Carotti, Anal. Chem. Vol. 72, No. 8, April 15, 2000.
- (3) Ekborg-Ott, Liu and Armstrong, Chirality 10: 434-483 (1999)

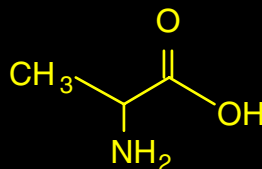
Selectivity Comparison T vs TAG for Aliphatic α -Amino Acids

CHIROBIOTIC T



50/50: EtOH/H₂O
1.0 mL/min.
 $\alpha=1.80$

Alanine



CHIROBIOTIC TAG

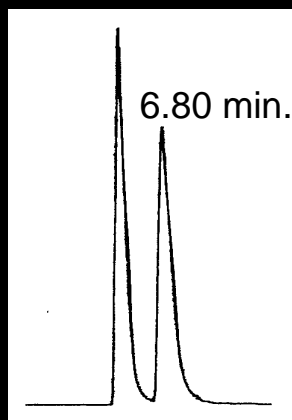


30/70: MeOH/H₂O
1.0 mL/min.
 $\alpha=5.19$

Selectivity Comparison T vs TAG for Aromatic α -Amino Acids

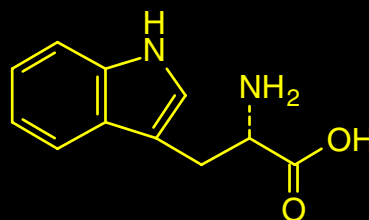
CHIROBIOTIC T

5.63 min.



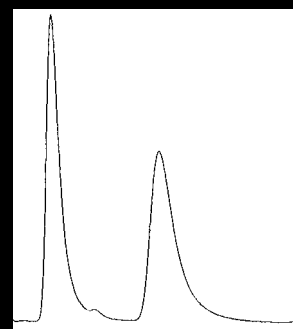
30/70: EtOH/H₂O
1.0 mL/min.
 $\alpha = 1.42$

Tryptophan



CHIROBIOTIC TAG

7.11 min.

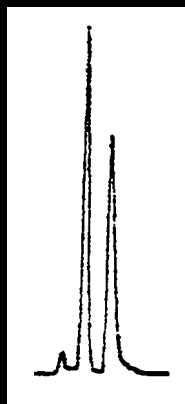


60/40: MeOH/H₂O
1.0 mL/min.
 $\alpha = 1.82$

Selectivity Comparison T vs TAG for Basic α -Amino Acids

CHIROBIOTIC T

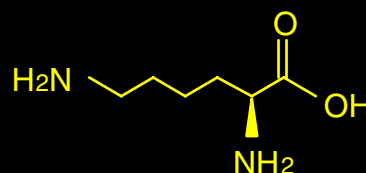
4.81 min.



5.44 min.

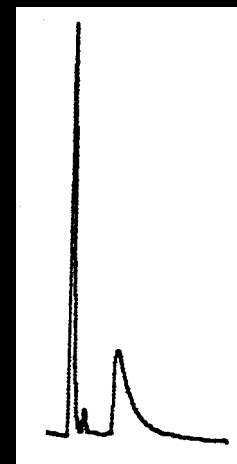
20/80: EtOH/100mM NaH₂PO₄
1.0 mL/min.
 $\alpha = 1.32$

Lysine



CHIROBIOTIC TAG

7.07 min.

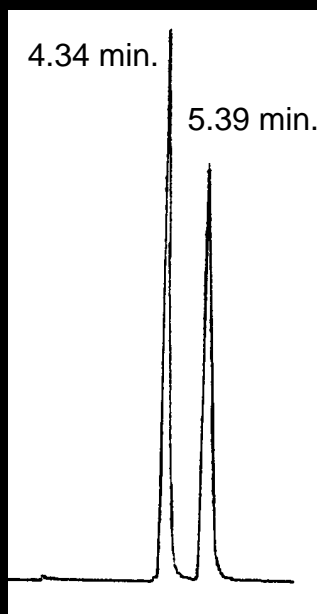


10.17 min.

20/80: MeOH/100mM NaH₂PO₄
1.0 mL/min.
 $\alpha = 1.80$

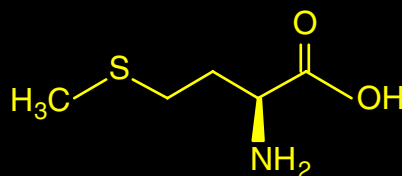
Selectivity Comparison T vs TAG for Sulfur Containing α -Amino Acids

CHIROBIOTIC T

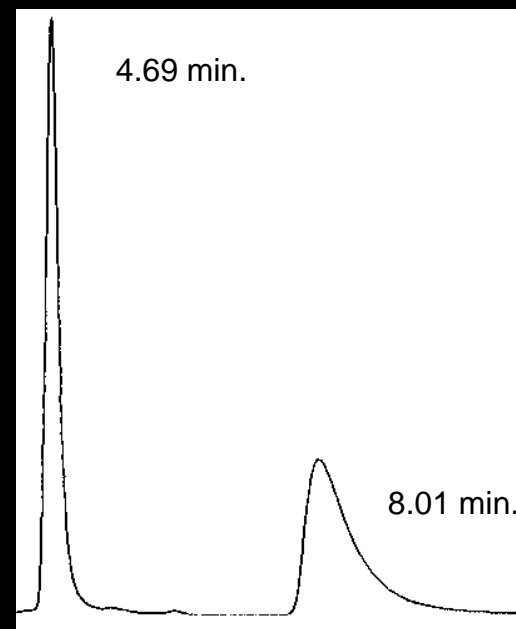


20/80: EtOH/H₂O
1.0 mL/min.
 $\alpha = 1.32$

Methionine



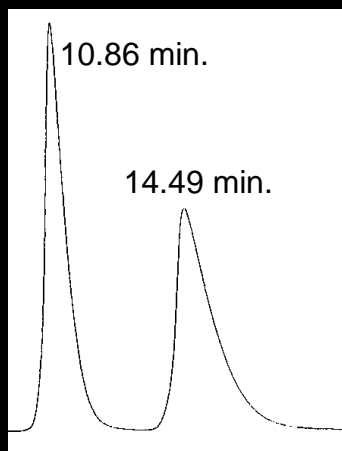
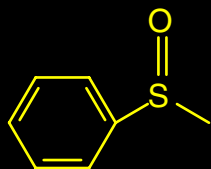
CHIROBIOTIC TAG



30/70: MeOH/H₂O
1.0 mL/min.
 $\alpha = 2.98$

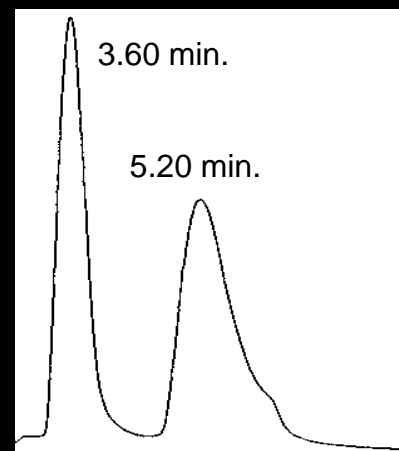
Selectivity of Bonded Teicoplanin Aglycone for Sulfur Containing Racemates

Methyl-phenyl sulfoxide



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

Cysteine



60/40: MeOH/H₂O
1.0 mL/min.

