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*Advanced Separation Technologies Inc.*

510-7P

New Modified Bonded Macrocyclic Glycopeptide  
Chiral Stationary Phases with Enhanced Chiral  
Selectivity for Important Drugs and Drug  
Metabolites

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# Abstract

LC chiral stationary phases (CSPs) made by bonding the macrocyclic glycopeptides vancomycin, teicoplanin, ristocentin A and teicoplanin aglycone have demonstrated very wide chiral selectivity and excellent robustness since their introduction in 1995.

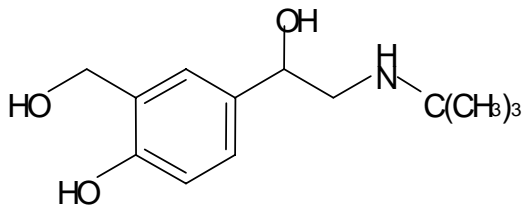
It has been demonstrated that a number of mechanisms are at work in obtaining chiral selectivity on these CSPs. The type and number of linkage bonds and the position of these linkages can be modified to enhance or diminish selectivity for certain classes of molecules in certain mobile phase conditions. In addition, the use of certain blocking groups to protect possible interactive sites has contributed to a greater understanding of the separation mechanism. A study of silica gel supports with different pore size also demonstrated an impact on retention, selectivity and loading capacities for racemates. As a result, better resolution and higher capacity were obtained in a number of cases.

Chirality has long been regarded as one of the critical issues in drug design and the discovery processes. During the development stages, the pharmacokinetic aspects of the chiral drugs need to be addressed because each enantiomer can behave differently in terms of absorption, distribution, metabolism and excretion in clinical studies. With new modified CSPs, not only two enantiomers of many important chiral drugs were separated, but also their metabolites were resolved in the same run. In certain cases, the combination of two chiral columns was utilized. This presentation will give examples of the most important applications in the reversed phase mode and polar ionic mode, both suitable for LC/MS platforms.

