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Advanced Separation Technologies Inc.

Novel Applications of Macrocyclic Glycopeptide Chiral Stationary Phases for Achiral Separations

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Abstract

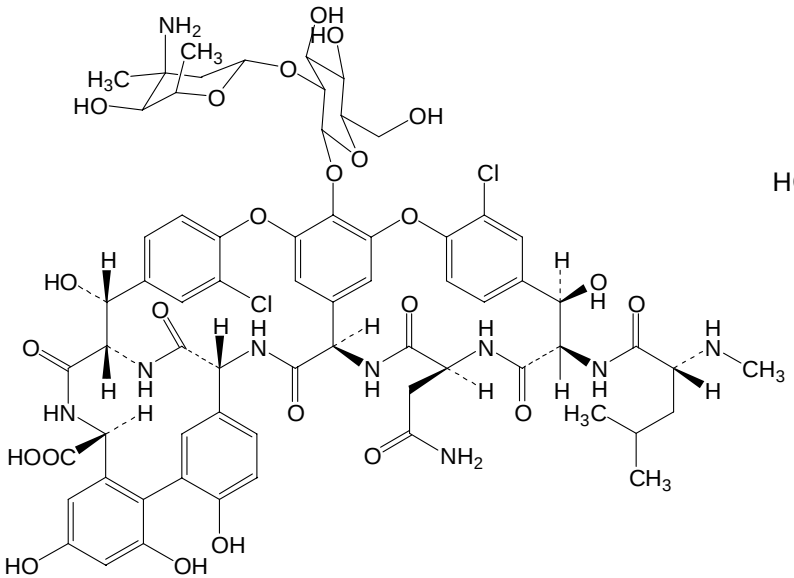
Chiral stationary phases (CSPs) prepared by bonding the macrocyclic glycopeptides vancomycin, teicoplanin, teicoplanin aglycone and ristocetin A, have demonstrated broad selectivity in LC chiral separations since their introduction by Dr. D.W. Armstrong in 1994. Their complex structures allow them to interact with chiral molecules through many different kinds of forces including ionic (electrostatic) interaction, π-π interaction, hydrogen bonding, inclusion complexation, hydrophobic interaction as well as steric (repulsive) hindrance.

These potential interactions make macrocyclic glycopeptide CSPs perfect for the separation of a broad range of analyte structures, both chiral and achiral. In today's drug discovery process, a large percentage of the compounds generated that require analysis are highly polar. They have been a great challenge for conventional reversed phase stationary phases. Aided with unique ionic interaction, macrocyclic glycopeptide CSPs have demonstrated great resolution power towards both polar basic and polar acidic compounds. This presentation will demonstrate a number of successful achiral assays that outperform the traditional achiral stationary phases such as C18, C8 and the so called embedded phases.

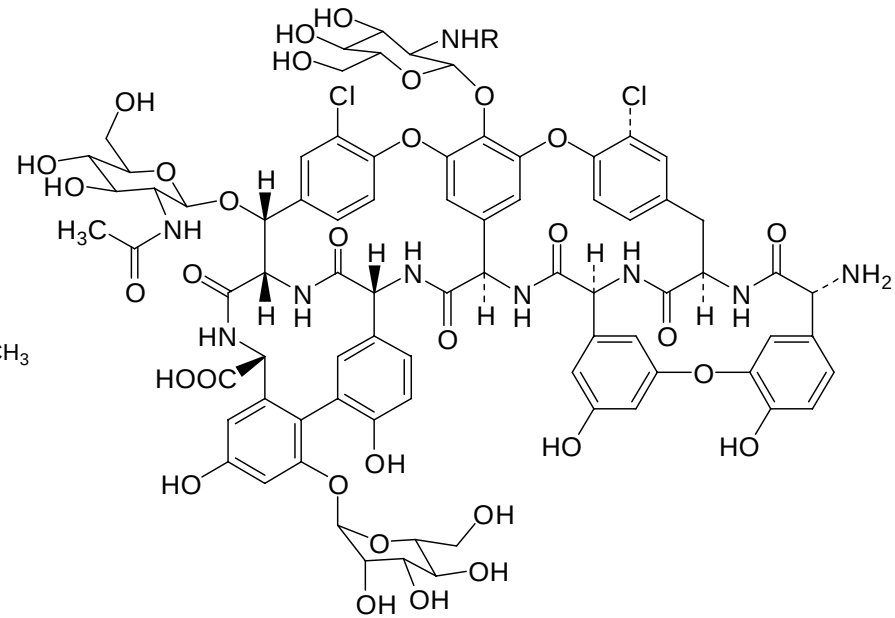
This study will include separations in both the reversed phase mode and a very useful polar ionic mode© for both preparative and LC/MS applications. The advantages of using macrocyclic glycopeptide CSPs in terms of mobile phase design, asymmetry values and selectivity will be presented.

Proposed Structures of Macrocyclic Glycopeptide Chiral Stationary Phases

Vancomycin



Teicoplanin



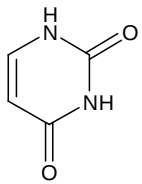
Why Using CHIROBIOTIC Columns for Nonchiral Separations?