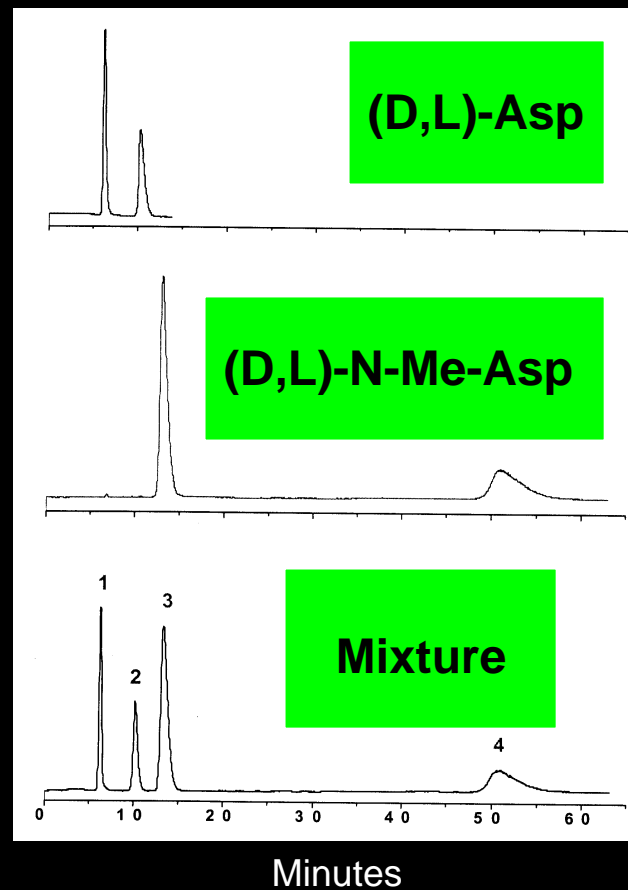
Acidic Amino Acids (D, L)-Asp and (D, L)-N-Me-Asp

Column: CHIROBIOTIC TAG (250x4.6 mm I.D.)

Eluent: 85/15, MeOH/10 mM NH₄OAc, pH =3.8

Flow rate: 1 mL/min,

Detector: ELSD (T = 80 C, P = 3.1 bar)



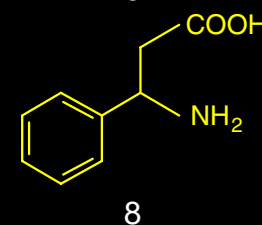
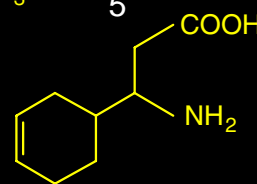
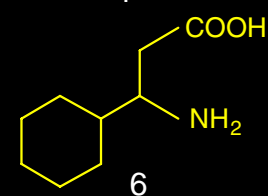
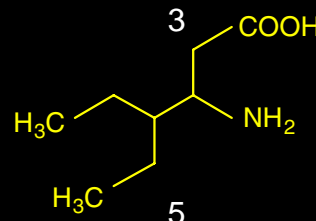
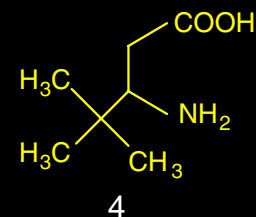
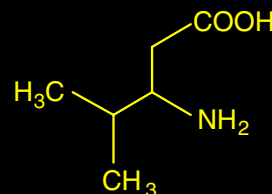
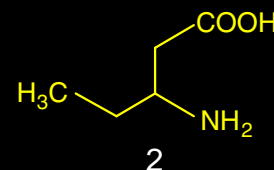
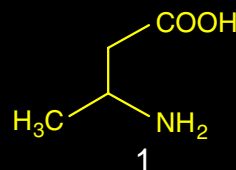
Separation of β -Amino Acids

- (1) 3-Amino-butanoic acid
- (2) 3-aminopentanoic acid
- (3) 3-amino-4-methylpentanoic acid
- (4) 3-amino-4, 4 dimethylpentanoic acid
- (5) 3-amino-4-ethylhexanoic acid
- (6) 3-amino-3-cyclohexyl-propanoic acid
- (7) 3-amino-3-(3-cyclohexene-1-yl)propanoic acid
- (8) and 3-amino-3-phenylpropanoic acid

A. Peter et al. J. Chromatogr. A 926 (2001)
229-238

CHIROBIOTIC T – Separates 2, 3, 4, 5, 6 & 8

CHIROBIOTIC R – Separates 1, 2, 4 & 8

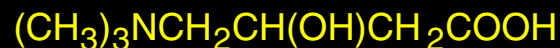


Reversed Phase Separations CHIROBIOTIC TAG

No significant change has been observed for use of this phase in the reversed phase mode.

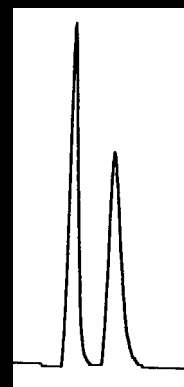
All of the points made under the CHIROBIOTIC T are applicable here, including the selectivity of methanol as the primary organic component.

Carnitine



Peak 1: 12.19 min.

Peak 2: 14.89 min.



85/15:MeOH/25mM NH₄OAc, pH 6.0

1.0 mL/min.

Detector: ELSD, T=80C

Nitrogen Flow: 1.0 SLPM

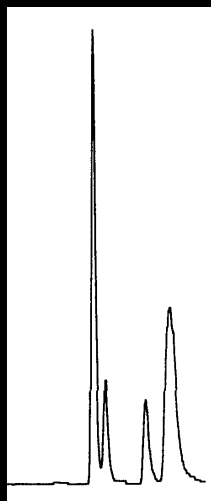
Amino Acid Diastereomers

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.

