

Modified Macrocyclic Glycopeptides for Enhanced Chiral Selectivity

J.T. Lee, Thomas E. Beesley

Advanced Separation Technologies Inc. (Astec)

37 Leslie Court, P.O. Box 297, Whippany, NJ 07981 USA

Tel: 973.428.9080 Fax: 973.428.0152

E-mail: astecusa@aol.com Website: www.astecusa.com

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Orland, FL



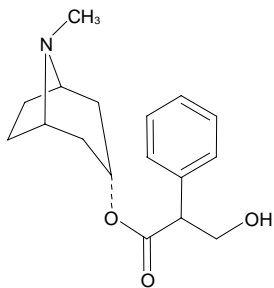
Abstract

Chiral stationary phases made by bonding the macrocyclic glycopeptides vancomycins, teicoplanin, teicoplanin aglycone and restocetin A have demonstrated very wide chiral selectivity by HPLC since their introduction by Dr. D. W. Armstrong in 1994. In the largest number of separations, very large alpha values (>2) were obtained at very low retention times for a broad range of molecule types in a number of different solvent modes. For certain structural types of racemates, however, the margin of selectivity was less than 1.5 and, in other cases, the load capacity for the preparative purifications was not as great as anticipated from the alpha values.

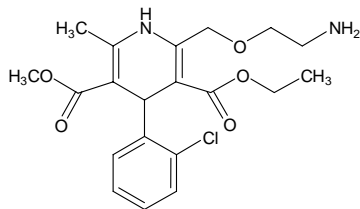
It had been suggested in several published papers that a number of mechanisms are at work in obtaining chiral selectivity on these CSPs, and much effort has been exerted to identify those particular areas of the glycopeptides responsible. Both the type of linkage, number of linkage bonds and position of these linkages indicated that certain interactions can be modified to enhance or diminish selectivity for certain classes of molecules. In addition, the use of certain blocking groups to protect possible interactive sites has contributed to a greater understanding of the mechanisms. The use of different pore sizes of the silica support (200A vs. 100A) also showed big impact on the retention, selectivity and the loading of racemates due to the changes of bonding chemistry. As a result, cases of separations with better resolution and higher capacity were obtained.

Identifying the type of interaction as a function of the type of analyte and the chemistry used to influence the selectivity outcome will be the primary focus of this presentation. The study will include separations in both the normal phase, reversed phase and the most important application in the polar organic mode suitable for LC/MS applications.

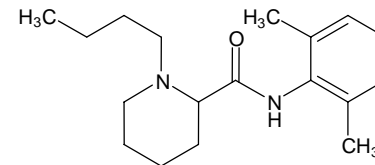
Basic Molecules



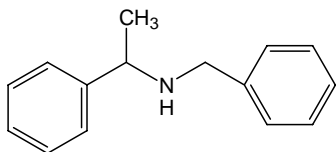
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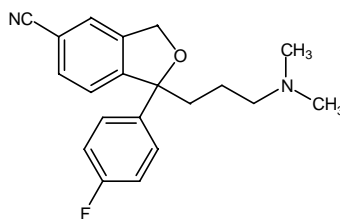
Amlodipine



