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# Chiral Method Development in LC/MS Using Macrocyclic Glycopeptide CSPs

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# Abstract

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Chirality has long been a key issue in drug design and discovery processes in the pharmaceutical related industries. The advancement of mass spectrometry (LC/MS/MS) has given researchers/scientists an attractive and powerful tool in the area of chromatography. Due to its sensitivity, robustness and efficiency for qualitative and quantitative determinations of enantiomers, the specificity of tandem MS platforms has been harnessed for analysis of chiral molecules in biological/tissue extracts. Therefore, in HPLC, the demands for compatible mobile phase systems have been on the rise. The mobile phase systems that have been applied in this technique include 100% methanol with small addition of ammonium trifluoroacetate and ammonium formate. In addition, methanol or acetonitrile with aqueous buffer containing the aforementioned additives with proper pH adjustment can be employed effectively. This presentation will focus on applying the column coupling technique onto the MS platform for screening purpose. The column dimensions and the corresponding separation conditions will be mentioned. The feasibility of simultaneous analysis of compound mixtures will also be presented.

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# Introduction

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In HPLC, column coupling technique has been employed in certain areas for the separation of achiral and/or chiral molecule simultaneously<sup>1,2</sup>. For enantiomeric resolution, the macrocyclic glycopeptides vancomycin, teicoplanin and ristocetin A are among the most powerful CSPs for resolving a wide variety of racemic compounds<sup>3-5</sup>. Since the inception of coupling these three chiral stationary phases<sup>6</sup>, it is becoming a popular tool for screening and method development purposes in HPLC. Recently, the advances of mass spectrometry have rendered it a powerful and versatile tool among researchers. This presentation will utilize the mass platform with the chiral column coupling technique to demonstrate the possibility of this combination.

<sup>1</sup>K. Kristensen et al, J. Chromatogr., 666, 283 (1994).

<sup>2</sup>D. Johnson and I. Wainer, Chirality, 8, 551 (1996).

<sup>3</sup>D. Armstrong et al, Anal. Chem. 66, 1473 (1994).

<sup>4</sup>D. Armstrong et al, Chirality, 7, 474 (1995).

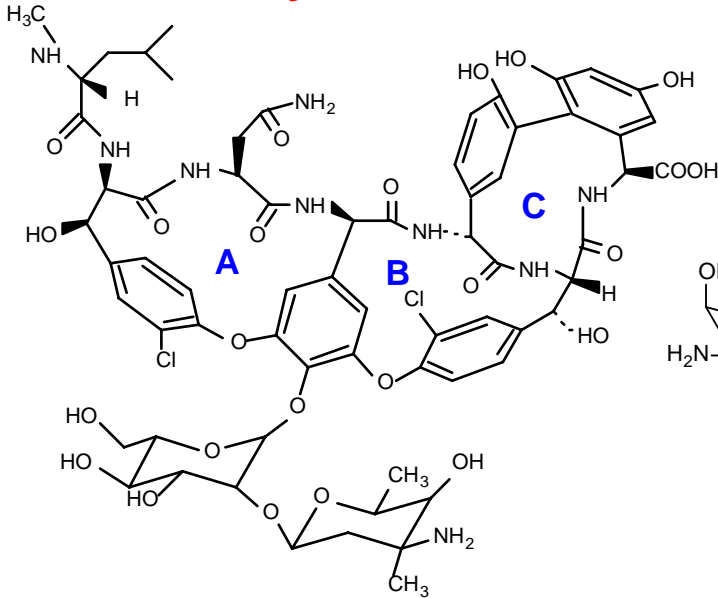
<sup>5</sup>K. Ekborg-Ott et al, Chirality, 10, 434 (1998).

<sup>6</sup>A. Wang et al, LCGC, 18, 626-639 (2000).

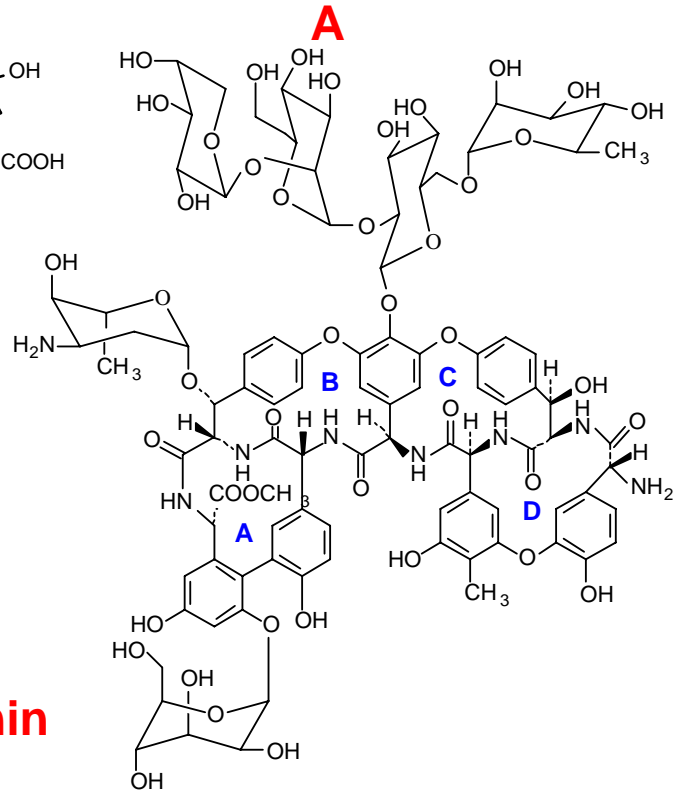
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# Proposed Structures of Glycopeptide CSPs