Isoleucine

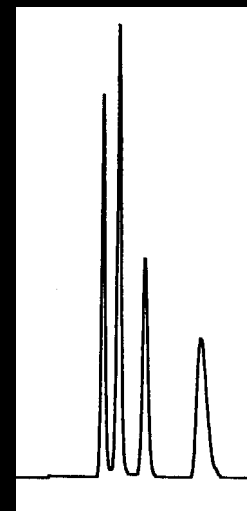
Peak 1/4: L/D-Isoleucine
Peak 2/3: L/D-Allo-Isoleucine



CHIROBIOTIC R, 250x4.6mm
80/20: MeOH/H₂O @ 0.6 mL/min.
Detector: ELSD, T=80°C
Nitrogen Flow: 0.8 SLPM

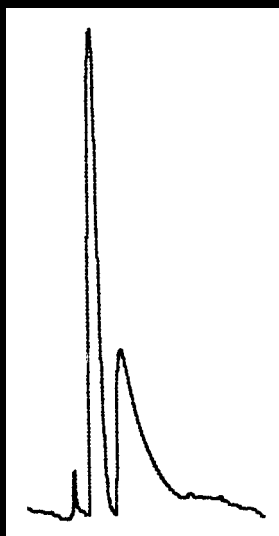
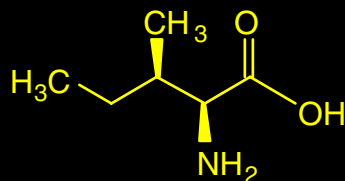
Threonine

Peak 1/3: L/D-Threonine
Peak 2/4: L/D-Allo-Threonine



CHIROBIOTIC TAG, 250x4.6mm
60/40: MeOH/H₂O @ 1.0 mL/min.
Detector: ELSD, T=85°C
Nitrogen Flow: 0.9 SLPM

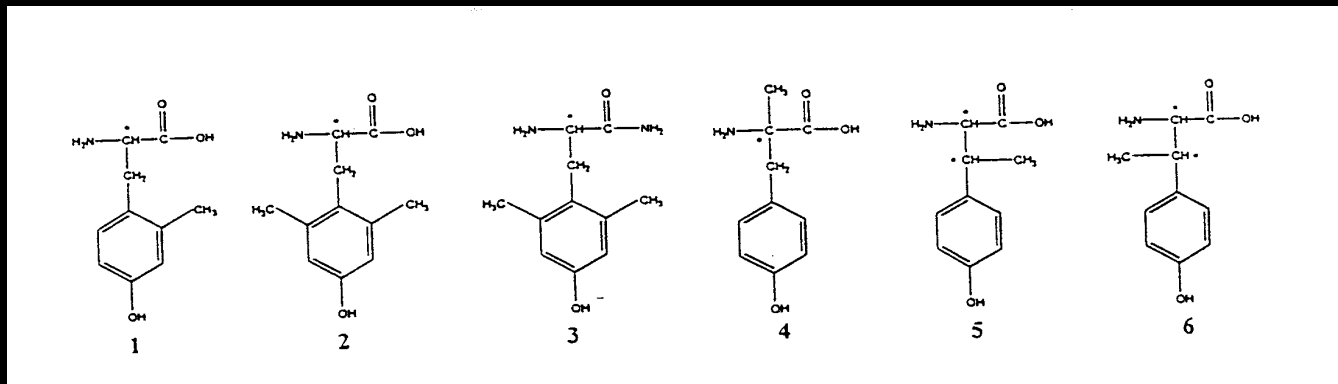
Preparative Purification of Isoleucine on Teicoplanin Aglycone



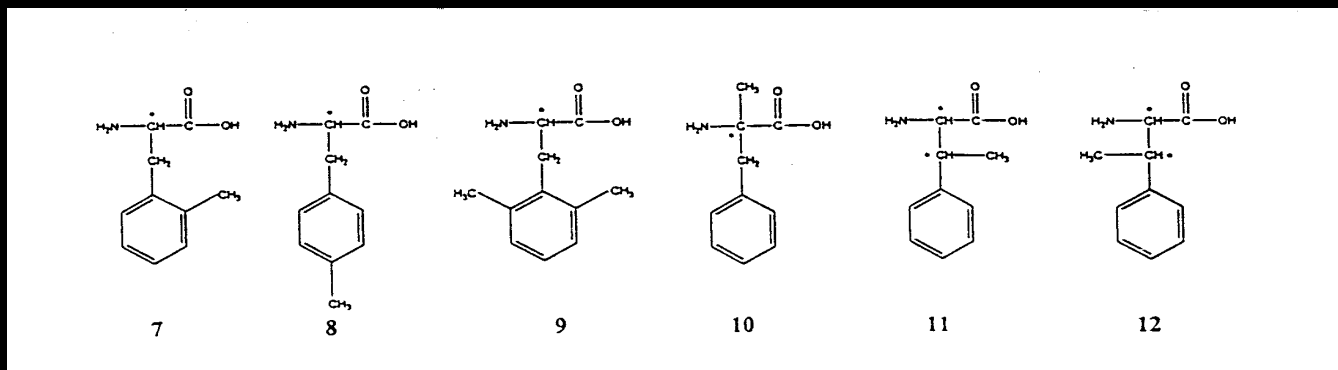
Column:	CHIROBIOTIC TAG
Size:	250 x 22.1 mm
Mobile Phase:	30/70: MeOH/H ₂ O
Flow Rate:	20 mL/min
Injection:	170 mg in 10 mL

Chiral Separation on Unusual Amino Acids – (1)

