
Unique Chiral Ionic Interaction Mechanism on Macrocyclic Glycopeptide CSPs in HPLC

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Abstract

Macrocyclic glycopeptide-based chiral stationary phases (CSPs) are becoming more popular due to their multimodal capability, ruggedness and broad selectivity. Due to their multi-functional complex structures, different type of chiral interactions can be promoted between the analyte and CSPs under different mobile phase conditions. There are at least six possible interactions available within the structure of these CSPs. These include ionic, π - π , hydrogen bonding, hydrophobic, dipole-dipole and steric repulsion.

This presentation will focus on the ionic interactions due to the fact that it is the most specific and effective force for chiral recognition. In this study, LC phases prepared by covalently bonding Vancomycin, Teicoplanin and Ristocetin A are tested with a variety of ionizable molecules under reversed and polar organic phase systems. In each mobile phase type, ionic interaction(s) can be realized by altering the acid/base ratio in the polar organic mode or by changing the pH of the buffer in the reversed phase mode. Consequently, the retention, peak efficiency and selectivity will be affected.

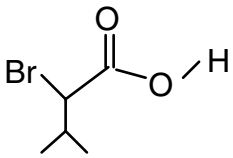
Detailed information on the relationship between the structure of the analytes and the glycopeptide CSPs will be discussed. Also, the differences between the mechanism for aqueous versus organic mobile phase systems for the availability of the ionic interaction site of the glycopeptides will be summarized. Examples of enantiomer repulsion where the analyte elutes before the void volume marker will be shown. Examples of reversal of elution order by altering the mobile phase system will also be presented.

Objectives

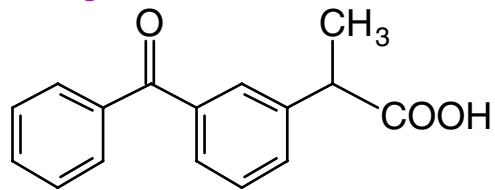
- t To study the effect of acid/base in the mobile phase for the retention and the selectivity of the macrocyclic CSPs, i.e. Vancomycin, Teicoplanin and Ristocetin A.
- t To further understand the relationship between classes (structures) of the molecules and the CSPs in different mobile phase systems.
- t To decipher the key separation mechanisms involved between the molecules and the CSPs under mobile phases of polar organic mode and reversed phase mode.

