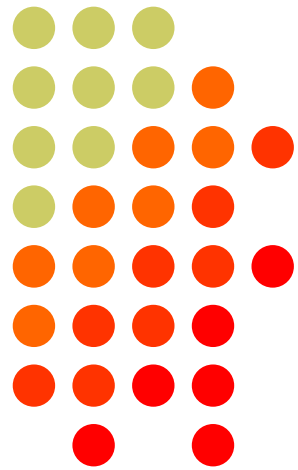
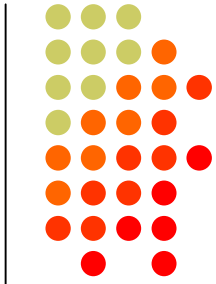
Non-Chiral Applications Using Macrocyclic Glycopeptide Chiral Stationary Phases

J.T. Lee, Thomas E. Beesley
Advanced Separation Technologies Inc. (Astec)
37 Leslie Court, P. O. Box 297
Whippany, New Jersey 07981 USA
Phone: (973) 428-9080; Fax: (973) 428-0152
Website: www.astecusa.com; Email: info@astecusa.com

Poster # 15700-500P



Abstract



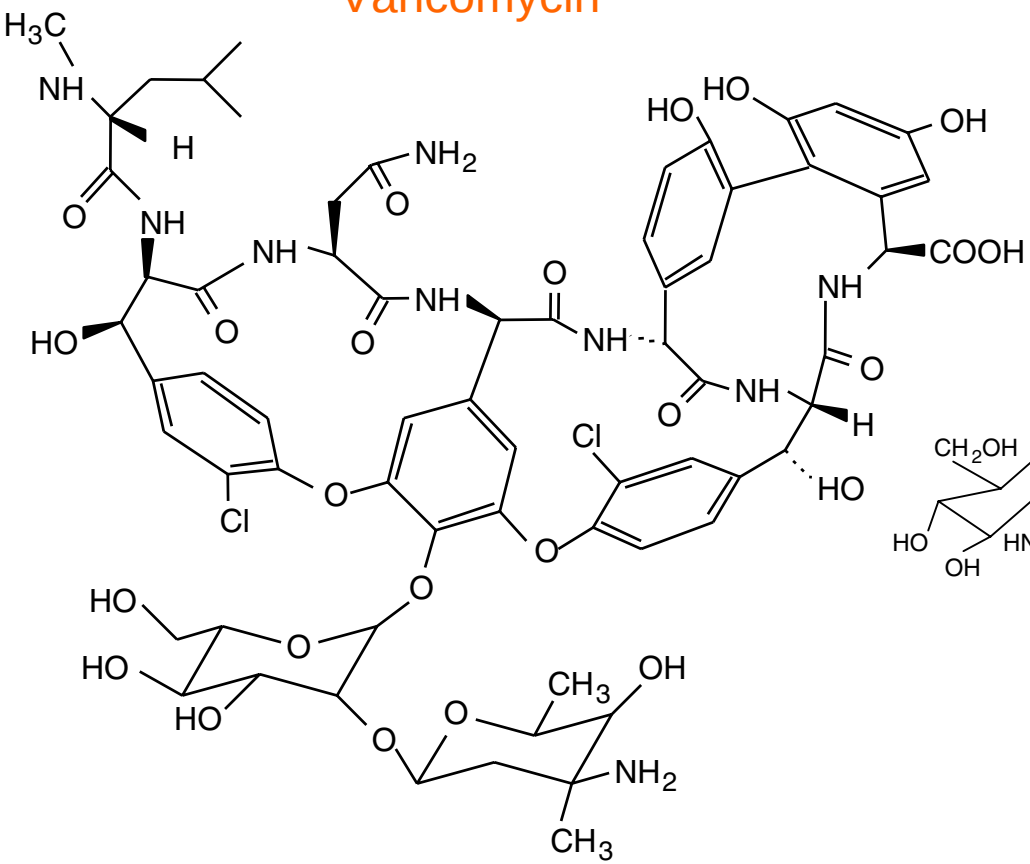
Chiral stationary phases (CSPs) made by bonding macrocyclic glycopeptides, such as vancomycin, teicoplanin, teicoplanin aglycone and ristocetin A, have demonstrated very wide chiral selectivity by HPLC since their introduction by Dr. D. W. Armstrong in 1994. In the largest number of separations, very significant alpha values (>2) were obtained at very low retention times for a broad range of molecule types in a number of different mobile phase modes.

With the current output of combinatorial chemistry, many more polar compounds have been generated that are unretained by conventional reversed phase columns. Although amides phases have some successes, the macrocyclic glycopeptides have been found to be much more effective in solving these problems due to their ionic characters and layers of hydrogen bonding groups. Aided with unique ionic interaction characters, these types of CSPs have a strong resolution power towards polar basic and acidic analytes. Thus, a number of successful non-chiral assays have been generated that outperform the traditional achiral stationary phases like C18, C8 and amide phases. Identifying the type of interaction as a function of the type of analytes and the bonding chemistry used to influence the selectivity outcomes will be the primary focus of this presentation.

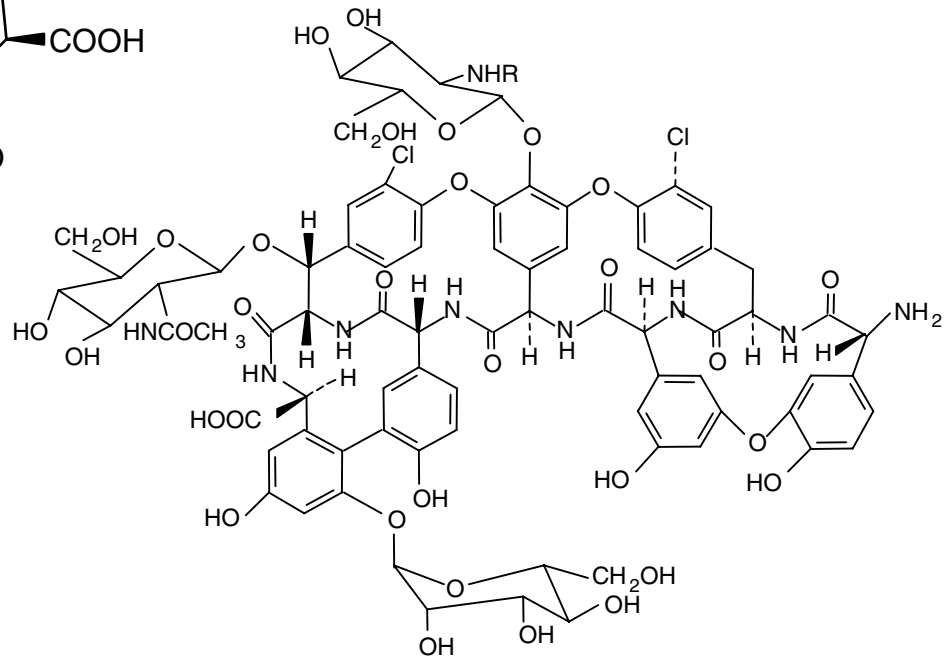
This study will focus on the separations in both the reversed phase mode and the polar ionic mode suitable for potential LC/MS applications. The advantages of using these chiral stationary phases in terms of mobile phase designs, peak shapes and selectivity will also be presented.

