



astec

Advanced Separation Technologies Inc.

**Enhanced Chiral Selectivity by
Chemical Modification of
Chiral Stationary Phases For Pharmaceutically
Important Drugs and Drug Metabolites**

**J.T. Lee, Brian He, Thomas E. Beesley
Advanced Separation Technologies Inc.**

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

Abstract

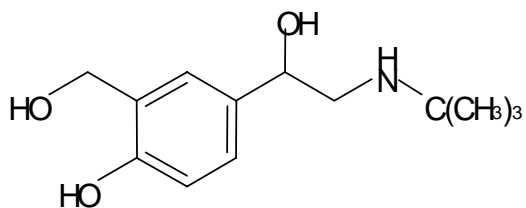
Chiral stationary phases for LC made by bonding the macrocyclic glycopeptides vancomycin, teicoplanin, ristocentin A and teicoplanin aglycone have demonstrated very wide chiral selectivity and excellent robustness since their introduction in 1995.

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 different pore size silica gel supports (200Å vs. 100Å) also demonstrated an impact on retention, selectivity and loading capacities for racemates. As a result, a number of cases with better resolution and higher capacity were possible.

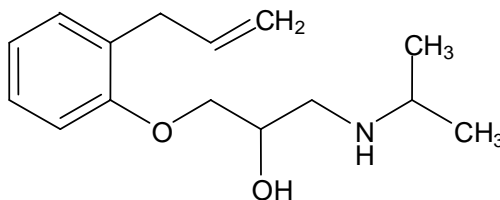
Chirality has long been regarded as one of the critical issues in drug design and the discovery processes. During the development stages, an understanding of pharmacokinetic aspects of the chiral drugs need to be addressed because each enantiomer can behave differently in terms of absorption, distribution, metabolism and excretion in clinical studies. In order to separate both target drug and its metabolite in one LC run, the combination of two chiral columns can be utilized in certain cases. 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