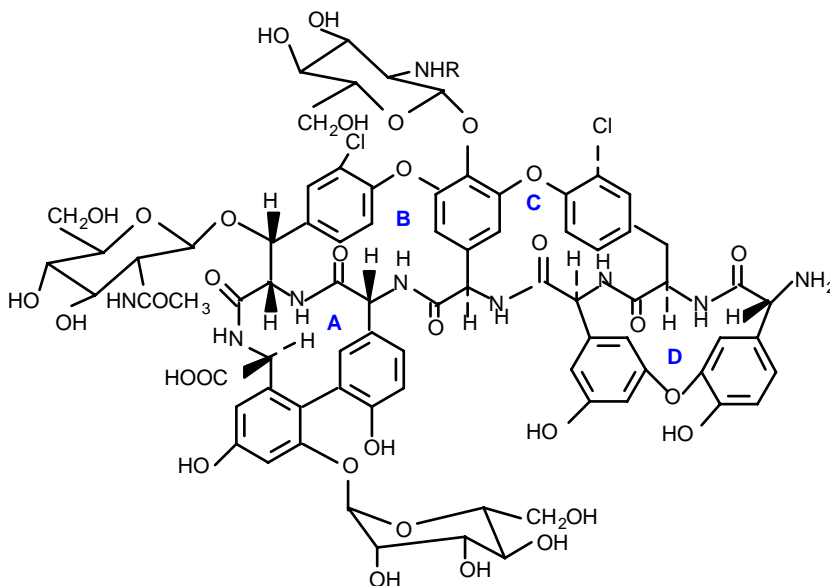
## Vancomycin



## Ristocetin

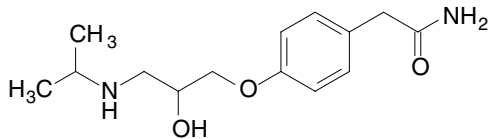


## Teicoplanin

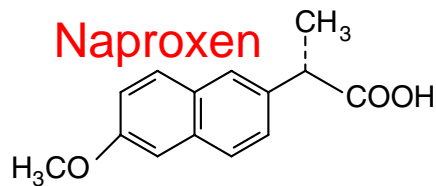


# Analytes

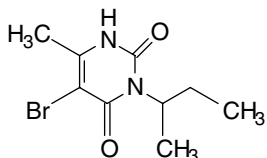
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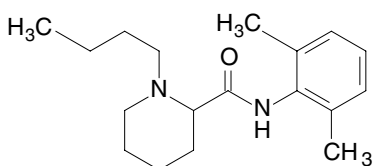
Naproxen



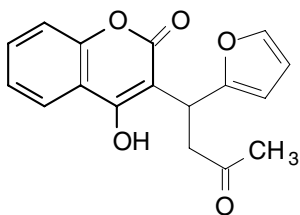
Bromacil



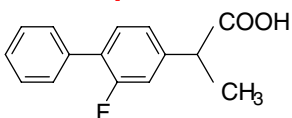
Bupivacaine



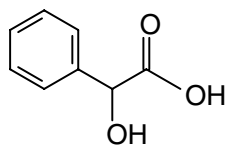
Coumafuryl



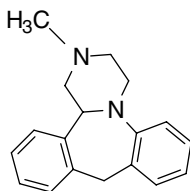
Flurbiprofen



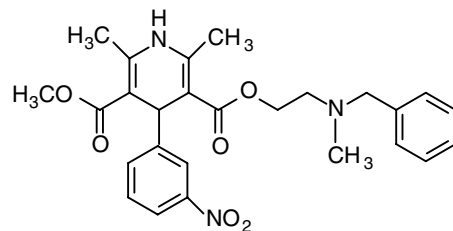
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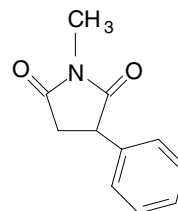
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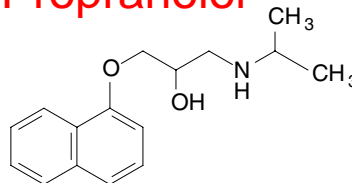
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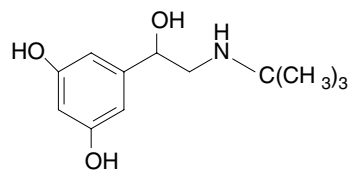
Phensuximide



