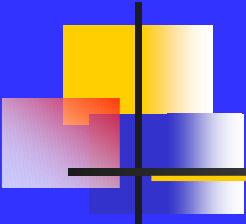


# Capacity vs Throughput on Modified Macrocylics in Reversed Phase, Polar Ionic Mode<sup>©</sup> and Normal Phase



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Denise Wallworth, J T Lee & Thomas E Beesley,

Advanced Separation Technologies

Chromatographic Society Meeting,  
GSK, Stevenage. March 2003

*astec*

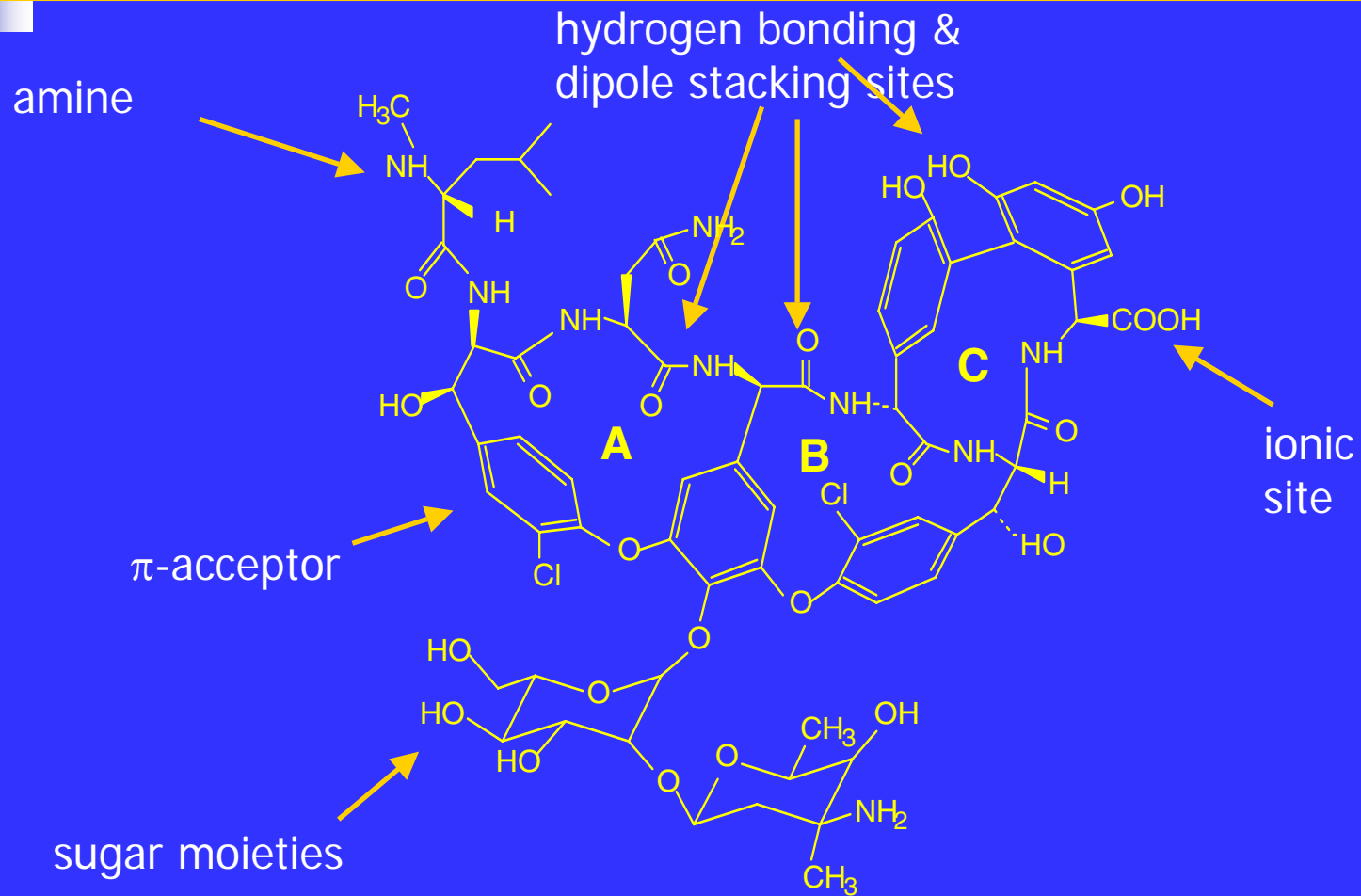


# Mechanisms for broad chiral selectivity on macrocyclic glycopeptides

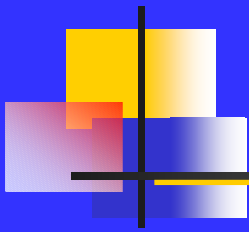
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- Ionic interaction
- Hydrogen bonding
- Inclusion
- $\pi$ - $\pi$  interaction
- Dipole stacking
- Dispersive interactions
- Steric interaction