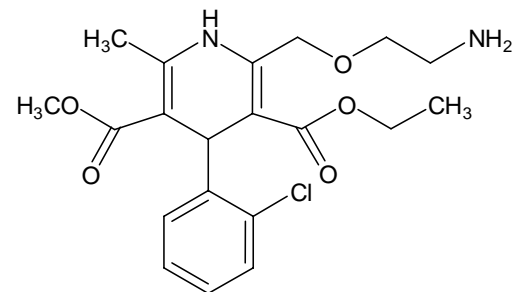
■ Albuterol



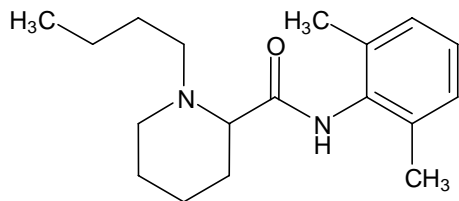
■ Alprenolol



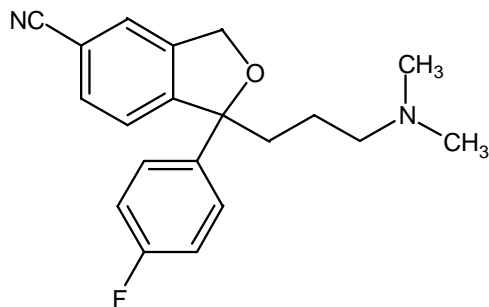
■ Amlodipine



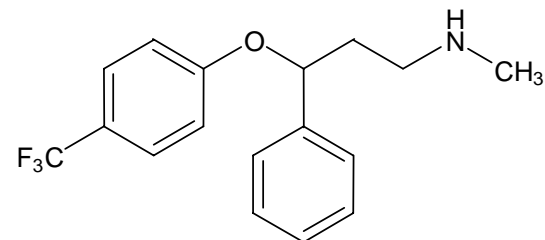
■ Bupivacaine



■ Citalopram

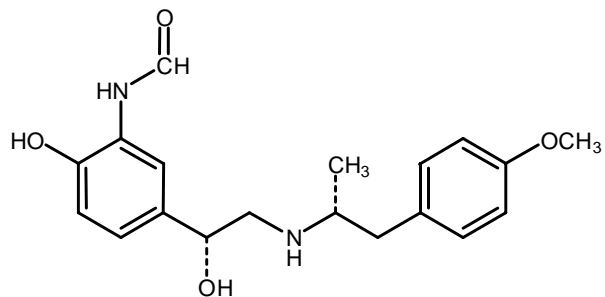


■ Fluoxetine

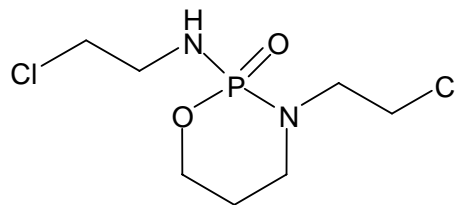


Structures of Compounds Studied

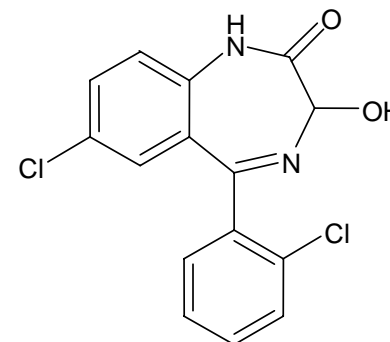
■ Formoterol



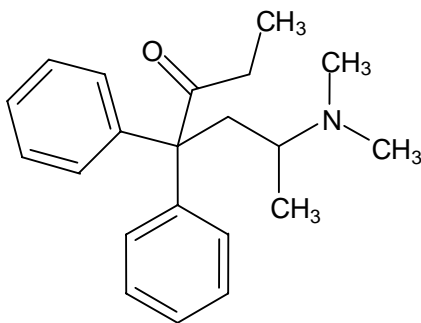
■ Ifofamide



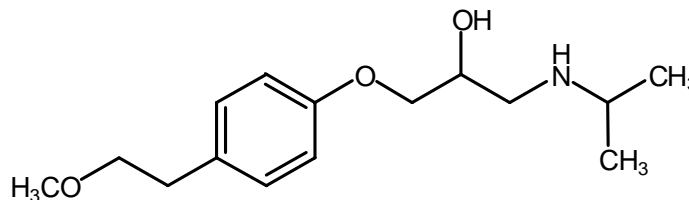
■ Lorazepam



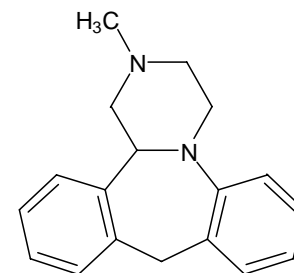
■ Methadone



■ Metoprolol

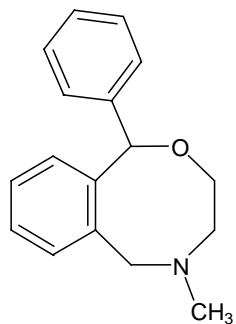


■ Mianserin

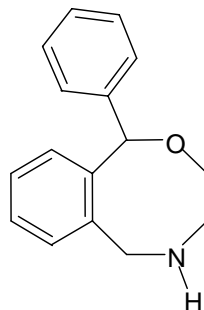


Structures of Compounds Studied

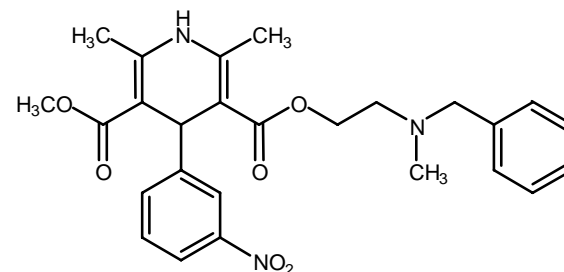
■ Nefopam



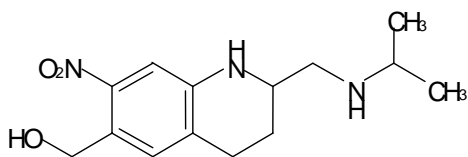
■ N-Desmethyl nefopam



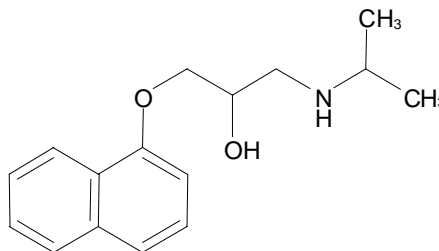
■ Nicardipine



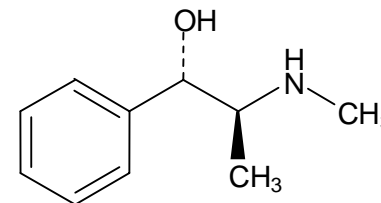
■ Oxamniquine



■ Propranolol

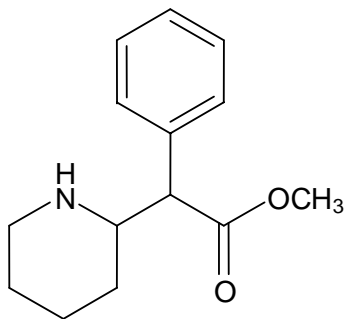


■ Pseudo-ephedrine

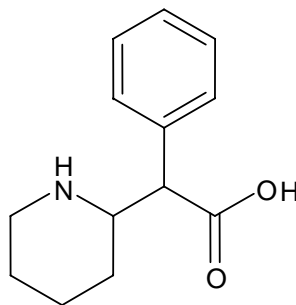


Structures of Compounds Studied

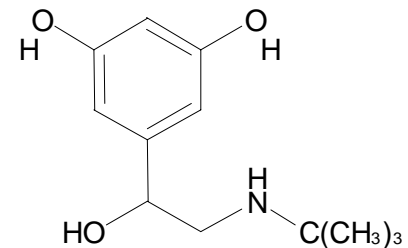
■ Ritalin



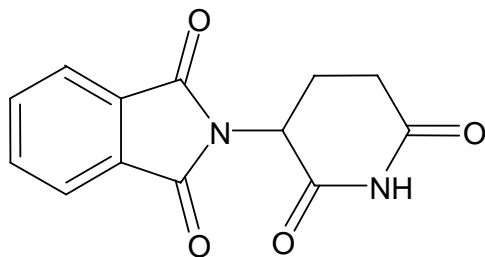
■ Ritalinic acid



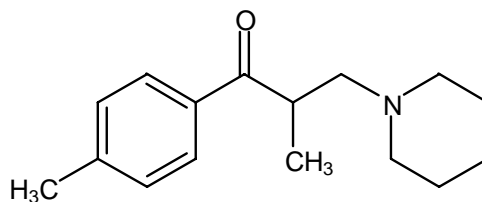
■ Terbutaline



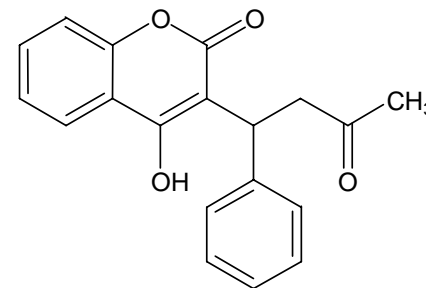
■ Thalidomide