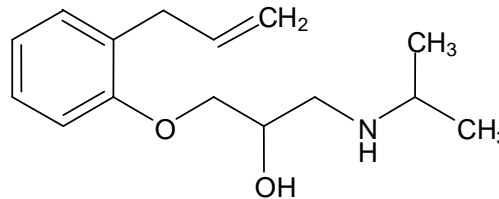


# Structures of Compounds Studied

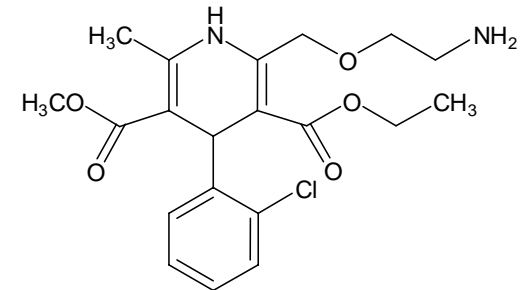
## ■ Albuterol



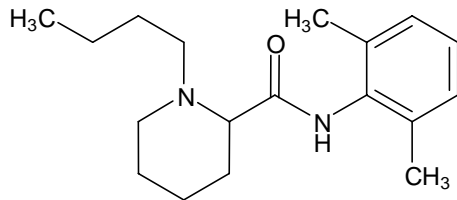
## ■ Alprenolol



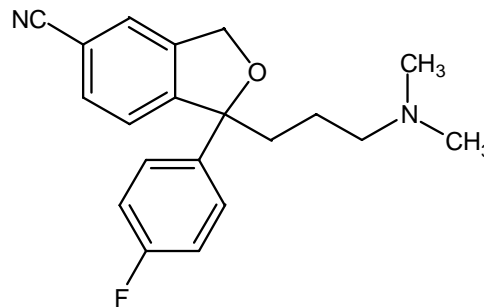
## ■ Amlodipine



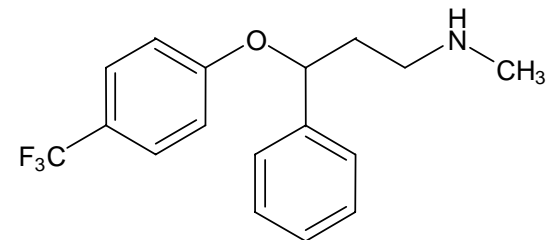
## ■ Bupivacaine



## ■ Citalopram

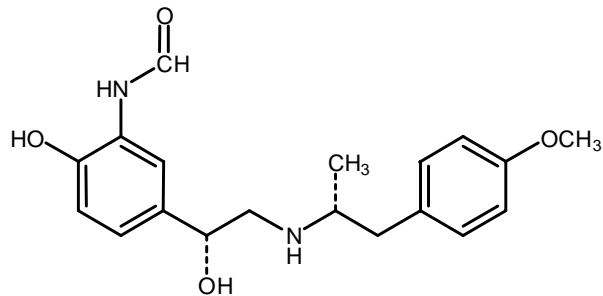


## ■ Fluoxetine

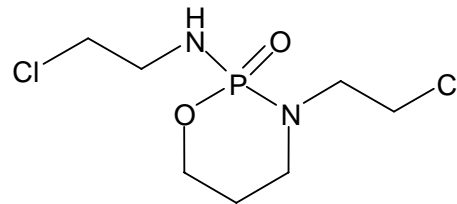


# Structures of Compounds Studied

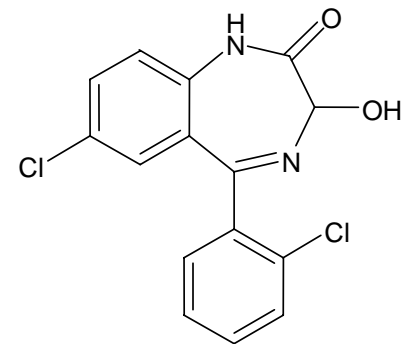
■ Formoterol



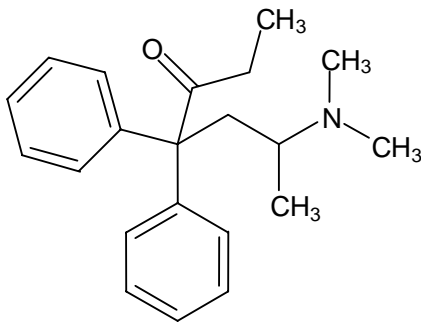
■ Ifosfamide



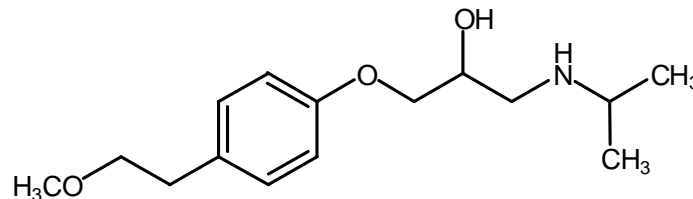
■ Lorazepam



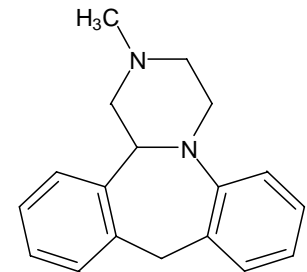
■ Methadone



■ Metoprolol

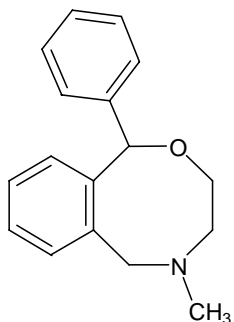


■ Mianserin

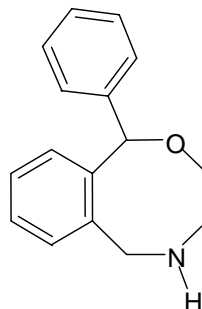