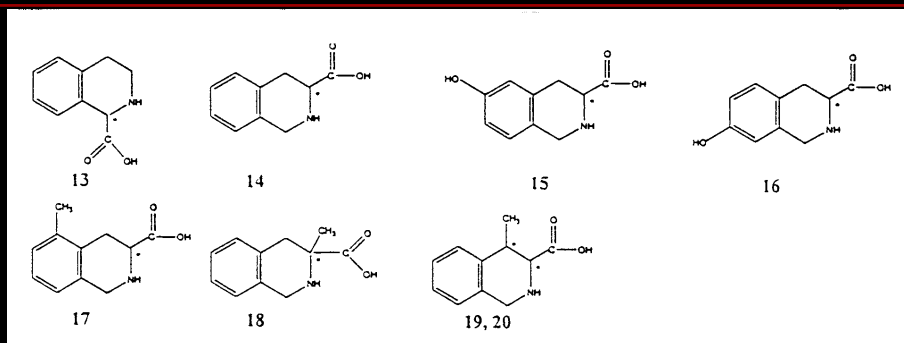
A. Tyrosine analogs



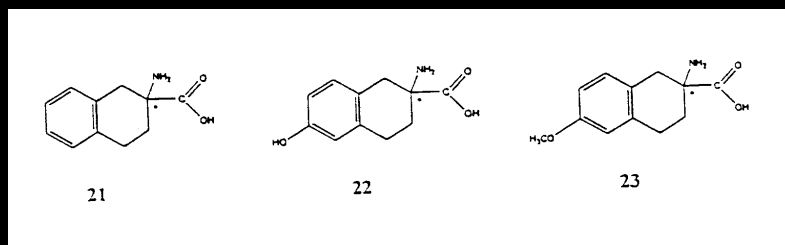
B. Phenylalanine analogs



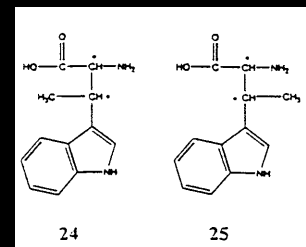
Chiral Separation on Unusual Amino Acids – (2)



C. Tetrahydroisoquinoline analogs



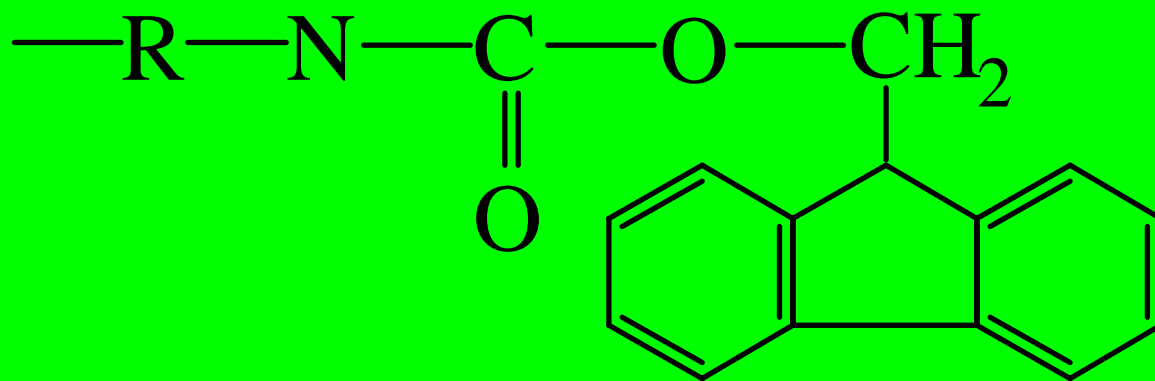
D. Amino tetralin analogs



E. Tryptophan analogs

1. A. Peter et al., J. of Chromtography A. 793 (1998) 283 – 296
2. G. Torok et al., Chirality 13 (2001) 648 - 656

1. N-FMOC (9-Fluorenyl methyl chloroformate) amino acids



Resolution of N-FMOC D, L-Amino Acids

Compound	Mobile Phase	Column	k1	α	Rs
Alanine	50/50, MeOH/20mM NH4OAc 40/60, MeOH/1% TEAA, pH 4.1 100/0.02w%, MeOH/NH4OAc	R	0.38	3.89	3.5
		T	1.26	2.27	5.5
		R	0.57	2.37	2.2
Arginine	20/80, MeOH/0.1%TEAA, pH 6.8 100/0.1w%, MeOH/NH4TFA	R	3.28	1.46	1.6
		R	1.69	2.95	4.6
Asparagine	100/1/1, MeOH/HOAc/TEA 40/60, MeOH/1% TEAA, pH 4.1 100/0.1w%, MeOH/NH4TFA 30/70, MeOH/20mM NH4OAc	T	0.63	1.81	1.7
		T	4.41	1.22	3.0
		R	1.55	1.49	1.3
		R			1.8
Aspartic acid	20/80, MeOH//0.1%TEAA, pH 6.8 40/60, MeOH/0.1% TEAA, pH 4.1 100/0.1w%, MeOH/NH4TFA	R	0.46	1.68	2.0
		T	2.59	1.23	1.8
		R			1.3
Citrulline	40/60, MeOH/0.1% TEAA, pH 4.1 100/1/1, MeOH/HOAc/TEA 30/70, MeOH/20mM NH4OAc	T	1.07	2.50	4.0
		T	1.34	2.05	3.0
		R			2.6
Cysteine	65/35, EtOH/Hexane	R			1.7
Glutamic acid	20/80, MeOH/0.1%TEAA, pH 6.8 100/1/1, MeOH/HOAc/TEA 40/60, MeOH/0.1% TEAA, pH 4.1	R	1.07	1.60	1.6
		T			1.3
		T			3.8
Glutamine	20/80, MeOH/0.1%TEAA, pH 6.8 65/35, EtOH/Hexane 40/60, MeOH/0.1% TEAA, pH 4.1 100/0.1w%, MeOH/NH4OAc 30/70, MeOH/20mM NH4OAc	R	0.61	2.85	1.6
		R	1.90	2.04	1.3
		T	0.93	2.46	5.0
		R			3.8
		R			3.6
Histidine	20/80, MeOH/0.1%TEAA, pH 4.1	R			1.0

Resolution of N-FMOC D, L-Amino Acids (continued)

