Novel Chiral Separations by Hydrophilic Interaction on Macrocyclic Glycopeptide Phases

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Abstract

Macrocyclic glycopeptide based chiral stationary phases (CSPs) are becoming more popular due to their multi-modal capability, broad selectivity and ruggedness. Owing to their complex structures, different type of chiral interactions can be promoted between the analyte and CSPs under different mobile phase conditions. The forces of ionic interaction, hydrogen bonding, hydrophobic association, Π-Π complexation, dipole-dipole interaction and steric repulsion are accessible within the structure of these chiral stationary phases.

Routinely, these phases have been operated in an anhydrous polar organic, reversed and normal phase condition for a wide variety of compounds. However, for extremely polar molecules like underivatized amino acids, hydrophilic interaction chromatography becomes a viable technique to obtain sufficient retention and resolution. Unlike traditional reversed phase mode, this mobile phase system contains more organic solvent (>60%) than the aqueous counter part. Therefore, this system has rendered itself to facilitate electrospray mass spectrometry and evaporative light scattering detection for compounds with out UV chromophore.

Detailed comparisons on the relationship between the structure of the analytes and the glycopeptide CSPs will be presented. The effects of the buffer type and concentration together with pH will also be discussed. Finally, the chiral recognition mechanisms involved in this type of chromatography will be proposed.

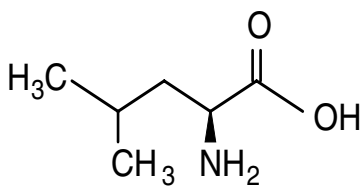
Introduction

Hydrophilic interaction chromatography[1] provides a general utility for extremely polar compounds that cannot be analyzed by traditional normal phase, reversed phase or polar organic mode in HPLC. Thus, the multi-modal capability of CHIROBIOTIC columns were utilized to demonstrate the strong retention and effective resolution of polar racemic compounds like underivatized amino acids. The high content of organic solvent in the mobile phase facilitates the retention of the molecules investigated. This and the chiral ionic interaction make the enantiomeric resolution possible.[2]

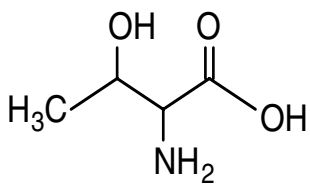
[1]A. Alpert, J. Chromatogr. 1990, **499**, 177-196.
[2]D. Risley and M. Strege, Anal. Chem. 2000, **72**, 1736-1739.

Analytes/Structures

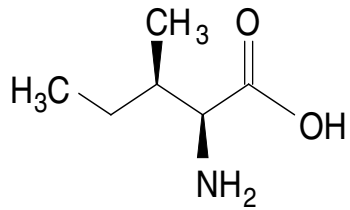
Leucine



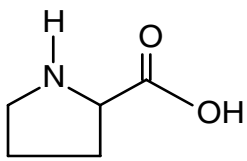
Isoleucine (w/allo)



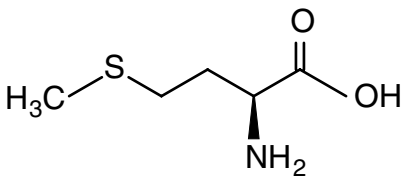
Threonine (w/allo)