■ Tolperisone



■ Warfarin



Best Conditions on CHIROBIOTIC V2

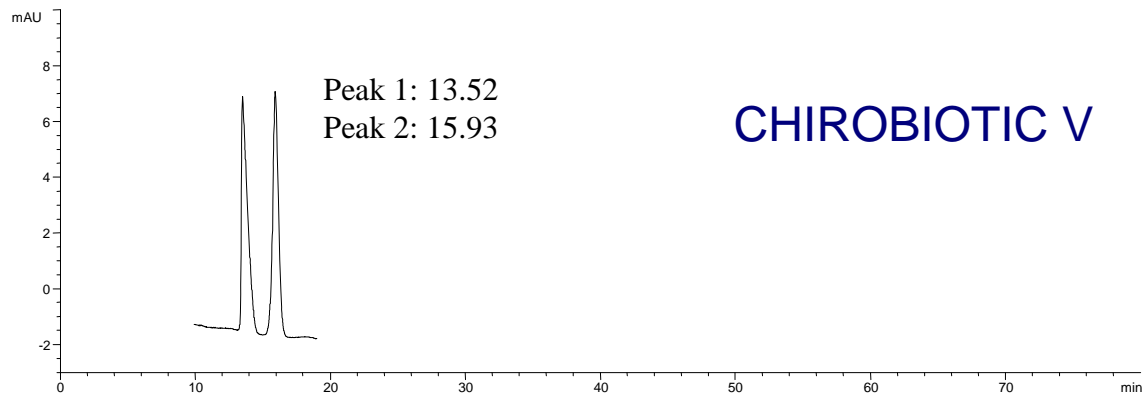
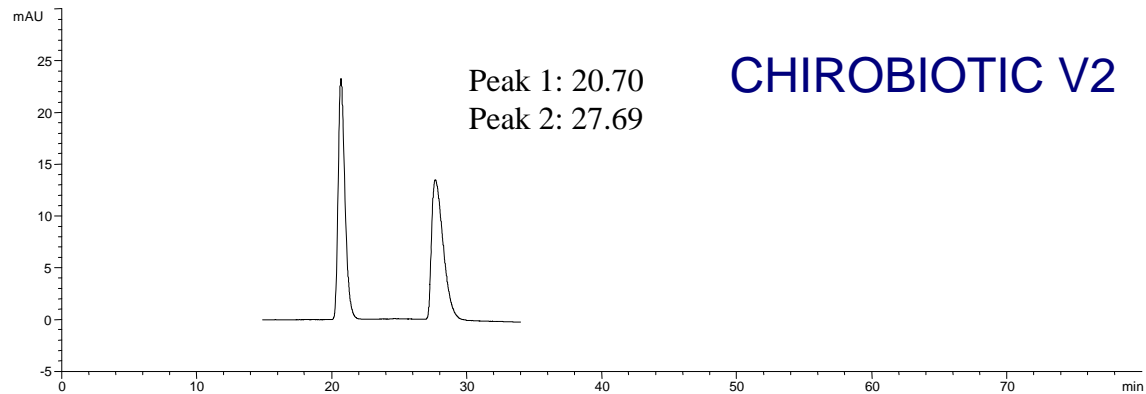
Compounds	Mobile Phase	K1'	α	Rs
Amlodipine	100/0.1w%, MeOH/NH4TFA	3.2	1.11	1.5
Bupivacaine	100/0.1w%, MeOH/NH4TFA	0.8	1.40	2.5
Fluoxetine	100/0.1w%, MeOH/NH4TFA	1.5	1.27	2.5
Methadone	100/0.04/0.007, MeOH/HAc/NH ₄ OH	4.7	1.08	1.5
Methylphenidate	100/0.1w%, MeOH/NH4TFA	2.2	1.15	1.6
Mianserin	100/0.1w%, MeOH/NH4TFA	2.9	1.55	5.0
Nefopam	100/0.1w%, MeOH/NH4TFA	2.3	1.15	1.5
N-Desmethyl Nefopam	100/0.1w%, MeOH/NH4TFA	2.0	1.17	1.7
Nicardipine	100/0.02w%,MeOH/NH4TFA	0.9	1.79	4.5
Oxamniquine	100/0.1w%, MeOH/NH4TFA	2.7	1.23	2.2
Ritalin	100/0.1w%,MeOH/NH4Formate	1.4	1.50	3.0
Thalidomide	100% MeOH	0.5	3.44	8.0
Tolperisone	100/0.1w%, MeOH/NH4TFA	1.8	2.33	2.7
Trimipramine	100/0.1w%, MeOH/NH4TFA	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	K1'	α	Rs
Albuterol	100/0.1 w% ,MeOH/NH4TFA	2.5	1.33	3.1
Alprenolol	100/0.1 w% ,MeOH/NH4TFA	2.1	1.13	1.5
Atropine	100/0.1 w% ,MeOH/NH4TFA	8.0	1.13	1.5
Clenbuterol	100/0.1 w% ,MeOH/NH4TFA	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/H2O	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	100/0.1 w% ,MeOH/NH4TFA	2.3	1.14	1.5
Propranolol	100/0.1 w% ,MeOH/NH4TFA	2.7	1.10	1.5
Pseudo-ephedrine	100/0.1 w% ,MeOH/NH4TFA	2.7	1.13	1.7
Terbutaline	100/0.1 w% ,MeOH/NH4TFA	2.2	1.93	6.5