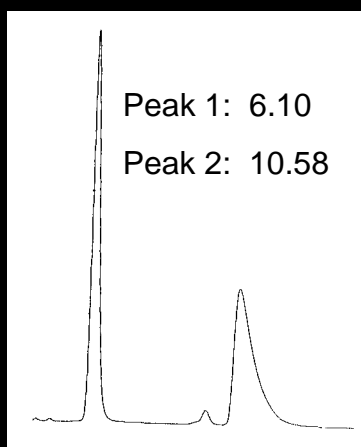
Isoleucine	40/60, MeOH/0.1% TEAA, pH 4.1 100/0.1w%, MeOH/NH4OAc 30/70, MeOH/20mM NH4OAc	T	1.08	1.78	2.2
		R	0.45	1.87	2.3
		R	2.32	1.85	1.6
Isoleucine,allo	100/0.1w%, MeOH/NH4OAc	R	0.53	1.57	2.0
Isoleucine	65/35, EtOH/Hexane 50/50, MeOH/1% TEAA, pH 5.5	R			1.5
		T			4.5
Leucine	40/60, MeOH/0.1% TEAA, pH 4.1 100/0.1w%, MeOH/NH4TFA	T	1.03	2.45	5.0
		R	0.46	2.41	3.5
Lysine	50/50, MeOH/1% TEAA, pH 5.5 100/0.1w%, MeOH/NH4TFA	T	0.79	2.12	1.4
		R			3.4
Methionine	40/60, MeOH/0.1% TEAA, pH 4.1 100/1/1, MeOH/HOAc/TEA 100/0.1w%, MeOH/NH4TFA 50/50, MeOH/20mM NH4OAc	T	0.96	3.43	6.0
		T	0.94	2.70	3.0
		R	0.27	5.77	4.5
		R			5.4
Norleucine	40/60, MeOH/0.1%TEAA, pH 4.1 100/0.1w%,MeOH/NH4TFA 30/70, MeOH,20mM NH4OAc	T	1.20	2.87	6.5
		R	0.50	2.0	3.0
		R	2.92	2.15	3.0
Norvaline	100/0.1w%, MeOH/NH4TFA 30/70, MeOH/20mM NH4OAc 40/60, MeOH/0.1% TEAA, pH 4.1	R	0.61	2.56	3.5
		R	2.12	3.56	6.5
		T	0.99	3.19	5.5
Ornithine	50/50, MeOH/1% TEAA, pH 5.5 100/0.1w%, MeOH/NH4TFA	T	1.22	1.72	1.4
		R			3.0
Phenylalanine	100/1/1, MeOH/HOAc/TEA 40/60, MeOH/0.1% TEAA, pH 4.1 100/0.02w%, MeOH/NH4OAc 50/50, MeOH/20mM NH4OAc	T	1.54	2.82	3.0
		T	0.45	6.65	6.0
		R	0.12	8.53	5.0
		R			6.0

CHIROBIOTIC T (Reversed Phase Mode)

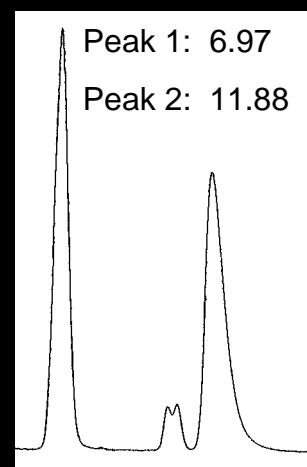
LC→LC/MS Compatible Mobile Phase

Example: Fmoc Leucine

Mobile Phase: MeOH/ Buffer*, 40/60



*Buffer: 0.1% TEAA, pH=4.1



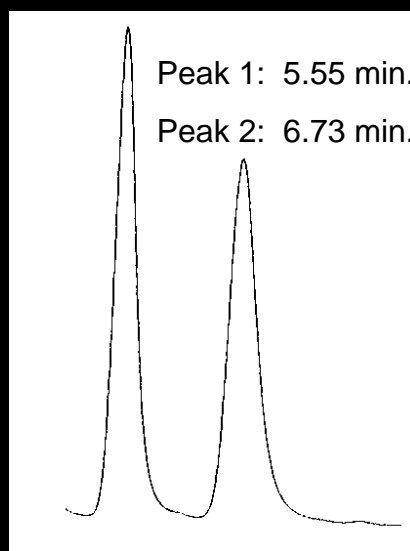
*Buffer: 20mM NH₄OAc, pH=4.1

N-FMOC (9-Fluorenylmethyl Chloroformate) Amino Acids

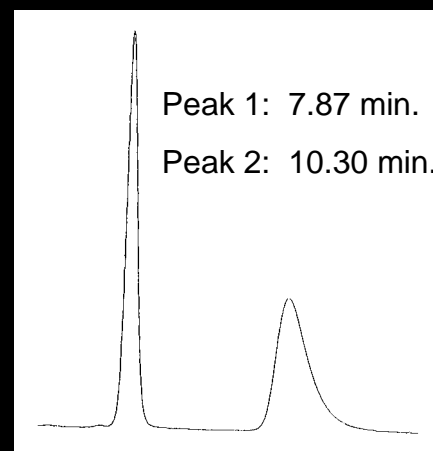
Column: CHIROBIOTIC T (Reversed Phase Mode)



Alanine



Aspartic Acid

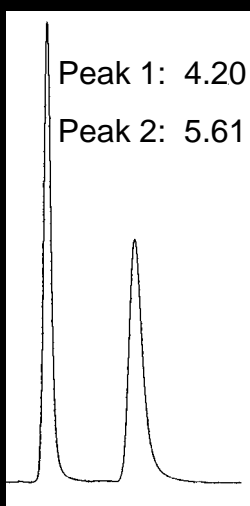


Glutamic Acid

Mobile Phase: 40/60: MeOH/0.1% TEAA, pH=4.1

N-FMOC (9-Fluorenylmethyl Chloroformate) Amino Acids

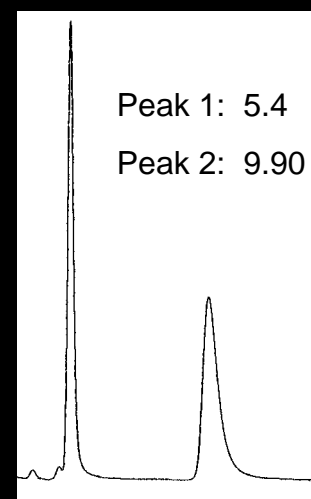
Column: CHIROBIOTIC R (Polar Organic Mode)



Norleucine



Norvaline

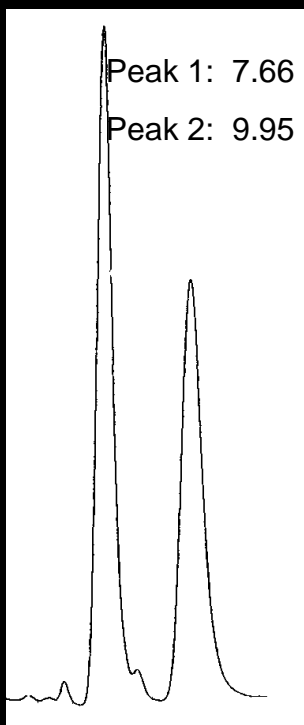


Methionine

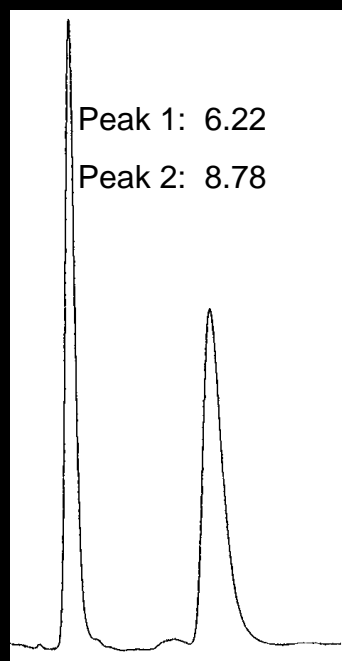
Mobile Phase: 100/0.1w%: MeOH/NH₄TFA (LC/MS Compatible)

