Experimental Protocols for Preparative Purifications Using Macrocyclic Glycopeptide Chiral Stationary Phases

Introduction

CHIROBIOTIC phases offer unique opportunities for preparative purifications. Key factors are:

1. No solvent limitations. Halogenated solvents as well as very polar solvents are well tolerated on these CSPs. This solvent tolerance is especially useful when optimizing for sample solubility.

2. Same CSP can be run in four distinctly different mobile phase types. Use of acid/base on any one of these CSPs does not preclude their use in other mobile phases. Mobile phases listed here in the order of success:

    a. Polar ionic mode©:
    MeOH/Acid/Base or
    MeOH/NH_4 salt

    b. Reversed phase mode:
    ACN/Buffer

Please note efficient workup procedures available for the reversed phase mode. Method outlined for analytical C_{18} column. See workup following Example 3: Warfarin

    c. Polar organic mode;
    MeOH, ACN or combination

    d. Normal phase mode:
    Heptane/EtOH

**Note: The chiral recognition mechanism is different in each of these mobile phase types.*

3. Very long term stability with these CSPs due, in part, to the multiple linkages used in anchoring the CSP and, secondly, to the mild conditions typically required.

4. Range of capacities is compound dependent. Significantly overlaps cellulose and amylase phases based on throughput primarily because separations on these CSPs are usually very fast. Capacities on CHIROBIOTIC V2/T2 phases have been 2.5mg/gm with an alpha of 1.5. Maximum capacity achieved was 300mg ″on column″ using a 250x21.2mm column with an alpha of 2.0.

5. Excellent economics especially with the polar organic and polar ionic modes. Ionic interactions play a significant role in the chiral recognition mechanism on these phases. Solvents here are anhydrous and more volatile and less toxic than the typical normal phase mode.

Optimization Studies

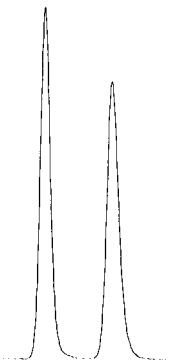
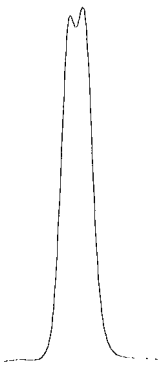
1. Prime consideration in dealing with preparative applications is the balance between selectivity and sample solubility. See Example 3.

2. Check the CHIROBIOTIC V2 and T2 if the polar organic or polar ionic modes have been chosen. These two CSPs may offer increased resolution and increased capacity in these modes only. For neutral molecules, the polar organic mode will work best eliminating the need for acid/base or volatile salt.

3. When operating in the polar ionic mode check carefully the choice of volatile salt as it may effect selectivity dramatically. Ammonium trifluoroacetate usually favors bases while ammonium acetate favors acids.

CASE STUDY: Nicardipine

Optimization for Prep
Column: CHIROBIOTIC V
FLOW RATE: 1.0 mL/min.

100/0.1w%, MeOH/NH_4TFA	100/0.1w%, MeOH/NH_4OAc
 Peak 1 – 4.07 Peak 2 – 4.69	 Peak 1 – 3.41 Peak 2 – 3.47
α = 1.50	α = 1.09

Issues on Mass Overload

1. Study injection volume and concentration effects:
   Compromise between injection volume and analyte concentration is typically required. For neutral molecules a smaller volume can be used but for separations in the Polar Ionic Mode samples have to be more dilute and larger volumes injected.

2. Salts of chiral drugs:
   a. Increased buffer strength. When a large amount of sample is injected onto the column consideration has to be given to the quick disassociation of the sample. Usually increasing the buffer strength by a factor of 2 will accomplish this task.
   b. Solubility. If the salt reduces the solubility of the sample, water may be added for all modes but especially in the polar ionic mode. See example 6.