- Great retention and selectivity for highly polar compounds
- Symmetric peak shape for basic compounds (generally skew<1.1)
- No extreme pH needed for basic compounds
- LC/MS compatible mobile phase (no phosphate buffer needed)
- Multiple separation modes, i.e. reversed phase mode and polar ionic mode
- Great stability under all separation conditions

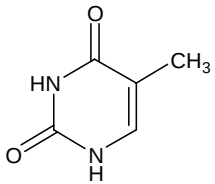
Structures of Compounds Studied

5 Nucleic Acid Bases

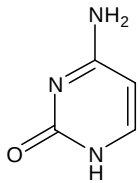
Uracil



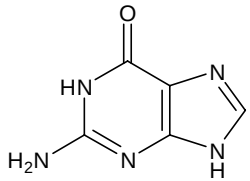
Thymine



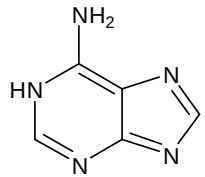
Cytosine



Guanine

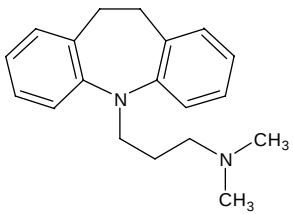


Adenine

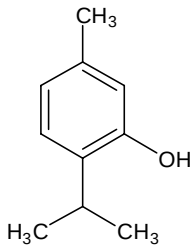


Acidic, Basic and Neutral Drug Mixture

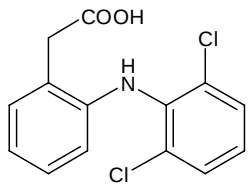
Imipramine



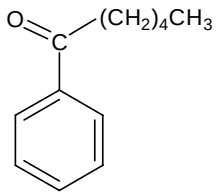
Thymol



Diclofenac



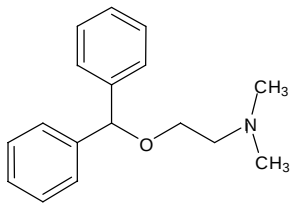
Hexanophenone



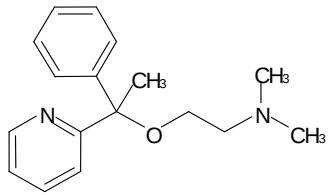
Structures of Compounds Studied

Sleeping aids:

Diphenhydramine

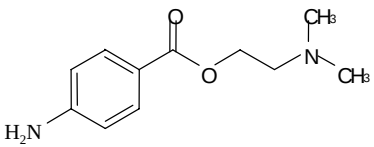


Doxylamine

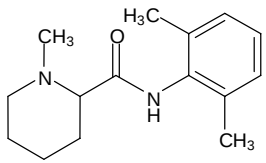


Anesthetics:

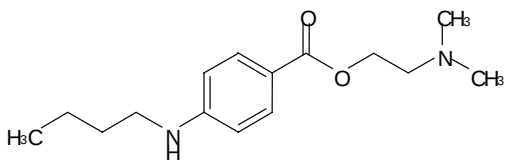
Procaine



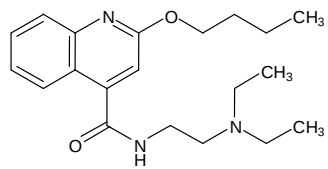
Mepivacaine



Tetracaine



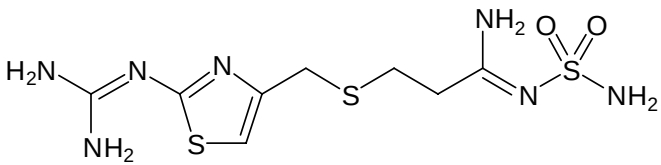
Dibucaine



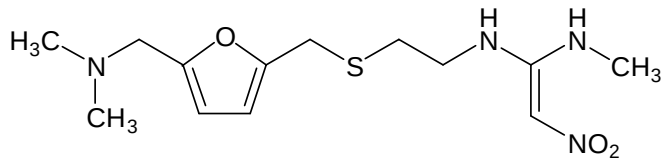
Structures of Compounds Studied

Anti-ulcerative drugs

Famotidine

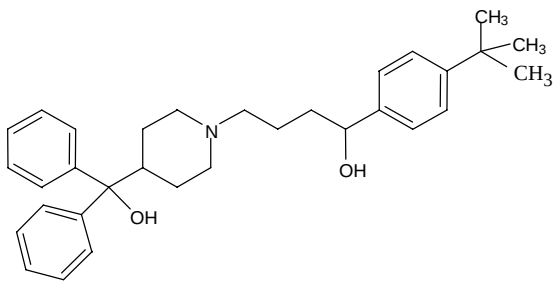


Ranitidine

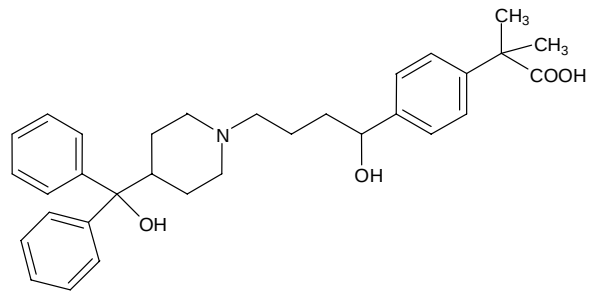


Terfenadine and its metabolite (fexofenadine):