N-FMOC (9-Fluorenylmethyl Chloroformate) Amino Acids

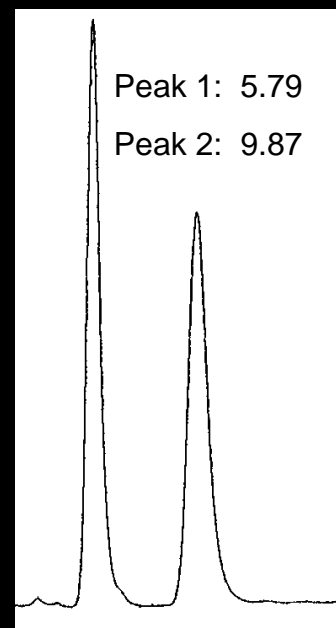
Column: CHIROBIOTIC R (Reversed Phase Mode)



Asparagine



Glutamine



Serine

Mobile Phase: 30/70: MeOH/20mM NH₄OAc (LC/MS Compatible)

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.

Compound	Mobile Phase	Column	k ₁	α	R _s
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 .1	R	1.67	1.25	1.6
	10/90, ACN/20mM NH ₄ OAc	RSP	2.00	1.54	1.6

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

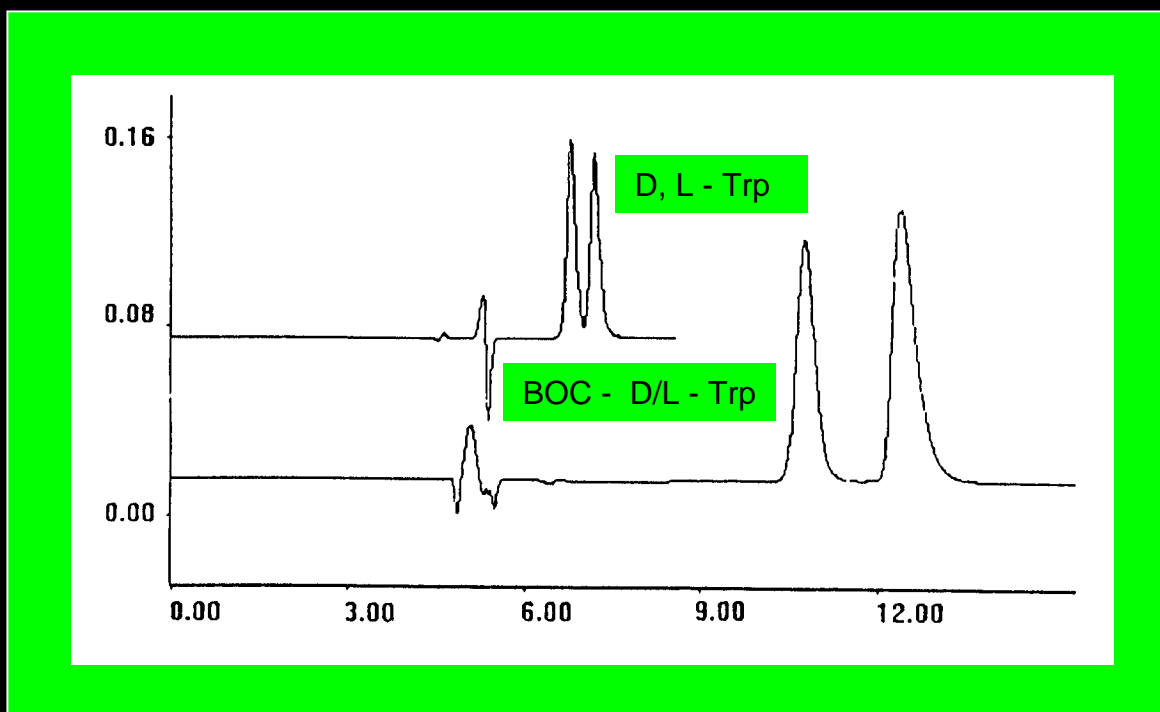
Methionine	20/80, MeOH/0.1%TEAA, pH 6.0 20/80, MeOH/0.1%TEAA, pH 4.1 10/90, ACN/20mM NH4OAc	R	0.34	20.3	12.0
		T	0.48	3.90	5.5
		RSP	1.20	4.09	10.0
Phenylalanine	20/80, MeOH/0.1%TEAA, pH 6.0 10/90, MeOH/0.1%TEAA, pH 6.0 10/90, ACN/20mM NH4OAc	R	1.02	3.30	4.8
		T*	0.44	1.55	1.6
		RSP	1.90	1.16	1.2
Phenylglycine	20/80, MeOH/0.1%TEAA, pH 6.0 20/80, MeOH/0.1%TEAA, pH 4.1 10/90, ACN/20mM NH4OAc	R	0.15	9.24	5.0
		T	0.52	4.65	3.5
		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 20/80, MeOH/0.1%TEAA, pH 4.1 10/90, ACN/20mM NH4OAc	R	0.61 (D)	3.89	5.4
		T	0.73 (D)	2.17	2.2
		RSP	1.46 (D)	2.96	5.7
p-Tyrosine	20/80, MeOH/0.1%TEAA, pH 6.0 10/90, MeOH/0.1%TEAA, pH 6.0 10/90, ACN/20mM NH4OAc	R	0.79	4.31	5.5
		T*	0.24	1.77	1.4
		RSP	1.05	1.16	1.3
Valine	20/80, MeOH/0.1%TEAA, pH 4.1 10/90, ACN/20mM NH4OAc	R	1.44	1.26	2.0
		RSP	1.64	1.45	1.6

Legend: TEAA = Triethylammonium acetate
T = CHIROBIOTIC T
R = CHIROBIOTIC R

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



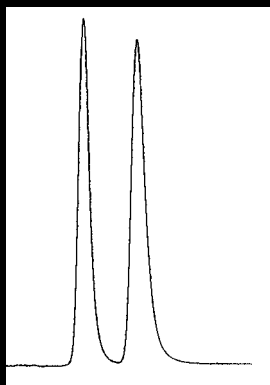
Mobile Phase: 20/80, Acetonitrile/1% Triethylamine Acetate, pH 4.1