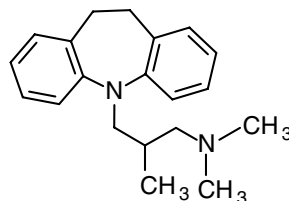
Propranolol



Terbutaline



Trimipramine



# Solvents Compatible for API-MS

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## Suitable for ES and APCI

Methanol  
Ethanol  
Propanol  
Isopropanol  
Butanol  
Acetonitrile  
Water  
DMF  
DMSO  
Acetic Acid  
Formic Acid  
Acetone  
CH<sub>2</sub>Cl<sub>2</sub>  
CHCl<sub>3</sub>

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## Suitable for only APCI

Toluene  
Benzene  
Hydrocarbons  
(e.g., Hexane)  
Styrene  
CCl<sub>4</sub>  
CS<sub>2</sub>  
Cyclic Hydrocarbons  
(e.g.,  
Cyclohexane)

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Note: Macrocyclic glycopeptide CSPs are compatible with all solvents listed above.

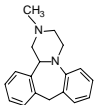
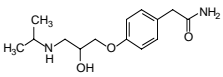
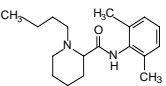
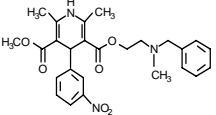
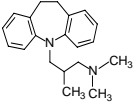
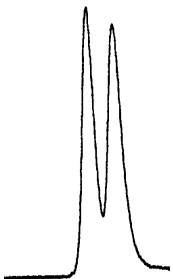
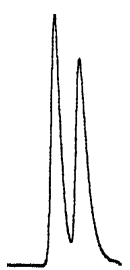
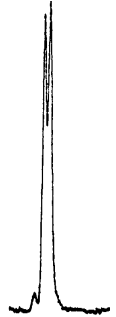
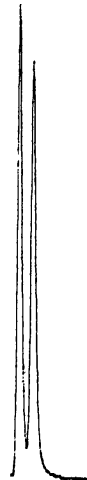

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# Considerations for Using Macrocyclic Glycopeptides in LC/MS/MS Screening Studies

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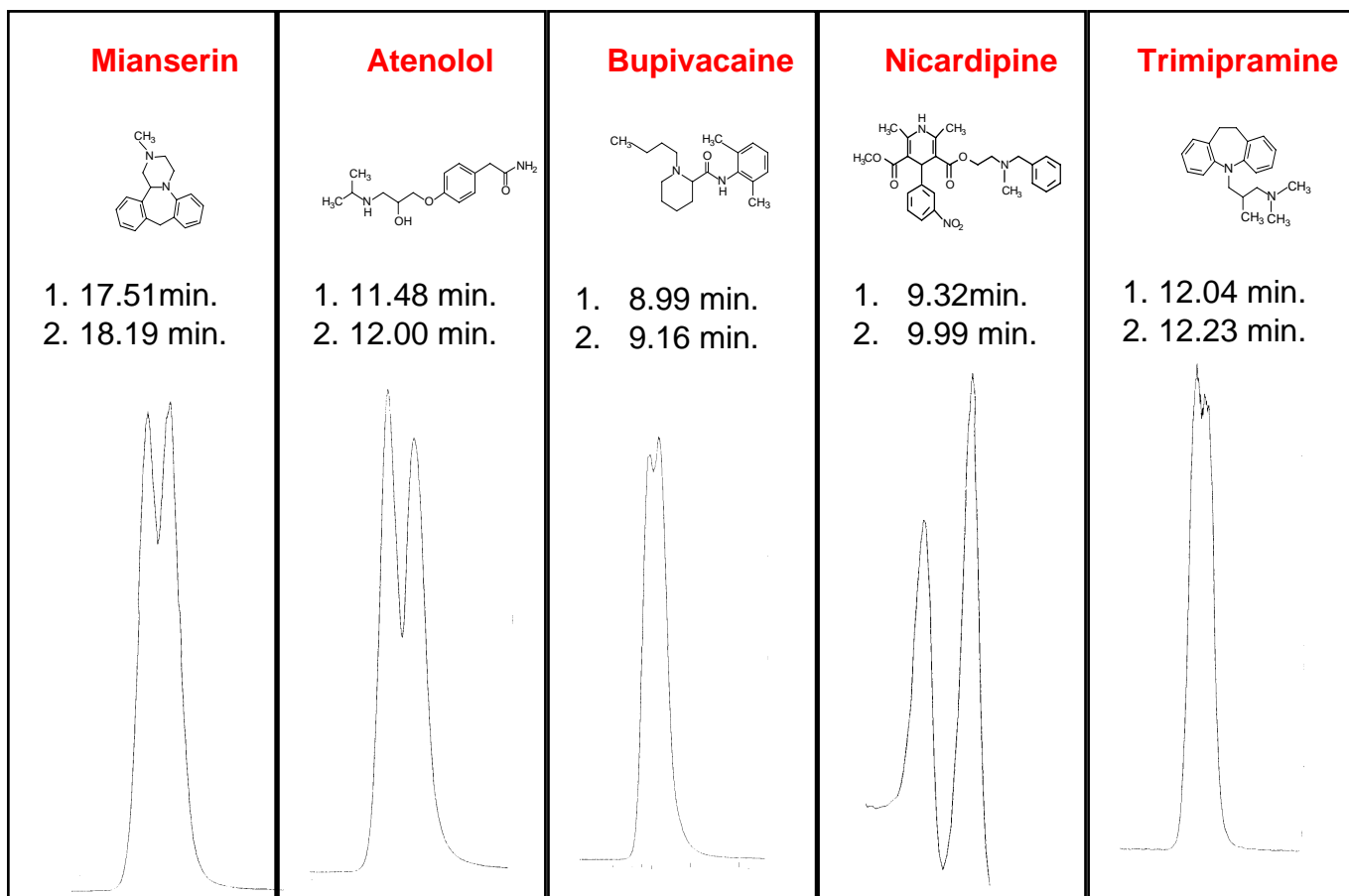
- Efficient separations
  - Faster method development, simple optimization
  - Polar organic mode uses 100 parts methanol in <1 part each acid and base for selectivity. This system has:
    - Lower boiling point than heptane or hexane
    - Less toxic
    - Higher evaporation rate
  - Can use ammonia and TFA or acetic acid modifiers for LC/MS/MS compatibility. The salts of ammonium trifluoroacetate, ammonium formate and ammonium acetate are very useful for this purpose.
  - No solubility problem for salts
-