# Proposed structure of Vancomycin & enantioselective interactions



A, B, C are inclusion pockets (weak)



# Selectivity for current bonded macrocyclic glycopeptides

---

- A large number of separations have alpha values  $> 2$  with low retention times over a broad range of molecular types
- Certain racemates exhibit lower alpha values ( $< 1.5$ ) and load capacity is lower than predicted



# Bonding effects on chiral selectivity for macrocyclic glycopeptides

---

- Type and number of linkages for covalent bonding of the glycopeptide to silica
  - Site of linkages
  - Effect of length & type of linkage on rigidity
- Effect of amount of glycopeptide bonded
  - Bonding of Vancomycin dimer



# Effects on chiral selectivity for macrocyclic glycopeptide modifications

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- Blocking of certain sites studied
  - Methylation of phenolic sites (no effect)
  - Linking through amino sites (variable effect)
- Effect of pore size
  - Accessibility to the glycopeptide important
  - Effect on glycopeptide rigidity



# Study of modified macrocyclic glycopeptides

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- Effect of increasing pore size studied for a range of molecules
- Study of interactions with respect to type of molecule
- Effect on selectivity and capacity



# Compounds evaluated

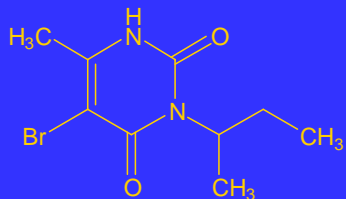
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## Basic Molecules

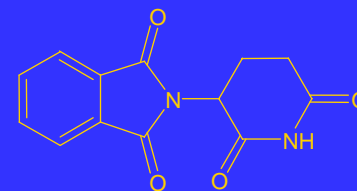
- Atropine
- Amlodipine
- Bupivacaine
- N-Benzyl- $\alpha$ -methylbenzylamine
- Citalopram
- Fluoxetine
- Formoterol
- Metoprolol
- Mianserin
- Mosapride
- Nefopam
- Nicardepine
- Propranolol
- Terbutaline
- Tolperisone
- Verapamil

# Compounds evaluated

## Neutral Molecules



- Bromacil
- Coumachlor
- Lorazepam
- Mephenytoin
- Warfarin
- 4-Benzyl-2-oxazolidinone
- 5-Methyl-5-phenyl hydantoin
- 4-Phenyl-2-oxazolidinone
- Mandelic benzyl ester
- Thalidomide



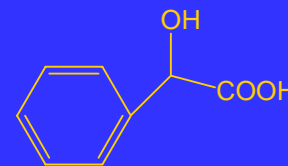
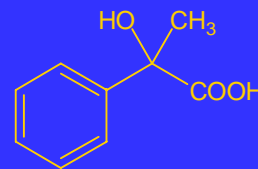


# Compounds evaluated

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## Acidic Molecules

- Atrolactic acid
- Carnitine
- Mandelic acid
- Methionine
- 2-Phenyl propionic acid
- Phenylalanine





# Differentiation of polar organic from polar ionic © mode

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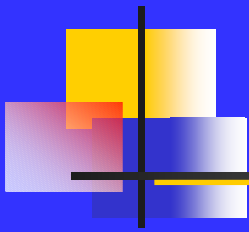
- The Polar Organic Mode terminology was developed originally by Dr D W Armstrong and Astec in '85 for use with CYCLOBOND cyclodextrin technology
- The Polar Organic Mode terminology is now frequently applied to other chiral phases, but in this case it means 100% organic only, usually MeOH/EtOH



# Differentiation of polar organic from polar ionic<sup>©</sup> mode

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- The mechanism (predominantly hydrogen bonding) for the POM on other phases is *different* from that on the CHIROBIOTIC phases and the optimisation process is *different*