3. Overload studies:
   Minimize detector overload by using a less sensitive wavelength. It is necessary to evaluate column overload not detector overload. The analytical column is injected with increasing amounts of sample until resolution decreases to about 85%. This number can then be used for direct scale up. From a 250x4.6mm column to a 21.2mm id is a factor of 20x. From the analytical to a 50mm id column is a factor of 120x.

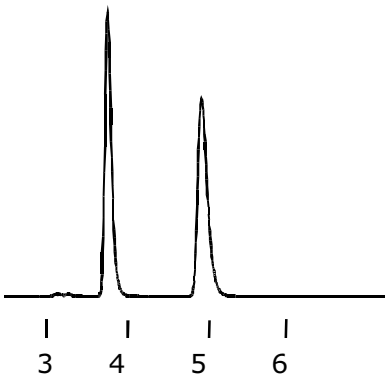
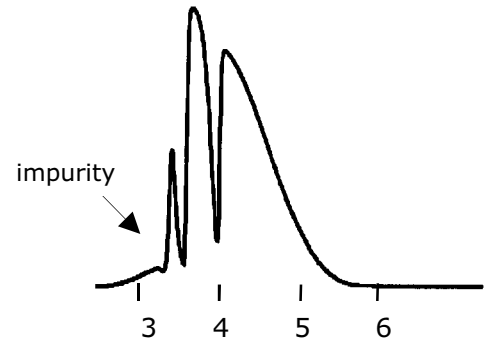
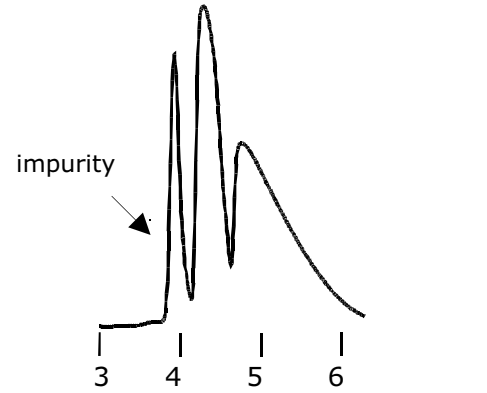
4. Non-linear adsorption isotherms are observed when overloading, i.e., retention decreases with increasing load and peak splitting can result at some critical point.

5. Connecting tubing. To minimize band spread keep tubing connections to 0.01" (0.25mm) or 0.02"(0.5mm) especially if recycle and shave methodologies are used.

The following examples were chosen as simple demonstrations of some of the points covered above. They do not represent the best or the worst examples of what is currently a broad base of experience. Please read CHIROBIOTIC V2/T2 literature for more detailed information about these phases.

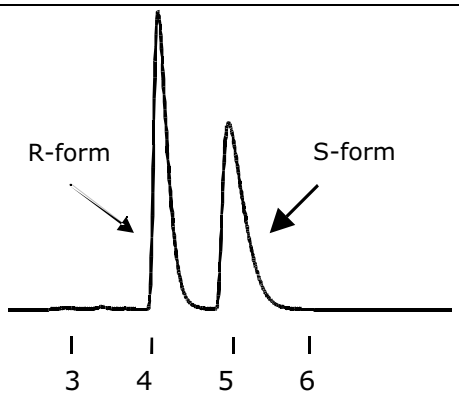
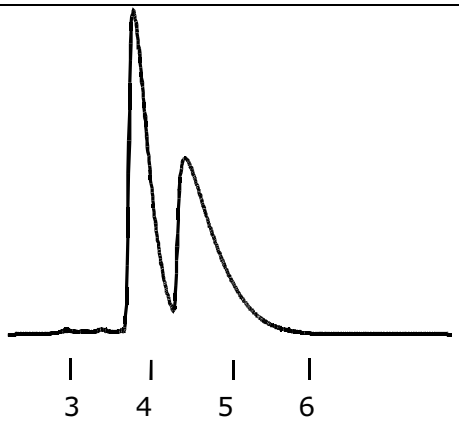
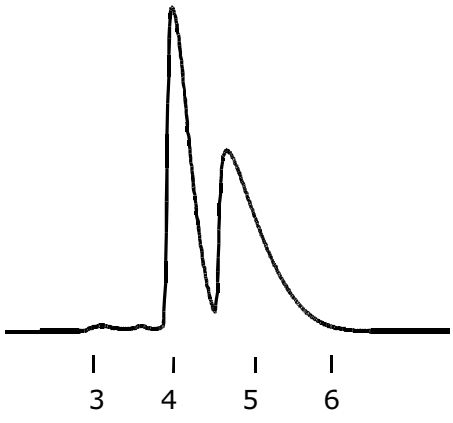
Example 1: 5-Methyl-5-phenyl hydantoin

