

# Benefits of Preparative Chiral Separations with Macrocyclic Glycopeptide CSPs in Reversed-Phase and Polar Ionic/Polar Organic Modes

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## Abstract

The traditional normal-phase preparatory separations of chiral compounds are known to bring about a medley of obstacles. Due to complications arising from poor sample solubility and the creation of large amounts of toxic organic waste associated with normal-phase methods, reversed-phase and polar-ionic/polar-organic preparatory approaches are currently becoming more appealing to scientists. Several widely used chiral stationary phases (CSPs) are unable to accommodate a highly-polar aqueous reversed-phase environment; however the exception has been the CSPs termed “CHIROBIOTIC™,” macrocyclic glycopeptides bonded to high-purity silica particles. The CHIROBIOTIC phases operate in any solvent system, including very polar and halogenated solvents, allowing the user to optimize the preparative separation based on a balance between enantioselectivity and sample solubility.

## Abstract (contd.)

The macrocyclic glycopeptide CSPs also have desirable preparative attributes of predictable scale-up because the same chemistry is employed on all particle sizes.

The following presentation will demonstrate the preparative performance of macrocyclic glycopeptide CSPs in reversed-phase and polar-ionic/polar-organic mobile phase systems as well as the post-separation methods used to isolate the purified enantiomers.