Fexofenadine



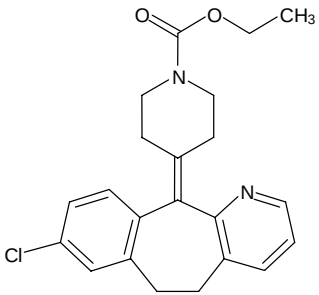
Terfenadine



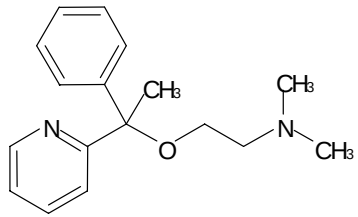
Structures of Compounds Studied

Antihistamine:

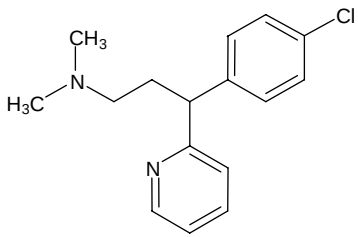
Loratadine



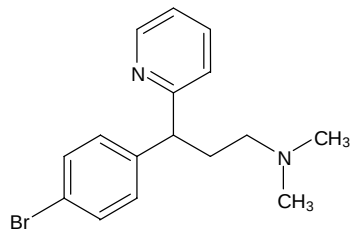
Doxylamine



Chlorpheniramine



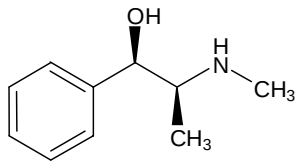
Brompheniramine



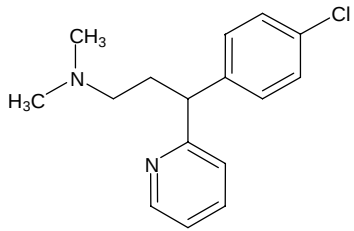
Structures of Compounds Studied

Sinus Medications:

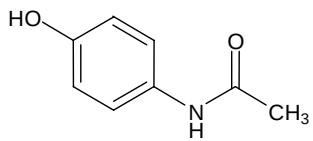
Pseudoephedrine



Chlorpheniramine

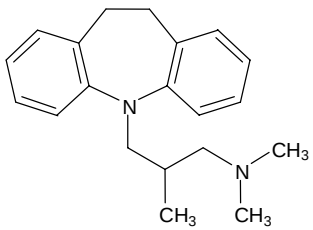


Acetaminophen

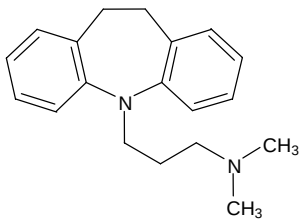


Tricyclic Antidepressants:

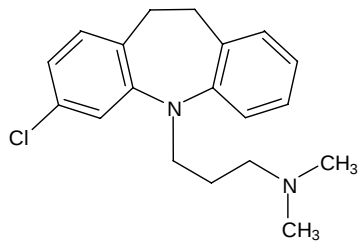
Trimipramine



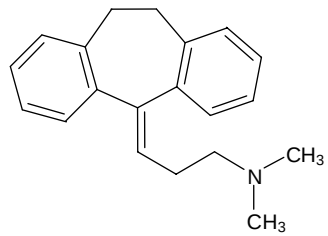
Imipramine



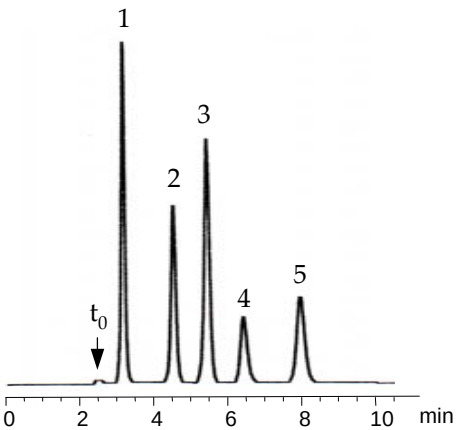
Clomipramine



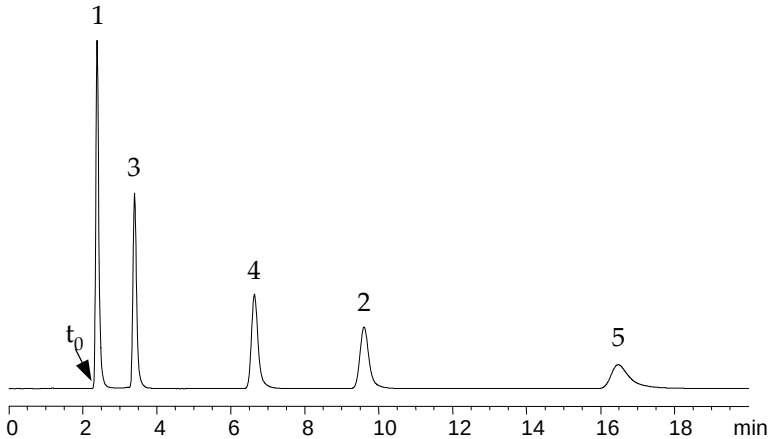
Amitriptyline



CHIROBIOTIC V vs. Conventional C18



Peak List:
1. Uracil
2. Thymine
3. Cytosine
4. Guanine
5. Adenine



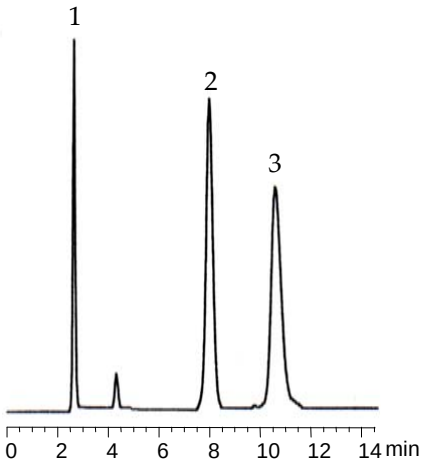
Column: CHIROBIOTIC V
Astec Catalog #: 11023
Dimensions: 150 x 4.6 mm
Particle size: 5 µm
Pore size: 100 Å