Solubility: >80 mg/mL in MeOH
Column: CHIROBIOTIC T
Mobile Phase: 100% MeOH
Selectivity: 2.1

Analytical: 250x4.6mm, 5µM Load: 2 mg/mL x 5µL (10 µg) Flow Rate: 1 mL/min UV: 220nm Peak 1: 3.83 min Peak 2: 5.05 min	
Analytical: 250x4.6mm, 5µM Load: 80mg/mL x 0.1mL (8 mg) Flow Rate: 1mL/min UV: 270 nm	
Prep: 250x21.2mm, 5µM Load: 80mg/mL x 1.5mL (120 mg) Flow Rate: 18 mL/min UV: 270 nm Throughput: 20 mg/g CSP/hr	

Example 2: 4-Phenyl-2 oxazolidinone

Solubility: >80mg/mL in MeOH
Column: CHIROBIOTIC T2
Mobile Phase: 100% MeOH
Selectivity: 1.6

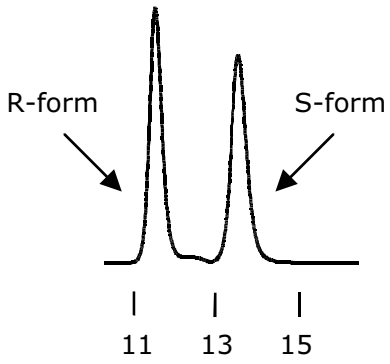
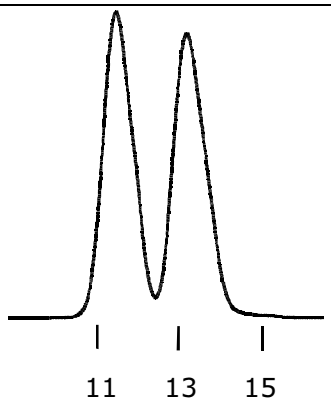
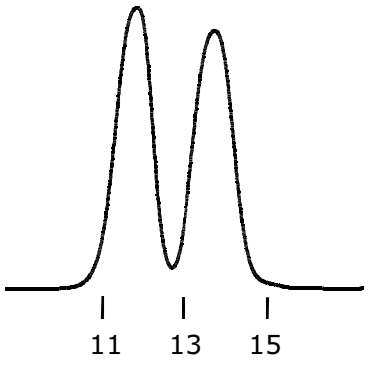
Analytical: 250x4.6mm, 5µ M Load: 2mg/mL x 5µL (10 µg) Flow Rate: 1 mL/min UV: 220nm Peak 1: 4.16 min Peak 2: 4.95 min	
Analytical: 250x4.6mm, 5µM Load: 80mg/mL x 0.02mL (1.6 mg) Flow Rate: 1 mL/min UV: 254 nm	
Prep: 250x21.2mm, 5µM Load: 80mg/mL x 0.5mL (40 mg) Flow Rate: 18 mL/min UV: 254 nm Throughput: 6 mg/g CSP/hr	

Example 3: Warfarin

Column: CHIROBIOTIC V

Solubility vs. Selectivity

EtOH/0.1% TEAA, pH 4.1	k_1	Solubility mg/mL	Selectivity
20/80	4.94	0.1	1.50
30/70	2.19	0.3	1.60
40/60	1.31	0.6	1.47
50/50	0.29	3.0	1.45
80/20	0.1	15.0	1.0