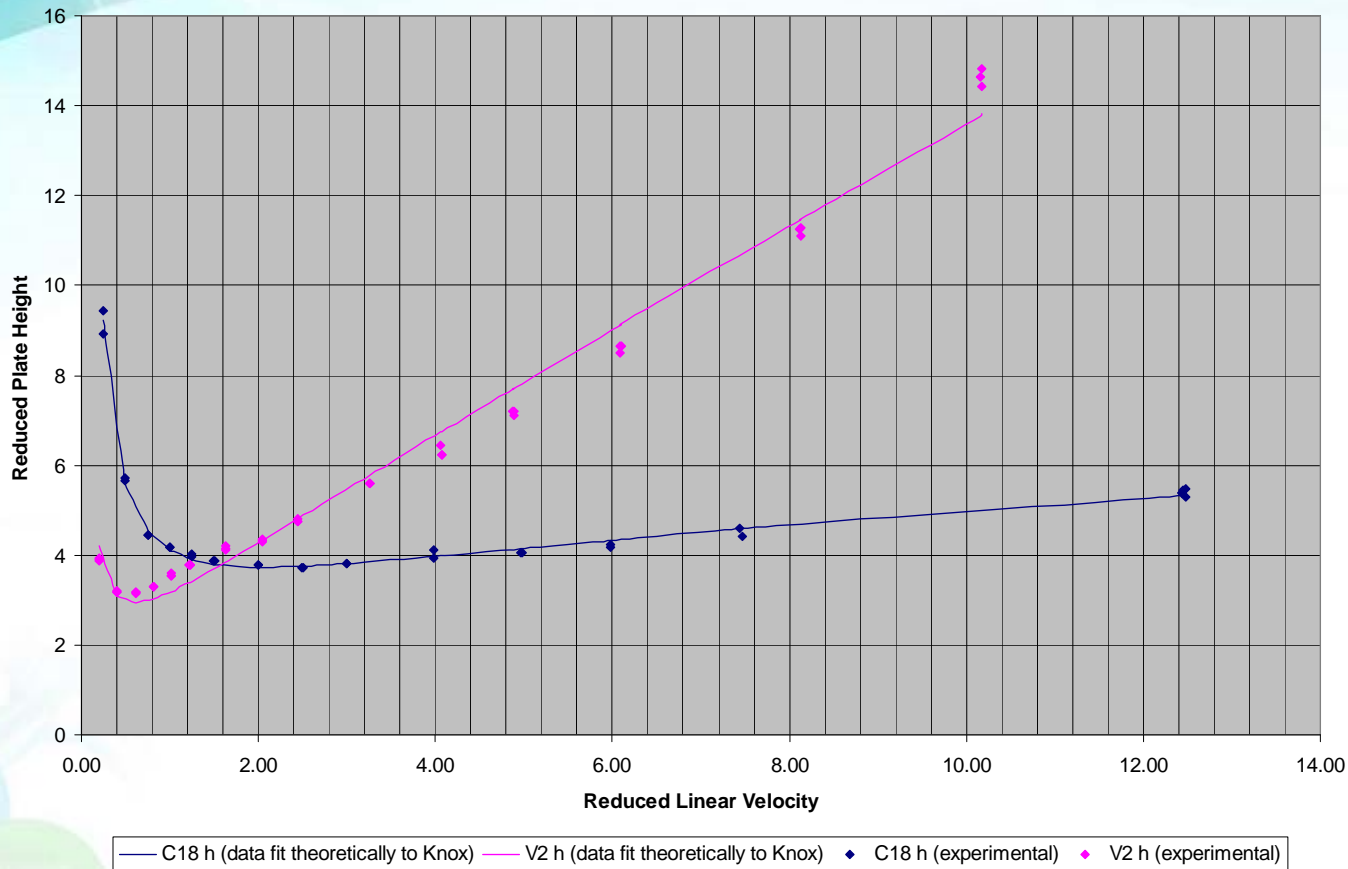
# Introduction

- Benefits of Preparatory Separations in Reversed-Phase
  - Reversed-Phase is **GREENER** than Normal-Phase.
    - Reversed Phase: mobile phase is mostly aqueous.
      - Better for the environment than toxic organic solvents.
    - Normal Phase: 100% organic solvents and usually between 70% and 100% hexane or heptane.
      - Hexane has been known to produce neurotoxic effects<sup>1</sup>.
  - Faster Sample Recovery
    - Aqueous fractions containing analyte can be run through a C18 flash cartridge.
    - Analyte adheres to cartridge and can be washed with water to remove mobile phase additives.
    - Analyte eluted from flash cartridge using minimal amount of MeOH, that can be removed quickly *in vacuo*.

## Introduction (contd.)

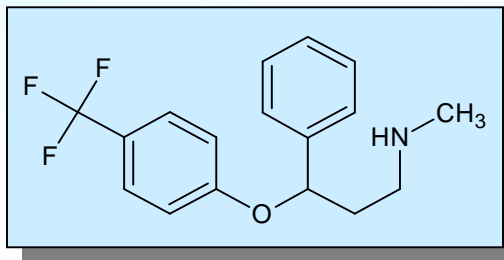
- Benefits of using Low Flow Rates
  - Low Flow is **GREENER**: Less Solvent Wasted.
    - Stacked injections are used, so only time/solvent lost is that between first injection and when very first peaks elute.
  - Greater Efficiency at Low Flow on the CHIROBIOTIC Phases.
    - As seen in Figure 1, the optimal linear velocity on the CHIROBIOTIC V2 is significantly lower than that observed on the Ascentis C18. Therefore, the CHIROBIOTIC columns produce optimal efficiency at flow rates lower than that observed with traditional non-chiral columns.

# Figure 1. The Knox plot of Fluoxetine on the CHIROBIOTIC V2 vs. the Ascentis<sup>®</sup> C18<sup>2</sup>



As seen in Figure 1, the optimal reduced linear velocity on the V2 is 0.61, which translates into a flow rate of 0.15 mL/min. on the analytical column used in this study.