Conditions:
Mobile phase: 2.5 mM NH_4OAc (pH 4.1)
Flow: 1.0 ml/min
Temp: ambient
Det: UV@254 nm

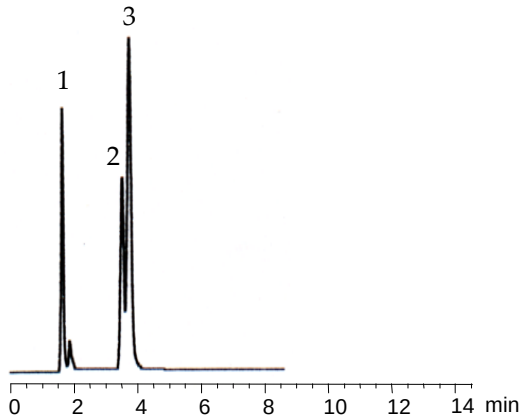
Column: C18
Astec Catalog #: 55023
Dimensions: 150 x 4.6 mm
Particle size: 5 µm
Pore size: 100 Å

Conditions:
Mobile phase: 2.5 mM NH_4OAc (pH 5.7)
Flow: 0.6 ml/min
Temp: ambient
Det: UV@254 nm

CHIROBIOTIC V vs. Conventional C18



Peak List:
1. Uracil
2. Famotidine
3. Ranitidine



Column: CHIROBIOTIC V
Astec Catalog #: 11023
Dimensions: 150 x 4.6 mm
Particle size: 5 µm
Pore size: 100 Å

Conditions:
Mobile phase:
20/80 MeOH/20 mM NH_4OAc (pH 4.1)
Flow: 1.0 ml/min
Temp: ambient
Det: UV@254 nm

Column: C18
Astec Catalog #: 55023
Dimensions: 150 x 4.6 mm
Particle size: 5 µm
Pore size: 100 Å

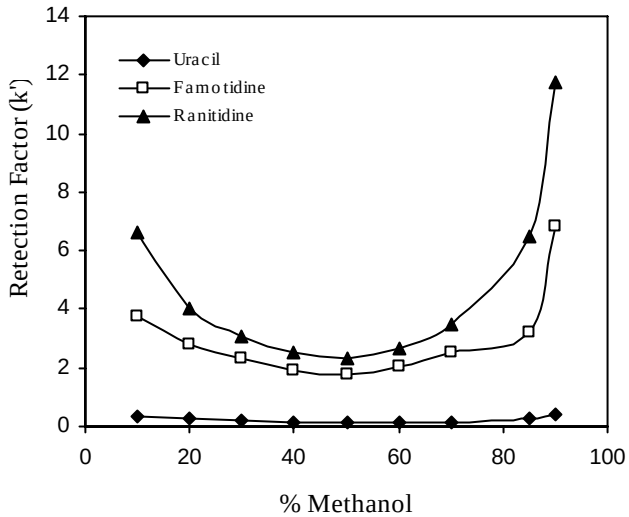
Conditions:
Mobile phase:
20/80 ACN/0.5% (w/v) NH_4OAc (pH7.2)
Flow: 1.0 ml/min
Temp: ambient
Det: UV@254 nm

Retention Behavior of Basic Drugs

Antiulceratives

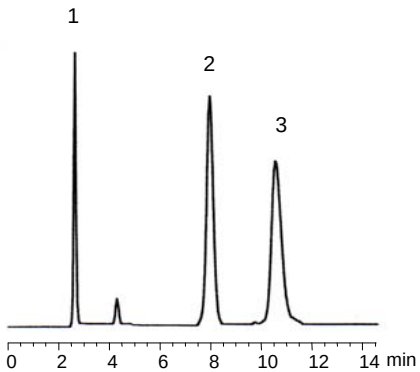
1. Uracil
2. Famotidine
3. Ranitidine

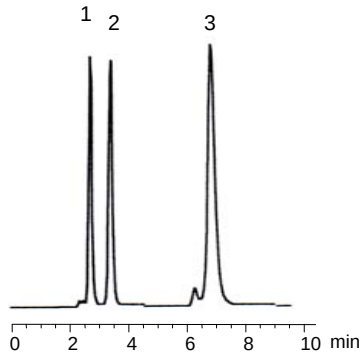
Column: CHIROBIOTIC V
Astec Catalog #: 11023
Dimensions: 150 x 4.6 mm
Particle size: 5 µm
Pore size: 100 Å



Reversed Phase Mode

Mobile phase:
20/80 MeOH/ 20 mM NH_4OAc (pH 4.1)
Flow: 1.0 ml/min
Temp: ambient
Det: UV@254 nm