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

N-t-BOC Amino Acids

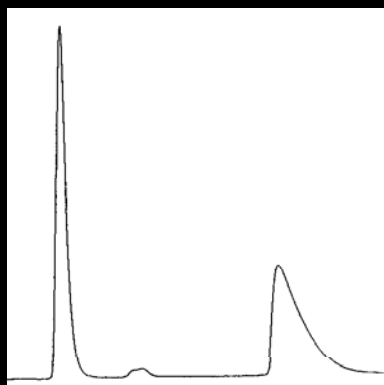
Column: CHIROBIOTIC R

Peak 1: 5.43
Peak 2: 6.32



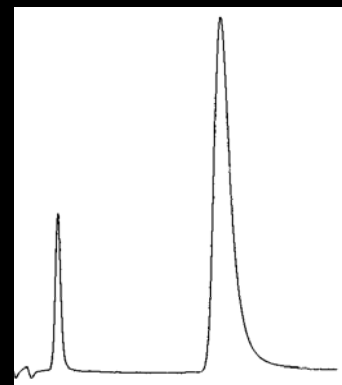
Histidine

Peak 1: 6.06
Peak 2: 13.24



Phenylalanine

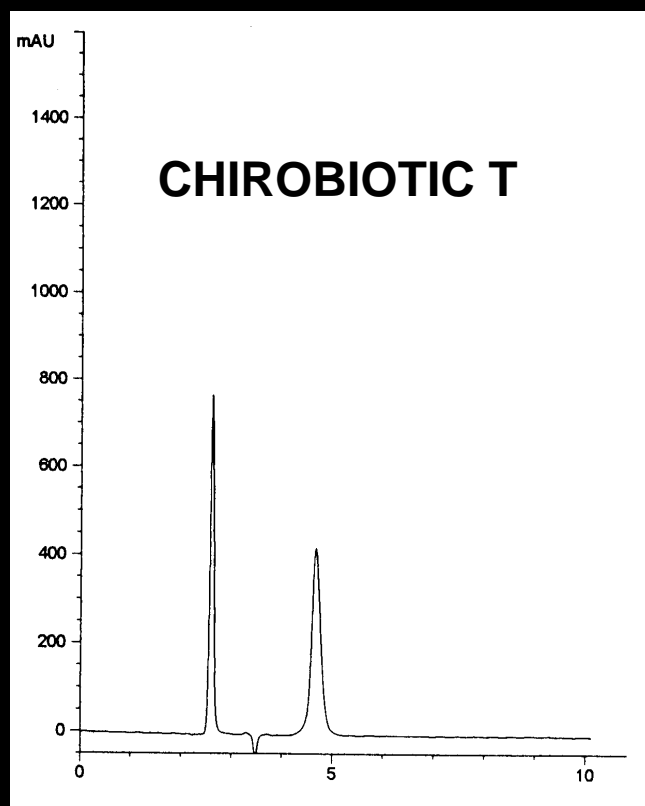
Peak 1: 4.84
Peak 2: 10.12



Tryptophan

Mobile Phase: 20/80: MeOH/0.1% TEAA, pH=6.0

3. Enantioseparation of N-acetyl Serine



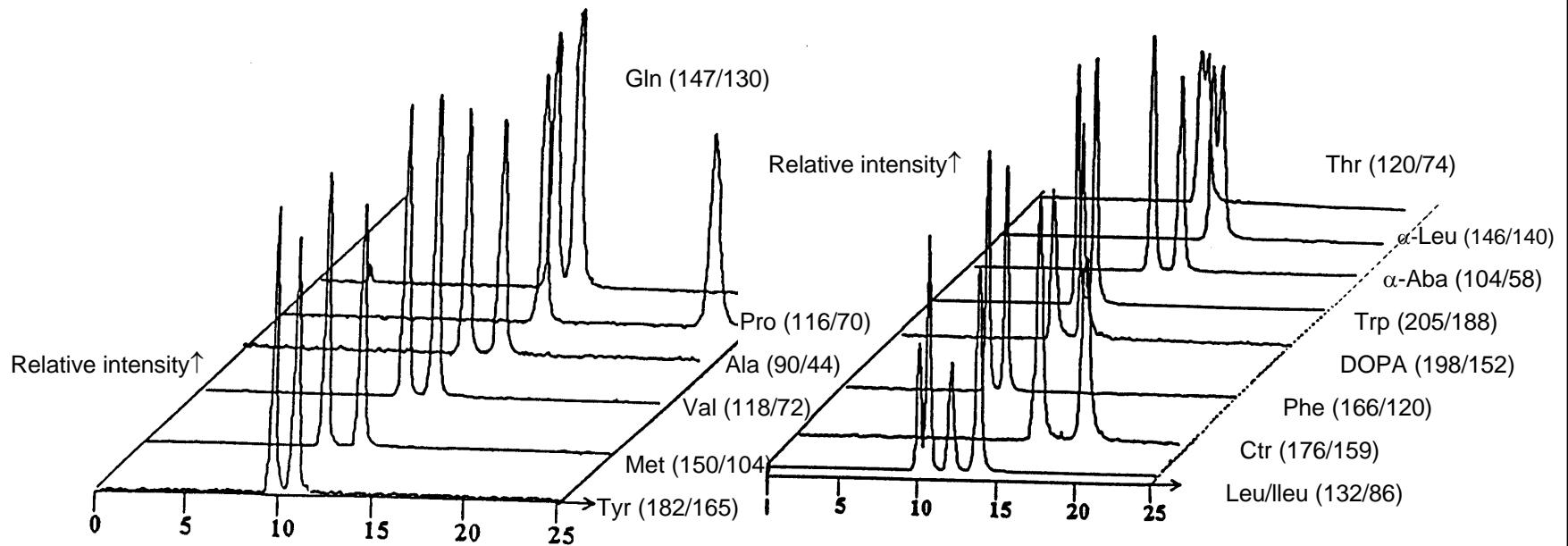
30/70: Methanol/0.1% Triethylamine Acetate, pH 4.1 @ 1.0 mL/min.

Enantioseparation of N-acetyl Amino Acids

N-Acetyl Amino Acid	Column	k_1	R_s	Mobile Phase
Tryp	CHIROBIOTIC T	0.19	2.5	30/70: MeOH/0.1% TEAA, pH 4.1
Tryp	CHIROBIOTIC TAG	0.51	5.0	100/0.1w%, MeOH/NH ₄ OAc
Tryp	CHIROBIOTIC TAG	1.23	3.0	30/70: MeOH/ 20mM NH ₄ OAc, pH 6.0
Val	CHIROBIOTIC R	0.42	1.5	50/50: ACN/H ₂ O
2-fluoro-Phe	CHIROBIOTIC R	0.52	1.5	50/50: ACN/H ₂ O
3-fluoro-Phe	CHIROBIOTIC T	0.55	2.5	50/50/ 0.2/0.2: ACN/MeOH/HOAc/TEA
4-fluoro-Phe	CHIROBIOTIC T	1.43	5.6	20/80: MeOH/1% TEAA, pH 4.1

Simultaneous Isocratic Analysis of 15 Underivatized Amino Acids by LC-ISP-MS-MS on CHIROBIOTIC T

75/25: ACN/H₂O



Petritis, K., J. of Chromatogr. A, 913 (2001) 331-340.