Analytical: 250x 4.6 mm, 5µM Mobile Phase: 40/60, EtOH/0.1% TEAA, pH 4.1 Selectivity: 1.47 Flow Rate: 0.5 mL/min Load: 2mg/mL x 5µL (10 µg) UV: 230 nm Peak 1: 11.33 min Peak 2: 13.64 min	
Analytical: 250 x 4.6 mm, 5µM Mobile Phase: 40/60, EtOH/0.1% TEAA, pH 4.1 Flow Rate: 0.5 mL/min Load: 7.5mg/mL x 0.05mL (0.37 mg) UV: 330 nm	
Prep: 250 x 21.2mm, 5µM Mobile Phase: 40/60, EtOH/0.1% TEAA, pH 4.1 Flow Rate: 10 mL/min Load: 7.5mg/mL x 1 mL (7.5 mg) UV: 330 nm Throughput: 0.5 mg/g CSP/hr	

Recovery From Reversed Phase Systems

The degree of hydrophobicity of the analyte is the main criterion in applying this technique. The following procedure can be used to ascertain the capacity of the C18 column and the size column required for recovery of separated analytes from a full scale prep run.

1. Equilibrate an analytical C18 column with HPLC grade water.

2. Pump the recovered chiral column eluant* containing the enantiomers through the column until the compound breaks through.

3. Wash column with water to remove any buffer present and elute with an appropriate organic solvent. Methanol is often the best choice but ethanol or acetonitrile can be used as well. Measure the volume of collected solvent and assay for recovered analyte.

4. If recovered amount falls below anticipated capacity it is always possible to further dilute the eluant that is being charged with water. In addition, larger size C18 columns can be used.

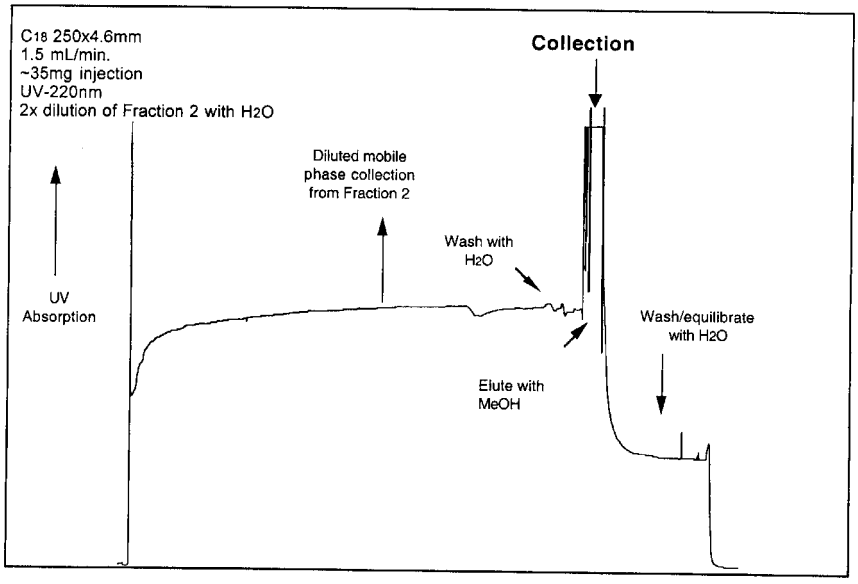
5. After elution of the compound of interest the column is equilibrated with water for the next addition.

6. From analytical runs it is possible to calculate the size of the column required for a larger scale run.

**Note: Adjust pH to increase hydrophobicity and convert all cations and anions to neutral salt. Principle is to suppress the ionization of the analyte.*

Typically doubling the column diameter is equivalent to four times the volume.