Proposed Structures of Macrocyclic Glycopeptide Chiral Stationary Phases

Vancomycin



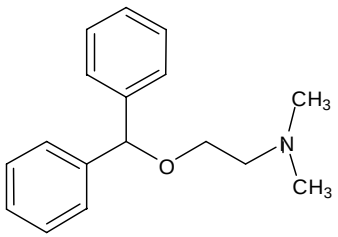
Teicoplanin



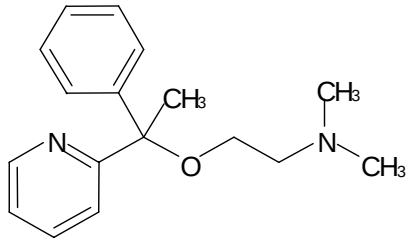
Structures of Compound Classes Investigated:

Sleeping aids:

Diphenhydramine

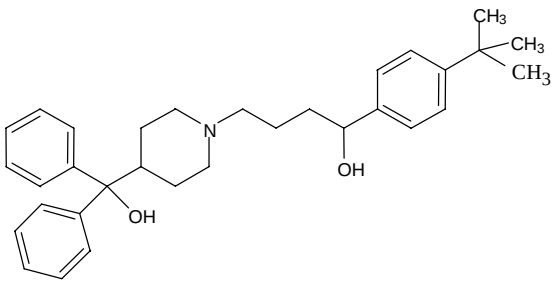


Doxylamine

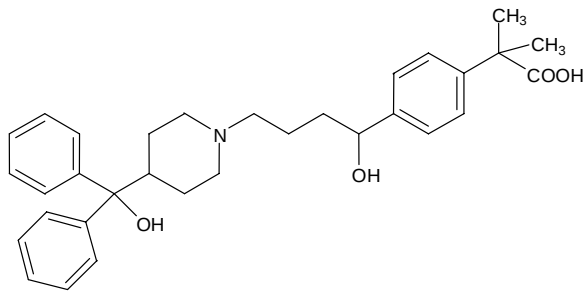


Terfenadine and its metabolite (fexofenadine):

Fexofenadine



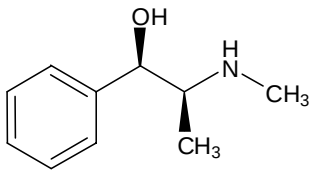
Terfenadine



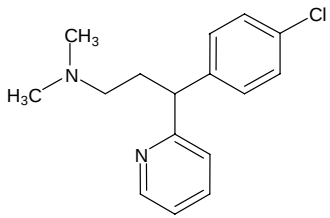
Structures of Compound Classes Investigated:

Sinus Medications:

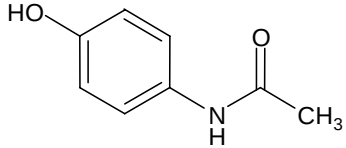
Pseudoephedrine



Chlorpheniramine

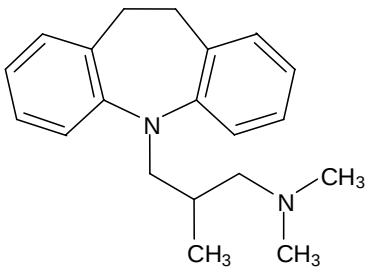


Acetaminophen

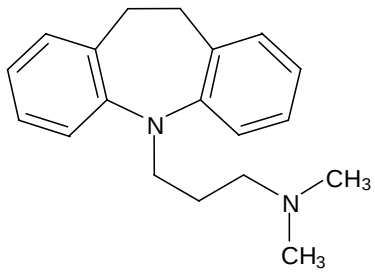


Tricyclic Antidepressants:

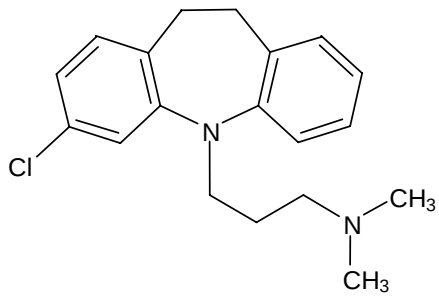
Trimipramine



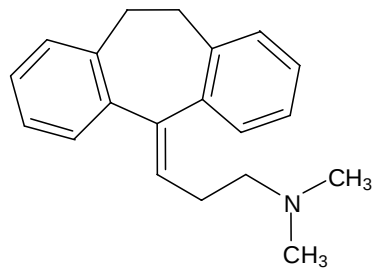
Imipramine



Clomipramine



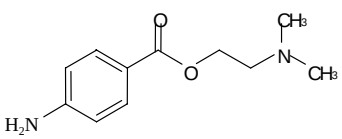
Amitriptyline



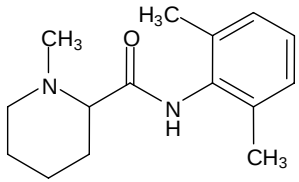
Structures of Compound Classes Investigated:

Anesthetics:

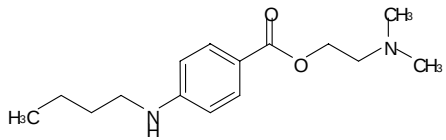
Procaine



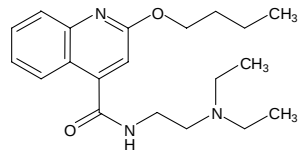
Mepivacaine



Tetracaine

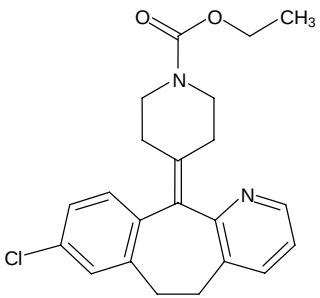


Dibucaine

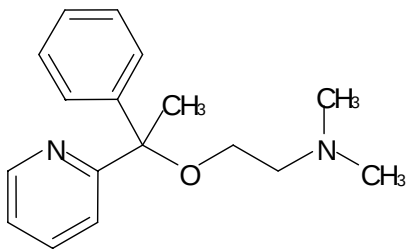


Antihistamine:

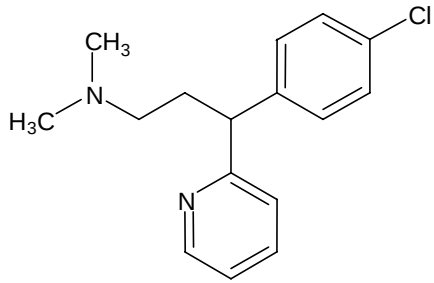
Loratadine



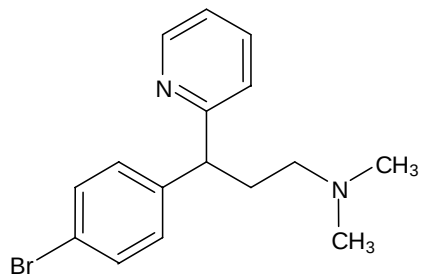
Doxylamine



Chlorpheniramine



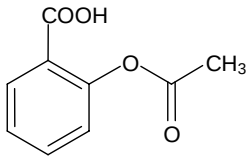
Brompheniramine



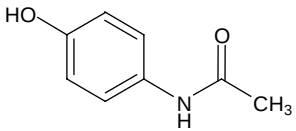
Structures of Compound Classes Investigated:

Analgesics:

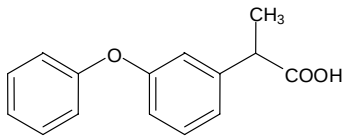
Aspirin



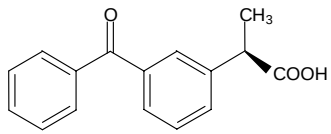
Acetaminophen



Fenoprofen

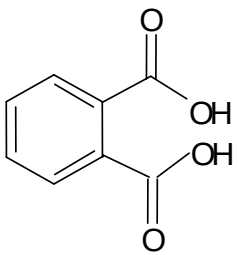


(-) Ketoprofen

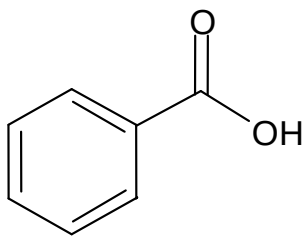


Aromatic Acids:

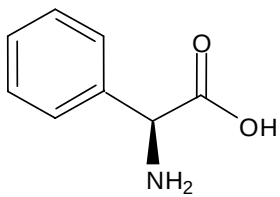
Phthalic Acid



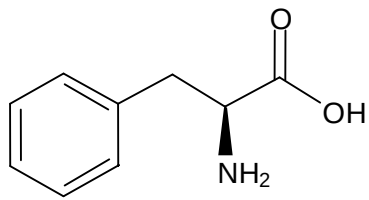
Benzoic Acid



L-Phenylglycine



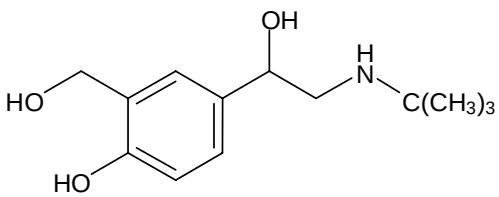
L-Phenylalanine



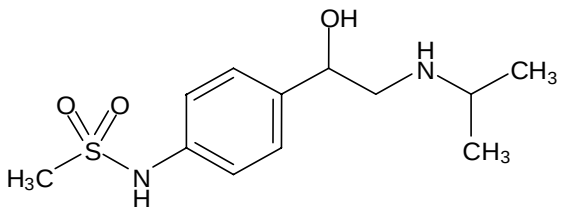
Structures of Compound Classes Investigated:

Beta Blockers:

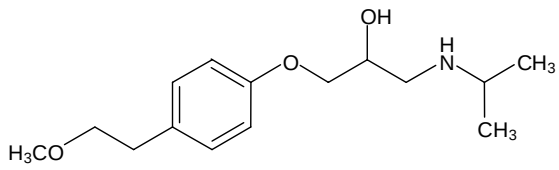
Albuterol



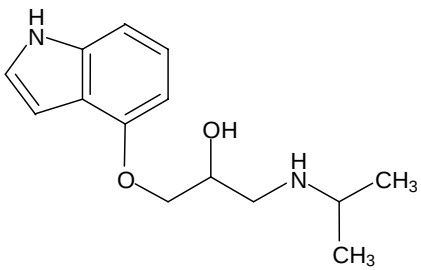
Sotalol



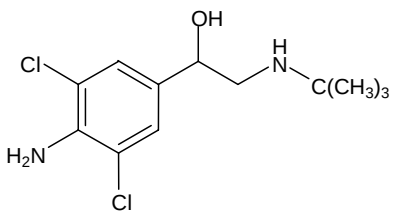
Metoprolol



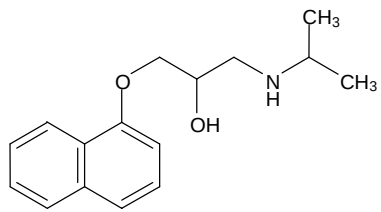
Pindolol



Clenbuterol



Propranolol

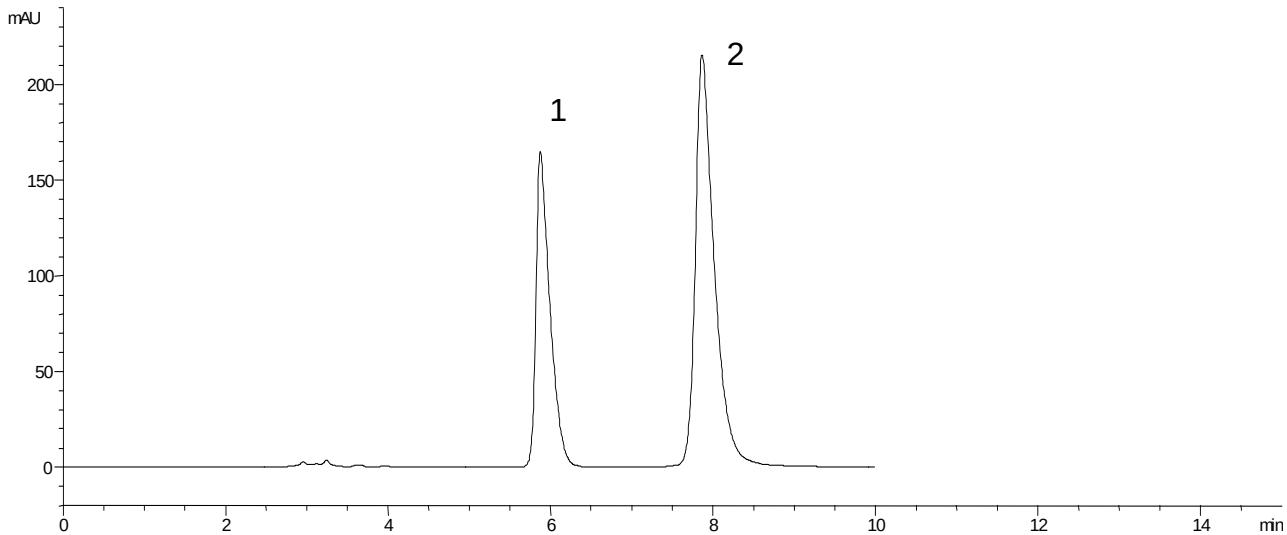


Application

Sleeping Aid:

1. Diphenhydramine
2. Doxylamine

Mobile Phase: 100/0.1w%, MeOH/NH_4 Formate
Column: CHIROBIOTIC V, 250x4.6mm, 5µ
Flow Rate: 1.0 mL/min.