# Structures of Compounds Studied

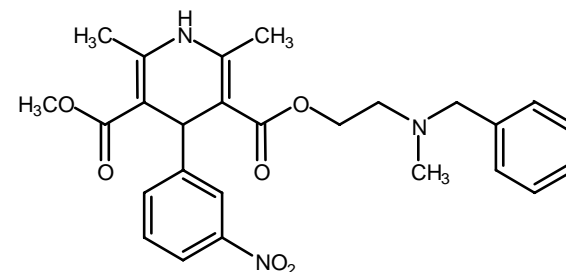
■ Nefopam



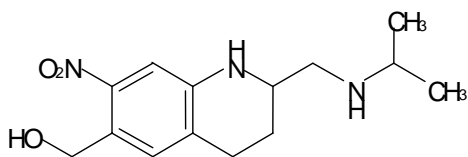
■ N-Desmethyl nefopam



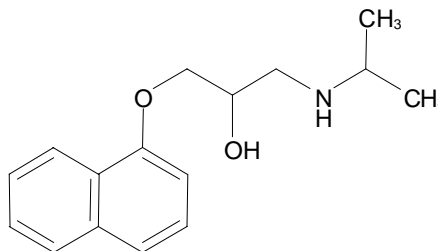
■ Nicardipine



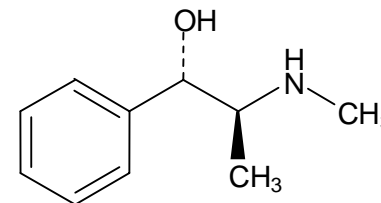
■ Oxamniquine



■ Propranolol

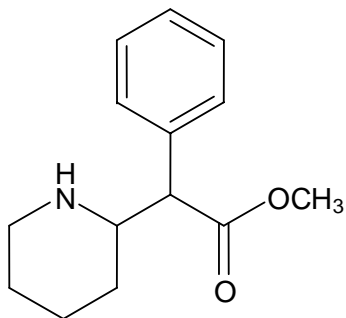


■ Pseudo-ephedrine

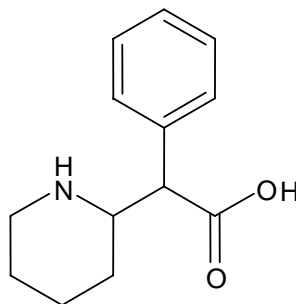


# Structures of Compounds Studied

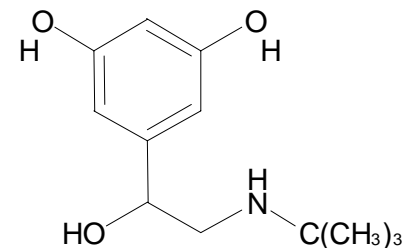
■ Ritalin



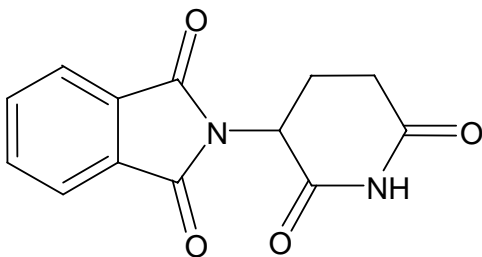
■ Ritalinic acid



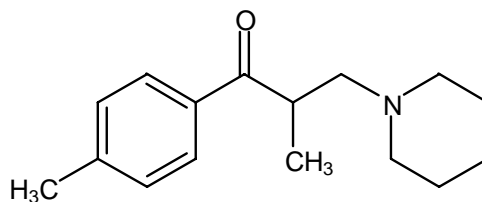
■ Terbutaline



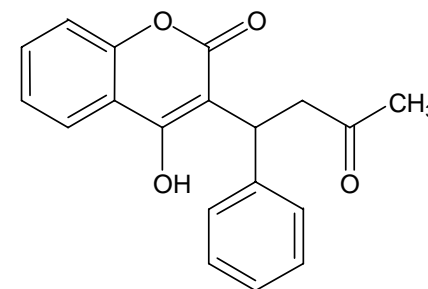
■ Thalidomide



■ Tolperisone

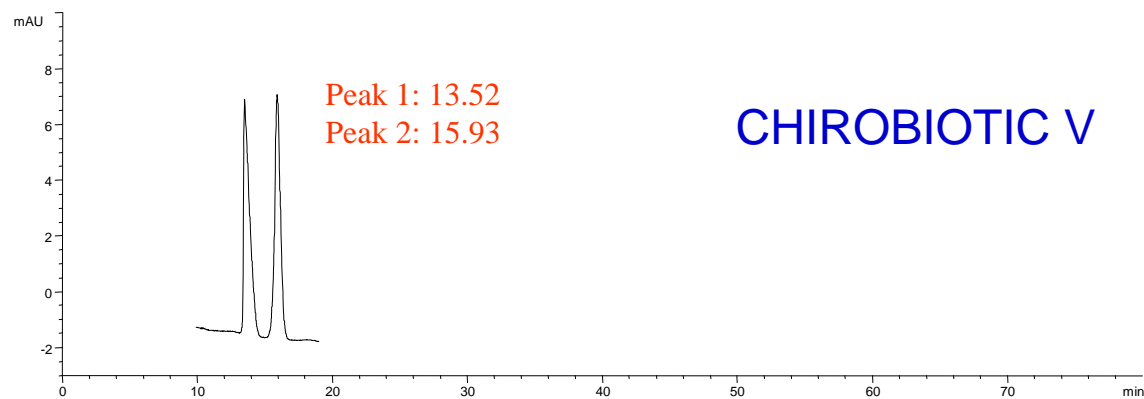
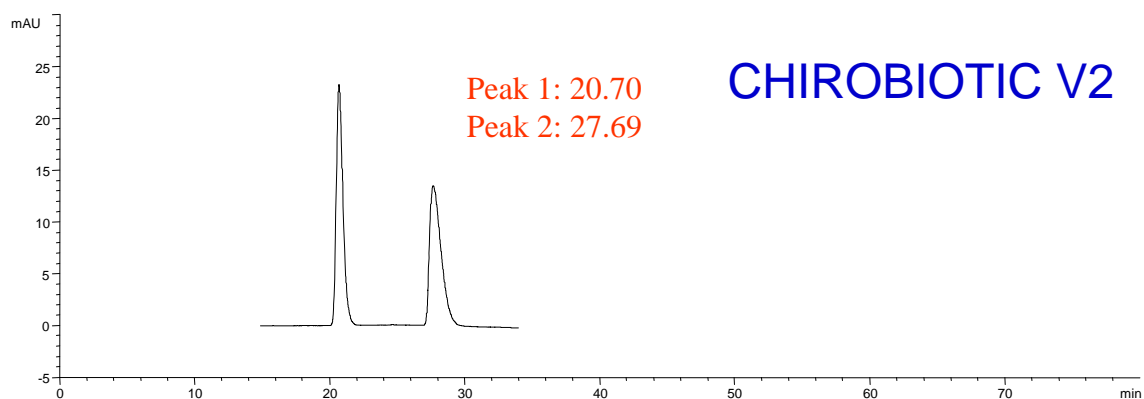


■ Warfarin



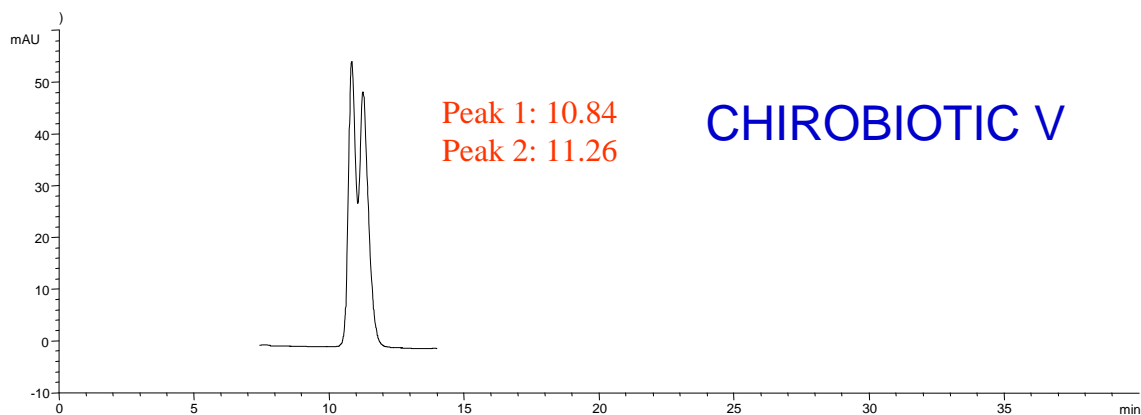
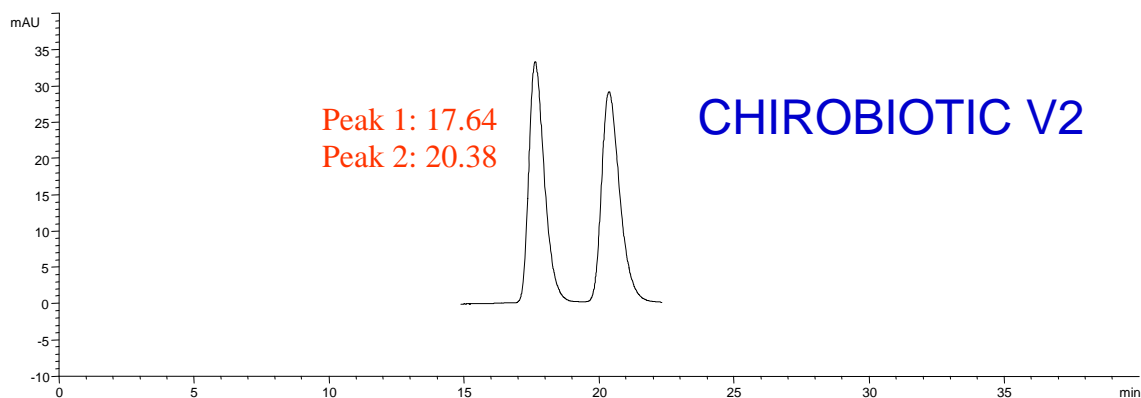
# CHIROBIOTIC V2 vs. V