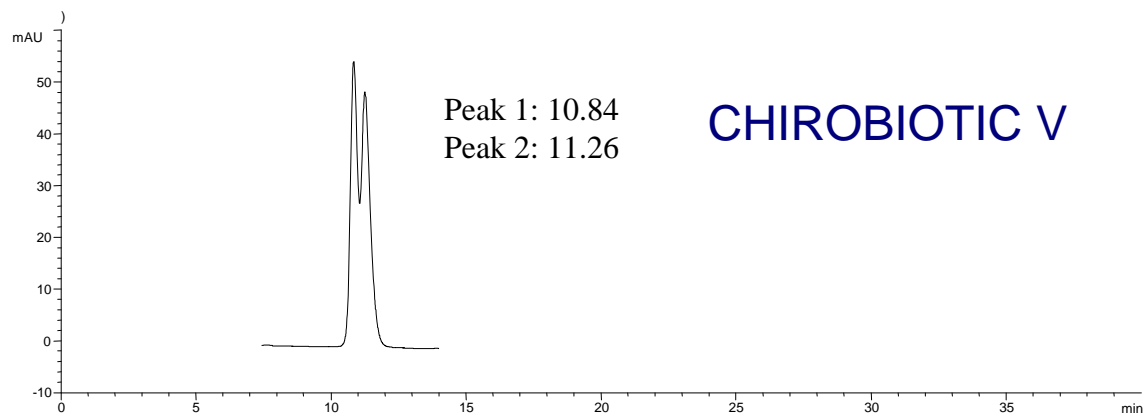
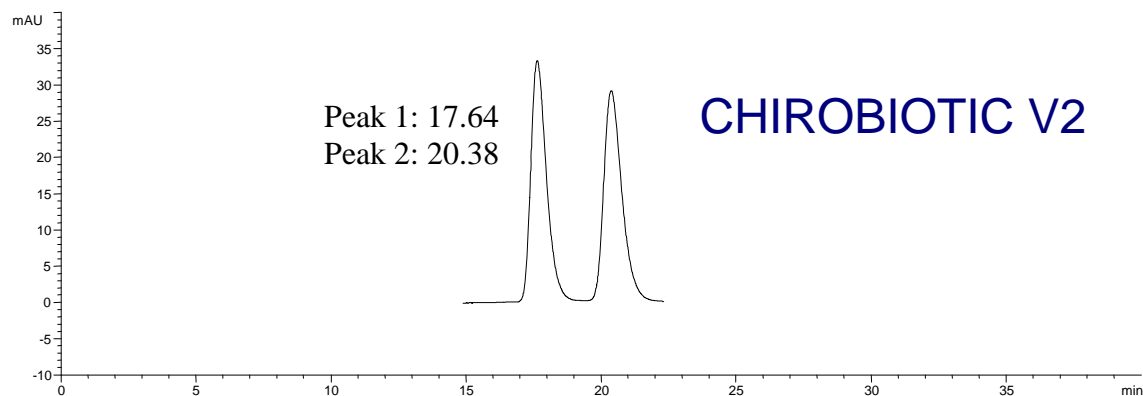
CHIROBIOTIC V2 vs. V