Samples Investigated

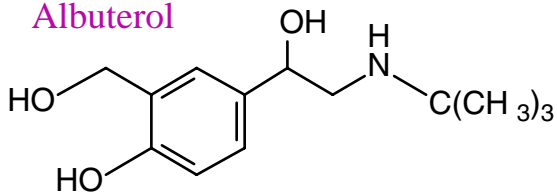
2-Br-3-Me-Butyric Acid



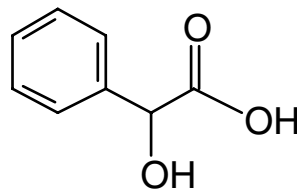
Ketoprofen



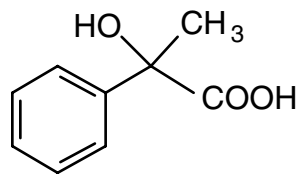
Albuterol



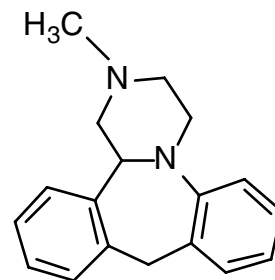
Mandelic Acid



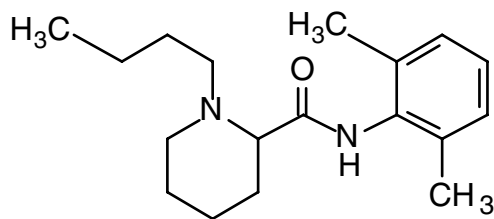
Atrolactic Acid



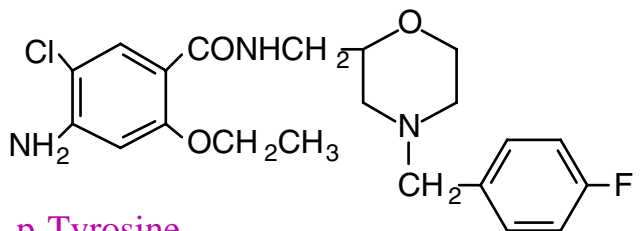
Mianserin



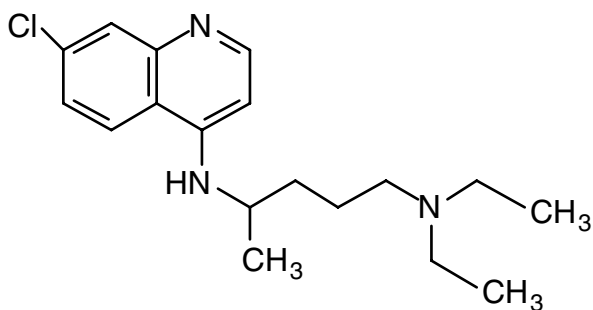
Bupivacaine



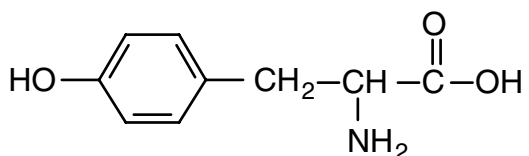
Mosapride



Chloroquine



p-Tyrosine



Polar Organic Mode

MeOH/HOAc/TEA

CHIROBIOTIC V (150x4.6mm)

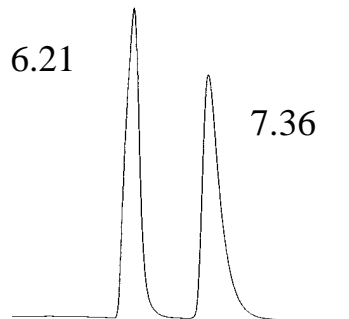
V/V/V	100/0.1/0.1			100/0.15/0.05			100/0.05/0.15		
Compound s	k ₁		Rs	k ₁		Rs	k ₁		Rs
2-Br-3Me-Butyric Acid	N/A		--	N/A		--	N/A		--
Albuterol	0.88	1.17	1.3	1.38	1.16	1.14	1.67	1.14	1.4
Atrolactic Acid	0.18	1.0	--	0.19	1.0	--	0.40	1.0	--
Bupivacaine	0.71	1.14	1.0	1.37	1.14	1.4	0.19	1.0	--
Chloroquine	No Peak		--	No Peak		--	5.95	1.13	1.3
Ketoprofen	0.61	1.0	--	0.45	1.0	--	0.40	1.0	--
Mandelic Acid	0.89	1.0	--	1.03	1.0	--	0.52	1.0	--
Mianserin	2.00	1.25	2.0	3.97	1.48	4.0	0.63	1.12	0.8
Mosapride	0.40	1.0	--	0.62	1.28	1.3	0.29	1.0	--
p-Tyrosine	1.15	1.0	--	1.19	1.0	--	1.03	1.0	--