Polar Ionic Mode

Mobile phase:
100% MeOH with 20 mM NH_4OAc
Flow: 1.0 ml/min
Temp: ambient
Det: UV@254 nm

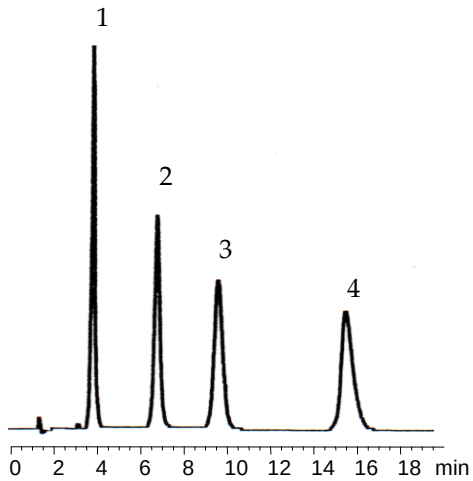
pH Effect

Peak List:

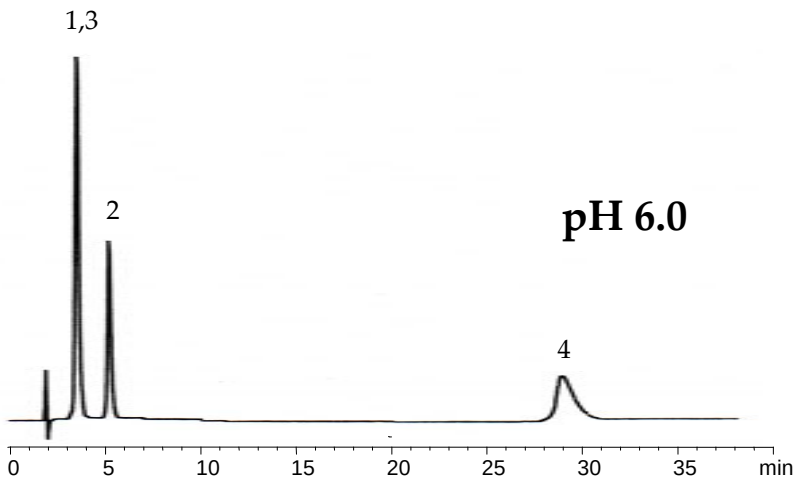
1. Thymol (Hydrophilic)
2. Hexanophenone (Hydrophobic)
3. Diclofenac (Acidic)
4. Imipramine (Baisc)

Column: CHIROBIOTIC V
Astec Catalog #: 11023
Dimensions: 150 x 4.6 mm
Particle size: 5 mm
Pore size: 100 Å

Conditions:
Mobile phase: ACN/20 mM NH_4OAc
Flow: 1.0 ml/min
Temp: ambient
Det: UV@254 nm



pH 4.1



pH 6.0

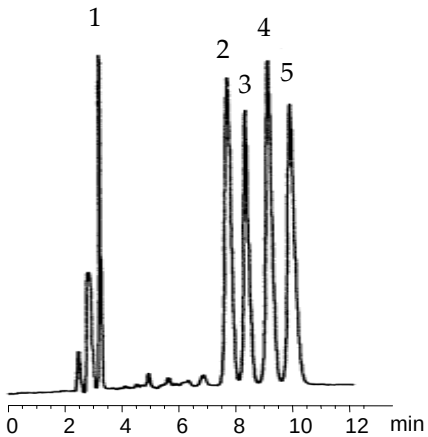
pH Effect

Tricyclic Antidepressants:

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

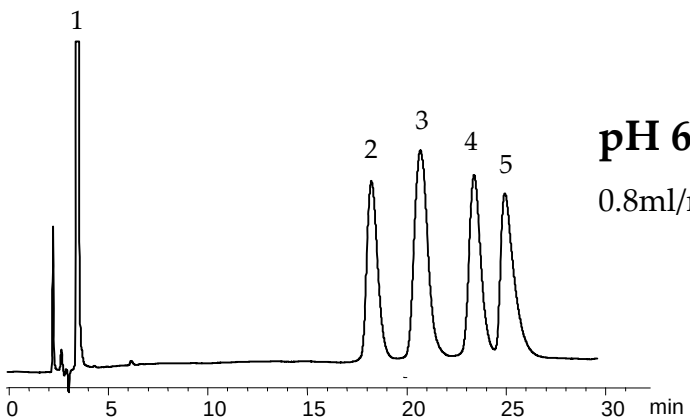
Column: CHIROBIOTIC V
Astec Catalog #: 11024
Dimensions: 250 x 4.6 mm
Particle size: 5 µm
Pore size: 100 Å