- **Sample:** Ritalin
- **Mobile Phase:** 95/5, MeOH/20mM NH<sub>4</sub>OAc, pH 4.1
- **Flow Rate:** 1 mL/min



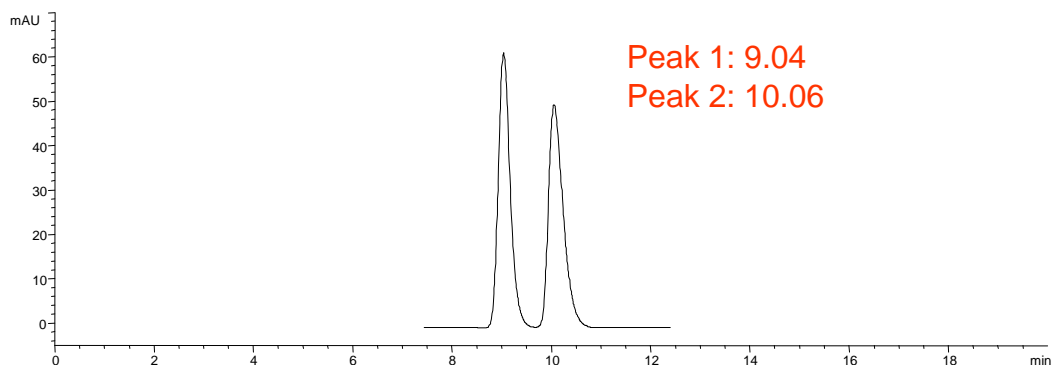
# CHIROBIOTIC V2 vs. V

- **Sample:** Oxamniquine
- **Mobile Phase:** 95/5, MeOH/20mM NH<sub>4</sub>OAc, pH 4.1
- **Flow Rate:** 2 mL/min

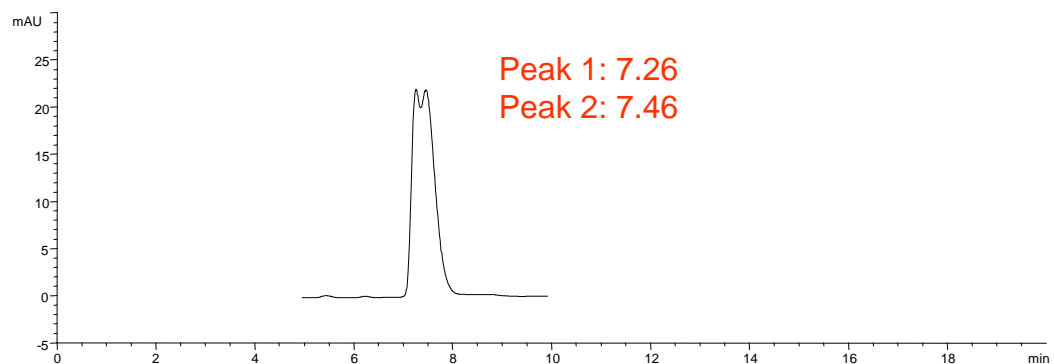


# Polar Ionic Mode vs. Reversed Phase Mode CHIROBIOTIC V2

- **Sample:** Ritalin
- **Mobile Phase:** 80/20, MeOH/20mM NH<sub>4</sub>OAc, pH 4.1
- **Flow Rate:** 1 mL/min

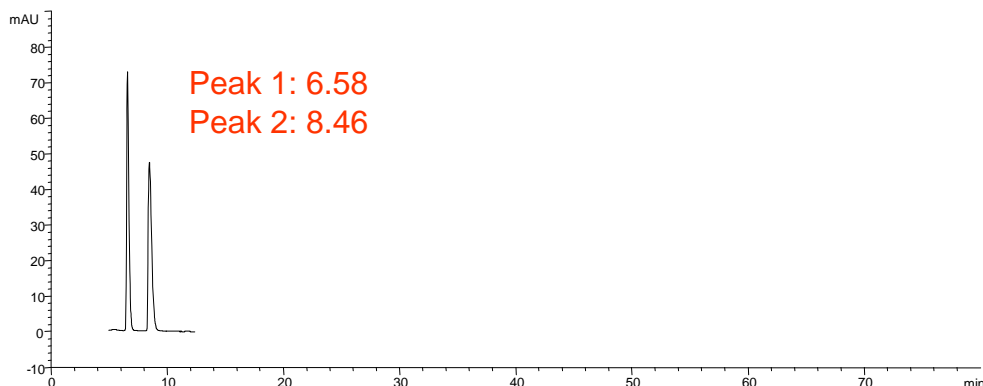


- **Mobile Phase:** 50/50, MeOH/20mM NH<sub>4</sub>OAc, pH 4.1
- **Flow Rate:** 1 mL/min