Acid/Base Effect on Polar Organic Mode

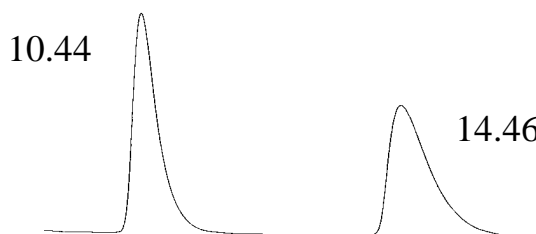
Example: Mianersin

Mobile Phase: MeOH/HOAc/TEA

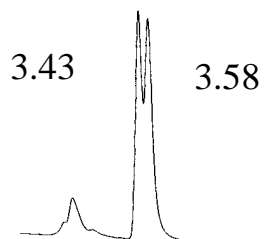
100/0.1/0.1



100/0.15/0.05



100/0.05/0.15

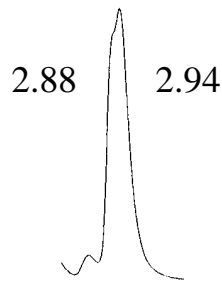


Acid/Base Effect on Polar Organic Mode

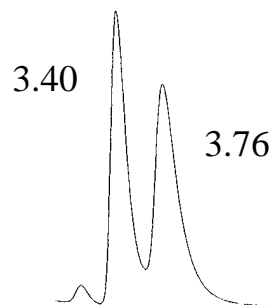
Example: Mosapride

Mobile Phase: MeOH/Acid/Base

100/0.1/0.1

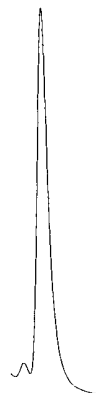


100/0.15/0.05



100/0.05/0.15

3.71



Reversed Phase

CHIROBIOTIC V (150x4.6mm)*

20/80: ACN/0.1% TEAA	pH4 .1			pH6 .5		
Compounds	k ₁		Rs	k ₁		Rs
2-Br-3Me-Butyric Acid	0.49	1.0	--	t ₁ -2.21 m in		--
Albuterol	0.60	1.0	--	No Peak		--
Atrolactic Acid	0.52	1.0	--	t ₁ -2.09 m in		--
Bupivacaine	No Peak		--	No Peak		--
Chloroquine	9.45	1.05	0.6	No Peak		--
Ketoprofen	2.6	1.0	--	0.58	1.0	--
Mandelic Acid	0.44	1.0	--	0.45	1.0	--
Mianserin	5.2	1.20	1.8	No Peak		--
Mosapride	5.78	1.35	2.1	8.08	1.19	1.2
p-Tyrosine	0.10	1.0	--	0.15	1.0	--

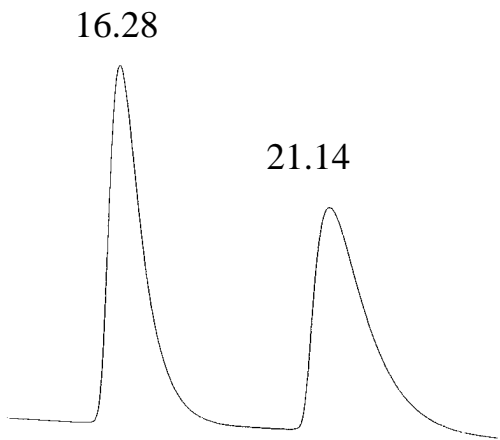
*t₀ = 2.40 min.