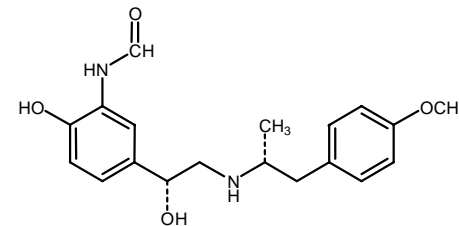
Bupivacaine



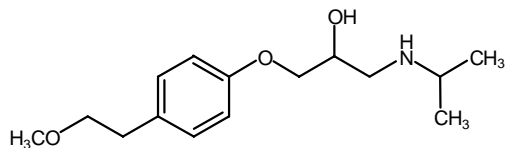
N-Benzyl- α -methylbenzylamine



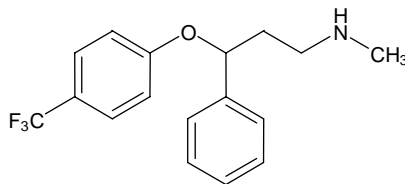
Citalipram



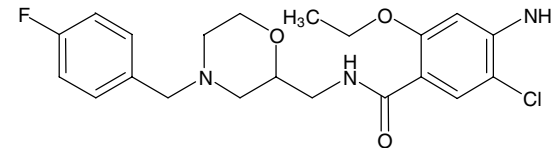
Formoterol



Metoprolol

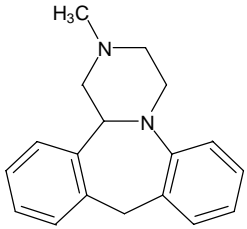


Fluoxetine

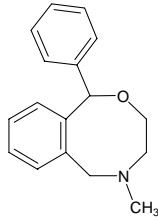


Mosapride

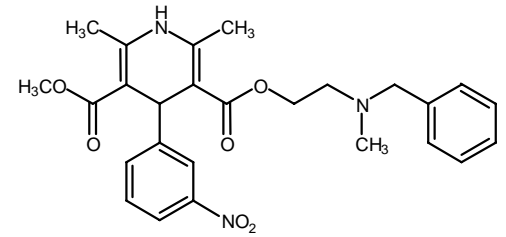
Basic Molecules



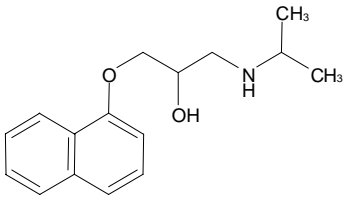
Mianserin



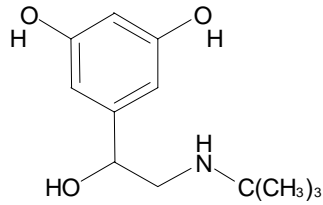
Nefopam



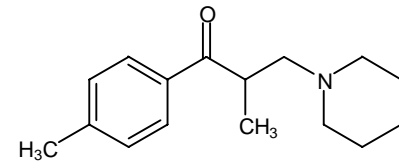
Nicardipine



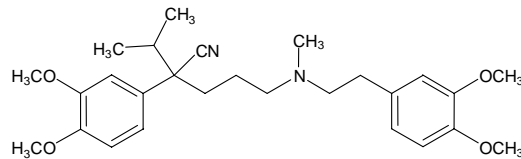
Propranolol



Terbutaline

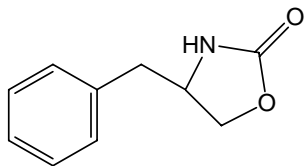


Tolperisone

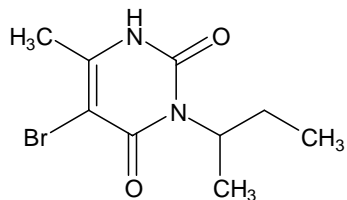


Verapamil

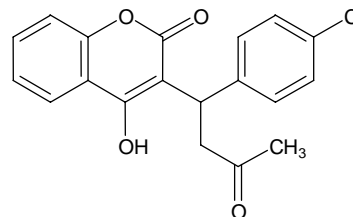
Neutral Molecules



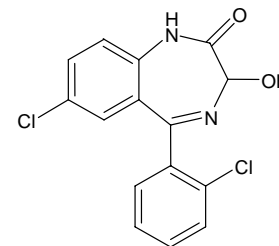
4-Benzyl 2-Oxazolidinone



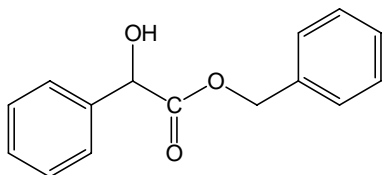
Bromacil



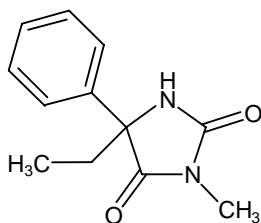
Coumachlor



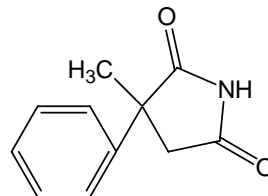
Lorazepam



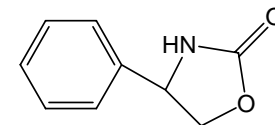
Mandelic benzyl ester



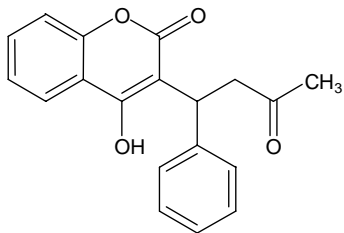
Mephentoin



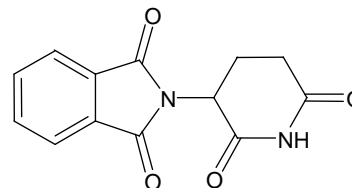
5-Methyl 5-phenyl hydantoin



4-Phenyl 2-Oxazolidinone

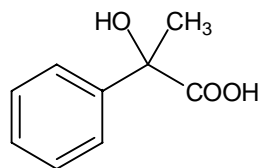


Warfarin

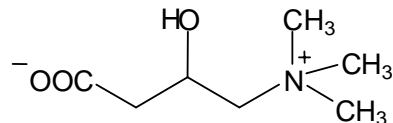


Thalidomide

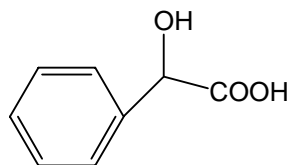
Acidic Molecules



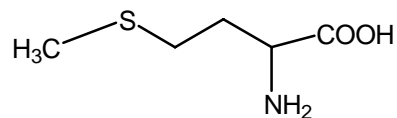
Atrolactic Acid



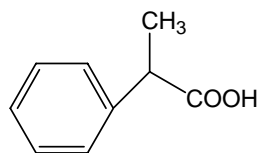