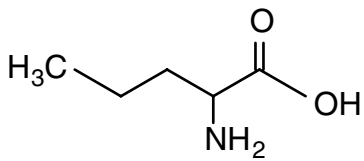
Proline



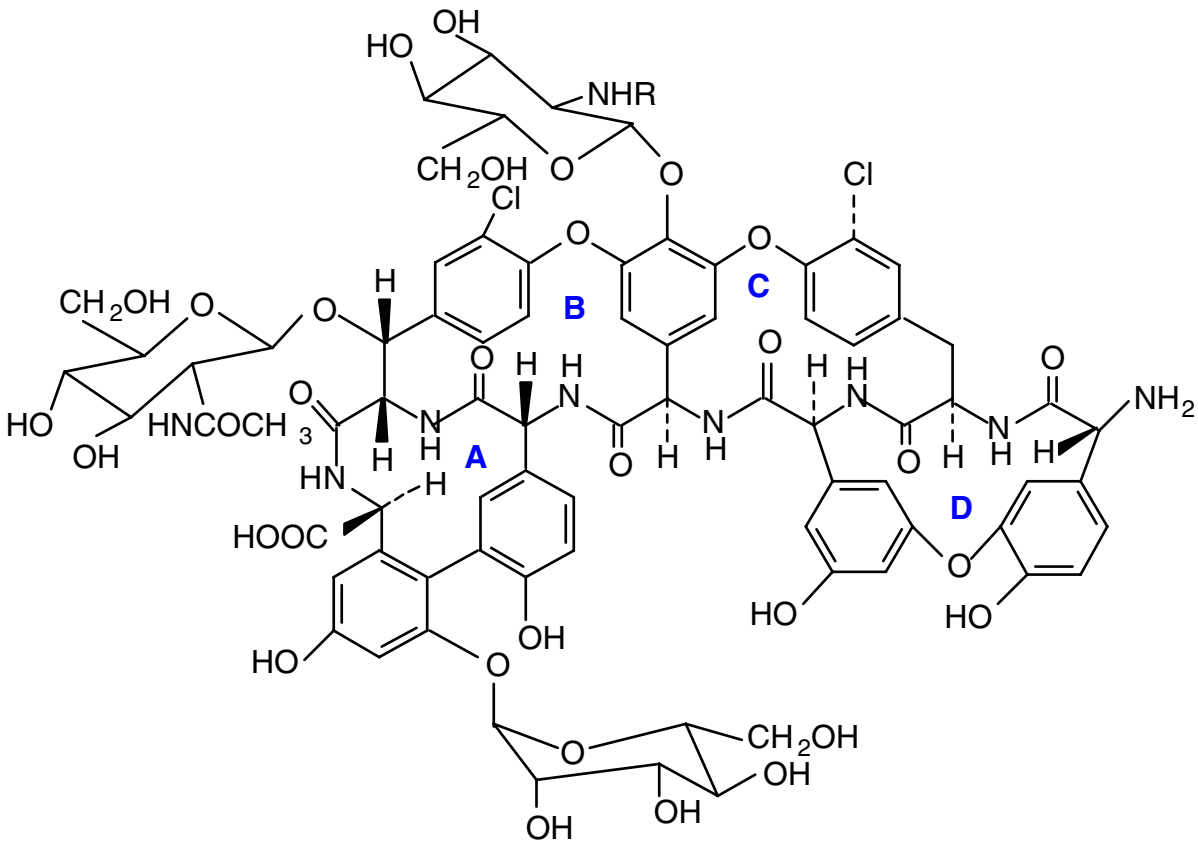
Methionine



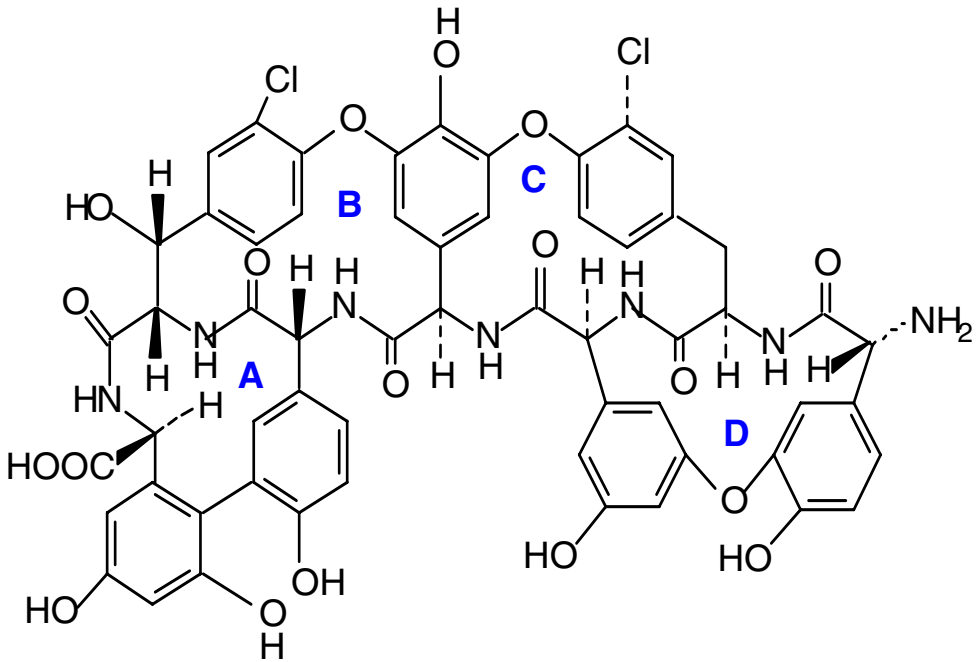
Norvaline



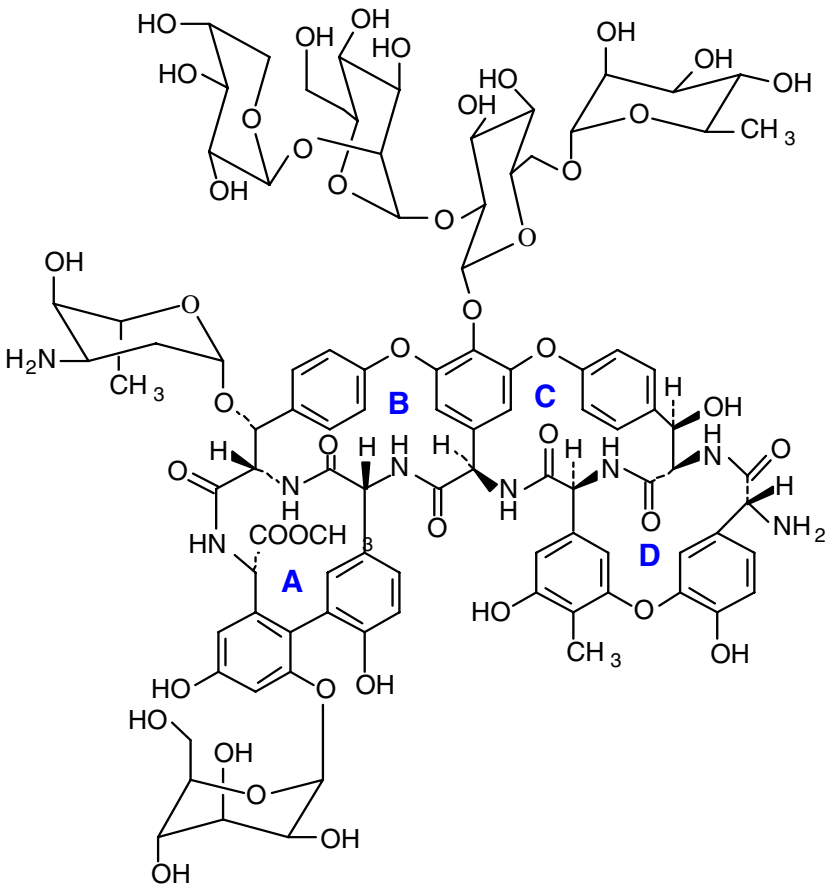
CHIROBIOTIC T Structure



CHIROBIOTIC TAG Structure



CHIROBIOTIC R Structure



CHIROBIOTIC TAG

Mobile Phase	60/40: ACN/Buffer			70/30: ACN/Buffer			80/20: ACN/Buffer		
Analyte	k_1	α	Rs	k_1	α	Rs	k_1	α	Rs
Leucine	0.46	2.69	4.5	1.03	2.30	6.2	2.62	2.37	7.0
Methionine	0.56	2.27	3.0	1.28	2.06	4.0	3.38	2.04	6.0
Norvaline	0.47	3.07	4.8	1.19	2.65	5.5	3.26	2.64	5.4
Proline	1.46	2.69	7.5	3.34	2.58	7.5	9.70	2.78	7.8