pH Effect on Reversed Phase

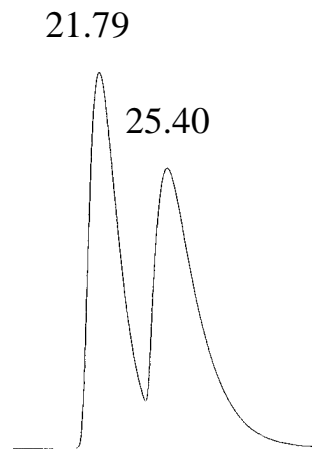
Example: Mosapride

Mobile Phase: 20/80: ACN/0.1% TEAA

pH 4.1



pH 6.5

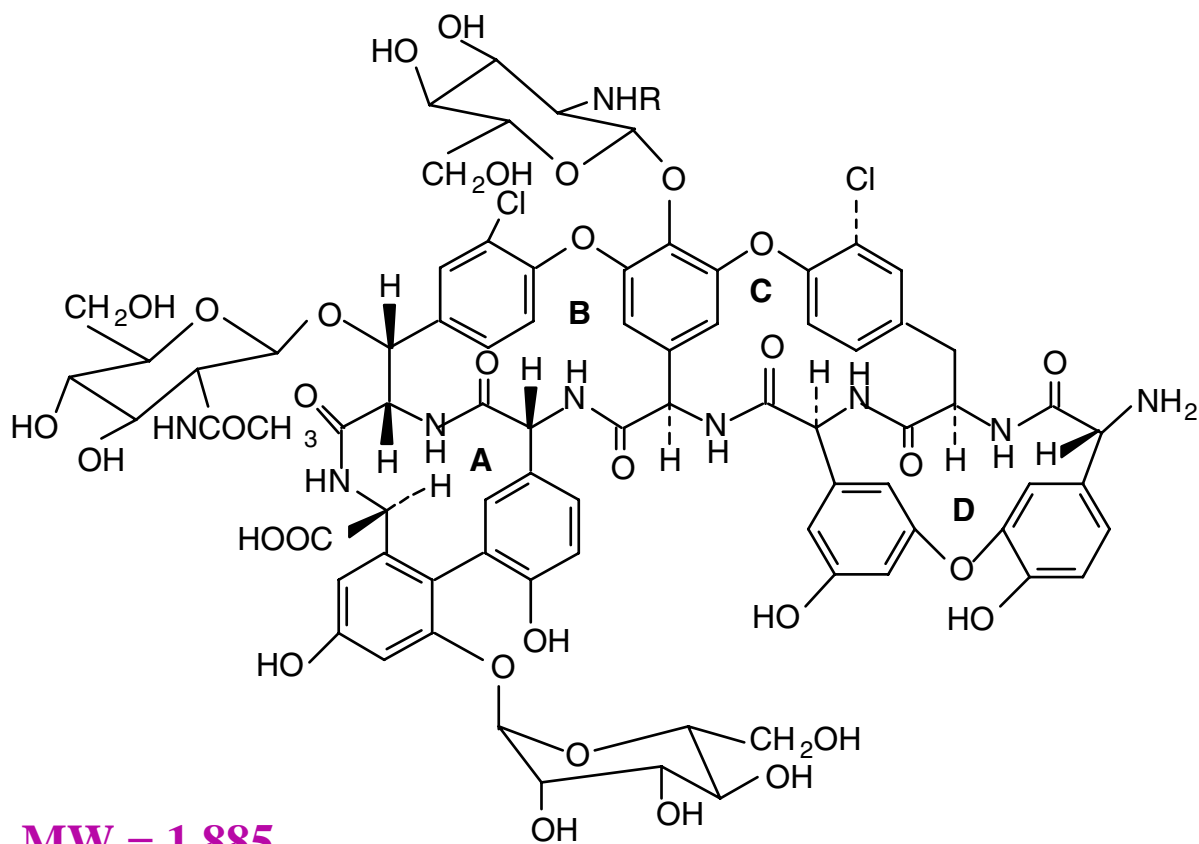


Observations on CHIROBIOTIC V

- ◆ The acid/base ratio and pH control both the selectivity and the retention.
- ◆ Ionic interactions play a significant role in the chiral recognition mechanism.
- ◆ In reversed phase mode, both amine and carboxyl groups of the CSP are involved in the interaction while in the polar organic mode, only the carboxyl group is accessible.
- ◆ For both modes, lower pH (more acid than base) gives better selectivity and resolution.*

*Profens are the only exception where THF/Na Citrate (pH 6.3) gives the best results.

Structure Teicoplanin



MW = 1,885

20 chiral centers

1 amino group

1 carboxyl group

P.I. = 3.8 ~ 6.5

Polar Organic Mode

MeOH/HOAc/TEA

CHIROBIOTIC T (150x4.6mm)

V/V/V	100/0.1/0.1			100/0.15/0.05			100/0.05/0.15		
Compound s	k ₁		Rs	k ₁		Rs	k ₁		Rs
3-Br-3 Me-Butyric Acid	N/A		--	N/A		--	N/A		--
Albuterol	2.87	1.20	2.5	3.50	1.17	2.5	3.01	1.20	2.5
Atrolactic Acid	0.30	3.75	6.0	0.28	3.01	4.0	0.25	4.32	6.0
Bup iva cai ne	1.33	1.07	0.7	2.93	1.05	0.75	0.27	1.0	--
Chloroquine	No P eak		--	No P eak		--	19.9	1.06	0.75
Ketoprofen	0.34	1.0	--		1.0	--	0.32	1.0	--
Mandelic Acid	0.42	7.19	10	0.22	5.46	6.0	0.32	7.59	8.0
Mianserin	3.13	1.09	1.3	0.43	1.11	1.8	1.00	1.0	--
Mos apride	0.51	1.0	--	3.83	1.0	--	0.33	1.0	--
p-Tyrosine	2.66	1.94	6.0	0.97	1.88	5.0	0.93	2.31	2.0