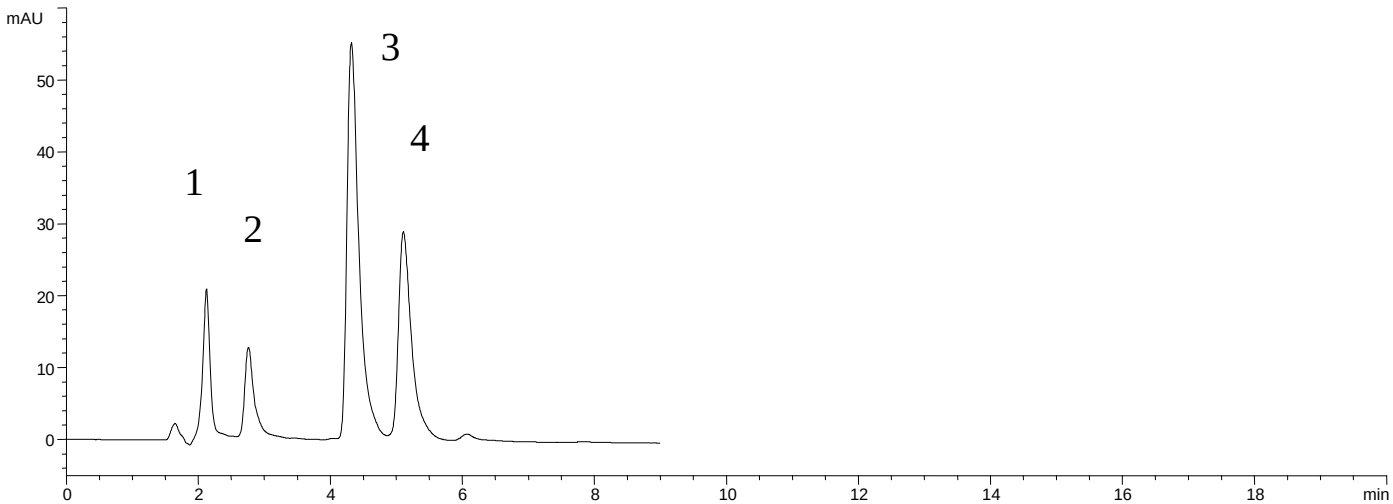


Application

Allegra Ingredients + Terfenadine:

1. MeOH
2. Pseudoephedrine
3. Fexofenadine
4. Terfenadine

Mobile Phase: 35/65, ACN/10mM NH_4OAc, pH 3.8
Column: CHIROBIOTIC V, 150x3.0mm, 3.5µ
Flow Rate: 0.5 mL/min.

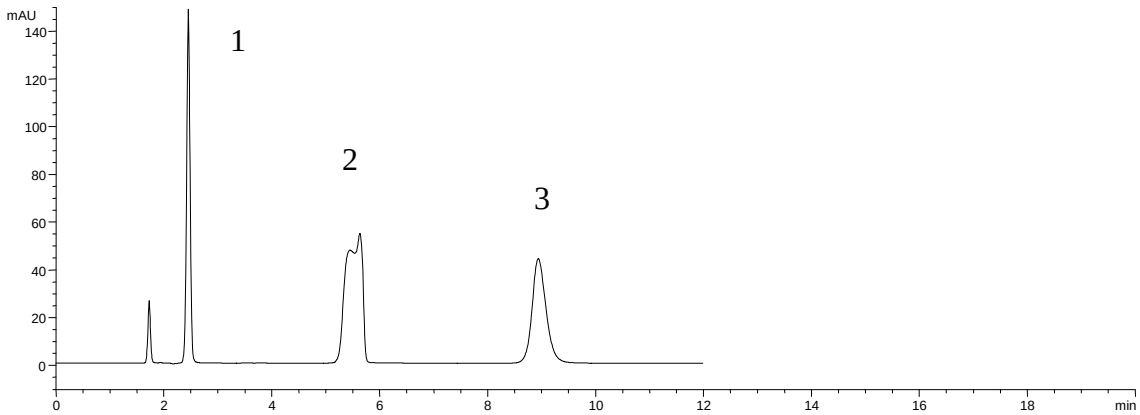


Application

Sinus Medication:

1. Acetaminophen
2. Pseudoephedrine
3. Chlorpheniramine

Mobile Phase: 35/65, ACN/10mM NH_4OAc, pH 5.0
Column: CHIROBIOTIC V, 150x3.0mm, 3.5µ
Flow Rate: 0.5 mL/min.

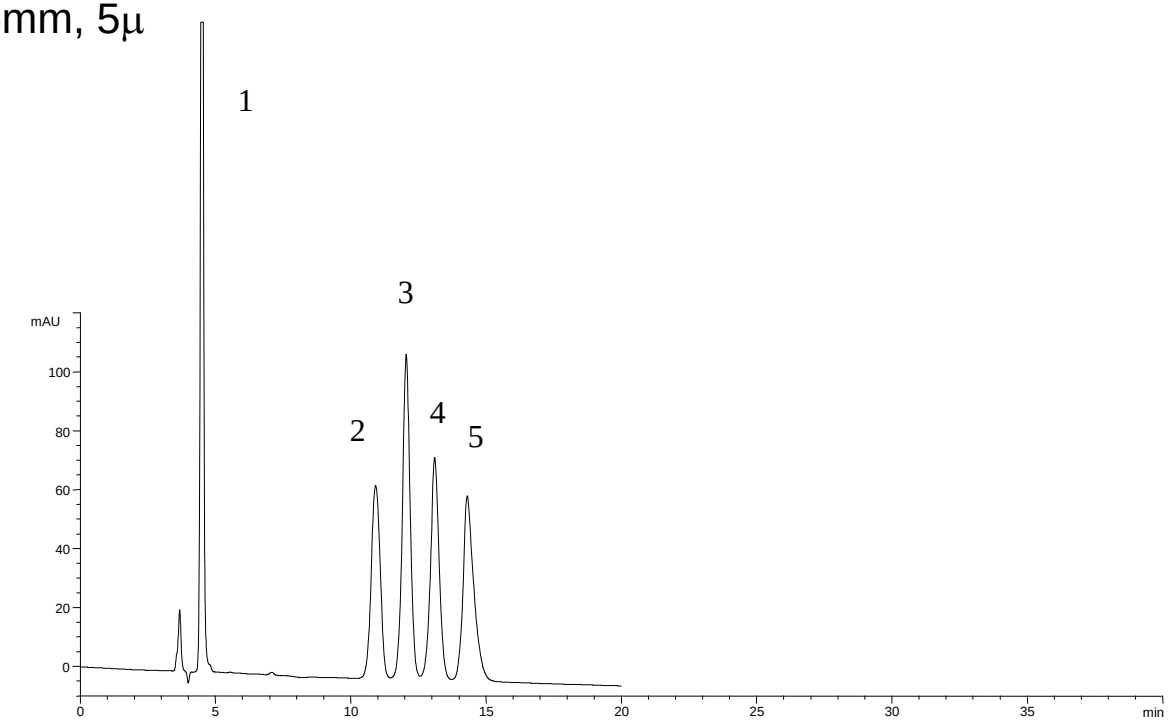


Application

Tricyclic Antidepressants:

1. Uracil
2. Trimipramine
3. Impramine
4. Clomipramine
5. Amitriptyline

Mobile Phase: 35/65, ACN/10mM NH_4OAc, pH 5.0
Column: CHIROBIOTIC V, 250x4.6mm, 5µ
Flow Rate: 0.8 mL/min.