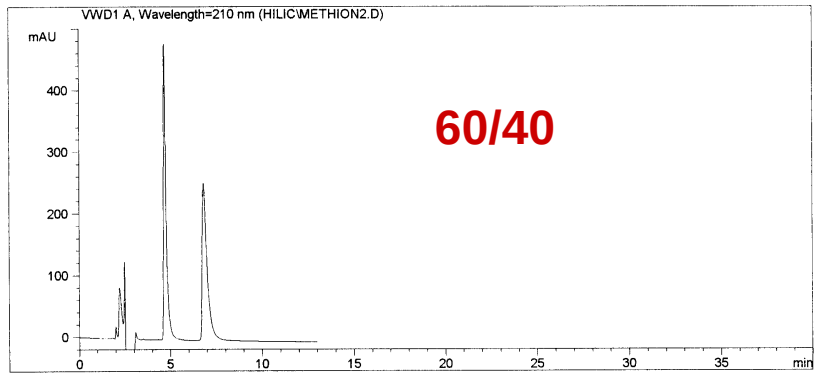
Buffer: 10mM NH_4OAc, pH 5.5

CHIROBIOTIC TAG

Typical mobile phase composition:
ACN/10mM NH_4OAc, pH 5.5
UV-220nm

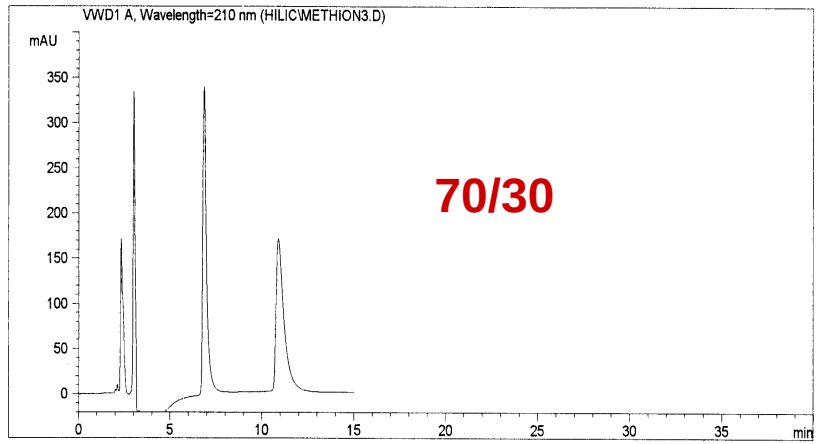
Analyte:
Methionine

$k_1 = 0.56$
$k_2 = 1.27$
$\alpha = 2.27$
Rs = 3.0



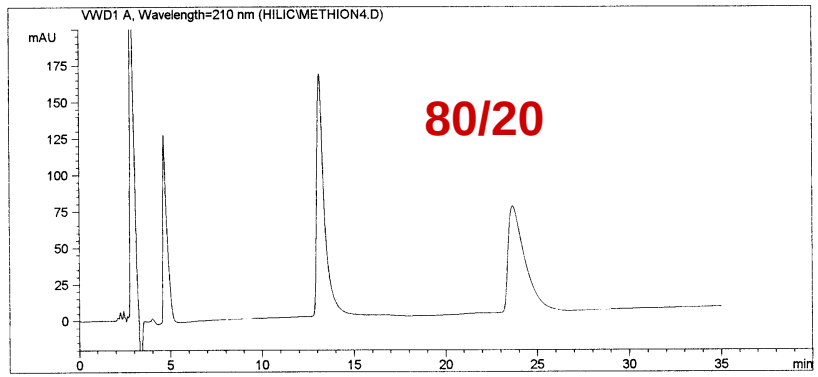
60/40

$k_1 = 1.28$
$k_2 = 2.63$
$\alpha = 2.06$
Rs = 4.0



70/30

$k_1 = 3.38$
$k_2 = 6.89$
$\alpha = 2.04$
Rs = 6.0



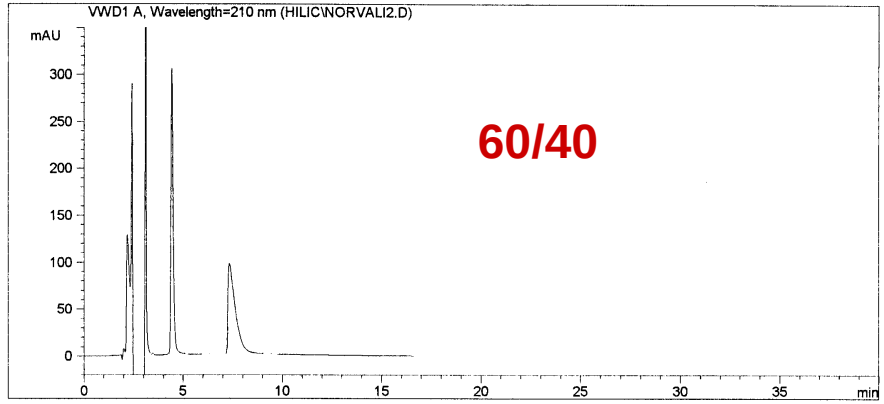
80/20

CHIROBIOTIC TAG

ACN/10mM NH_4OAc, pH 5.5
UV=210nm

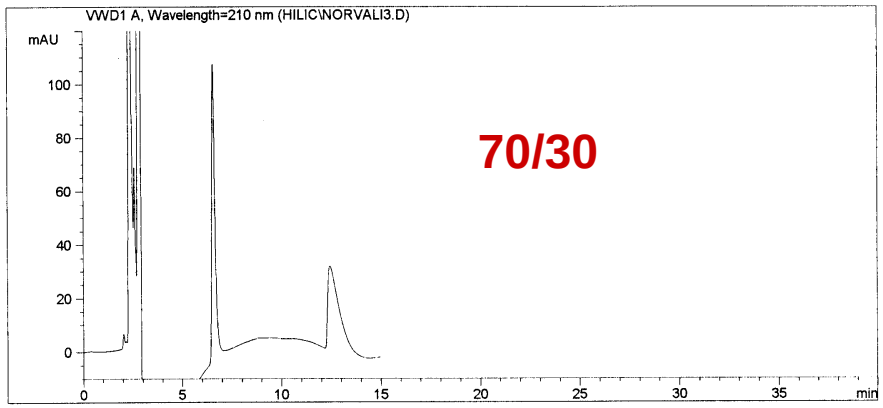
Analyte:
Norvaline

k_1 = 0.47
k_2 = 1.44
α = 3.07
Rs = 4.8