Conditions:
Mobile phase:
35/65 ACN/20 mM NH_4OAc,
Temp: ambient
Det: UV@254 nm



pH 4.0

1.0 ml/min



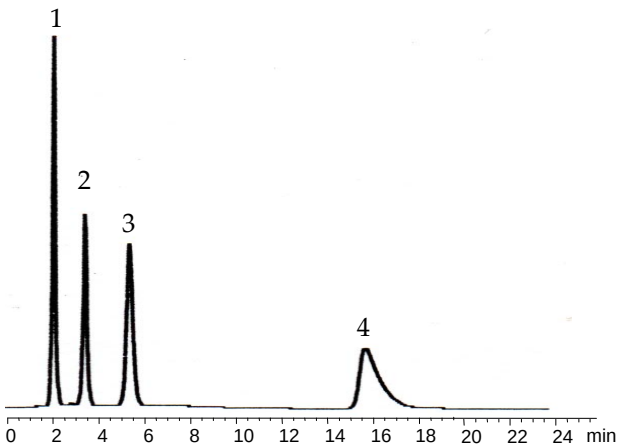
pH 6.0

0.8ml/min

MeOH vs ACN as Organic Additive

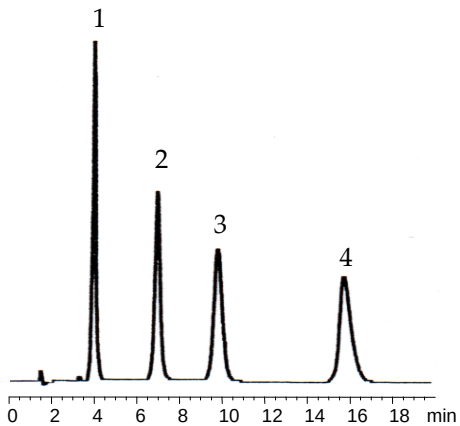
Peak List:
1. Thymol
2. Hexanophenone
3. Diclofenac
4. Imipramine

Conditions:
Mobile phase:
40/60 MeOH/0.1%TEAA, pH4.1
Flow: 1.0ml/min
Temp: ambient
Det: UV@254 nm



Column: CHIROBIOTIC V
Astec Catalog #: 11023
Dimensions: 150 x 4.6 mm
Particle size: 5 µm
Pore size: 100 Å

Conditions:
Mobile phase:
20/80 ACN/20 mM NH_4OAc, pH4.1
Flow: 1.0ml/min
Temp: ambient
Det: UV@254 nm



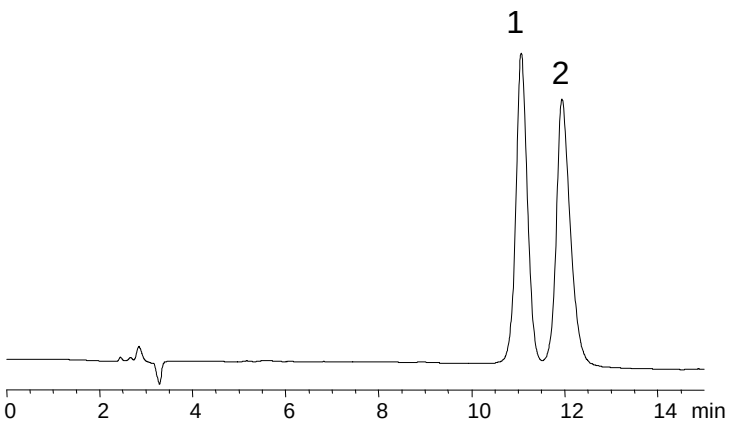
Reversed Phase Mode vs Polar Ionic Mode

Sleeping Aid:

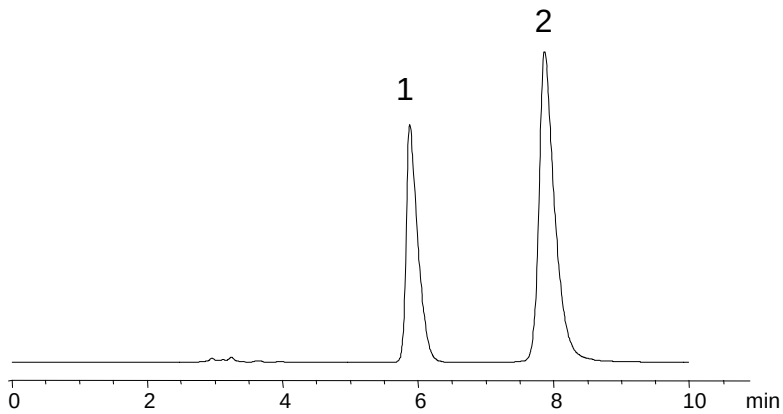
1. Diphenhydramine
2. Doxylamine

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

Reserved Phase Mode:
35/65, ACN/10mM NH4OAc, pH 5.0



Polar Ionic Mode::
100/0.1w%, MeOH/NH4 Formate



Method Development

Reversed Phase Mode:

Starting mobile phase/column:

1. 35/65, ACN/10mM NH_4OAc, pH 5.0/CHIROBIOTIC V
2. 20/80, 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 and T

Polar Ionic Mode:

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

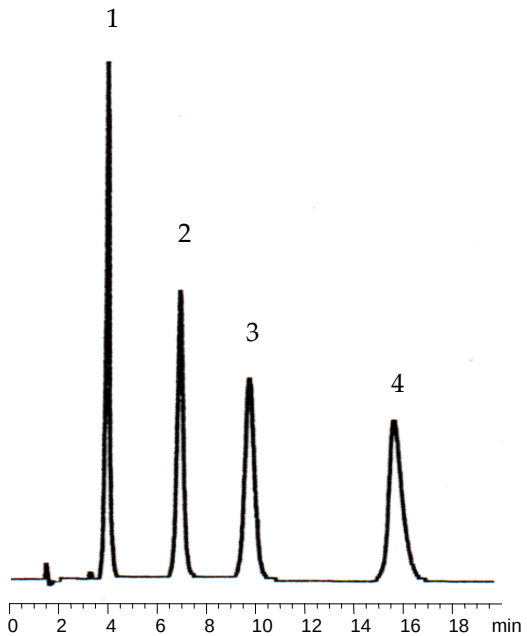
Simultaneous Analysis of Acidic, Basic and Neutral Drugs