# What is the Polar Ionic Mode<sup>©</sup> on CHIROBIOTIC phases?

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- 100 % MeOH with added acid and base (typically 0.1%, each with a range of 1.0 to 0.001%), or equivalent volatile salts
- The mechanism for the POM on the Chirobiotic phases is predominantly ionic, making it essential for acid and base to be added. Thus, *Polar Ionic Mode*<sup>©</sup>
- Very important mode for LC/MS and for prep



# Polar Ionic Mode<sup>©</sup>

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- **Methanol/Acid/Base Components**
  - Methanol (HPLC grade)
  - Acid: anhydrous TFA or glacial AcOH, formic acid
  - Base: TEA, DEA, NH<sub>3</sub>
  - Salts: NH<sub>4</sub>CF<sub>3</sub>COO, NH<sub>4</sub>CH<sub>3</sub>COO (test both; enantioselectivity is different in each case)



# Polar Ionic Mode<sup>©</sup> - Bases

---

Mobile phase: 100/0.1 w%, MeOH, NH<sub>4</sub>TFA

|             | Teicoplanin<br>200Å silica |      |                | CHIROBIOTIC T   |      |                |
|-------------|----------------------------|------|----------------|-----------------|------|----------------|
|             | k <sub>1'</sub>            | α    | R <sub>s</sub> | k <sub>1'</sub> | α    | R <sub>s</sub> |
| Atropine    | 12.57                      | 1.12 | 1.4            | 5.31            | 1.0  | -              |
| Formoterol* | 2.57                       | 1.21 | 1.5            | 1.83            | 1.06 | 0.6            |
| Metoprolol  | 2.7                        | 1.15 | 1.7            | 4.48            | 1.14 | 2.0            |
| Mianserin   | 4.54                       | 1.15 | 1.5            | 7.13            | 1.12 | 1.5            |
| Propranolol | 4.03                       | 1.13 | 1.6            | 4.2             | 1.14 | 1.9            |
| Terbutaline | 2.46                       | 1.93 | 4.4            | 3.84            | 1.33 | 3.0            |



# Polar Ionic Mode<sup>©</sup> - Acids

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Mobile phase: 100/0.1 w%, MeOH, NH<sub>4</sub>TFA

|                 | Teicoplanin<br>200Å silica |      |                | CHIROBIOTIC T   |      |                |
|-----------------|----------------------------|------|----------------|-----------------|------|----------------|
|                 | k <sub>1'</sub>            | α    | R <sub>s</sub> | k <sub>1'</sub> | α    | R <sub>s</sub> |
| Atrolactic acid | 0.18                       | 4.01 | 4.0            | 0.18            | 4.4  | 4.2            |
| Mandelic acid   | 0.25                       | 5.43 | 7.8            | 0.26            | 6.96 | 8.6            |
| Phenylalanine   | 1.16                       | 1.28 | 1.3            | 2.34            | 1.87 | 3.0            |

# Reversed Phase – Neutrals & Acids

Mobile phase: (1) 30/70 EtOH/H<sub>2</sub>O, (2) 20/80 MeOH/0.1% TEAA, pH 4.1  
(3) 85/15 MeOH/0.1% TEAA, pH 6

|                                       | Teicoplanin<br>200Å silica |      |                | CHIROBIOTIC T   |      |                |
|---------------------------------------|----------------------------|------|----------------|-----------------|------|----------------|
|                                       | k <sub>1'</sub>            | α    | R <sub>s</sub> | k <sub>1'</sub> | α    | R <sub>s</sub> |
| Bromacil <sup>1</sup>                 | 3.26                       | 1.26 | 1.9            | 1.13            | 1.24 | 1.5            |
| Methionine <sup>1</sup>               | 0.35                       | 2.5  | 3.9            | 1.44            | 2.15 | 5.8            |
| Mandelic acid <sup>2</sup>            | 0.37                       | 2.44 | 1.7            | 0.17            | 5.78 | 2.9            |
| 4-Benzyl 2-oxazolidinone <sup>2</sup> | 4.04                       | 1.36 | 1.5            | 2.67            | 1.42 | 1.6            |
| Benzyl mandelate <sup>2</sup>         | 0.51                       | 3.07 | 3.5            | 2.6             | 1.02 | 0.5            |
| Carnitine <sup>3</sup>                | 1.07                       | 1.25 | 1.4            | 4.38            | 1.09 | 0.9            |