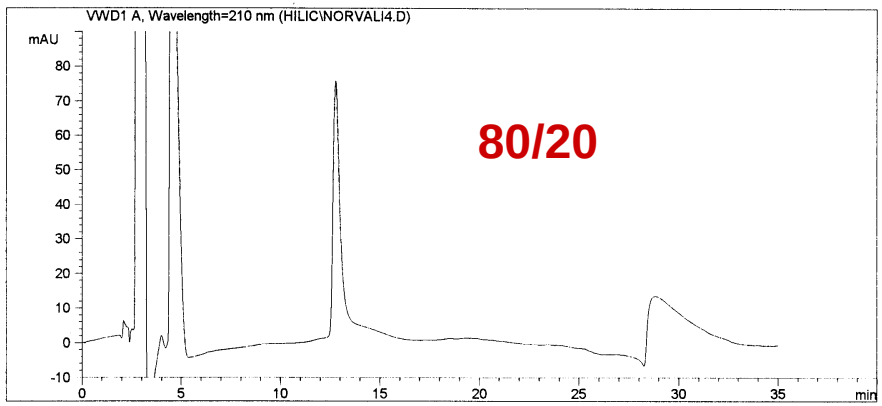
60/40

k_1 = 1.19
k_2 = 3.15
α = 2.65
Rs = 5.5



70/30

k_1 = 3.26
k_2 = 8.60
α = 2.64
Rs = 5.4



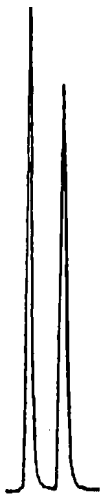
80/20

CHIROBIOTIC T vs CHIROBIOTIC TAG

Analyte: Methionine

CHIROBIOTIC T

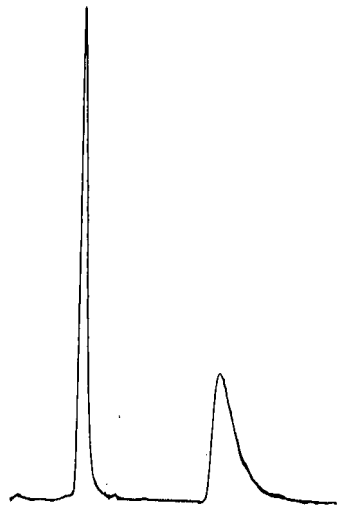
t_1 – 4.4
t_2 = 5.4



10/90: EtOH/H_2O @ 1.0 mL/min.
Detection: 210nm

CHIROBIOTIC TAG

t_1 – 4.7
t_2 = 8.0



30/70: MeOH/H_2O @ 1.0 mL/min.
Detection: 210nm

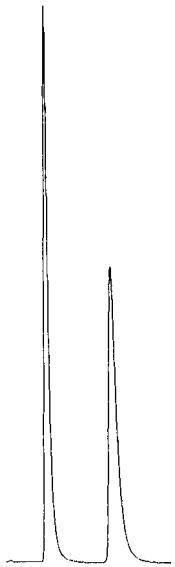
With or Without Buffer?

CHIROBIOTIC TAG

Example: Methionine

$k_1 = 0.56$
$k_2 = 1.27$

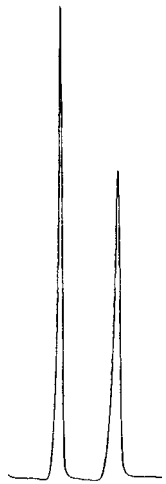