Carnitine



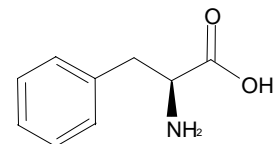
Mandelic Acid



Methionine



2-Phenyl proprionic Acid



Phenylalanine

Polar Ionic Mode©

Mobile Phase: 100/0.1w%, MeOH/NH₄TFA

Compounds	Teicoplanin, 200Å silica			CHIROBIOTIC T		
	k1'	α	Rs	k1'	α	Rs
Atrolatic Acid	0.18	4.01	4.0	0.18	4.40	4.2
Atropine	12.57	1.12	1.4	5.31	1.0	---
Formoterol*	2.57	1.21	1.5	1.83	1.06	0.6
Mandelic Acid	0.25	5.43	7.8	0.26	6.96	8.6
Metoprolol	2.70	1.15	1.7	4.48	1.14	2.0
Mianserin	4.54	1.15	1.5	7.13	1.12	1.5
Phenylalanine	1.16	1.28	1.3	2.34	1.87	3.0
Propranolol	4.03	1.13	1.6	4.20	1.14	1.9
Terbutaline	2.46	1.93	4.4	3.84	1.33	3.0

* Mobile Phase: 100/0.6/0.4, MeOH/HOAc/TEA

Reversed Phase Mode

	Teicoplanin, 200Å silica			CHIROBIOTIC T		
Compounds	k1'	α	Rs	k1'	α	Rs
Bromacil ¹	3.26	1.26	1.9	1.13	1.24	1.5
Methionine ¹	0.35	2.50	3.9	1.44	2.15	5.8
Phenylalanine ¹	0.79	1.13	0.8	0.75	1.36	1.5
4-Benzyl 2-Oxazolidinone ²	4.04	1.36	1.5	2.67	1.42	1.6
Benzyl mandelate ²	0.51	3.07	3.5	2.60	1.02	0.5
Mandelic acid ²	0.37	2.44	1.7	0.17	5.78	2.9
2 Phenyl propionic acid ²	3.25	1.13	1.1	0.92	1.09	1.0
Carnitine ³	1.07	1.25	1.4	4.38	1.09	0.9

1. Mobile phase: 30/70, EtOH/H₂O

2. Mobile phase: 20/80, MeOH/0.1% TEAA, pH 4.1

3. Mobile phase: 85/15, MeOH/0.1% TEAA, pH 6.0

Normal Phase Mode

Mobile Phase: 100% MeOH

	Teicoplanin, 200Å silica			CHIROBIOTIC T		
Compounds	k1'	α	Rs	k1'	α	Rs
Lorazepam	0.50	2.17	2.7	0.44	4.06	10.0
5-Methyl 5-phenyl hydantoin	0.28	2.48	3.9	0.37	2.17	4.9
4-Phenyl 2- oxazolidinone	0.53	1.68	2.2	1.02	1.20	1.6