# Normal Phase - Neutrals

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Mobile phase: 100% MeOH

|                             | Teicoplanin<br>200Å silica |          |       | CHIROBIOTIC T |          |       |
|-----------------------------|----------------------------|----------|-------|---------------|----------|-------|
|                             | $k_1'$                     | $\alpha$ | $R_s$ | $k_1'$        | $\alpha$ | $R_s$ |
| Lorazepam                   | 0.5                        | 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          | 1.2      | 1.6   |

# Polar Ionic Mode<sup>©</sup> - Bases on Vancomycin

Mobile phase: 100/0.1 w%, MeOH, NH<sub>4</sub>TFA

|                               | Vancomycin<br>200Å silica |      |                | CHIROBIOTIC V   |      |                |
|-------------------------------|---------------------------|------|----------------|-----------------|------|----------------|
|                               | k <sub>1'</sub>           | α    | R <sub>s</sub> | k <sub>1'</sub> | α    | R <sub>s</sub> |
| Citalopram                    | 3.64                      | 1.12 | 1.5            | 4.84            | 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            | 2.1             | 1.1  | 1.4            |
| Verapamil                     | 4.18                      | 1.1  | 1.1            | 3.92            | 1.08 | 1.0            |
| Tolperisone                   | 1.75                      | 1.33 | 2.7            | 2.03            | 1.17 | 1.5            |

# CASE STUDY: Nicardipine

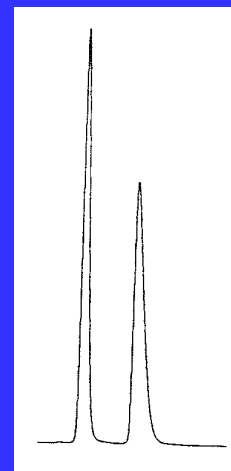


Column: CHIROBIOTIC V  
(250x4.6mm, 5 $\mu$ )

Mobile phase: 100/0.2/0.1, MeOH/AcOH/TEA

UV: 230 nm

Flow rate: 1mL/min



Peak 1: 4.66

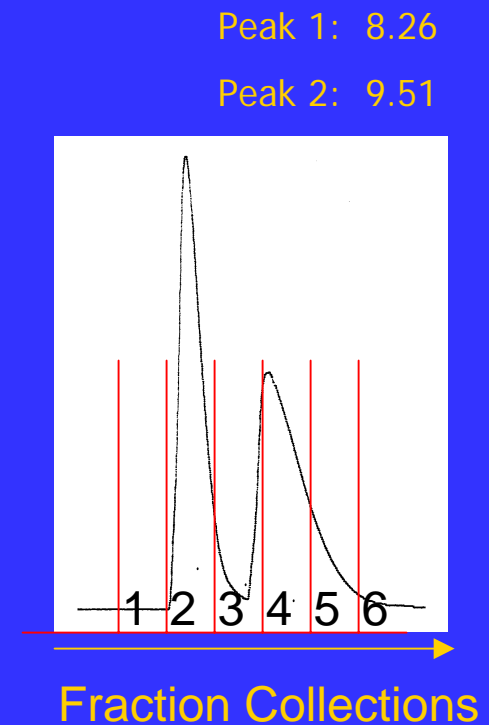
Peak 2: 5.57

$\alpha = 1.50$

# CASE STUDY: Nicardipine

Column: CHIROBIOTIC V  
(250 x 22.1 mm), 5 $\mu$ m)  
Load: 20mg in 4mL  
Mobile phase: 100/0.1w%,MeOH/NH<sub>4</sub>TFA  
UV: 230 nm  
Flow rate: 12 mL/min  
Throughput: 1.2 mg/g CSP/hr

Purity Peak 1: 99.67%  
Peak 2: 99.46%



# CASE STUDY: N-Acetyl Tryptophan

Column: CHIROBIOTIC TAG  
(250x22.1mm, 5 $\mu$ m)