- **Sample: Ritalin**
- Mobile Phase: 95/5, MeOH/20mM NH₄OAc, pH 4.1
- Flow Rate: 1 mL/min.



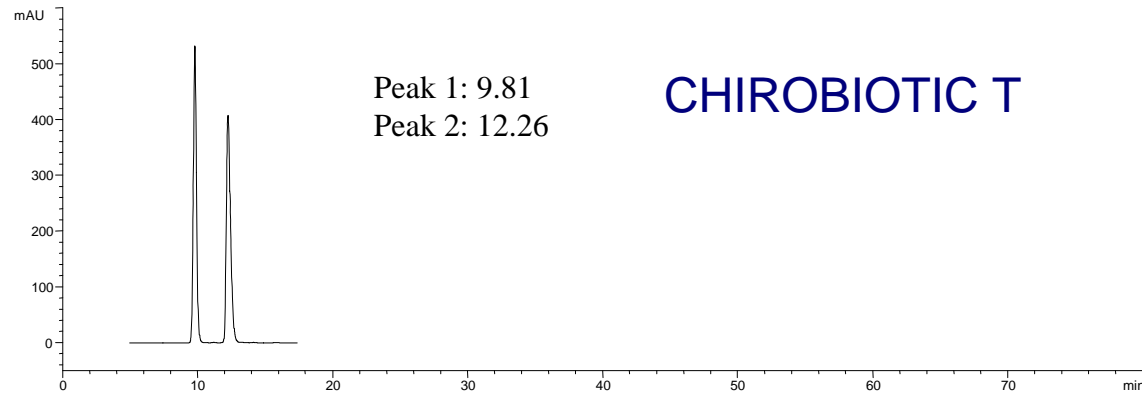
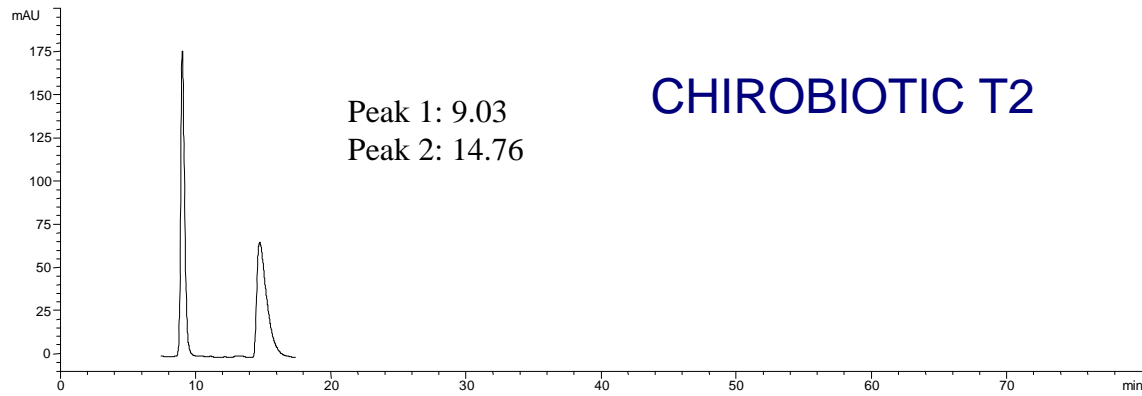
CHIROBIOTIC V2 vs. V

- **Sample: Oxamniquine**
- Mobile Phase: 95/5, MeOH/20mM NH₄OAc, pH 4.1
- Flow Rate: 2 mL/min.