Mandelic Acid vs Methyl Mendelate

Polar Organic Mode

100/0.2/0.1:
MeOH/HOAc/TEA

Mandelic Acid

3.95

10.33

Reversed Phase Mode

20/80:
MeOH/0.1% TEAA, pH 4.1

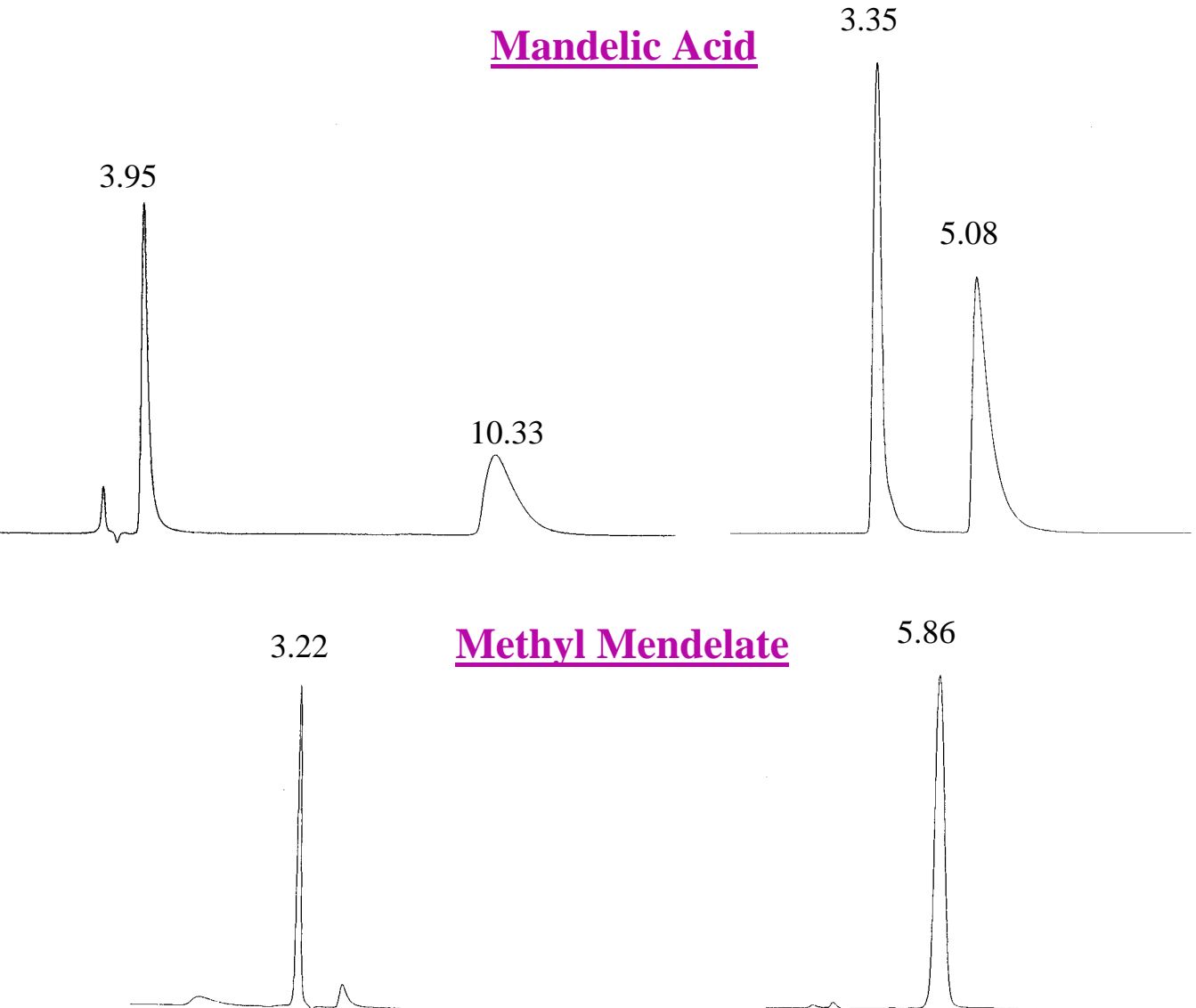
3.35

5.08

3.22

Methyl Mendelate

5.86

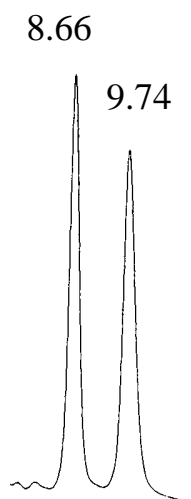


Polar Organic Mode vs Normal Phase Mode

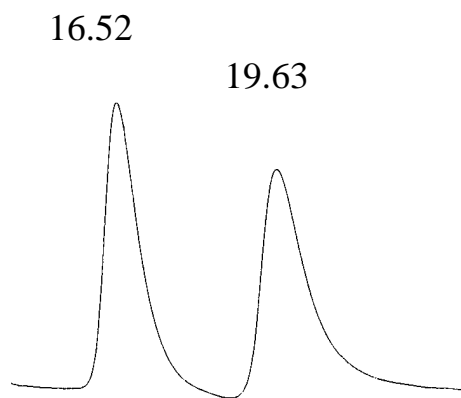
Example: Albuterol

k_1	= 2.09
k_2	= 2.48
α	= 1.19
R_s	= 1.8

k_1	= 3.71
k_2	= 4.06
α	= 1.24
R_s	= 1.8



CHIROBIOTIC T
250x4.6mm
100/0.2/0.1:
MeOH/HOAc/TEA



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

Reversed Phase

CHIROBIOTIC T (150x4.6mm)*

30/70: MeOH/0.1% TEAA	pH4.1			pH6.5		