Polar Ionic Mode©

Mobile Phase: 100/0.1w%, MeOH/NH₄TFA

	Vancomycin, 200Å silica			CHIROBIOTIC V		
Compounds	k1'	α	Rs	k1'	α	Rs
Citalopram	3.64	1.12	1.5	4.84	1.12	1.5
Nefopam	3.23	1.16	1.5	3.61	1.12	1.5
N-Benzyl α -methyl benzylamine	1.45	1.63	2.8	1.31	1.13	1.5
Bupivacaine	1.08	1.37	2.0	1.00	1.20	1.5
Fluoxetine	2.14	1.27	2.5	2.10	1.10	1.4
Verapamil	4.18	1.10	1.1	3.92	1.08	1.0
Tolperisone	1.75	1.33	2.7	2.03	1.17	1.5
Amlodipine	5.13	1.11	1.4	2.35	1.07	0.9
Nicardipine	0.79	2.02	4.8	0.58	1.55	3.3

Reversed Phase Mode

Mobile Phase: 30/70, ACN/0.1% TEAA, pH 4.1

	Vancomycin, 200Å silica			CHIROBIOTIC V		
Compounds	k1'	α	Rs	k1'	α	Rs
Coumachlor	5.59	1.48	3.5	2.08	1.45	3.2
Mosapride	6.96	1.09	1.4	3.06	1.32	3.3
Warfarin	3.07	1.36	2.3	1.42	1.28	2.5

Normal Phase Mode

	Vancomycin, 200A silica			CHIROBIOTIC V		
Compounds	k1'	α	Rs	k1'	α	Rs
5-Methyl,5-phenyl hydantoin ¹	0.34	1.43	1.8	0.32	1.56	2.0
Thalidomide ¹	0.51	3.47	6.6	0.65	3.44	7.0
Mephenytoin ²	1.92	1.15	1.4	3.42	1.15	1.4