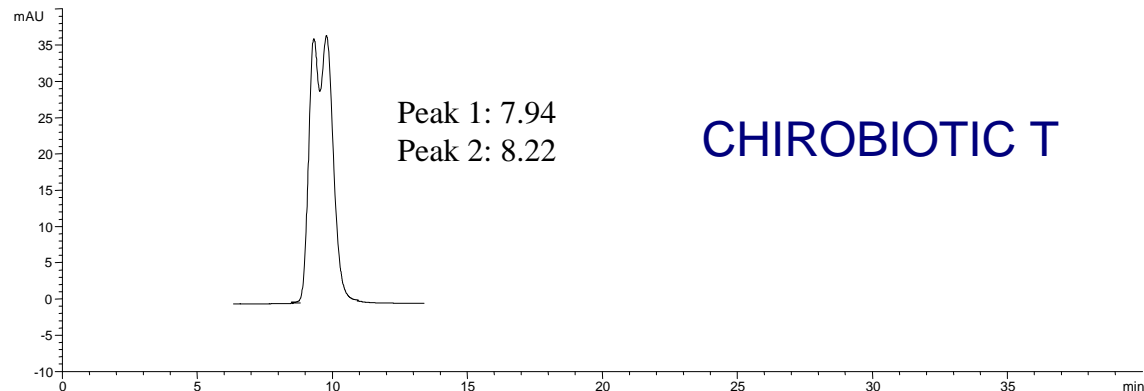
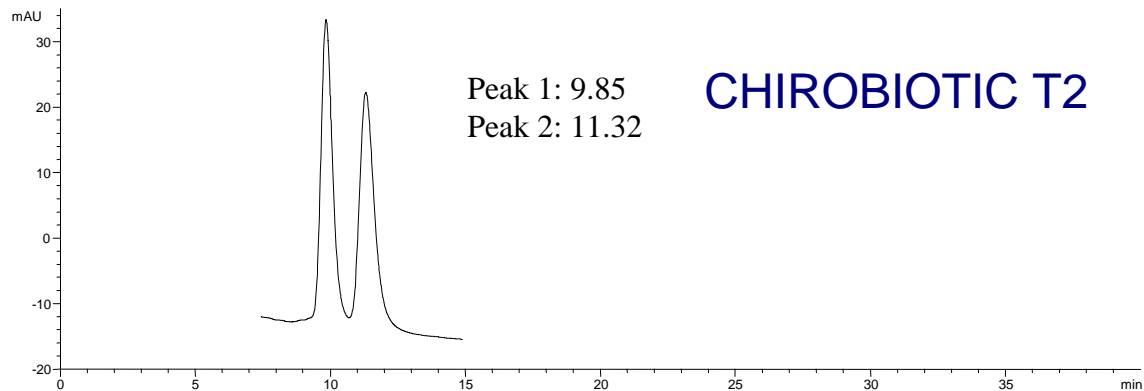
CHIROBIOTIC T2 vs. T

- **Sample: Terbutaline**
- Mobile Phase: 100/0.1w%, MeOH/NH₄TFA
- Flow Rate: 1 mL/min.