# Fluoxetine (Prozac™)

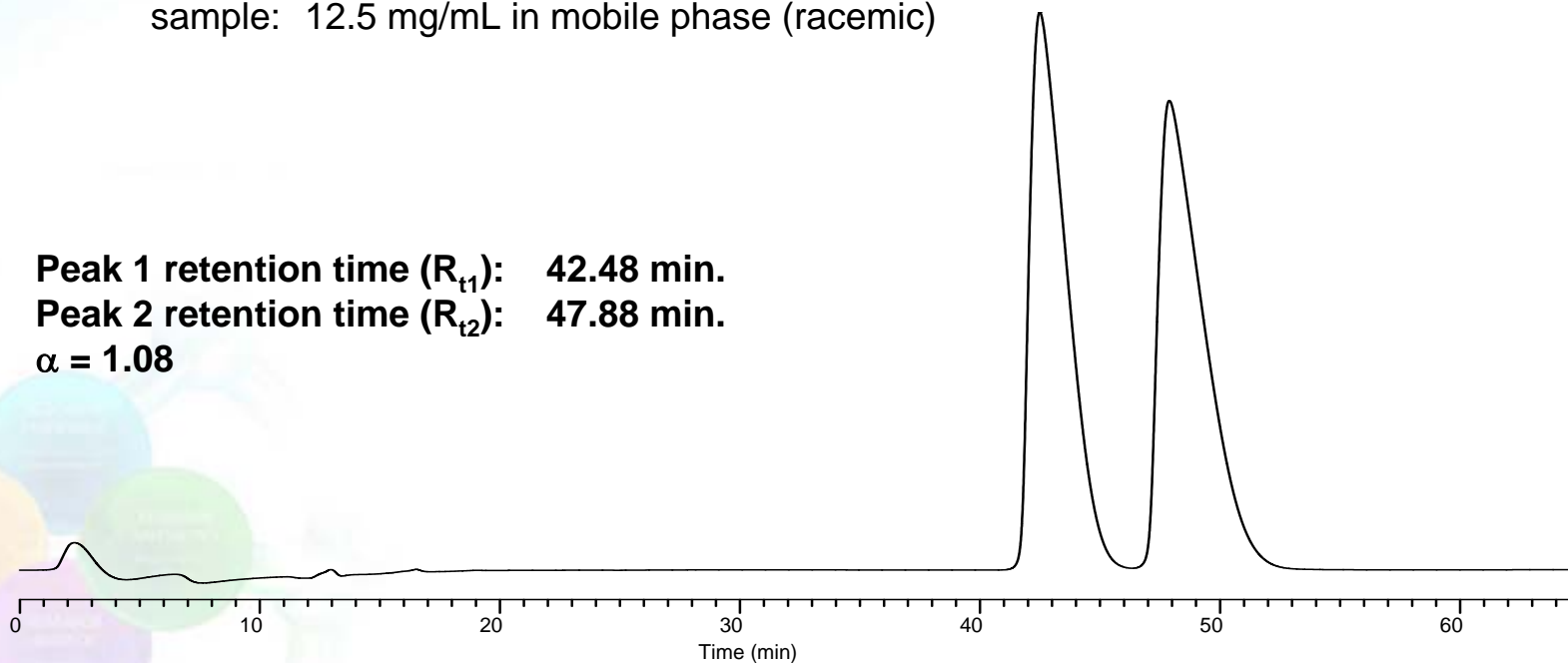


- The preparative separation of fluoxetine, a selective serotonin reuptake inhibitor, is an example of a successful chiral separation done in reversed-phase mode at low flow rates.<sup>3</sup>
- Recently, a chiral screen performed on racemic fluoxetine revealed enantiomeric selectivity on the CHIROBIOTIC V2 in reversed-phase mode.<sup>4</sup>
- Conditions were optimized on an analytical scale for the anticipated preparatory separation of Fluoxetine on the V2 in reversed-phase (See Figure 2).

## Figure 2. The Analytical Separation of Fluoxetine in Reversed-Phase

column: CHIROBIOTIC V2, 15 cm x 4.6 mm, 5  $\mu$ m particles  
mobile phase: 70:30, 20 mM NH<sub>4</sub>OAc (pH 4): ACN (Reversed-Phase Mode)  
flow rate: 0.15 mL/min  
temp.: 10 °C  
det.: UV at 230 nm  
injection: 2  $\mu$ L  
sample: 12.5 mg/mL in mobile phase (racemic)

Peak 1 retention time ( $R_{t1}$ ): 42.48 min.  
Peak 2 retention time ( $R_{t2}$ ): 47.88 min.  
 $\alpha = 1.08$