Peak List:

1. Thymol (Hydrophilic)
2. Hexanophenone (Hydrophobic)
3. Diclofenac (Acidic)
4. Imipramine (Baisc)

Column: CHIROBIOTIC V
Astec Catalog #: 11023
Dimensions: 150 x 4.6 mm
Particle size: 5 µm
Pore size: 100 Å

Conditions:
Mobile phase: 20/80 ACN/20 mM NH_4OAc (pH 4.1)
Flow: 1.0 ml/min
Temp: ambient
Det: UV@254 nm



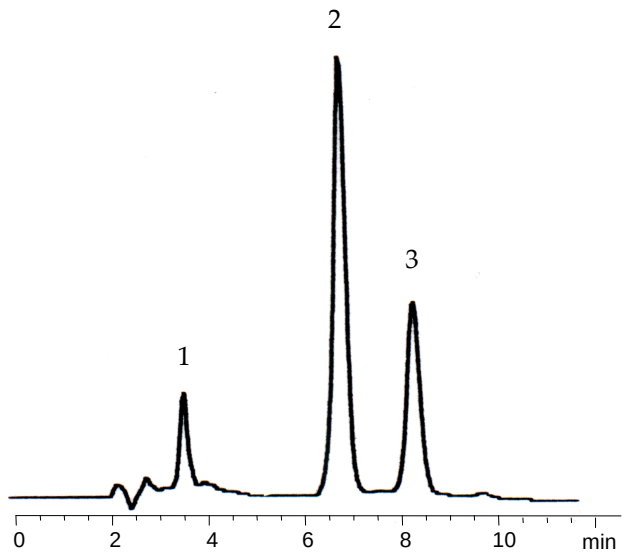
Application

Allegra Ingredients + Terfenadine:

1. Pseudoephedrine
2. Fexofenadine (basic)
3. Terfenadine (acidic)

Column: CHIROBIOTIC V
Dimensions: 150 x 4.6 mm
Particle size: 5 µm
Pore size: 100 Å

Conditions:
Mobile phase:
35/65 ACN/20 mM NH_4OAc, pH 4.1
Flow: 0.8 ml/min
Temp: ambient
Det: UV@254 nm



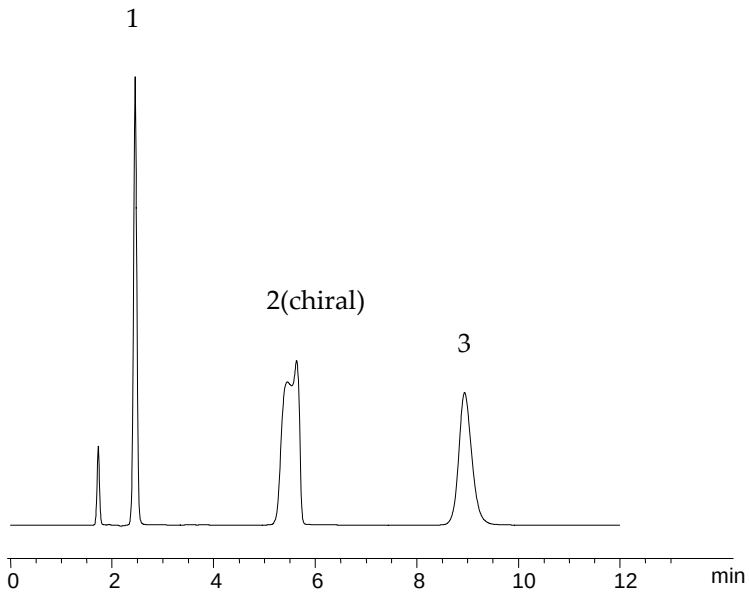
Application

Sinus Medication:

1. Acetaminophen
2. Pseudoephedrine
3. Chlorpheniramine

Column: CHIROBIOTIC V
Dimensions: 150 x 3.0 mm
Particle size: 3.5 µm
Pore size: 100 Å

Conditions:
Mobile phase:
35/65 ACN/10 mM NH_4OAc, pH 5.0
Flow: 0.5 ml/min
Temp: ambient
Det: UV@254 nm