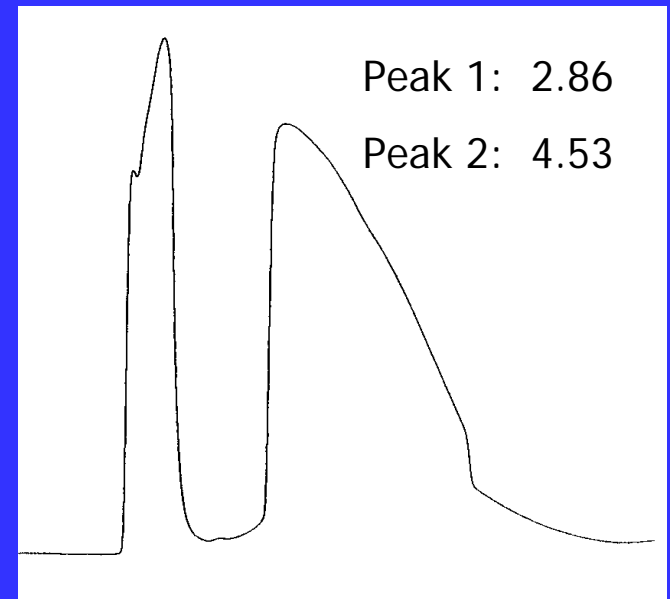
Load: 200mg in 6mL

Mobile phase: 100/0.1w%, MeOH/NH<sub>4</sub> OAc

UV: 300 nm

Flow rate: 35 mL/min

Throughput: 20 mg/g CSP/hr



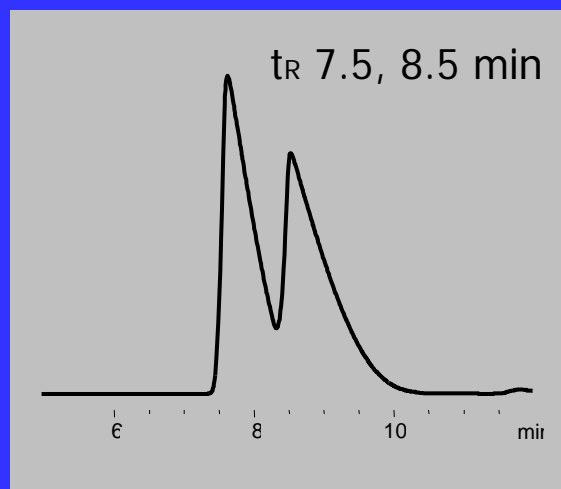
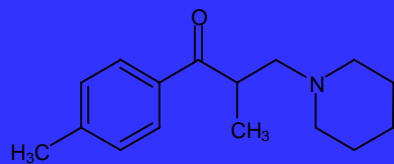
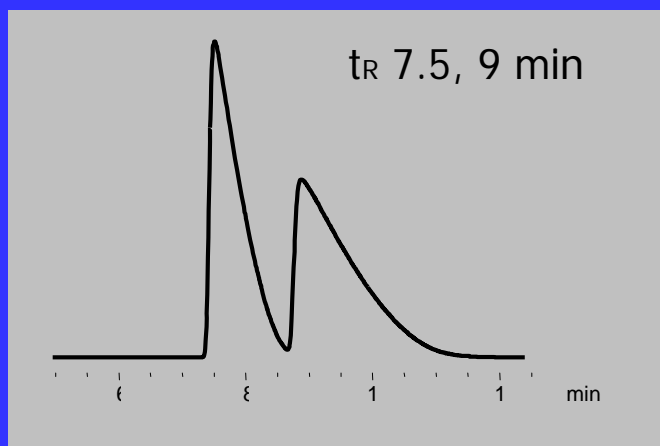
Capacity very high for amino acids and AA building blocks