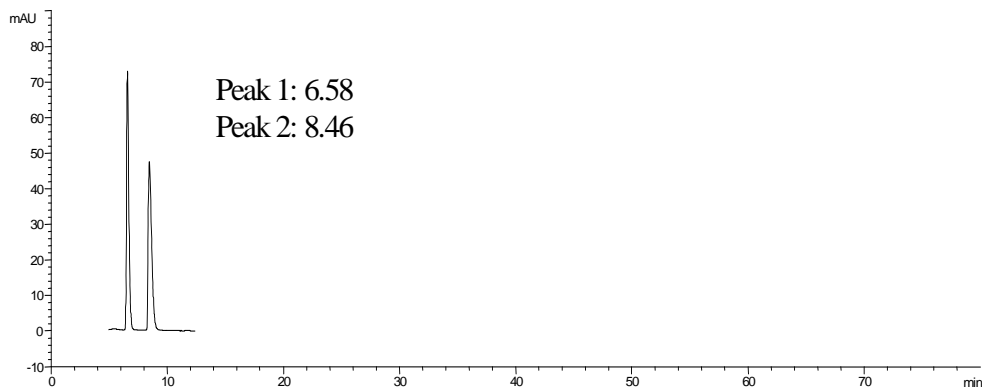
CHIROBIOTIC T2 vs. T

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

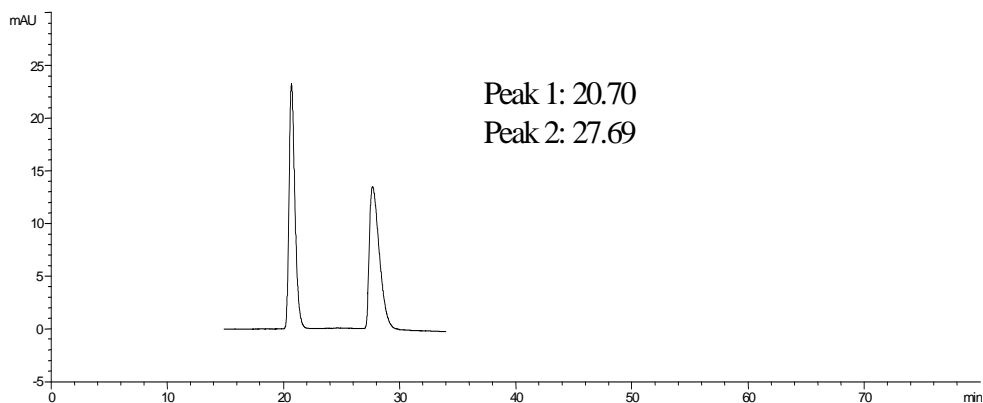


From Polar Ionic to Reversed Phase Mode CHIROBIOTIC V2

- **Sample: Ritalin**
- 100/0.1w%, MeOH/NH₄Formate
- Flow Rate : 1 mL/min.

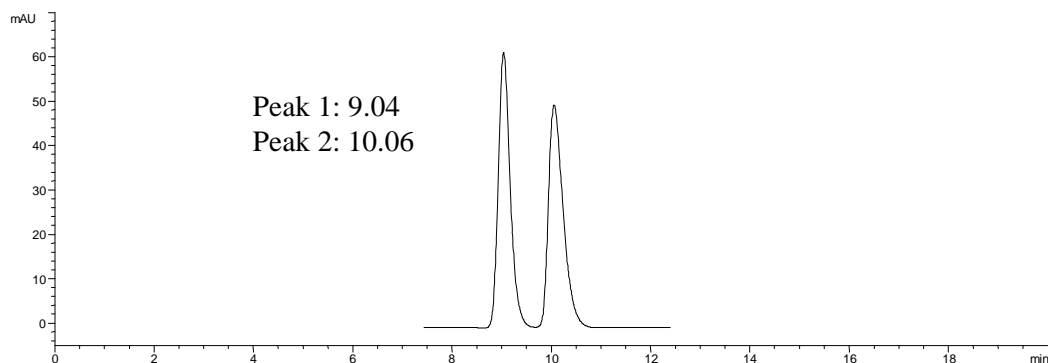


- 95/5, MeOH/20mM NH₄OAc, pH 4.1
- Flow Rate : 1 mL/min.

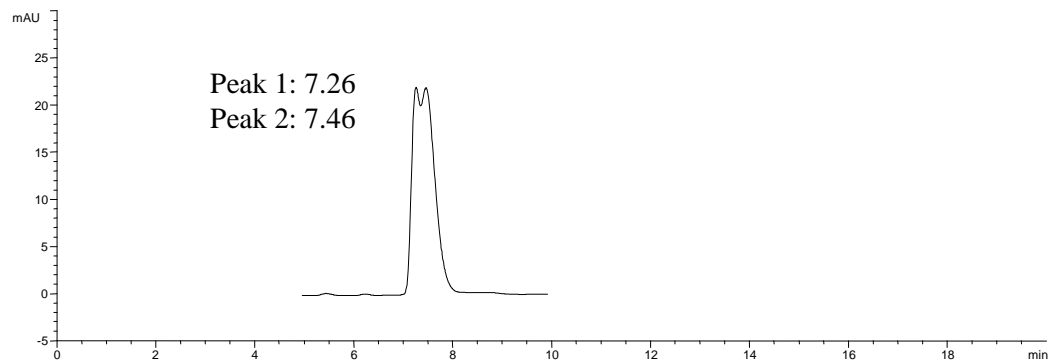


From Polar Ionic to Reversed Phase Mode CHIROBIOTIC V2 (cont'd)

- **Sample : Ritalin**
- 80/20, MeOH/20mM NH₄OAc, pH 4.1
- Flow Rate : 1 mL/min.