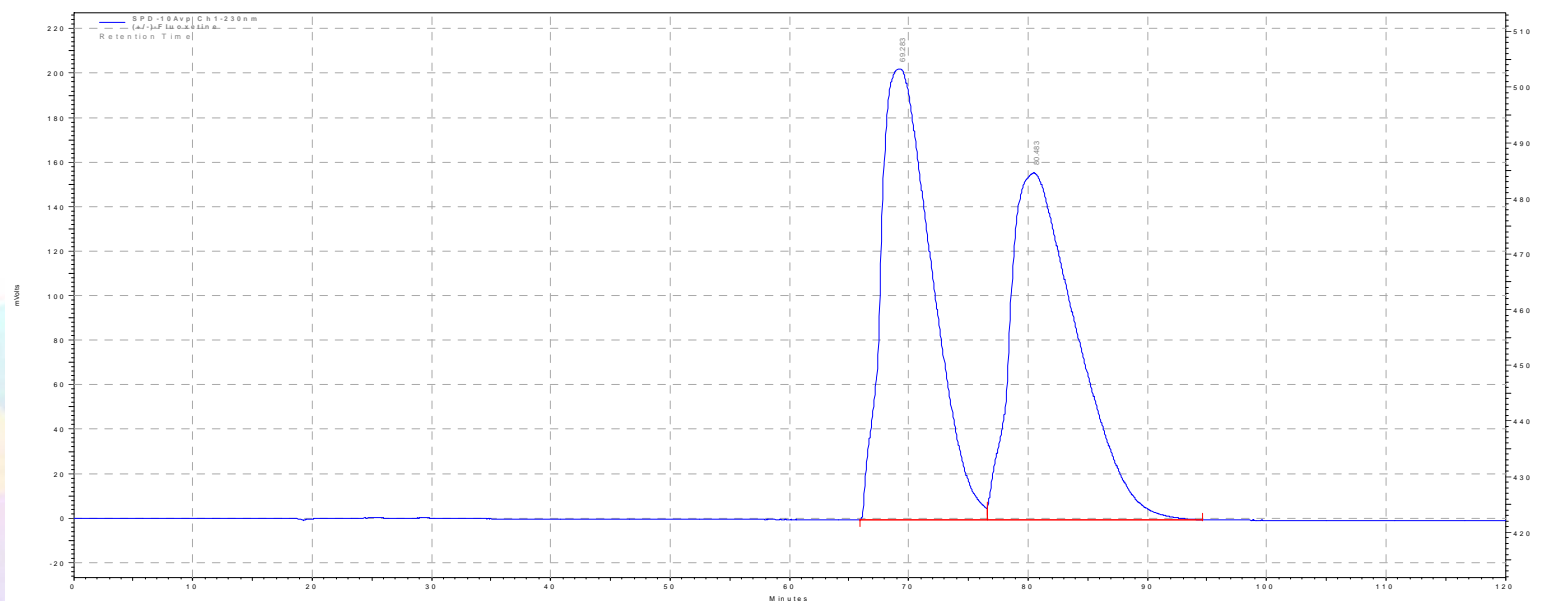


## Scaleup of Injection Size

- From a loading study done on an analytical V2 column, it was estimated that approximately 1 mg of material could be injected on a 250 x 21.2 mm prep column while maintaining good resolution of the peaks.
- As seen in Figure 3, it was experimentally determined that an injection of 4.4 mg, more than 4 times greater than the amount estimated by the loading study, could be made while maintaining resolution.

# Figure 3. First Injection Made on the CHIROBIOTIC V2 Preparative Column

column: CHIROBIOTIC V2, 25 cm x 21.2 mm, 5  $\mu$ m particles  
mobile phase: 70:30, 20 mM NH<sub>4</sub>OAc (pH 4): ACN (Reversed-Phase Mode)  
flow rate: 3.2 mL/min  
temp.: 10 °C  
det.: UV at 230 nm  
injection: 88  $\mu$ L (4400  $\mu$ g of analyte)  
sample: 50 mg/mL in mobile phase (racemic)



# Translation to Prep Scale

## Analytical Conditions:

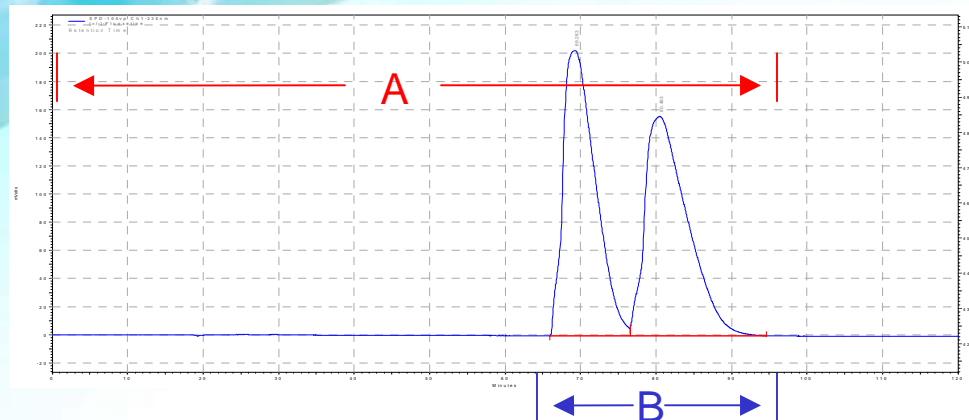
column: CHIROBIOTIC V2,  
15 cm x 4.6 mm I.D.  
mobile phase: 70:30, 20 mM NH<sub>4</sub>OAc  
(pH 4): ACN  
flow rate: 0.15 mL/min,  
temp.: 10 °C  
det.: UV at 230 nm  
injection: 2 µL  
sample: 25 mg/mL in mobile phase