# HPLC Column Coupling: CHIROBIOTIC R+V+T New Polar Organic Mode

Mianserin	Atenolol	Bupivacaine	Nicardipine	Trimipramine
				
1. 9.99 min. 2. 10.66 min.	1. 14.36 min. 2. 15.62 min.	1. 4.70 min. 2. 4.88 min.	1. 3.69 min. 2. 4.05 min.	1. 11.27 min. 2. 11.54 min.
				

Mobile Phase: 100/0.02/0.01: MeOH/HOAc/TEA  
Flow Rate: 2 mL/min.  
Time Window: 25 minutes

# LC/ES/MS Column Coupling: CHIROBIOTIC R+V+T New Polar Organic Mode

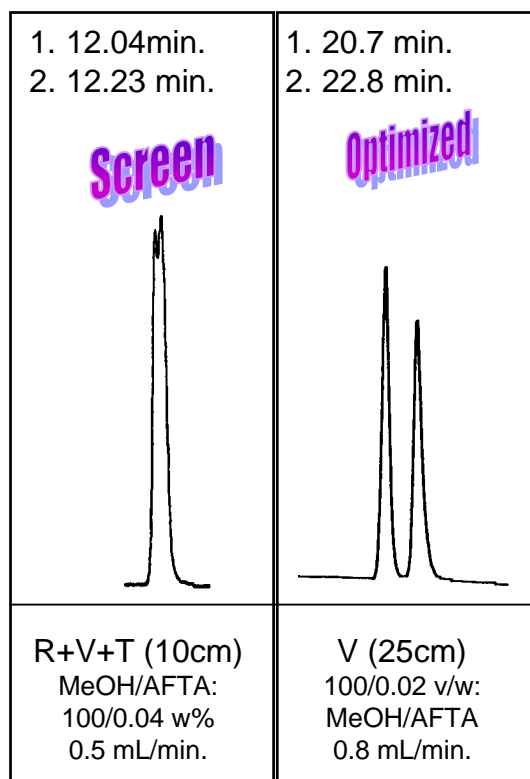
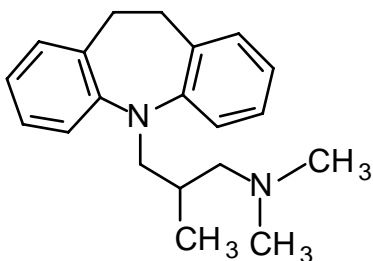


Mobile Phase: 100/0.04w%: MeOH/NH<sub>4</sub>TFA  
Flow Rate: 0.5 mL/min.  
Time Window: 25 minutes