$= 2.27$
$Rs = 3.0$



60/40: ACN/Buffer

$k_1 = 0.40$
$k_2 = 1.47$

$= 2.87$
$Rs = 3.0$



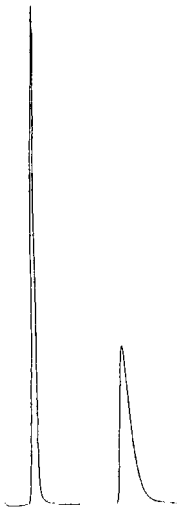
60/40: ACN/H_2O

With or Without Buffer?

CHIROBIOTIC TAG

Example: Norvaline

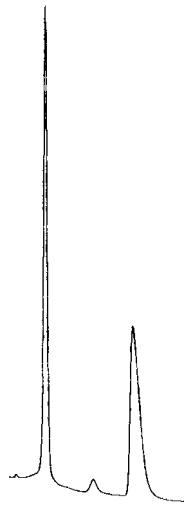
$k_1 = 0.47$
$k_2 = 1.44$
$\alpha = 3.07$
Rs = 4.8



60/40: ACN/Buffer

$k_1 = 0.51$
$k_2 = 1.46$

$= 2.87$
Rs = 4.8



60/40: ACN/H_2O

CHIROBIOTIC TAG vs CHIROBIOTIC R

CSPs	CHIROBIOTIC TAG			CHIROBIOTIC R		
Analyte	k_1	α	Rs	k_1	α	Rs
Leucine	0.68	4.96	6.5	0.38	1.66	2.2
Methionine	1.08	3.95	6.4	0.55	1.75	2.2
Norvaline	0.74	6.90	6.0	0.44	2.27	3.0
Proline	5.63	3.64	6.0	1.67	5.64	6.5