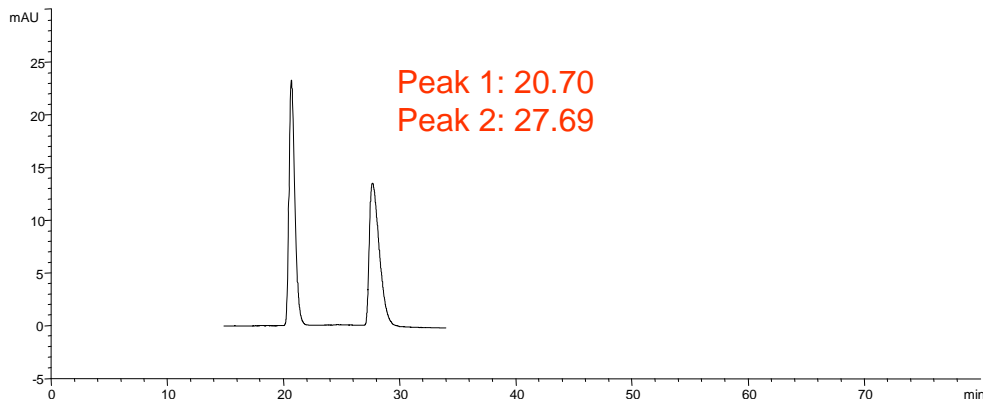


# Polar Ionic Mode vs. Reversed Phase Mode CHIROBIOTIC V2 (cont'd)

- Sample: Ritalin
- Mobile Phase: 100/0.1w%, MeOH/NH<sub>4</sub>Formate
- Flow Rate: 1 mL/min



- Mobile Phase: 95/5, MeOH/20mM NH<sub>4</sub>OAc, pH 4.1
- Flow Rate: 1 mL/min



# Improved Resolution and Capacity CHIROBIOTIC V2

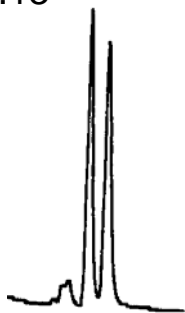
- **Sample:** Basic compound

## Analytical Separation

Peak 1: 10.51 min

Peak 2: 11.53 min

$\alpha$  -1.13



**CHIROBIOTIC V** (250x4.6 mm)

100/0.2/0.1 MeOH/HOAc/TEA

0.9 mL/min

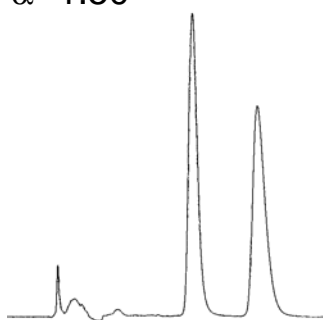
Injection: 10mg

## Analytical Separation

Peak 1: 8.83 min

Peak 2: 11.76 min

$\alpha$  -1.50



**CHIROBIOTIC V2** (250x4.6 mm)

100/0.5/0.5 MeOH/HOAc/TEA

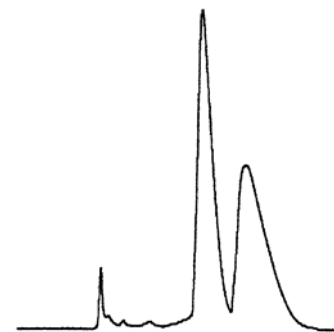
1.0 mL/min

Injection: 50mg

## Preparative Separation

Peak 1: 10.22 min

Peak 2: 12.54 min



**CHIROBIOTIC V2** (250x21.2 mm)

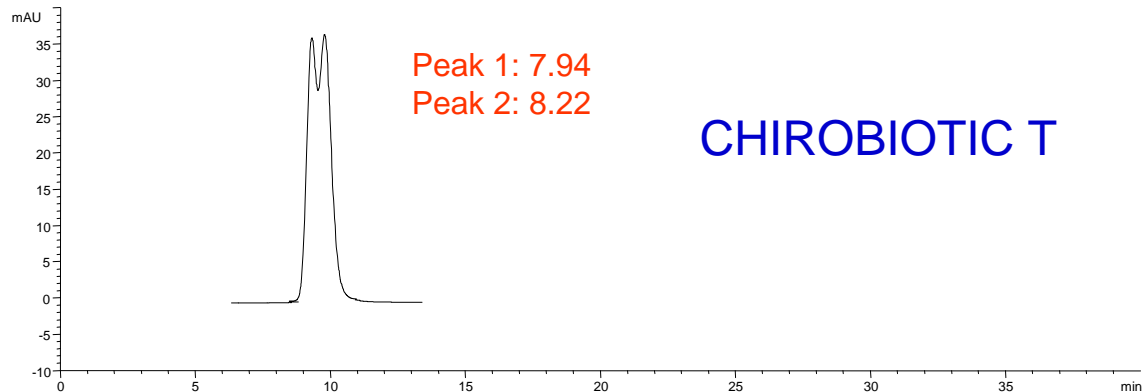
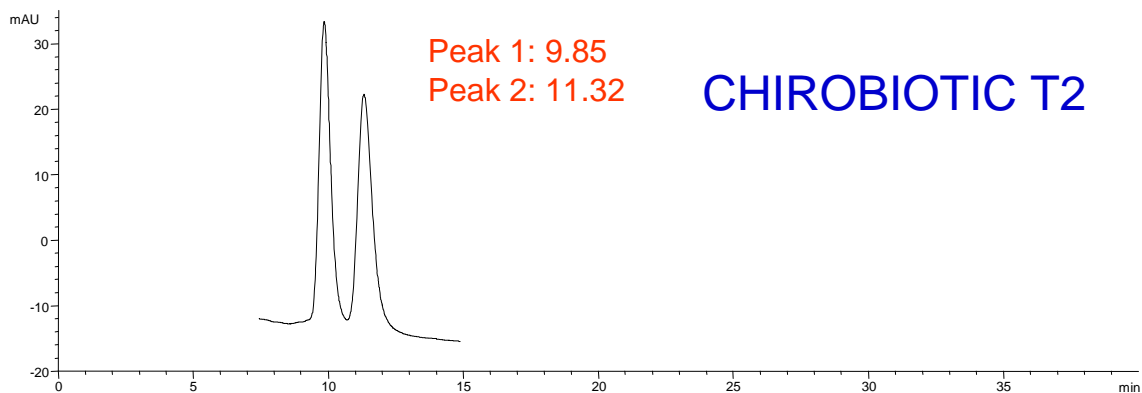
100/0.5/0.5 MeOH/HOAc/TEA