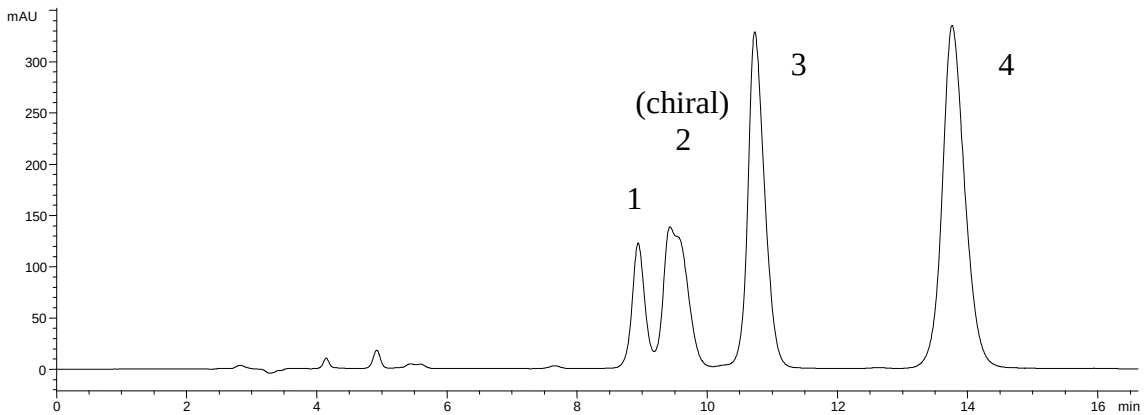


Application

Anesthetics:

1. Procaine
2. Mepivacaine (chiral)
3. Tetracaine
4. Dibucaine

Mobile Phase: 35/65, ACN/10mM NH_4OAc, pH 5.0
Column: CHIROBIOTIC V, 250x4.6mm, 5µ
Flow Rate: 1.0 mL/min.

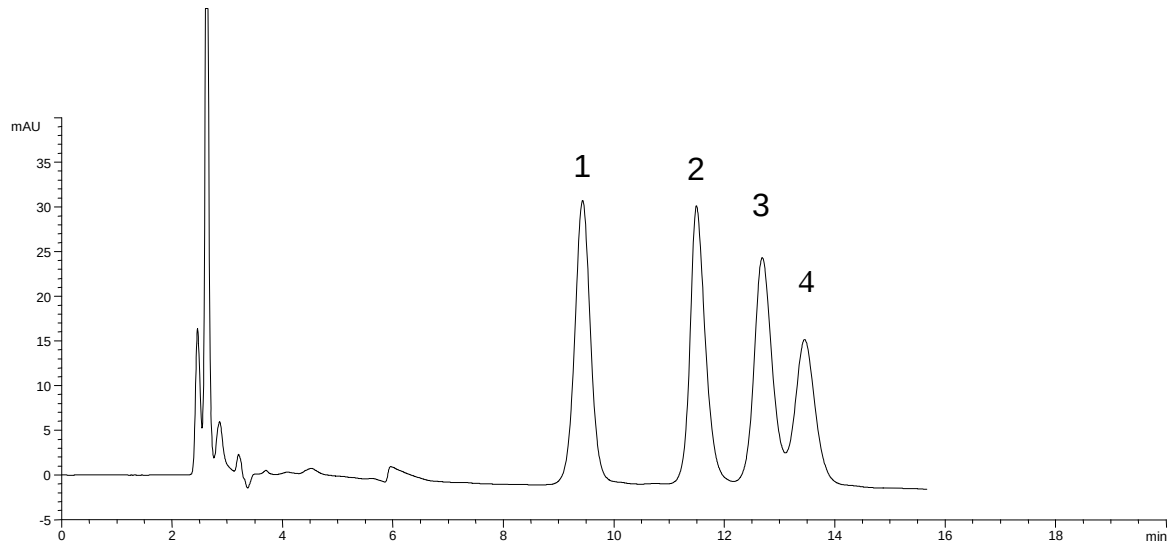


Application

Antihistamines:

1. Loratadine
2. Doxylamine
3. Chlorpheniramine
4. Brompheniramine

Mobile Phase: 35/65, ACN/10mM NH_4OAc, pH 5.0
Column: CHIROBIOTIC V, 250x4.6mm, 5µ
Flow Rate: 1.0 mL/min.

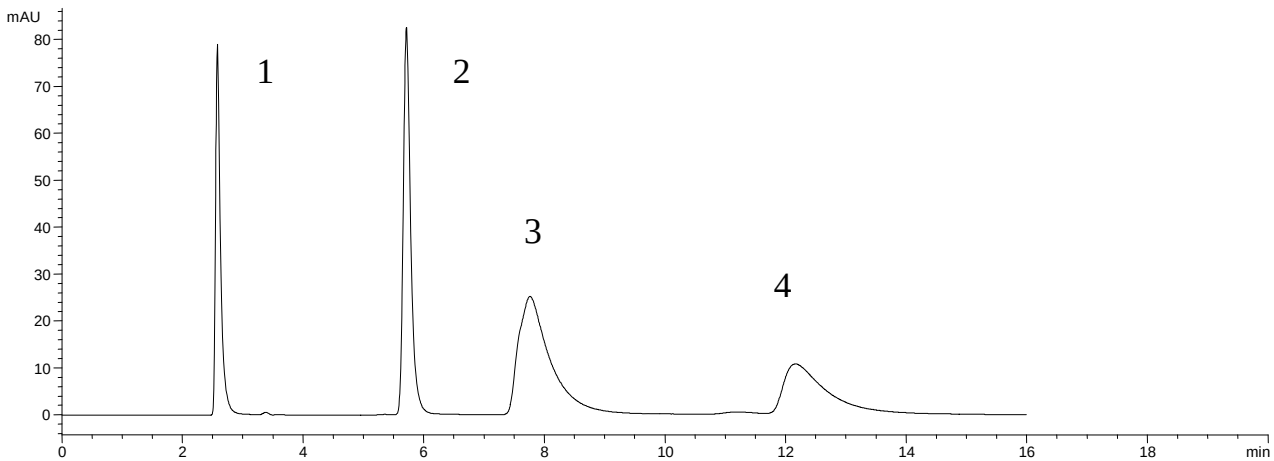


Application

Analgestics:

1. Aspirin
2. Acetaminophen
3. Fenoprofen
4. (-) Ketoprofen

Mobile Phase: 10/90, MeOH/10mMNH_4OAc, pH 5.0
Column: CHIROBIOTIC T, 150x4.6mm, 3.5µ
Flow Rate: 0.6 mL/min.