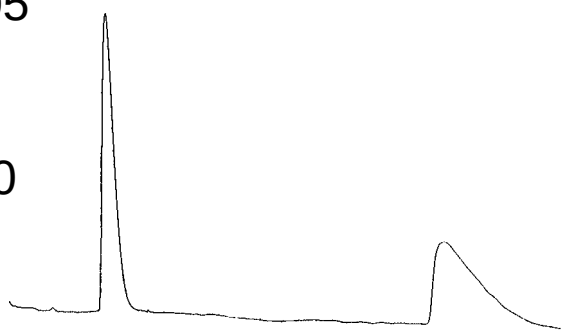
Proline

Mobile Phase: 80/20: MeOH/H_2O
UV = 210nm

CHIROBIOTIC TAG

k_1 = 5.63
k_2 = 2.05

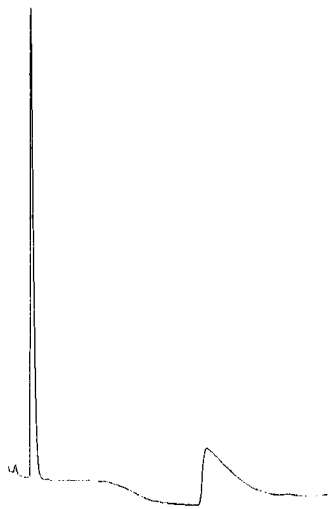
= 3.64
Rs = 6.0



CHIROBIOTIC R

k_1 = 1.67
k_2 = 9.43

= 5.64
Rs = 6.5

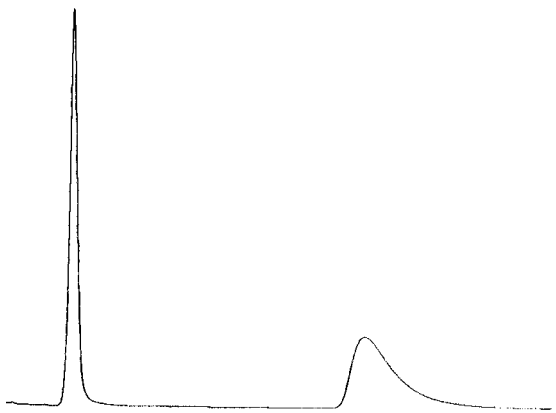


Methionine

Mobile Phase 80/20: MeOH/H_2O
UV = 220nm

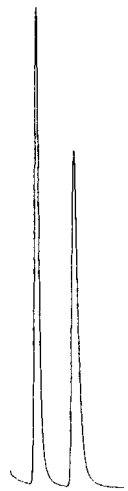
CHIROBIOTIC TAG

k_1 = 1.08
k_2 = 4.27
α = 3.95
Rs = 6.4



CHIROBIOTIC R

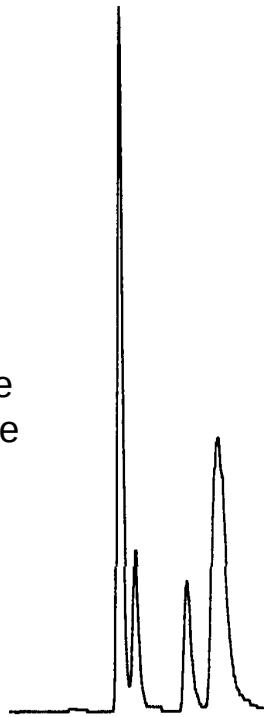
k_1 = 5.47
k_2 = 0.96
α = 1.75
Rs = 2.2



Evaporative Light Scattering Detector

Isoleucine

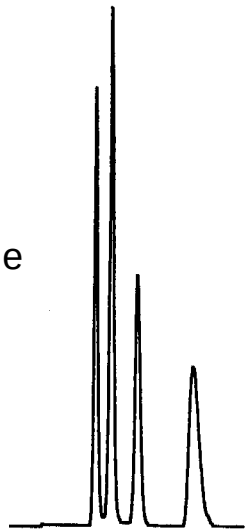
Peak 1 – L-Isoleucine
Peak 2 – L-Allo-Isoleucine
Peak 3 – D-Allo-Isoleucine
Peak 4 – D-Isoleucine