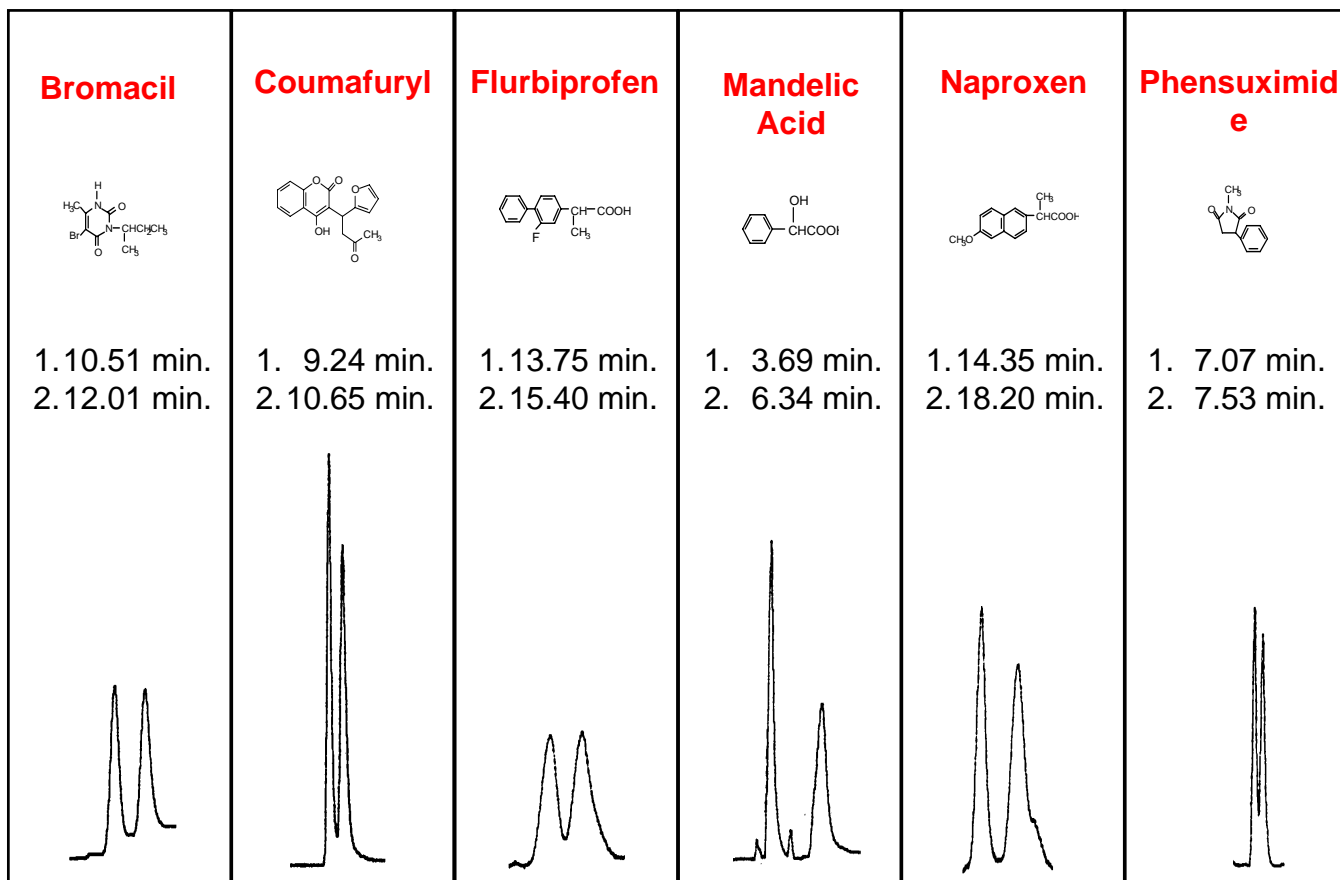
# Optimization in the New Polar Organic Mode for LC/MS

1. Use a single analytical column with screening mobile phase.
2. Evaluate acid and base type, if required (TFA, NH<sub>4</sub>OH, etc.).
3. Investigate acid/base ratios between 4/1 to 1/4.
4. Evaluate the use of salts like ammonium trifluoroacetate, ammonium formate or ammonium acetate and the concentration of acid and base (0.001% to 1%).
5. Evaluate flow rate; lower flow often results in higher resolution.