## Prep Conditions:

column: CHIROBIOTIC V2,  
25 cm x 21.2 mm I.D.  
mobile phase: 70:30, 20 mM NH<sub>4</sub>OAc  
(pH 4): ACN  
flow rate: 3.2 mL/min  
temp.: 10 °C  
det.: UV at 230 nm  
injection: 88 µL  
sample: 50 mg/mL in mobile phase

- Injections are stacked to conserve both time and solvent.
- Figure 4 describes the calculations performed to successfully time the stacked injections.

## Figure 4. Calculation of Stacked Injections



**A = total run time = 92 min.**

**B = time for analytes to elute = 26 min.**

**C = time for injector to make injection  
= 0.4 min.**

$$\text{Possible Loops} = \frac{A}{B} = \frac{92 \text{ min.}}{26 \text{ min.}} = 3.5 \quad (\text{Integer} = 3)$$

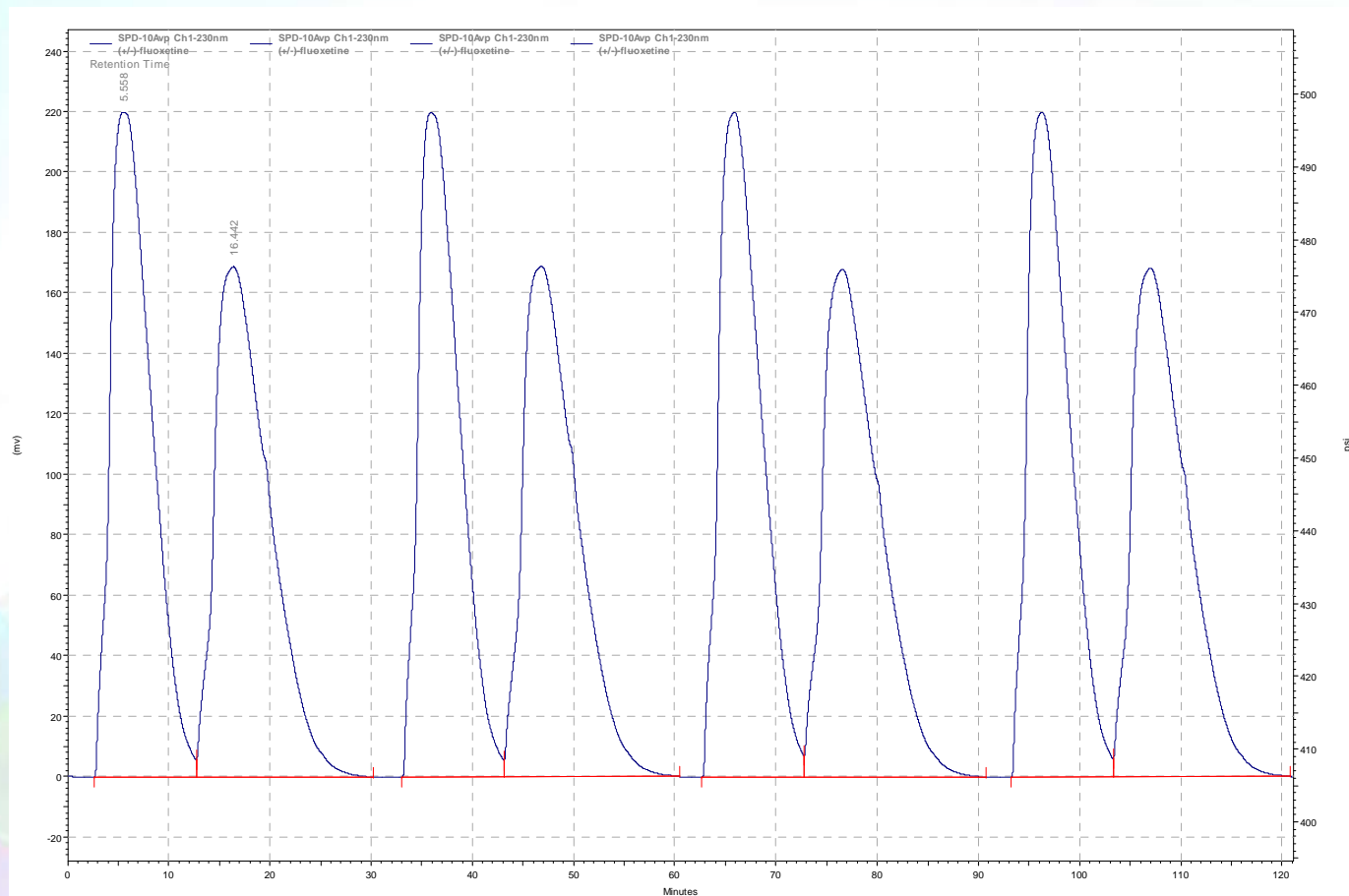
Therefore, 3 injections can be made in the time taken for the first injection to elute.