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

Threonine

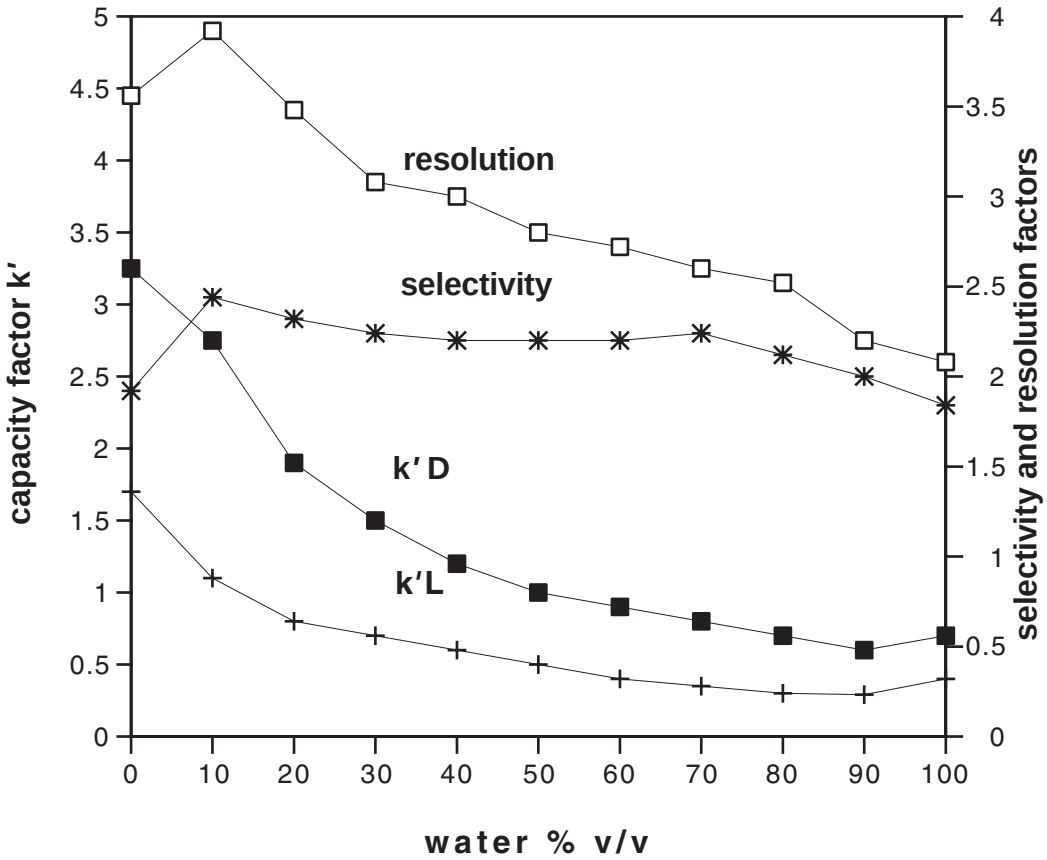
Peak 1,3 – DL-Threonine
Peak 2,4 – DL-Allo-Threonine



CHIROBIOTIC TAG, 250x4.6mm
60/40: MeOH/H_2O @ 0.6 mL/min.
Detector: ELSD
T=85°C, Nitrogen Flow: 0.9 SLPM

Effect of Alcohol Modifier on Retention, Selectivity and Resolution

Example Methionine



Summary

Enantioresolution of Underivatized α-Amino Acids

R-CH-COOH | NH_2		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	$-(CHC)_3-NH-CNH-NH_2$	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	$-CH_2OH$	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

Legend to Summary

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/H_2O

   R: 50/50: MeOH/H_2O or 50/50 ACN/H_2O

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_2PO_4

   TAG: 50/50: MeOH/100mM NaH_2PO_4

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, 123-127 (1996).

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

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

Conclusions

1. For underivatized neutral amino acids the use of alcohol/H_2O or ACN/H_2O systems is the best choice.
2. An increase in the organic content of the mobile phase results in an increase in resolution due to an increase in retention while selectivity is largely unchanged.
3. Without the use of buffer, at low UV, the stable baseline can be reached quickly.
4. High organic content of mobile phase allows the use of evaporative light scattering detector to increase sensitivity.
5. For underivatized amino acids analyses the best column choice is CHIROBIOTIC TAG followed by CHIROBIOTIC T and R.
6. The key chiral interaction site is the amino group on the end of the aglycone structure in all three CSPs.
7. CHIROBIOTIC series columns can be operated in any type of mobile phase including hydrophilic interaction chromatography.