Compounds	k ₁		Rs	k ₁		Rs
2-Br-3-Me-Butyric Acid	t ₁ = 2.28 t ₂ = 2.60		0.9	t ₁ = 1.68 t ₂ = 2.62		1.8
Albuterol	4.0	1.0	--	No Peak		--
Atrolactic Acid	0.21	2.68	1.5	t ₁ = 1.71 t ₂ = 2.41		2.0
Bupivacaine	No Peak		--	No Peak		--
Chloroquine	No Peak		--	No Peak		--
Ketoprofen	4.97	1.07	0.8	0.45	1.36	0.8
Mandelic Acid	0.07	8.9	2.0	t ₁ = 1.71 t ₂ = 2.61		3.5
Mianserin	No Peak		--	No Peak		--
Mosapride	No Peak		--	No Peak		--
p-Tyrosine	0.48	1.38	1.2	0.52	1.35	0.9

*t₀ = 2.40 min.

pH Effect on Reversed Phase

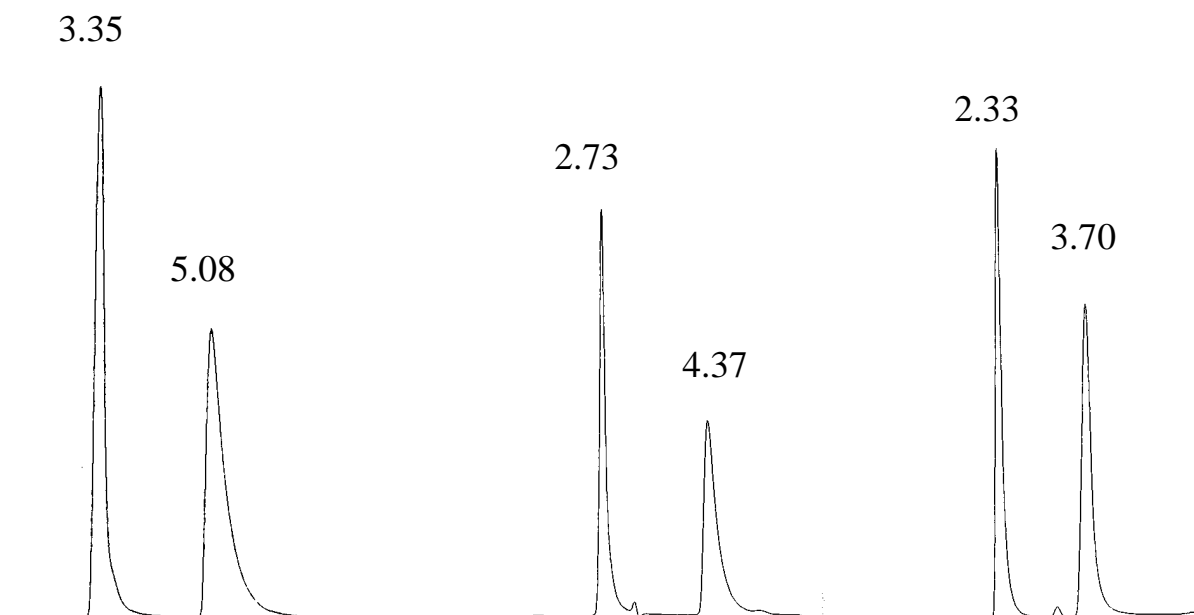
Example: Mandelic Acid

Mobile Phase*: 20/80: MeOH/0.1% TEAA

pH 4.1

pH 5.0

pH 6.5



* $t_0 = 3.2$ min.