15.0 mL/min

Injection: 140mg

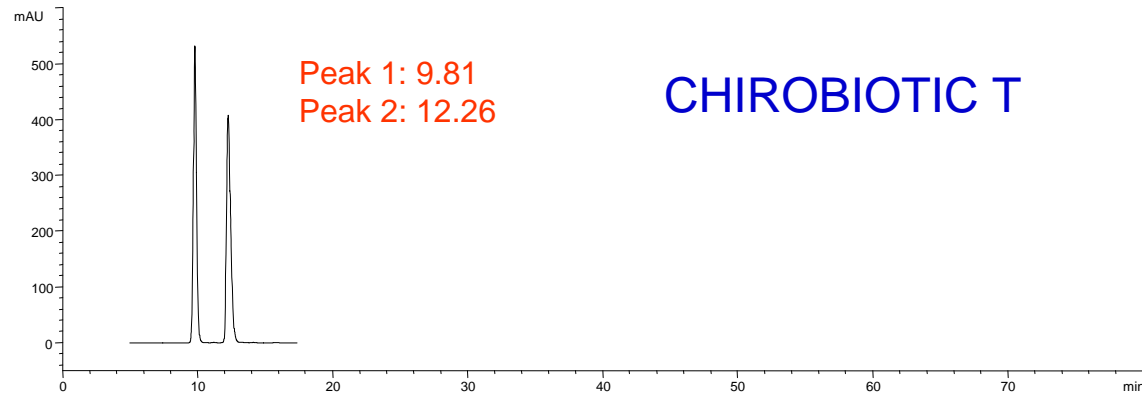
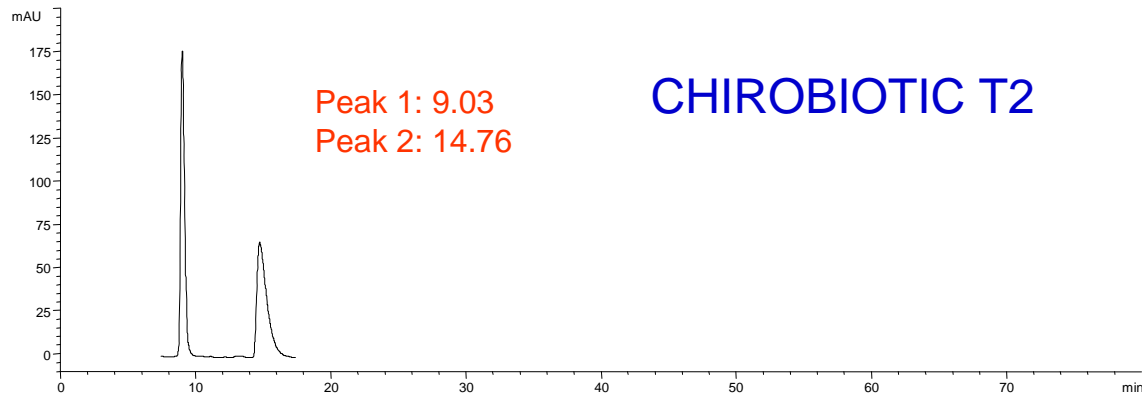
# CHIROBIOTIC T2 vs. T

- **Sample:** Formoterol
- **Mobile Phase:** 100/0.5/0.5, MeOH/HOAc/TEA
- **Flow Rate:** 1 mL/min



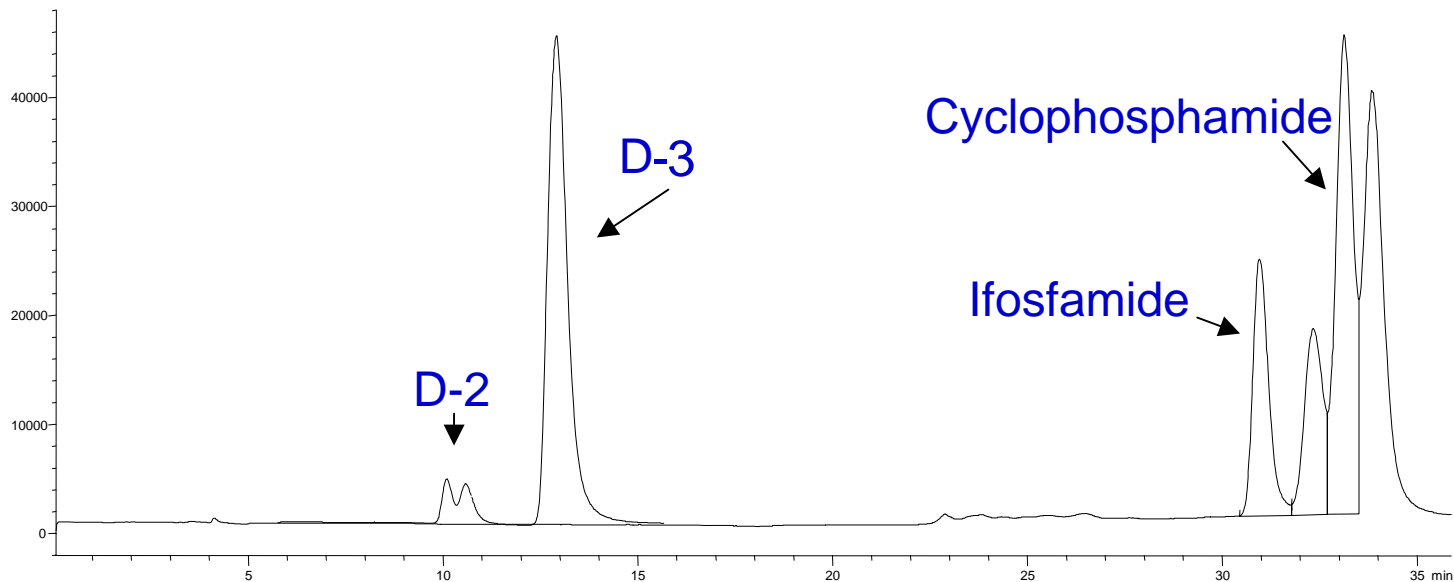
# CHIROBIOTIC T2 vs. T

- **Sample:** Terbutaline
- **Mobile Phase:** 100/0.1w%, MeOH/NH<sub>4</sub>TFA
- **Flow Rate:** 1 mL/min