# Enhanced selectivity & capacity: Polar Ionic Mode<sup>©</sup> - Tolperisone

Mobile phase: 100/0.1w%, MeOH/NH<sub>4</sub>TFA  
Flow rate: 1 ml/min

Vancomycin, 200Å  
250 x 4.6mm, 5μ  
Selectivity: 1.33  
Loading: 0.9 mg

CHIROBIOTIC V  
250 x 4.6mm, 5μ  
Selectivity: 1.17  
Loading: 0.2 mg



Throughput: 1.5mg/g CSP/hr

# CASE STUDY: Polar Ionic Mode<sup>©</sup>

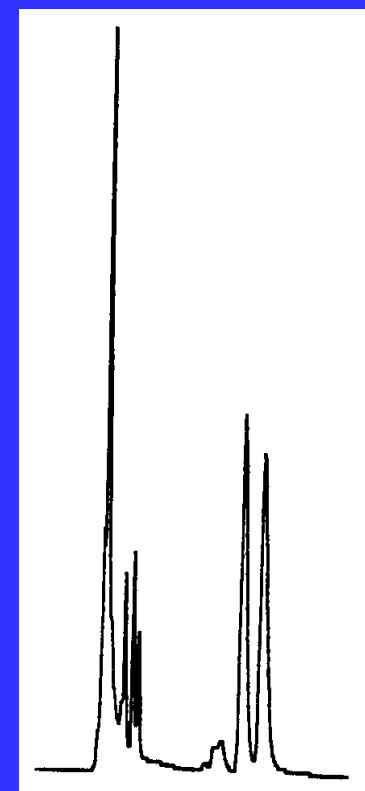
## BASIC COMPOUND

|               |   |
|---------------|---|
| Column:       | CHIROBIOTIC V, 5 $\mu$ m<br>250 x 4.6mm |
| Mobile phase: | 100/0.2/0.1,<br>MeOH/AcOH/TEA           |
| Flow rate:    | 0.9 mL/min                              |
| UV:           | 254 nm                                  |
| Inj:          | 2 $\mu$ L                               |