1. Mobile phase: 100% MeOH
2. Mobile phase: 10/90, EtOH/Hexane

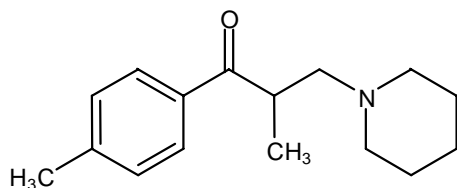
Loading Study Comparison

Polar Ionic Mode©

Mobile Phase: 100/0.1w% , MeOH/NH₄TFA

Flow Rate: 1 mL/min

Sample: Tolperisone



Vancomycin 200Å, 250x4.6mm, 5μ

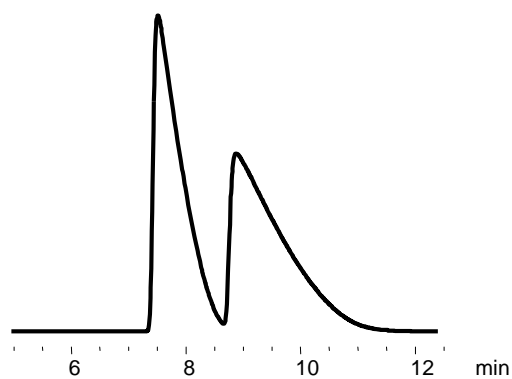
CHIROBIOTIC V, 250x4.6mm, 5μ

Selectivity: 1.33

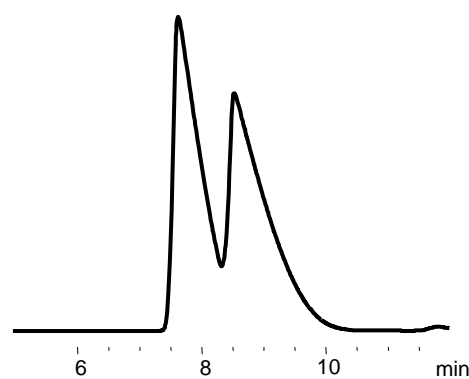
Selectivity: 1.17

Loading: 0.9 mg

Loading: 0.2 mg



Throughput: 1.5 mg/g CSP/hr



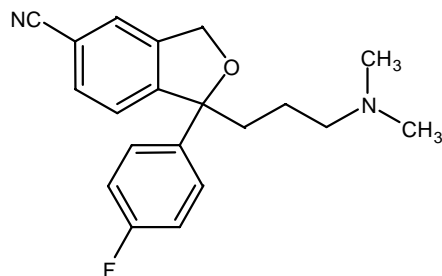
Loading Study Comparison

Polar Ionic Mode©

Mobile Phase: 100/0.1w% , MeOH/NH₄TFA

Flow Rate: 1 mL/min

Sample: Citalopram



Vancomycin 200Å, 250x4.6mm, 5μ

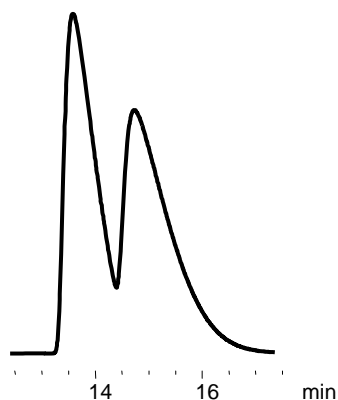
CHIROBIOTIC V, 250x4.6mm, 5μ

Selectivity: 1.12

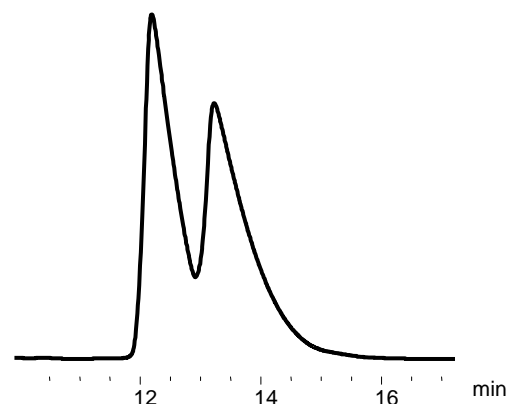
Selectivity: 1.12

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Loading: 0.12 mg



Throughput: 0.24 mg/g CSP/hr



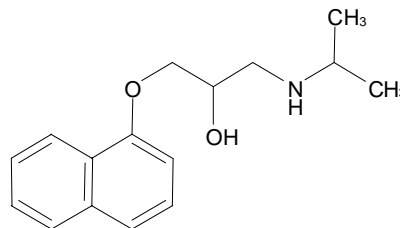
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Polar Ionic Mode©