## Trimipramine

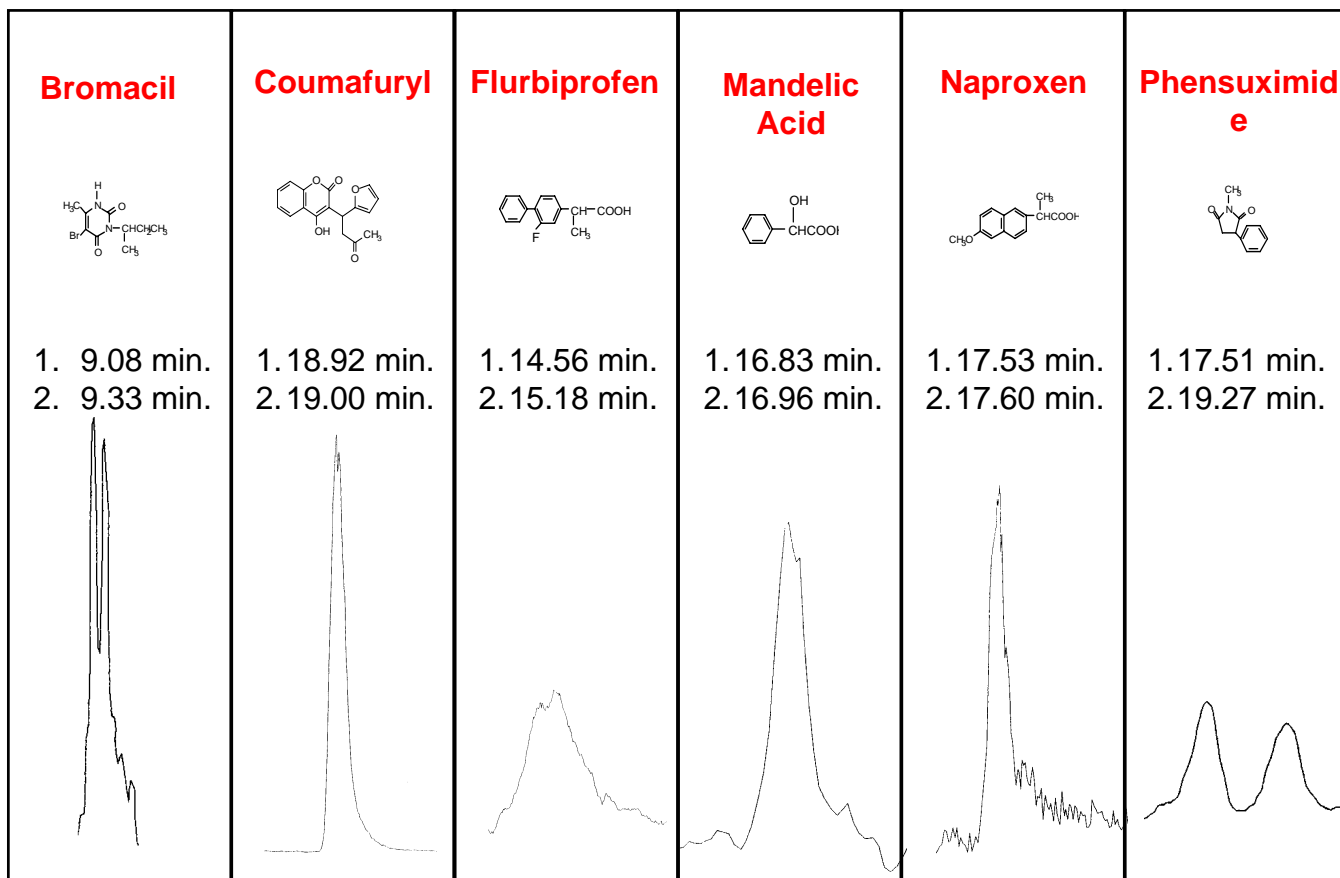


# HPLC Column Coupling: CHIROBIOTIC R+V+T Reversed Phase Mode



Mobile Phase: 25/75: MeOH/0.1% TEAA, pH 6.0  
Flow Rate: 1 mL/min.  
Time Window: 25 minutes

# LC/ES/MS Column Coupling: CHIROBIOTIC R+V+T Reversed Phase Mode



Mobile Phase: 25/75: MeOH/20mM Ammonium Acetate, pH 6.0  
Flow Rate: 0.5 mL/min.  
Time Window: 25 minutes