Product Recovery Case Study Warfarin



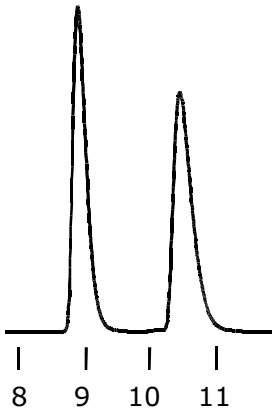
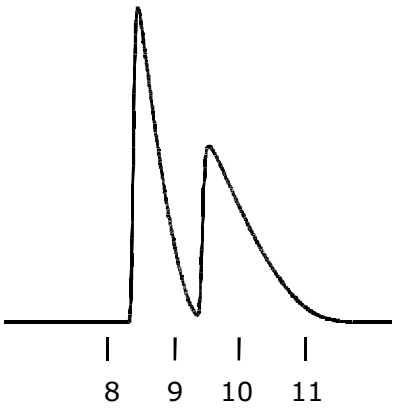
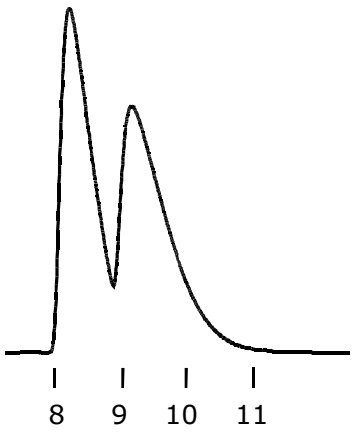
NOTE: C18 recovery method makes for efficient use of the reversed phase mode with CHIROBIOTIC phases. Also note speed of recovery of the separated enantiomers from a C18 is faster than evaporation of an equivalent volume of heptane.

Results:

Final Results	Peak 1	Peak 2
ee	98.5%	99%
Yield	90%	85%

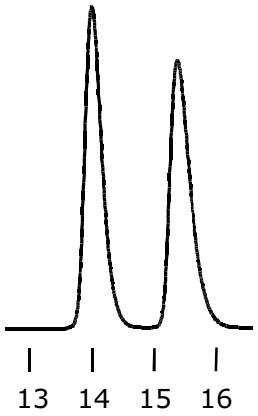
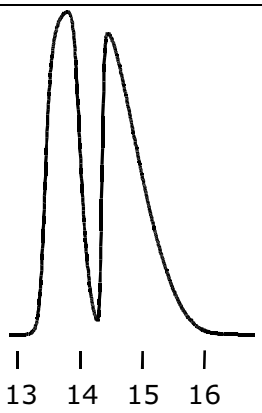
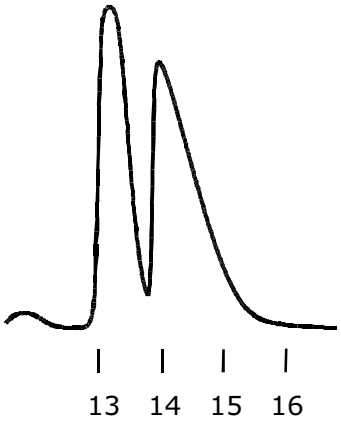
Example 4: Tolperisone

Solubility: >30mg/mL in MeOH
Column: CHIROBIOTIC V2
Mobile Phase: 100/0.1w%, MeOH/NH4TFA
Selectivity: 1.33

Analytical: 250x4.6mm, 5µM Load: 2mg/mL x 5µL (10 µg) Flow Rate: 1 mL/min UV: 230nm Peak 1: 8.80 min Peak 2: 10.69 min	
Analytical: 250x4.6mm, 5µM Load: 30mg/mL x 0.03 mL (0.9 mg) Flow Rate: 1mL/min UV: 265 nm	
Prep: 250x21.2mm, 5µM Flow Rate: 20 mL/min Load: 30mg/mL x 0.7mL (21 mg) UV: 265 nm Throughput: 1.8 mg/g CSP/hr	