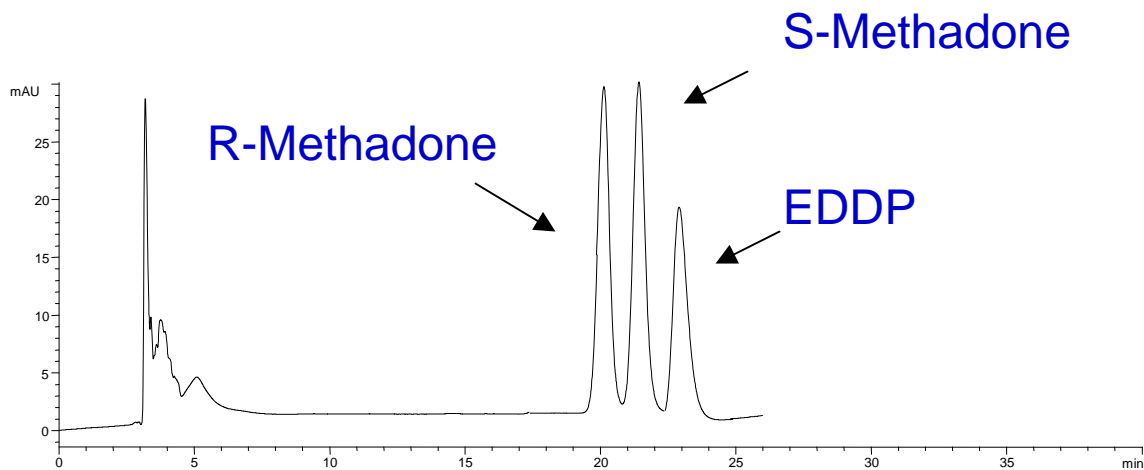
# Separation of Ifosfamide and Its Metabolites

- **Column:** CHIROBIOTIC T2 (150x4.6mm)+ Astec C18 (100x4.6mm)
- **Mobile Phase:** IPA/10mM NH<sub>4</sub>OAc, Gradient system
- **Flow Rate:** 0.8 mL/min



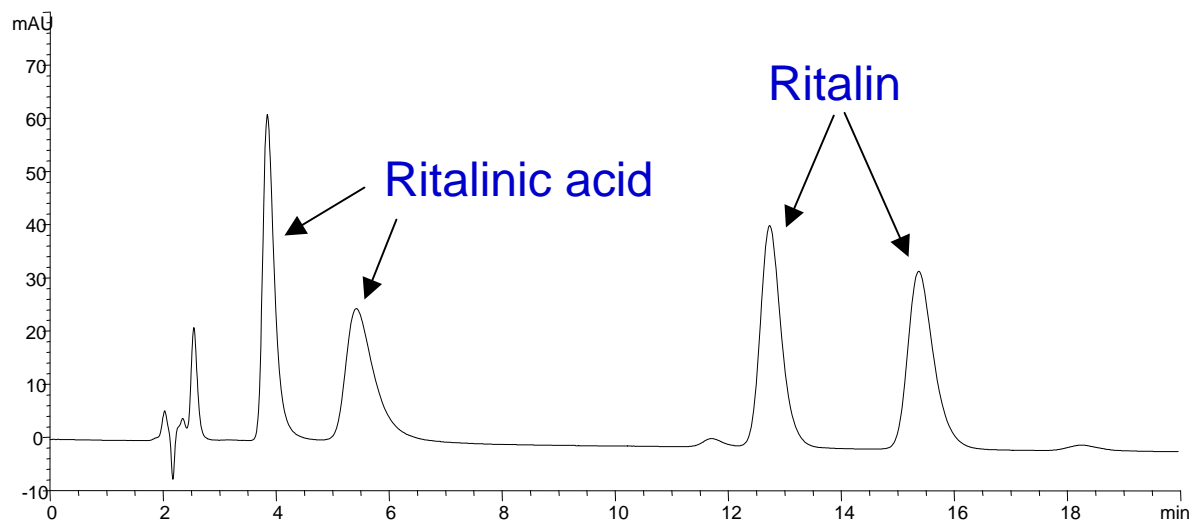
# Separation of Methadone and Its Metabolite

- **Column:** CHIROBIOTIC V2
- **Mobile Phase:** 100/0.04/0.007, MeOH/HOAc/NH<sub>4</sub>OH
- **Flow Rate:** 0.8 mL/min



# Separation of Ritalin and Its Metabolite

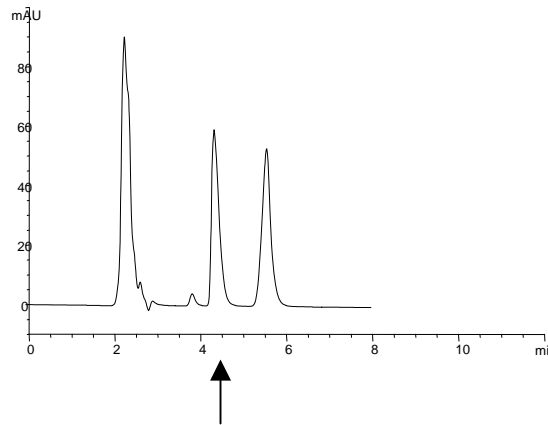
- **Column:** CHIROBIOTIC T2 Guard (20x4.0mm) + CHIROBIOTIC V2 (150x4.6mm)
- **Mobile Phase:** 93/7 MeOH/20 mM NH<sub>4</sub>OAc, pH4.1
- **Flow Rate:** 1.0 mL/min
- **Temperature:** 25 °C