Peak 1: 10.51 min

Peak 2: 11.53 min

$\alpha = 1.14$



# CASE STUDY: Polar Ionic Mode <sup>©</sup>

## BASIC COMPOUND

Column: CHIROBIOTIC V 5 $\mu$ m  
(Modified) 250 x 4.6mm

Mobile phase: 100/0.5/0.5,  
MeOH/AcOH/TEA

Flow rate: 1 mL/min

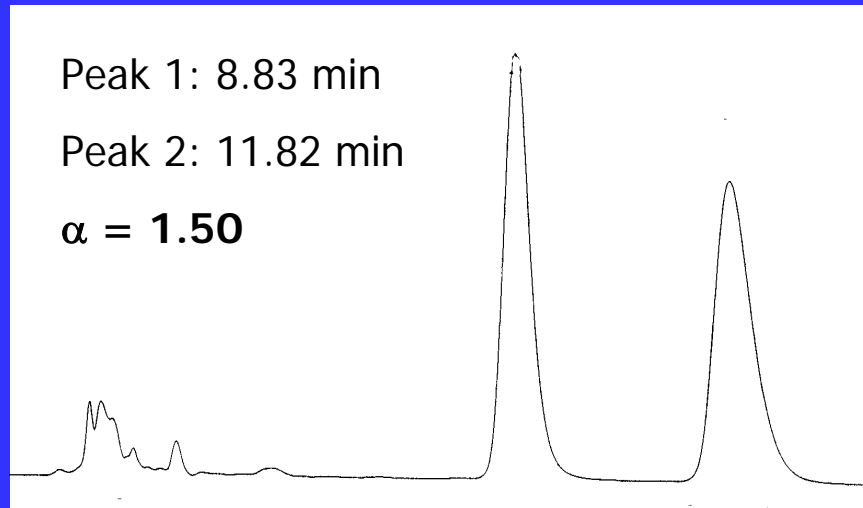
UV: 254 nm

Inj: 100 $\mu$ g

Peak 1: 8.83 min

Peak 2: 11.82 min

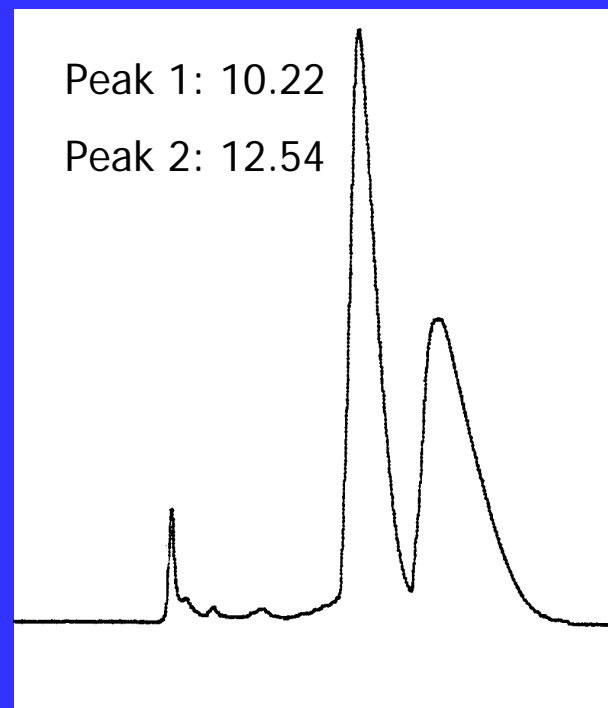
$\alpha = 1.50$



# CASE STUDY: Polar Ionic Mode ©

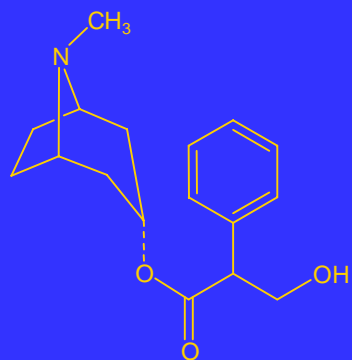
## BASIC COMPOUND

|               |   |
|---------------|---|
| Column:       | CHIROBIOTIC V, 5 $\mu$ m<br>(Modified) 250 x 22.1mm |
| Mobile phase: | 100/0.5/0.5,<br>MeOH/AcOH/TEA                       |
| Flow rate:    | 15 mL/min   |
| UV:           | 254nm   |
| Inj.          | 140mg (in 2ml MeOH)                                 |



Capacity increases from 25mg to 140mg on the modified CHIROBIOTIC V, for an  $\alpha$  change from 1.14 to 1.5

# New application: Atropine



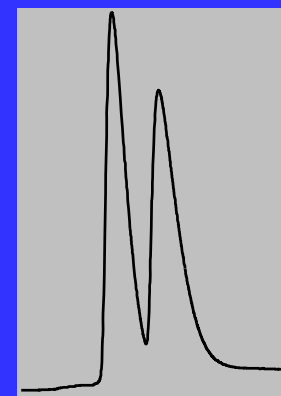
|            |       |
|------------|-------|
| $t_0$ :    | 1.4   |
| $K'_1$ :   | 12.57 |
| $K'_2$ :   | 14.09 |
| $\alpha$ : | 1.12  |

CHIROBIOTIC T modified, 200Å

Size: 250 x 4.6mm

Mobile phase: 100/0.1 w/%, MeOH/NH<sub>4</sub>TFA

Flow rate: 2 ml/min



# New application: Formoterol



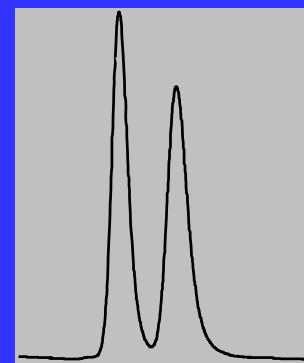
|            |      |
|------------|------|
| $t_0$ :    | 2.8  |
| $k'_1$ :   | 2.57 |
| $k'_2$ :   | 3.10 |
| $\alpha$ : | 1.21 |

CHIROBIOTIC T modified, 200Å

Size: 250 x 4.6mm

Mobile phase: 100/0.6/0.4, MeOH/AcOH/TEA

Flow rate: 1 ml/min





# Capacity

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- Highest capacity achieved to date on a 250 x 22 mm modified CHIROBIOTIC V was 300mg with an  $\alpha$  of 2.0, equivalent to 5mg/g CSP
- Average capacities to date have been 2.5mg/g CSP with an  $\alpha$  of 1.5
- Recently reported\* polysaccharide-based CSPs showed 2 to 6 mg/g CSP based on an  $\alpha$  of 1.5 to 2.4

\* Ref: Preparative Chromatographic Resolution of Enantiomers Using Polar Organic Solvents with Polysaccharide Chiral Phases, L Miller et al, ISCD, 1999



# Throughput

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- For the macrocyclic CSPs, the Polar Ionic Mode<sup>©</sup> gives higher efficiency and shorter retention times, enhancing throughput dramatically



# Conclusions

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- Polar Ionic Mode<sup>©</sup> characterised as the best mobile phase for analytical & prep on the CHIROBIOTIC CSPs
- Modification of the Teicoplanin bonded CSP by varying the amount and type of linkage had little or no change on capacity but some additional selectivity for basic compounds in the Polar Ionic Mode <sup>©</sup>



# Conclusions

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- Modification of the Vancomycin bonded CSP had a 2 to 20-fold increase in capacity and an increase in selectivity for basic molecules in the Polar Ionic Mode<sup>©</sup>
- Modifications of the T & V generally resulted in a decrease in selectivity for acids in the Polar Ionic Mode<sup>©</sup> and mixed results for selectivity and capacity for neutrals and acids in the reversed phase mode



# Conclusions

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- Capacities generally on the CHIROBIOTIC phases resulted in higher throughputs as a result of the speed of separation, coupled with good capacity
- Anhydrous 100% MeOH with volatile salts ideal for preparative LC