Determination of Pipecolic Acid in Plasma by LC/MS/MS

Peroxisomal Disorders:

Indicated by higher level of L-PA

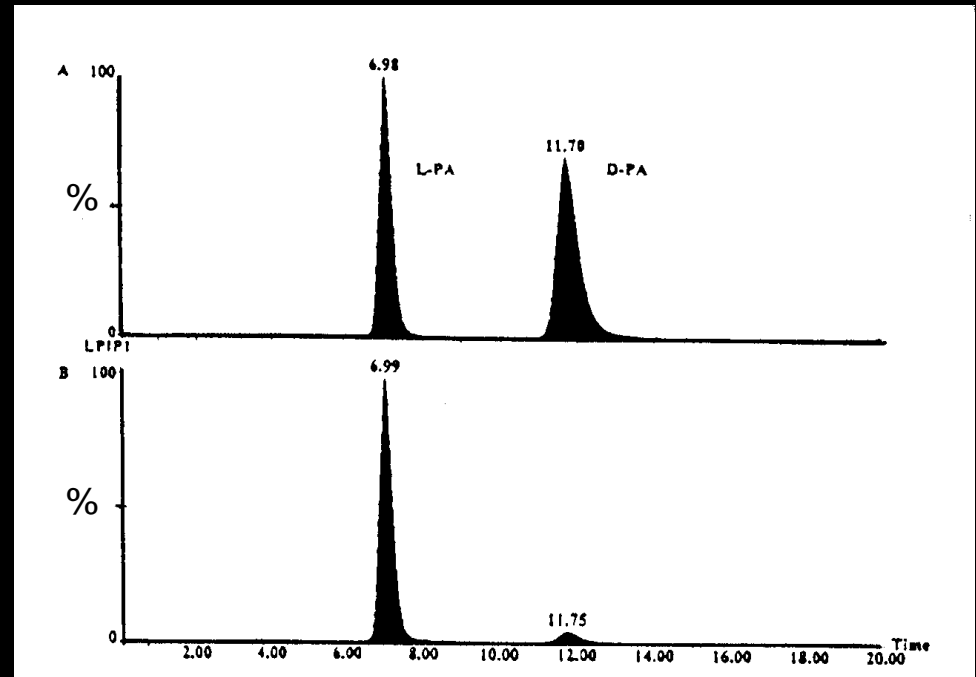
Higher concentration of D-PA indicated liver dysfunction.

LC/MS/MS methodology utilizes 50 μL of plasma, no derivatizing agents and no interfering substances utilizing the CHIROBIOITIC T, clearly distinguishing between healthy individuals and peroxisomal disease patients.

Linear calibration curves were obtained in the range of 0.5 – 80 $\mu\text{mol./L}$.

Transition: $m/z130 \rightarrow m/z84$

(A), mixture of D- and L-PA calibrators; (B) L-PA calibrator.



Determination of D and L 2-Hydroxyglutaric Acid

Two distinct inborn metabolic disorders:

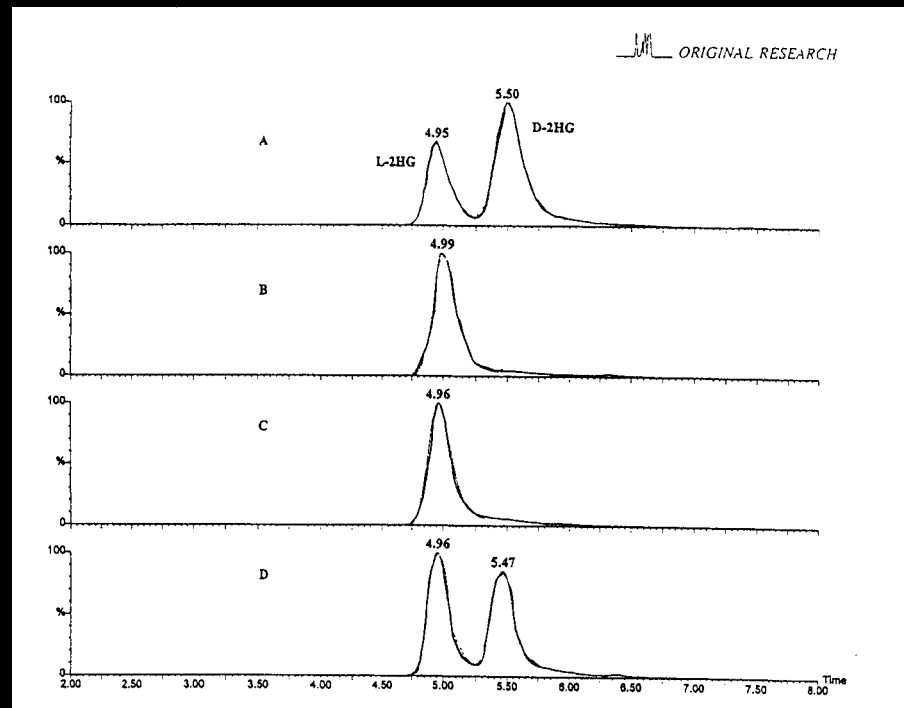
LC/MS/MS methodology utilizes 100 μ L urine + 900 μ L mobile phase and filtered through a 0.45 μ M membrane filter.

LC-MS/MS negative ion chromatograms obtained in the MRM mode of analysis using CHIROBIOTIC R column.

(A) A mixture of D- and L-2-HG

(B) and (C) urine samples from patients 1/2

(D) urine sample from patient 2 spiked with standard D-2-HG.



Ref. Rashed, M.S., AlAmoudi, M, Aboul-Enein, H.Y. Biomed. Chromatogr. 14, 317- 320 (2000).

Conclusions

- Macrocyclic glycopeptide CSPs offer the best solution for all kinds of amino acids chiral separation needs in reversed phase and polar organic mode.
- Mobile phases are LC/MS compatible for simultaneous detection of amino acids mixtures and for biological samples.
- The loading capacity for amino acids is fairly high and the eluent is easily removed for sample recovery.