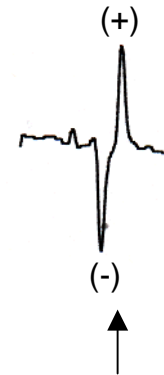
# Reverse Elution Orders on CHIROBIOTIC V2 and T2

- Sample: Ritalin
- Mobile Phase: 100/0.1 v/w, MeOH/NH<sub>4</sub>OAc

- CHIROBIOTIC V2 (150x4.6 mm)

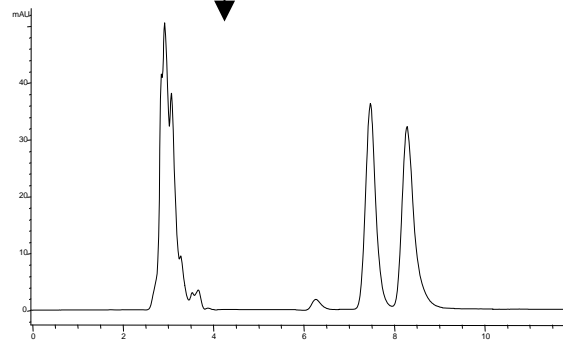


UV Detection @230 nm

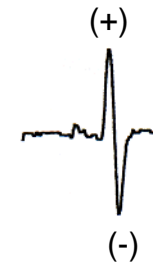


Chiral Detection (CHIRALYZER)

- CHIROBIOTIC T2 (250x4.6 mm)



PITTCON 2005



# Best Conditions on CHIROBIOTIC V2

Compounds	Mobile Phase	$K_1'$	$\alpha$	$R_s$
Amlodipine	100/0.1w%, MeOH/NH <sub>4</sub> TFA	3.2	1.11	1.5
Bupivacaine	100/0.1w%, MeOH/ NH <sub>4</sub> TFA	0.8	1.40	2.5
Fluoxetine	100/0.1w%, MeOH/ NH <sub>4</sub> TFA	1.5	1.27	2.5
Methadone	100/0.04/0.007, MeOH/HAc/NH <sub>4</sub> OH	4.7	1.08	1.5
Methylphenidate	100/0.1w%, MeOH/ NH <sub>4</sub> TFA	2.2	1.15	1.6
Mianserin	100/0.1w%, MeOH/ NH <sub>4</sub> TFA	2.9	1.55	5.0
Nefopam	100/0.1w%, MeOH/ NH <sub>4</sub> TFA	2.3	1.15	1.5
N-Desmethyl Nefopam	100/0.1w%, MeOH/ NH <sub>4</sub> TFA	2.0	1.17	1.7
Nicardipine	100/0.02w%,MeOH/ NH <sub>4</sub> TFA	0.9	1.79	4.5
Oxamniquine	100/0.1w%, MeOH/ NH <sub>4</sub> TFA	2.7	1.23	2.2
Ritalin	100/0.1w%,MeOH/NH <sub>4</sub> Formate	1.4	1.50	3.0
Thalidomide	100% MeOH	0.5	3.44	8.0
Tolperisone	100/0.1w%, MeOH/ NH <sub>4</sub> TFA	1.8	2.33	2.7
Trimipramine	100/0.1w%, MeOH/ NH <sub>4</sub> TFA	1.7	1.28	2.3
Warfarin	30/70, ACN/buffer, pH 4.1	3.5	1.31	2.8

# Best Conditions on CHIROBIOTIC T2

Compounds	Mobile Phase	$K_1'$	$\alpha$	$R_s$
Albuterol	100/0.1w%,MeOH/NH <sub>4</sub> TFA	2.5	1.33	3.1
Alprenolol	100/0.1w%,MeOH/ NH <sub>4</sub> TFA	2.1	1.13	1.5
Atropine	100/0.1w%,MeOH/ NH <sub>4</sub> TFA	8.0	1.13	1.5
Clenbuterol	100/0.1w%,MeOH/ NH <sub>4</sub> TFA	2.1	1.26	2.5
Formoterol	100/0.6/0.4, MeOH/HOAc/TEA	2.6	1.20	1.5
Ifosfamide	20/80, IPA/H <sub>2</sub> O	1.7	1.37	1.8
Lorazepam	100% MeOH	0.5	4.05	9.5
5-Methyl 5-phenyl Hydantoin	100% MeOH	0.3	2.30	3.5
Metoprolol	100/0.1w%,MeOH/ NH <sub>4</sub> TFA	2.3	1.14	1.5
Propranolol	100/0.1w%,MeOH/ NH <sub>4</sub> TFA	2.7	1.10	1.5
Pseudo-ephedrine	100/0.1w%,MeOH/ NH <sub>4</sub> TFA	2.7	1.13	1.7
Terbutaline	100/0.1w%,MeOH/ NH <sub>4</sub> TFA	2.2	1.93	6.5

# Conclusions

- CHIROBIOTIC V2 and T2 showed exceptional selectivity towards basic drugs, and they are complementary in nature. The selectivity between two enantiomers and other chiral/achiral impurities can be enhanced by coupling these two phases, one in a guard column format. In certain cases, the elution orders of two enantiomers were reversed on both columns.
- Elution order reversal has been noted for certain racemates.
- Multi-modal separation mechanisms allow the mobile phase to change from high aqueous to 100% organic to obtain desired separation.
- Unique mobile phase designs for ionic interactions can be tailored for LC/MS platforms useful for biological samples.
- Through multi-linkage, CHIROBIOTIC V2 and T2 also demonstrate very high stability and capacity in the more desirable polar ionic mode for preparative applications.