Observations on CHIROBIOTIC T

- t The acid/base ratio and pH control both the selectivity and the retention.
- t Ionic interactions play a significant role in the chiral recognition mechanism.
- t In the reversed phase mode, only amino groups of the CSP are involved in the interaction while in the polar organic mode, both amino and carboxyl groups are available.
- t In some cases, when the separation occurred, first peak eluted before void volume.

Structure Ristocetin A

MW = 2,066

38 chiral centers

2 amino groups

1 methyl ester

P.I. = 7.5

Polar Organic Mode

MeOH/HOAc/TEA

CHIROBIOTIC R (150x4.6mm)

V/V/V	100/0.1/0.1			100/0.15/0.05			100/0.05/0.15		
Compounds	k ₁		Rs	k ₁		Rs	k ₁		Rs
2-Br-3Me-Butyric Acid	0.77	1.56	3.0	1.03	1.59	3.0	0.43	1.59	2.4
Albuterol	0.22	1.0	--	0.12	1.0	--	0.43	1.0	--
Atrolactic Acid	1.24	1.96	2.8	1.40	1.89	2.5	0.77	1.97	2.8
Bupivacaine	0.19	1.0	--	0.24	1.0	--	0.09	1.0	--
Chloroquine	4.37	1.0	--	4.90	1.0	--	2.31	1.0	--
Ketoprofen	0.72	1.11	0.9	0.52	1.08	0.7	0.60	1.14	0.95
Mandelic Acid	1.58	3.39	8.0	2.03	3.17	7.0	0.97	3.56	6.0
Mianserin	0.75	1.0	--	0.98	1.0	--	0.50	1.0	--
Mosapride	0.23	1.0	--	0.25	1.0	--	0.23	1.0	--
p-Tyrosine	1.70	1.86	2.2	1.61	1.52	1.6	1.70	1.86	2.0

Acid/Base Effects on Polar Organic Mode

Example: Mandelic Acid

Mobile Phase: MeOH/HOAc/TEA

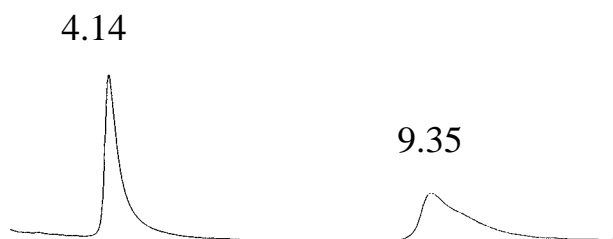
100/0.1/0.1



100/0.15/0.05



100/0.05/0.15

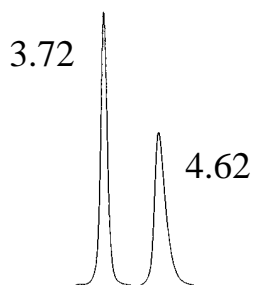


Acid/Base Effect on Polar Organic Mode

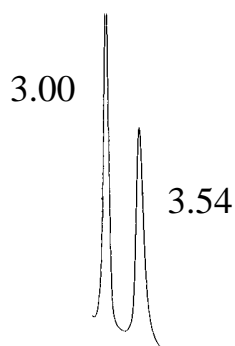
Example: 2-Br-3-Methyl Butyric Acid

Mobile Phase: MeOH/HOAc/TEA

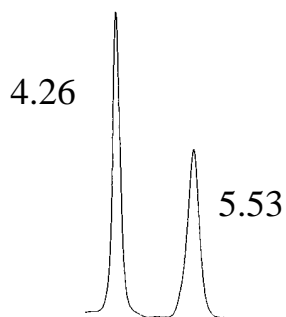
100/0.1/0.1



100/0.5/0.15



100/0.15/0.05



Reversed Phase

CHIROBIOTIC R (150x4.6mm)