Example 5: Propranolol

Solubility: >20mg/mL in MeOH
Column: CHIROBIOTIC T
Mobile Phase: 100/0.1w%, MeOH/NH4TFA
Selectivity: 1.14

Analytical: 250x4.6mm, 5µM Load: 2mg/mL x 5µL (10 µg) Flow Rate: 1 mL/min UV: 230nm Peak 1: 14.03 min Peak 2: 15.62 min	
Analytical: 250x4.6mm, 5µM Load: 15mg/mL x 0.7mL (1 mg) Flow Rate: 1mL/min UV: 270 nm	
Prep: 250x21.2mm, 5µM Flow Rate: 22 mL/min Load: 15 mg/mL x 1.6mL (24 mg) UV: 270 nm Throughput: 1.6 mg/g CSP/hr	

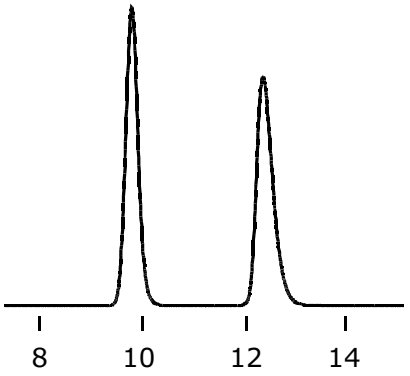
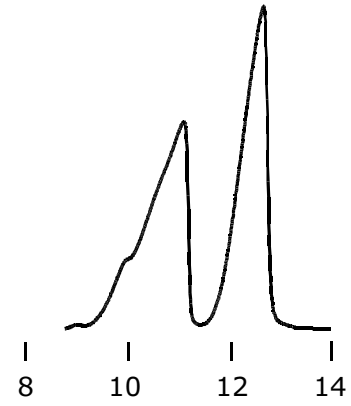
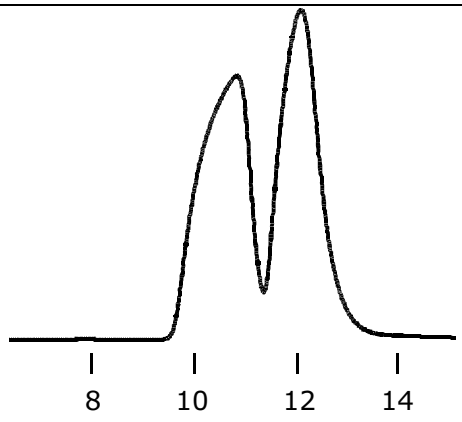
Example 6: Terbutaline (hemisulfate salt)

Column: CHIROBIOTIC T

Solubility vs. Selectivity

100/0.1w%, MeOH/NH4TFA	k_1	Solubility mg/mL	Selectivity
No H_2O*	2.50	2.2	1.37
Add 5% H2O (v/v)	2.76	12	1.29
Add 10% H2O (v/v)	3.50	21	1.24

**Adding water to increase sample solubility can sometimes be effective. This phenomena is sample dependent.*

Analytical: 250x 4.6 mm, 5µM Mobile Phase: 100/0.1w%, MeOH/NH_4TFA Selectivity: 1.37 Flow Rate: 1 mL/min Load: 2mg/mL x 5µL (10 µg) UV: 230 nm Peak 1: 9.81 min Peak 2: 12.26 min	
Analytical: 250 x 4.6 mm, 5µM Mobile Phase: 100/0.1w%, MeOH/NH_4TFA + 5% H_2O Selectivity: 1.29 Flow Rate: 1.0 mL/min Load: 8 mg/mL x 0.1mL (0.8 mg) UV: 254 nm	
Perp: 250 x 21.2mm, 5µM Mobile Phase: 100/0.1w%, MeOH/NH4TFA + 5% H2O Flow Rate: 20 mL/min Load: 7 mg/mL x 3mL (21 mg*) UV: 254 nm Throughput: 1.4 mg/g CSP/hr **Note: higher loading causes peak one to split but does not affect recovery.*	

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