Mobile Phase: 100/0.1w% , MeOH/NH₄TFA

Flow Rate: 1 mL/min

Sample: Propranolol



Teicoplanin 200Å, 250x4.6mm, 5μ

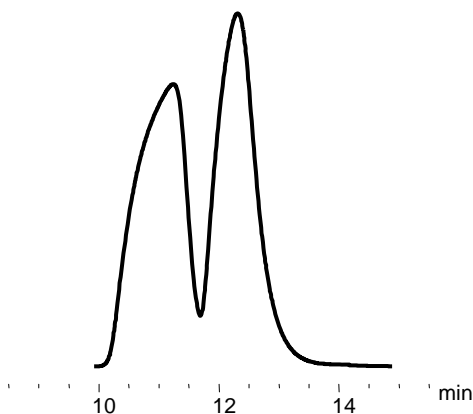
CHIROBIOTIC T, 250x4.6mm, 5μ

Selectivity: 1.13

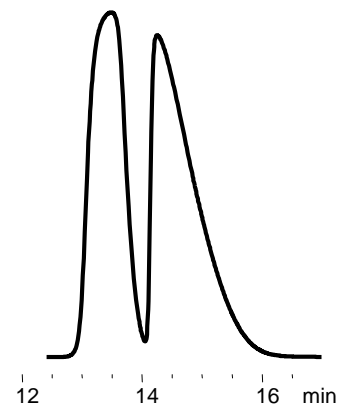
Selectivity: 1.14

Loading: 0.8 mg

Loading: 1.1 mg



Throughput: 1 mg/g CSP/hr

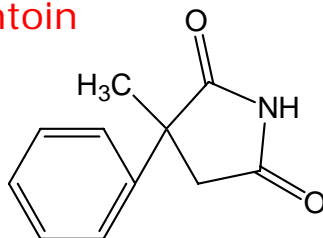


Throughput: 1.2 mg/g CSP/hr

Loading Study Comparison

Normal Phase Mode

Mobile Phase: 100% MeOH
Flow Rate: 1 mL/min
Sample: 5-Methyl 5-phenyl hydantoin



Teicoplanin 200Å, 250x4.6mm, 5μ

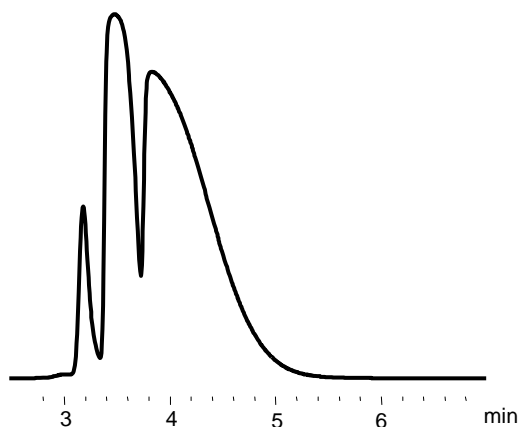
CHIROBIOTIC T, 250x4.6mm, 5μ

Selectivity: 2.48

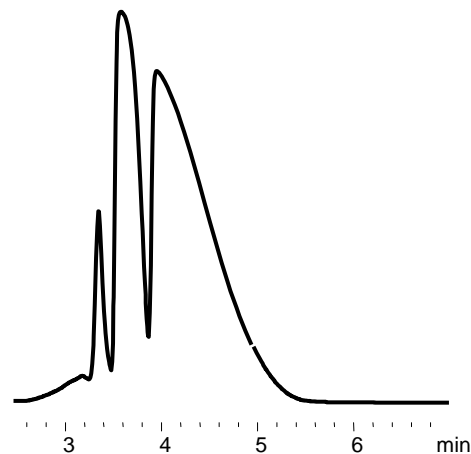
Selectivity: 2.17

Loading: 8.0 mg

Loading: 8.0 mg



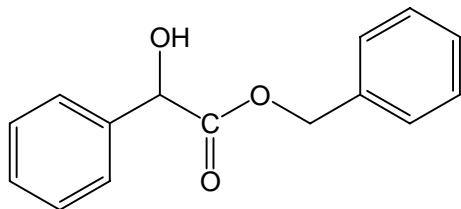
Throughput: 18 mg/g CSP/hr



Throughput: 20 mg/g CSP/hr

New LC Application Report

Sample: Mandelic Acid benzyl ester
Structure:



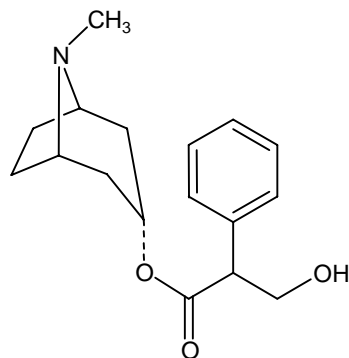
Peak 1: 4.54
Peak 2: 7.70



Conditions		Results	
Column:	Teicoplanin, 200A (Modified Chirobiotic T)	to	3.0
Dimension:	250 x 4.6mm	k' ₁	0.51
Catalog Number:	N/A	k' ₂	1.57
Mobile Phase:	20/80, MeOH/0.1% TEAA, pH 4.1	α	3.07
Flow Rate:	1 mL/min		
Temperature:	Ambient		
Chart Speed:	0.5 cm/min		
Detection:	230 nm		
Injection Volume:	10 μ L		
Sample Concentration:	2 mg/mL		