$$\text{Loop Time} = \left( \frac{A}{\text{Integer}} \right) - C = \left( \frac{92 \text{ min}}{3} \right) - 0.4 \text{ min} = 30.27 \text{ min. loop}$$

A new injection is made every 30.27 minutes.

- Figure 5 shows a portion of final prep chromatogram with stacked injections.

# Figure 5. Stacked Injections in the Fluoxetine Prep Separation



# Sample Recovery

- Reversed-Phase

- Because the reversed-phase mobile phase is composed mostly of water, traditional evaporation would be time consuming. Also, the resultant dried product would be contaminated with buffer salts.
- To eliminate these obstacles, aqueous fractions containing analyte can be run through a C18 flash cartridge.
  - The analyte adheres to cartridge and can be washed with water to remove mobile phase additives.
  - The analyte is then eluted from flash cartridge using a minimal amount of MeOH, that can be removed quickly *in vacuo*.

- Polar-Ionic Mode

- To remove the mobile phase additives resulting from prep work done in PIM, the mixture is concentrated to a residue, dissolved in diethyl ether, loaded onto a silica flash cartridge, and washed with diethyl ether. The final product is eluted with a minimal amount of methanol.

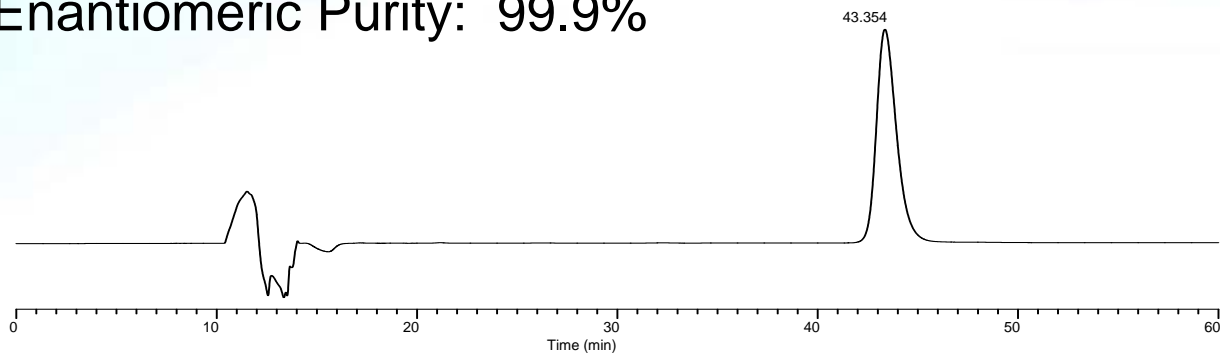
## Sample Recovery (contd.)

- For Peak 1
  - Connected VersaPak™ C18 flash cartridge (40 x 150 mm) to detector.
  - Equilibrated with mobile phase, 10 mL/min. for 30 min.
  - Combined fractions for peak one (total 1600 mL). Diluted to 3200 mL with water.
  - Loaded all of peak 1 onto cartridge at 10 mL/min.
  - Washed cartridge containing peak with water (1000 mL).
  - Eluted peak 1 from cartridge with 900 mL MeOH. Collected 300 mL of peak 1.
  - Concentrated *in vacuo* at 40 °C. Dissolved and re-suspended in Hexanes (3 times 5 mL) to remove residual moisture.
  - Transferred to a tared vial, and concentrated under nitrogen.
- A total of 257.2 mg of racemic HCl salt was processed to yield 102.6 mg of 99.9% enantiomerically pure peak 1 as the free base (89% recovery). Peak 2 had an enantiomeric purity of 99.4%. Refer to Figure 6 for chromatograms showing enantiomeric purity.