# LC/APCI/MRM Ion Chromatograms of Racemic Standard on Coupled CHIROBIOTIC R+V+T Columns

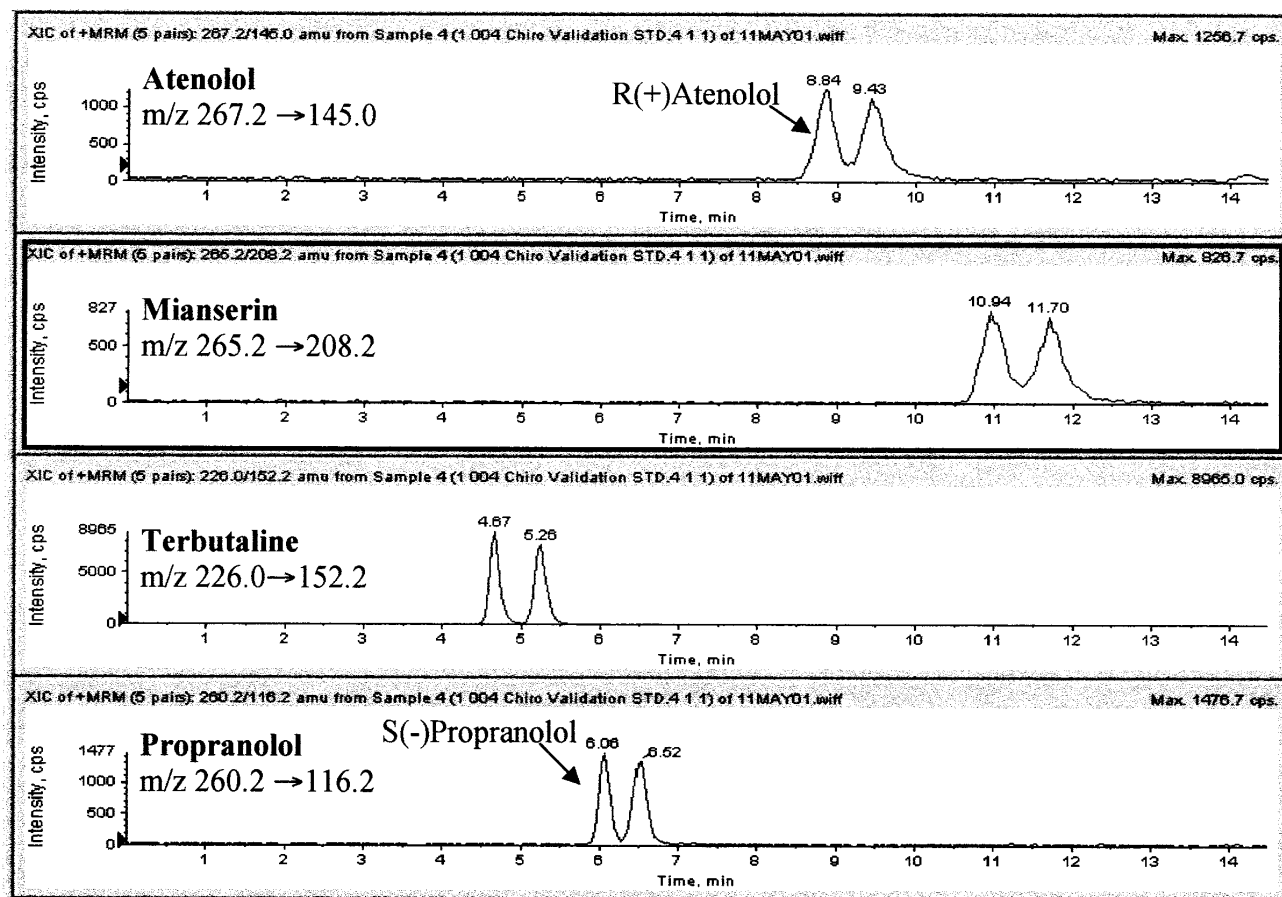


Figure 1. Net solution at 10 ng/mL of: Atenolol (m/z 267.2 → 145.0), Mianserin (m/z 265.2 → 208.2), Terbutaline (m/z 226.0 → 152.2), Propranolol (m/z 260.2 → 116.2).