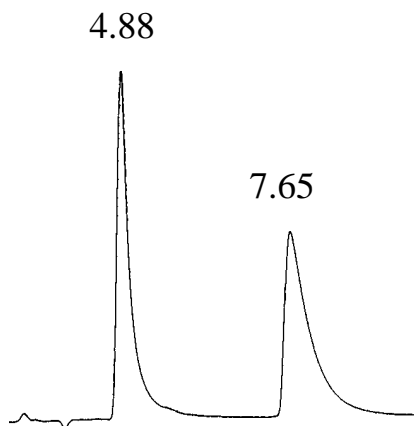
30/70: MeOH/0.1% TEAA	pH 4.1			pH 6.5		
Compounds	k ₁		Rs	k ₁		Rs
2-Br-3Me-Butyric Acid	1.58	1.69	2.6	0.8	1.80	2.2
Albuterol	t-2.24 min	1.0	--	0.48	1.0	--
Atrolactic Acid	1.51	1.48	1.6	0.32	1.85	1.45
Bupivacaine	NoPeak		--	NoPeak		--
Chloroquine	1.9	1.0	--	NoPeak		--
Ketoprofen	3.3	1.30	2.0	1.34	1.57	2.0
Mandelic Acid	1.49	2.62	4.0	0.39	2.32	2.4
Mianserin	1.28	1.0	--	NoPeak		--
Mosapride	2.90	1.0	--	10.77	1.0	--
p-Tyrosine	0.4	1.4	1.4	0.43	1.77	1.45

pH Effect on Reversed Phase

Example: Mandelic Acid

k_1	= 0.53
k_2	= 1.39
α	= 2.65
t_0	= 3.2

k_1	= 1.33
k_2	= 2.80
α	= 2.11
t_0	= 3.2

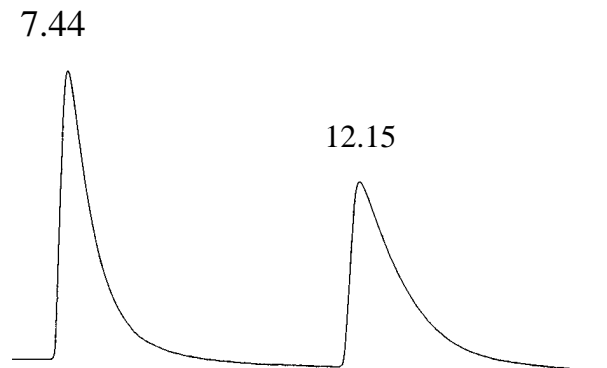


CHIROBIOTIC R

250x4.6mm

20/80:

MeOH/0.1% TEAA, pH 6.5



CHIROBIOTIC R

250x4.6mm

20/80:

MeOH/0.1% TEAA, pH 4.1

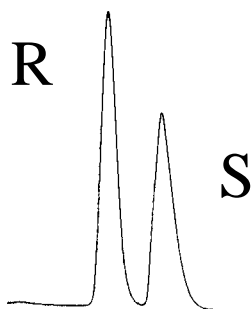
Observations on CHIROBIOTIC R

- t The acid/base ratio and pH control the selectivity, retentions and the peak efficiency.
- t Ionic interactions play a significant role in the chiral recognition mechanism.
- t Only amino group is available in the CSP.
- t In general, this CSP favors higher pH for improved selectivity and efficiency.

Reversal of Elution Order

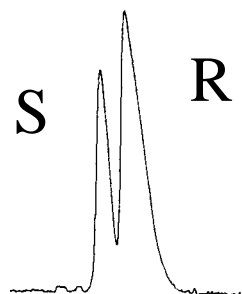
Example: N-Benzyl- α -methylbenzylamine
Column: CHIROBIOTIC V

Peak 1 - 17.09 min. (R)
Peak 2 - 18.82 min. (S)



100/0.02/0.01:
MeOH/HOAc/TEA

Peak 1 - 13.55 min. (S)
Peak 2 - 14.32 min. (R)



20/80:
ACN/0.1% TEAA, pH 4.1

Summary Ionizable Compounds vs CSPs

Molecules	Acidic (-)		Basic (+)	
	POM	RP	POM	RP
CHIROBIOTIC V				
CHIROBIOTIC T				*
CHIROBIOTIC R				

*only for hydrophilic interaction with high organic concentration.

Conclusions

- Polar organic mode is more efficient than reversed phase in terms of peak shape and retention time.
- Organic solvent and the concentration of acid/base control mostly retention time and peak efficiency.
- Acid/base ratio or pH affects both the ionic state of the CSPs and the molecules. Thus, the selectivity and the retention can be altered through the change of the addition of acid/base.
- In both operation modes, ion interaction(s) seem to be the most effective force for chiral selectivity.
- CHIROBIOTIC CSPs are complementary to one another in terms of selectivity.