## Figure 6. Purity of Peaks

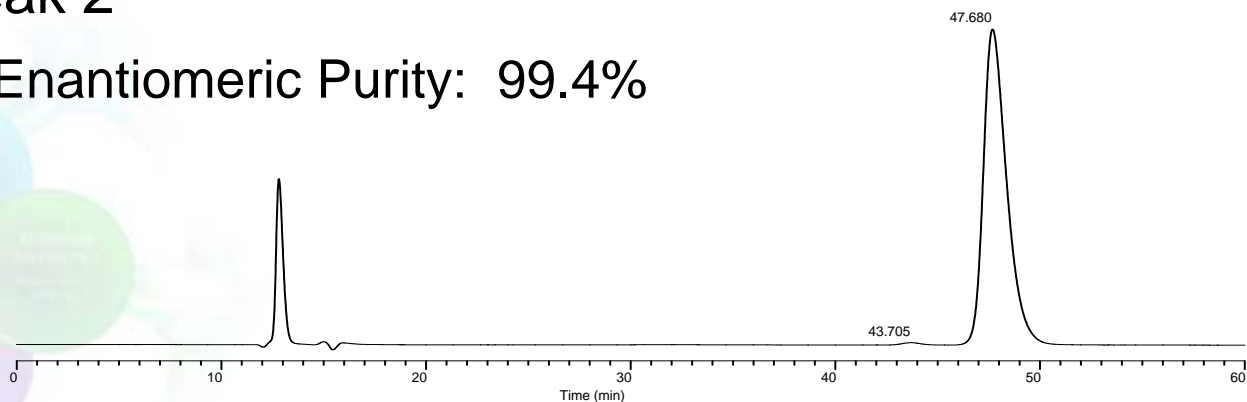
- Peak 1

- Enantiomeric Purity: 99.9%



- Peak 2

- Enantiomeric Purity: 99.4%



## Conclusions

- An environmentally friendly and less toxic low flow reversed-phase approach was applied to the preparative separation of (+/-)-Fluoxetine on the CHIROBIOTIC V2 in reversed-phase.
- Calculations and experimental analysis allowed for the successful scale-up of the optimized analytical separation to prep scale. Stacked injections were utilized to conserve both time and solvent usage.
- A total of 257.2 mg of racemic material was processed to yield 102.6 mg of 99.9% enantiomerically pure peak 1 (89% recovery). Peak 2 had an enantiomeric purity of 99.4%.
- Solvent consumption for the preparatory process totaled less than 6 L. Of this total, more than 4 L was aqueous based. Less than 2 L of organic solvent waste was produced. Therefore, preparatory separations done in reversed-phase mode produce minimal organic waste.

## Conclusions (contd.)

- Had this separation been done at the typical preparatory flow rate of 21 mL/min., it is estimated that twice the amount of solvent would have been used, taking into account the need for smaller injections due to a decrease in efficiency at higher flow rates (refer to Figure 1). Compared to high flow preparatory separations, preparatory work incorporating low flow rate produces less solvent waste.
- Additional benefits of using reversed-phase and polar ionic/polar organic modes over normal phase modes for prep separation include possible improvements in sample solubility, capacity, throughput, and yields.
- In summary, the use of reversed-phase and polar ionic/polar organic modes for prep separation at low flow rates are safe, efficient, and environmentally friendly ways to execute successful chiral preparatory separations.

## References

1. Proctor and Hughes' chemical hazards of the workplace, 3<sup>rd</sup> ed.; Van Nostrand Reinhold: New York, NY, 1991.
2. Li, J., Carr, P.W. Anal. Chem. **1997**, 69, 2530.
3. Ulrich, Sven. J. Chromatogr. B. **2003**, 783, 481-490.
4. Claus, J.E.; Lee, J.T. The Chiral Screening and Chromatographic Optimization of Racemic Fluoxetine Using Astec CHIROBIOTIC and CYCLOBOND™ Columns. Presented as a poster at The Chromatography Forum of Delaware Valley 2008 Spring Symposium, Fort Washington, PA, April 17, 2008.
5. CHIROBIOTIC Handbook, 5th ed.; Advanced Separation Technologies, Inc.: Whippany, NJ, 2004.