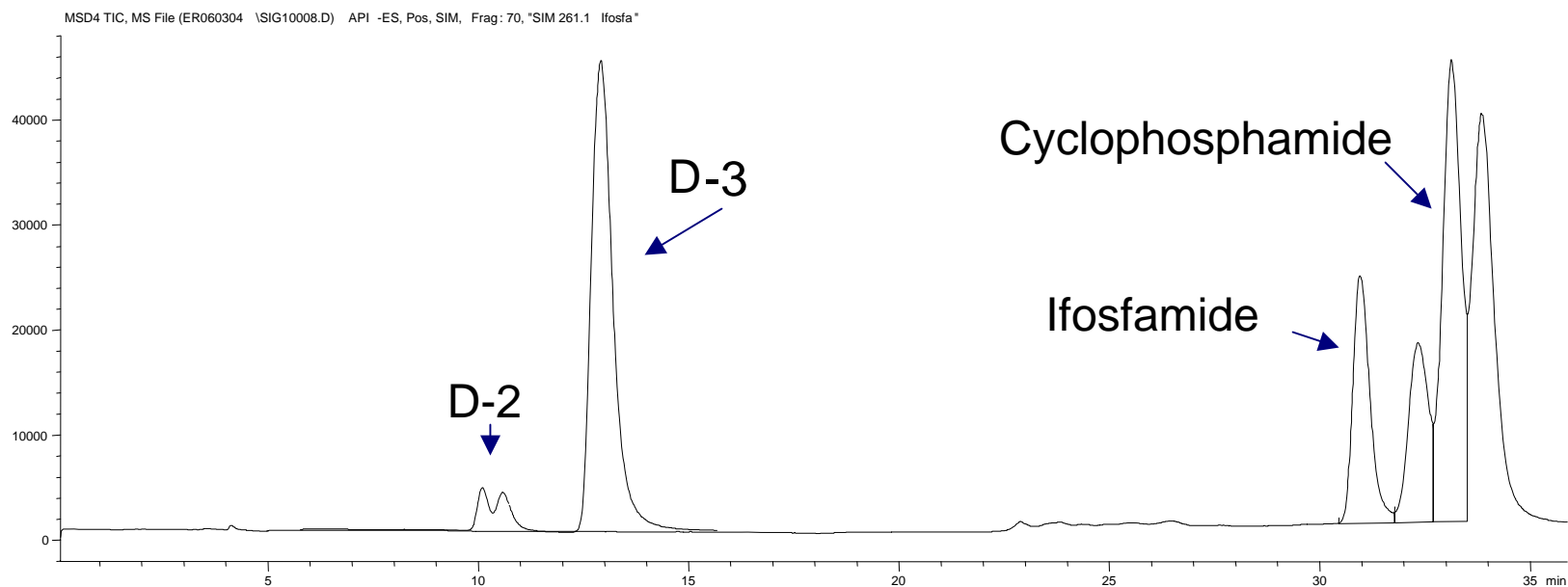


- 50/50, MeOH/20mM NH₄OAc, pH 4.1
- Flow Rate : 1 mL/min.



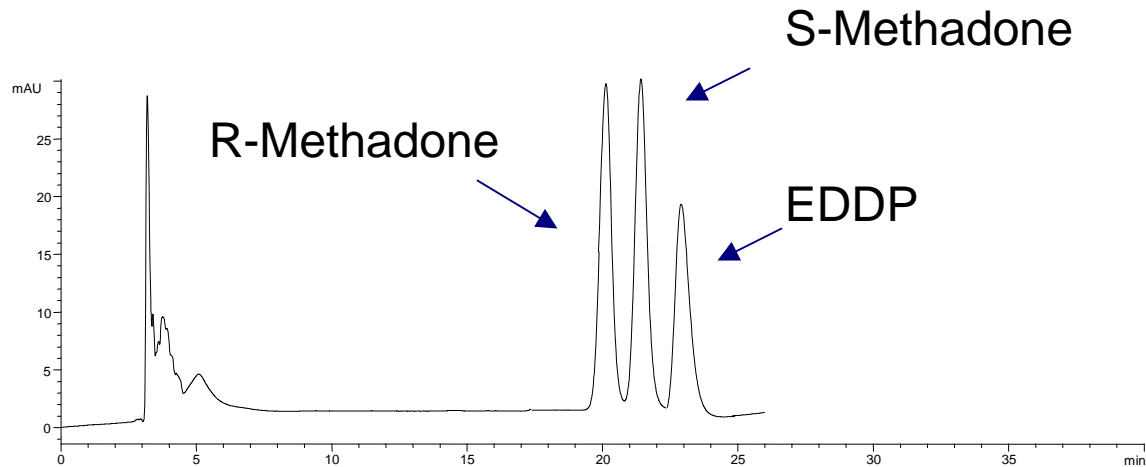
Ifosfamide and its Metabolites Separation

- Column: Chirobiotic T2 (150x4.6mm)+ Astec C18 (100x4.6mm)
- Mobile Phase: IPA/10mMNH₄OAc, Gradient system
- Flow Rate: 0.8 mL/min.



Methadone and its Metabolite Separation

- **Column: CHIROBIOTIC V2**
- **Mobile Phase: 100/0.04/0.007, MeOH/HOAc/NH₄OH**
- **Flow Rate: 0.8 mL/min.**



Conclusions

- CHIROBIOTIC V2 and T2 (200 Å) showed exceptional selectivity towards basic drugs, and there are complementary in nature.
- Unique multi-modal characteristics from high aqueous to 100% organic can be utilized to obtain best separation needs.
- Unique mobile phase designs for ionic interactions can be tailored for LC/MS platforms for biological samples.
- Through multi-linkage, CHIROBIOTIC V2 and T2 also demonstrate very high capacity in a more desirable polar ionic mode for preparative applications.