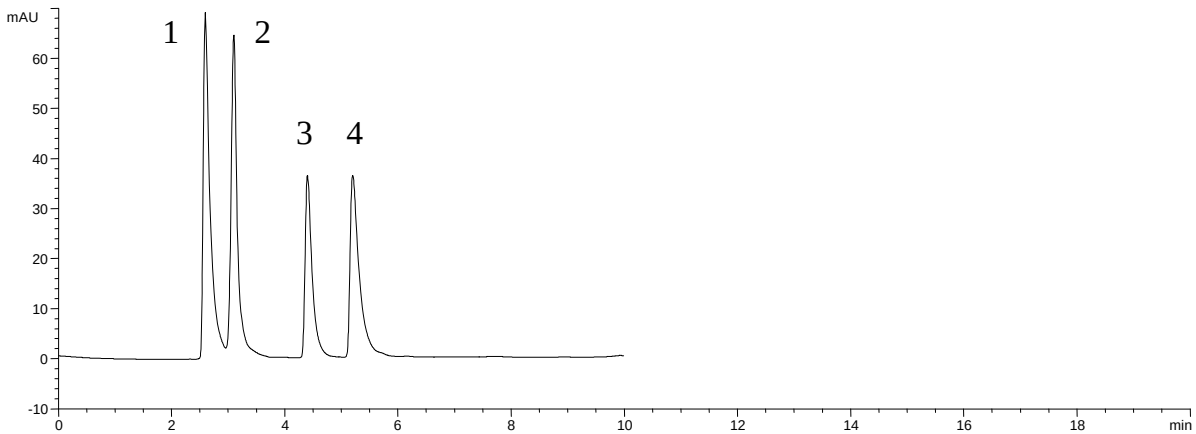


Application

Aromatic acids:

1. Phthalic acid
2. Benzoic acid
3. L-Phenylglycine
4. L-Phenylalanine

Mobile Phase: 10/90, MeOH/10mM NH_4OAc, pH 5.8
Column: CHIROBIOTIC T, 150x4.6mm, 3.5µ
Flow Rate: 0.6 mL/min.

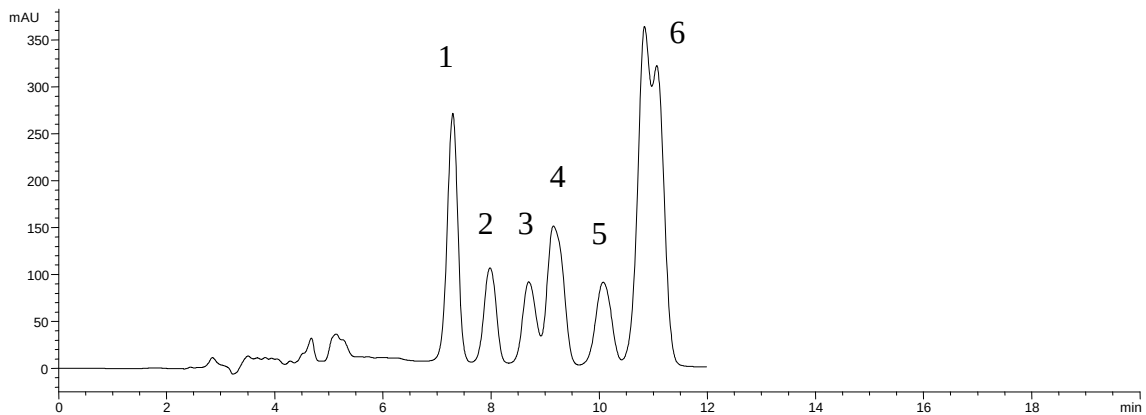


Application

Beta Blockers:

1. Albuterol
2. Sotalol
3. Metoprolol
4. Pindolol
5. Clenbuterol
6. Propranolol

Mobile Phase: 35/65, ACN/0.1% TEAA, pH 4.75
Column: CHIROBIOTIC V, 250x4.6mm, 5µ
Flow Rate: 1.0 mL/min.



pH Effect

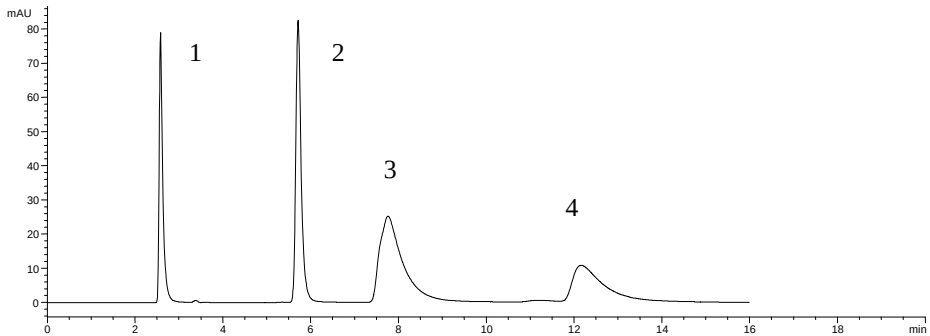
Analgesics:

1. Aspirin
2. Acetaminophen
3. Fenoprofen
4. (-) Ketoprofen

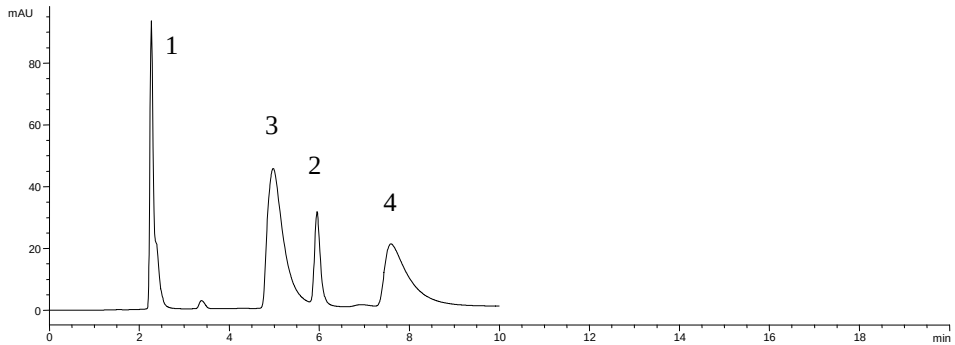
Mobile phase: 10/90, MeOH/10mM NH_4OAc

Column: CHIROBIOTIC T, 150x4.6mm, 3.5µ

pH 5.0



pH 5.8



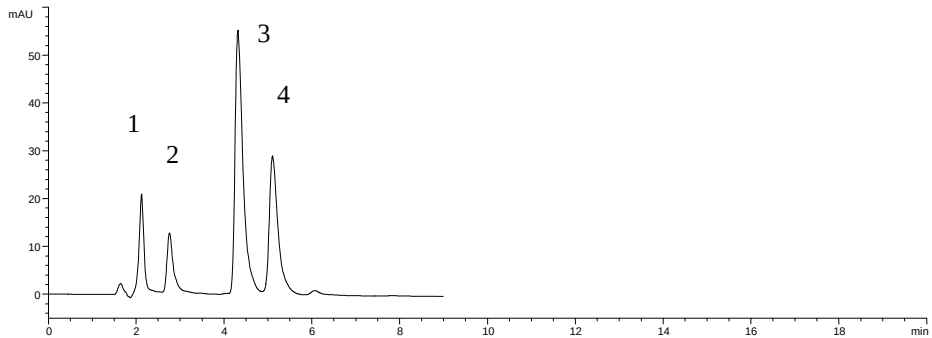
pH Effect

Allegra ingredients + Terfenadine:

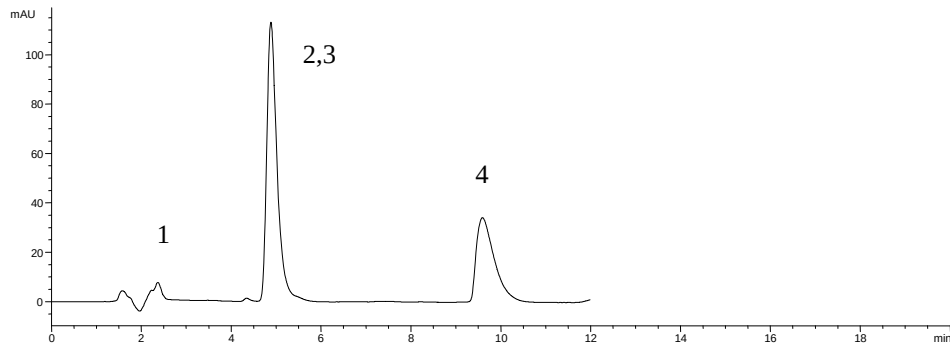
1. MeOH
2. Pseudoephedrine
3. Fexofenadine
4. Terfenadine

Mobile Phase: 35/65, ACN/10mM NH_4OAc
Column: CHIROBIOTIC V, 150x3.0mm, 3.5µ
Flow Rate: 0.5 mL/min.

pH 3.8



pH 5.0



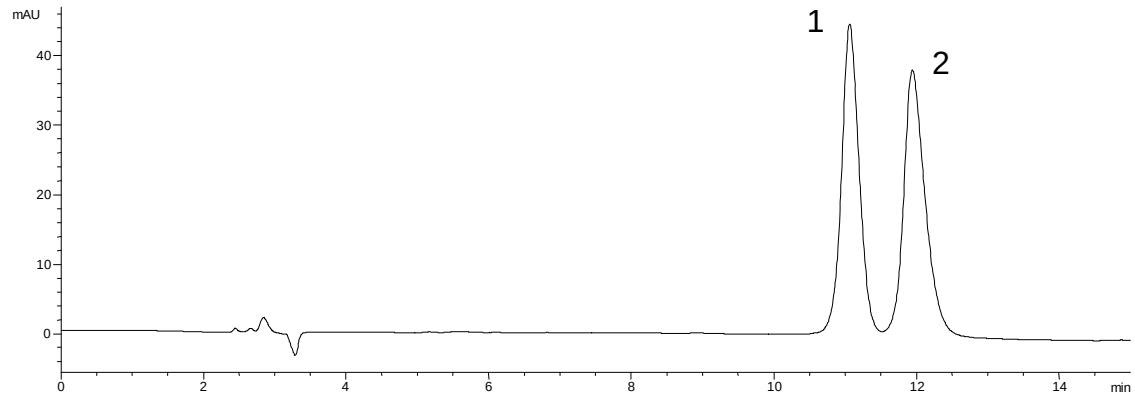
Reversed Phase Mode vs Polar Ionic Mode

Sleeping Aid:

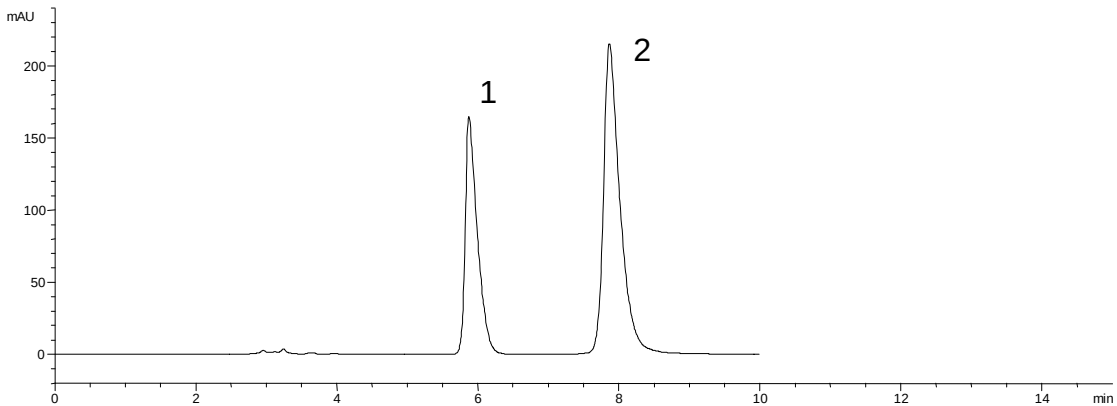
1. Diphenhydramine
2. Doxylamine

Column: CHIROBIOTIC V, 250x4.6mm, 5µ
Flow Rate: 1.0 mL/min.