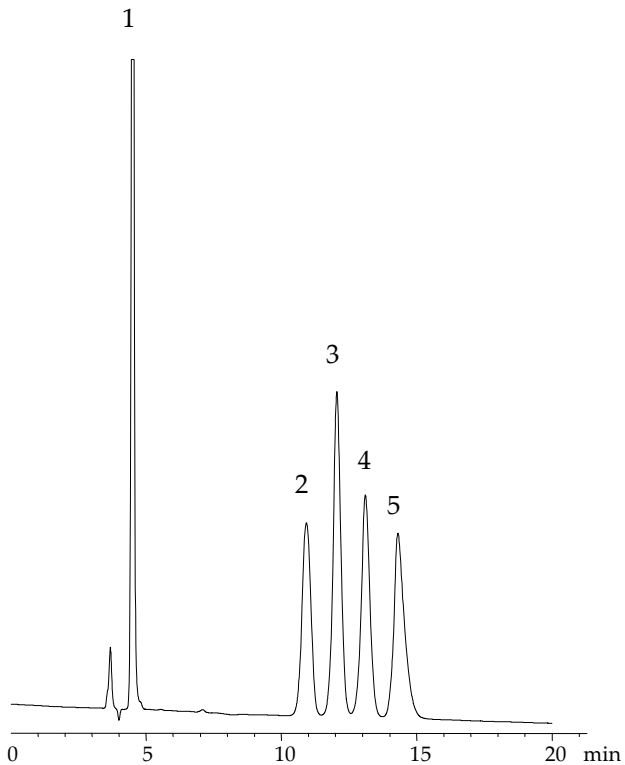
Application

Tricyclic Antidepressants:

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

Column: CHIROBIOTIC V
Astec Catalog #: 11024
Dimensions: 250 x 4.6 mm
Particle size: 5 µm
Pore size: 100 Å

Conditions:
Mobile phase:
35/65 ACN/10 mM NH_4OAc, pH5.0
Flow: 0.8 ml/min
Temp: ambient
Det: UV@254 nm



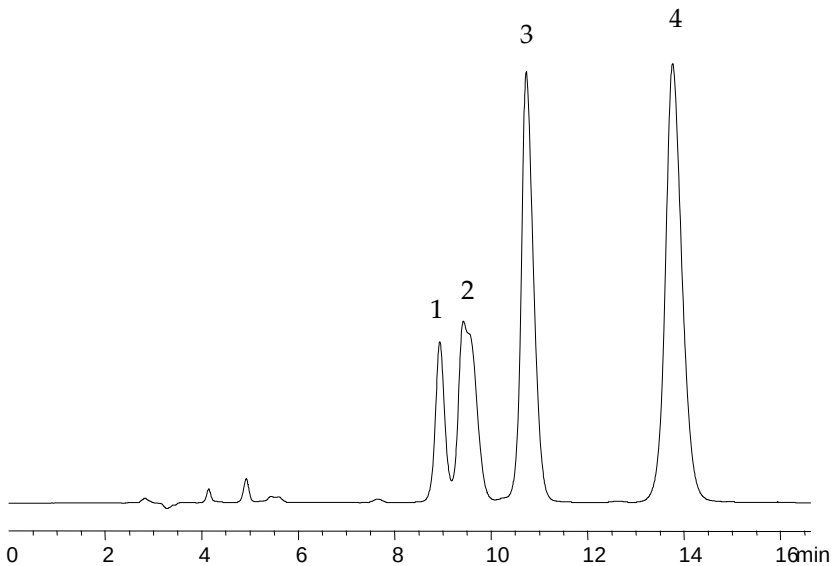
Application

Anesthetics:

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

Column: CHIROBIOTIC V
Astec Catalog #: 11024
Dimensions: 250 x 4.6 mm
Particle size: 5 µm
Pore size: 100 Å

Conditions:
Mobile phase:
35/65 ACN/10 mM NH_4OAc, pH 5.0
Flow: 0.5 ml/min
Temp: ambient
Det: UV@254 nm



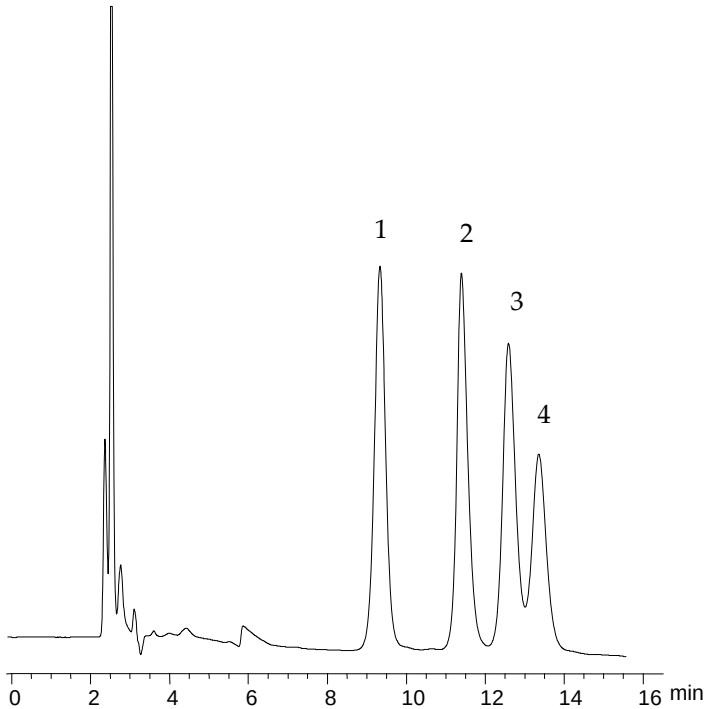
Application

Antihistamines:

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

Column: CHIROBIOTIC V
Astec Catalog #: 11024
Dimensions: 250 x 4.6 mm
Particle size: 5 µm
Pore size: 100 Å

Conditions:
Mobile phase:
35/65 ACN/10 mM NH_4OAc, pH 5.0
Flow: 1.0 ml/min
Temp: ambient
Det: UV@254 nm



Conclusions

- Macrocyclic glycopeptide 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.