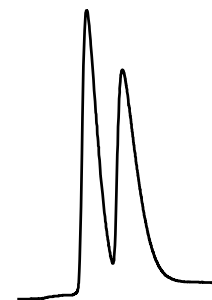
New LC Application Report

Sample: Atropine
Structure



Peak 1: 19.00

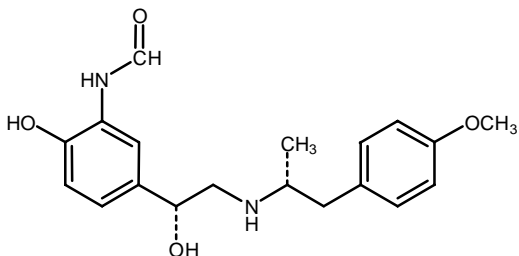
Peak 2: 21.13



Conditions		Results	
Column:	Teicoplanin, 200A (Modified Chirobiotic T)	to	1.4
Dimension:	250 x 4.6mm	k' ₁	12.57
Catalog Number:	N/A	k' ₂	14.09
Mobile Phase:	100/0.1 w%, MeOH/NH ₄ TFA	α	1.12
Flow Rate:	2 mL/min		
Temperature:	Ambient		
Chart Speed:	0.4 cm/min		
Detection:	230 nm		
Injection Volume:	10 uL		
Sample Concentration:	2 mg/mL		

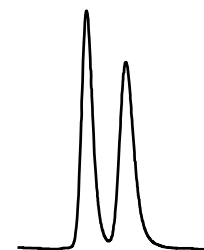
New LC Application Report

Sample: Formoterol
Structure:



Peak 1: 10.02

Peak 2: 11.49

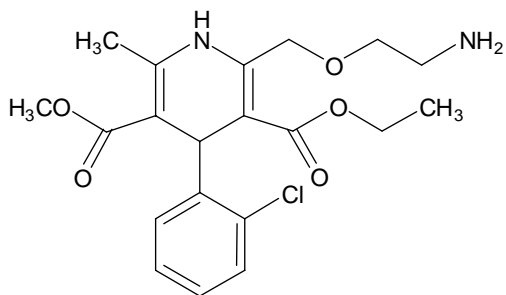


Conditions		Results	
		to	
Column:	Teicoplanin, 200Å (Modified Chirobiotic T)	to	2.8
Dimension:	250 x 4.6mm	k' ₁	2.57
Catalog Number:	N/A	k' ₂	3.10
Mobile Phase:	100/0.6/0.4, MeOH/HOAc/TEA	α	1.21
Flow Rate:	1 mL/min		
Temperature:	Ambient		
Chart Speed:	0.5 cm/min		
Detection:	230 nm		
Injection Volume:	10 µL		
Sample Concentration:	2 mg/mL		

New LC Application Report

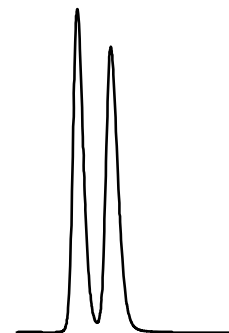
Sample: Amlodipine

Structure:



Peak 1: 14.68

Peak 2: 15.93



Conditions		Results	
Column:	Vancomycin, 200Å (Modified Chirobiotic V)	to	3.5
Dimension:	250 x 4.6mm	k' ₁	3.19
Catalog Number:	N/A	k' ₂	3.55
Mobile Phase:	100/0.1w%, MeOH/ NH ₄ TFA	α	1.11
Flow Rate:	0.8 mL/min		
Temperature:	Ambient		
Chart Speed:	0.5 cm/min		
Detection:	254 nm		
Injection Volume:	10 μ L		
Sample Concentration:	2 mg/mL		

Summary

Molecules	Basic		Neutral		Acidic	
	PIM ¹	RP ²	RP ²	NP ³	PIM ¹	RP ²
Modified T, 200Å	✓✓		✓	✓	✓	✓
CHIROBIOTIC T	✓		✓	✓	✓✓	✓
Modified V 200Å	✓✓	✓	✓	✓		
CHIROBIOTIC V	✓	✓✓	✓	✓		

1. Polar Ionic Mode©
2. Reversed Phase Mode
3. Normal Phase Mode

Conclusions

1. In Polar Ionic Mode, Teicoplanin 200Å showed unique selectivity towards some basic molecules while acids showed less desirable results than the CHIROBIOTIC T.
2. The loading capacity for Teicoplanin 200Å and CHIROBIOTIC T is similar.
3. Vancomycin 200Å showed equal or better selectivity towards basic molecules than CHIROBIOTIC V in Polar Ionic Mode while the loading capacity increases from 2 up to 20 fold.
4. For neutral molecules, there were pluses and minuses between the modified T and the CHIROBIOTIC T; and between the modified V and the CHIROBIOTIC V.