Mobile Phase:
35/65, ACN/10mM NH_4OAc, pH 5.0



Mobile Phase:
100/0.1w%, MeOH/NH_4 Formate

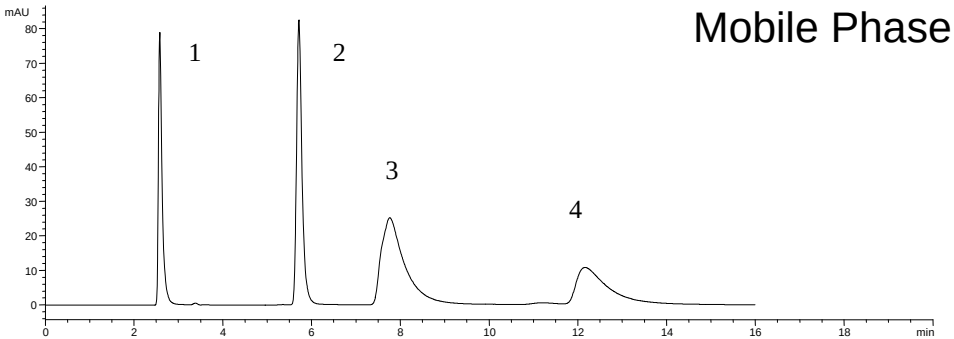


CHIROBIOTIC T vs CHIROBIOTIC V

Analgestics:

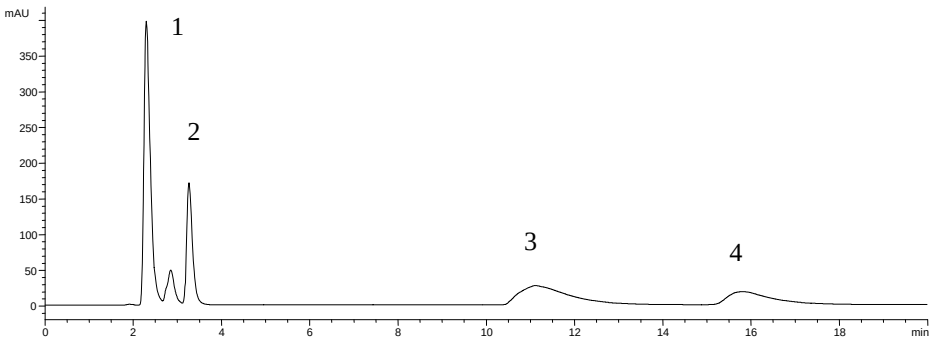
1. Aspirin
2. Acetaminophen
3. Fenoprofen
4. (-) Ketoprofen

Mobile Phase: 10/90, MeOH/10mM NH_4OAc, pH 5.0



CHIROBIOTIC T, 150x4.6mm, 3.5µ

Flow Rate: 0.6 mL/min.

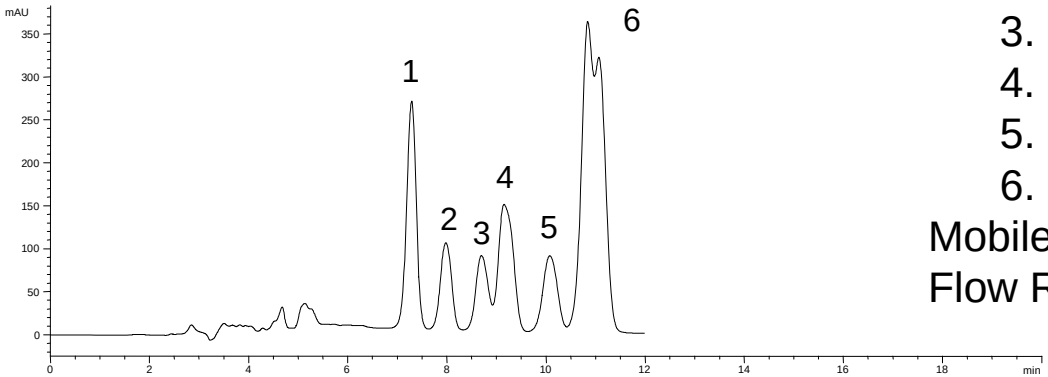


CHIROBIOTIC V, 150x3.0mm, 3.5µ

Flow Rate: 0.5 mL/min.

CHIROBIOTIC V vs CHIROBIOTIC V2

CHIROBIOTIC V, 250x4.6mm, 5µ



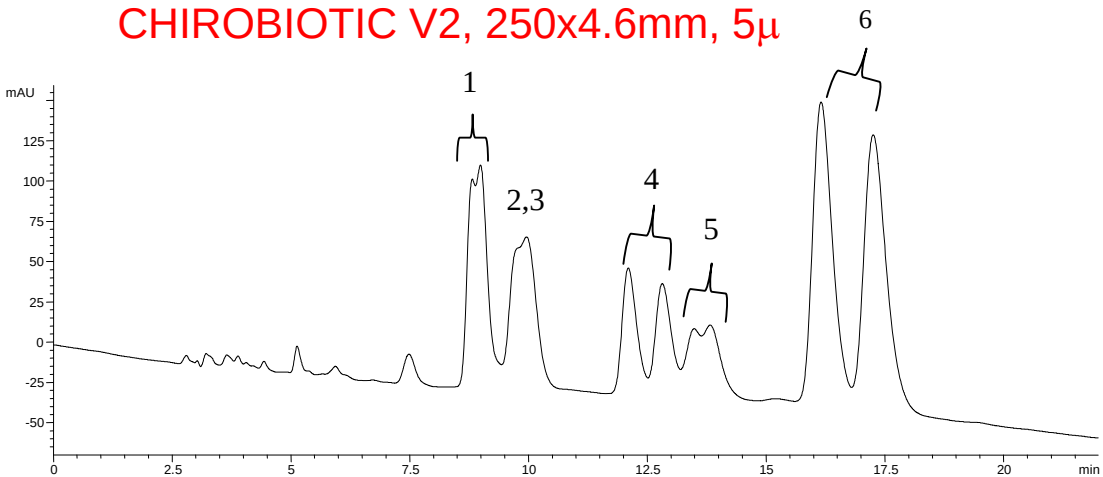
Beta Blockers (racemic):

1. Albuterol
2. Sotalol
3. Metoprolol
4. Pindolol
5. Clenbuterol
6. Propranolol

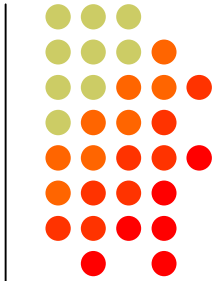
Mobile Phase: 35/65, ACN/0.1% TEAA, pH 4.75

Flow Rate: 1.0mL/min

CHIROBIOTIC V2, 250x4.6mm, 5µ



Methods Development



Reversed Phase Mode:

Starting mobile phase/column:

1. 35/65, ACN/10mM NH_4OAc, pH 5.0/CHIROBIOTIC V
2. 10/90, MeOH/10mM NH_4OAc, pH 5.0/CHIROBIOTIC T

Optimization:

1. Change pH between 3.8 to 6.8
2. Change organic composition
3. Complementary effect between CHRIOBIOTIC V/V2 and T

Polar Ionic Mode:

1. 100/0.1w%, MeOH/NH_4 Formate for CHIROBIOTIC V/V2 and T

Optimization:

1. 100/0.1w%, MeOH/NH_4TFA and/or 100/0.1w%, MeOH/NH_4OAc should be tried
2. Lower the flow rate if the retention is too short

Conclusions

- Macrocyclic Glycopeptides chiral stationary phases (CSPs) are suitable for nonchiral applications, especially for extremely polar molecules.
- These phases have unique selectivity towards ionizable molecules.
- The ionic characteristics of these CSPs together with subtle differences of the structures, can be utilized to resolve structure-related compound classes.
- The mobile phase designs are simple and the optimization steps are straightforward.
- The final methods usually are robust and LC/MS-friendly.