# LC/APCI/MRM Ion Chromatograms of Racemic Standard on CHIROBIOTIC T Teicoplanin Column

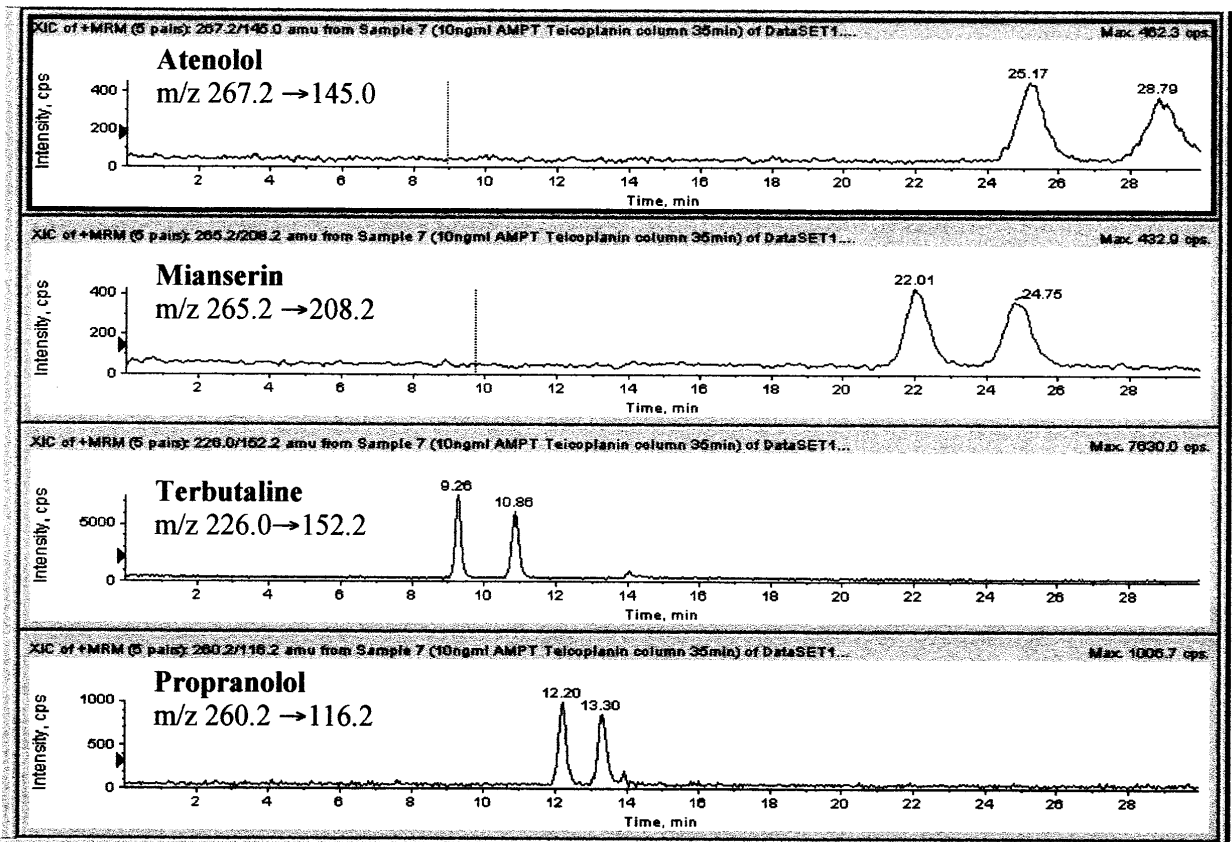


Figure 5. Plasma solution at 10 ng/mL of Atenolol (m/z 267.2 → 145.0), Mianserin (m/z 265.2 → 208.2), Terbutaline (m/z 226.0 → 152.2), Propranolol (m/z 260.2 → 116.2).

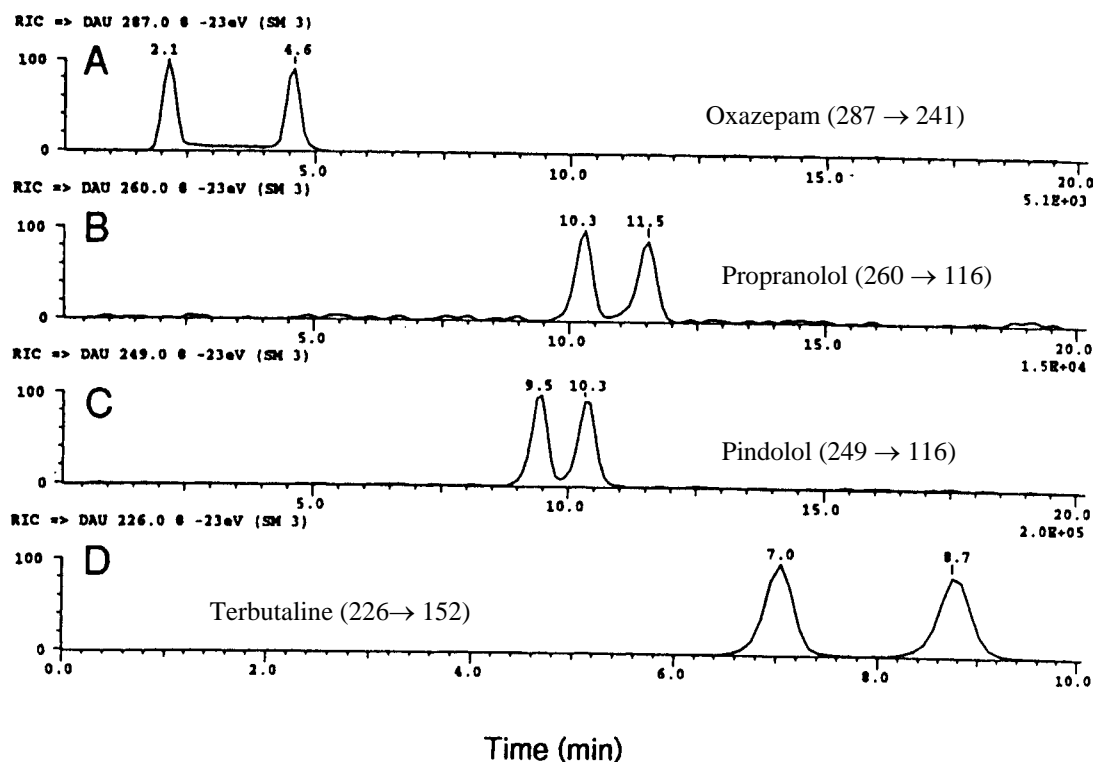
# High Throughput Chiral LC/APCI/MS/MS on Pharmaceuticals

## Simultaneous Detection of Mixtures

Column: CHIROBIOTIC T (150x4.6mm)

Mobile Phase: MeOH/Ammonium trifluoroacetate, 100/0.05 % by weight

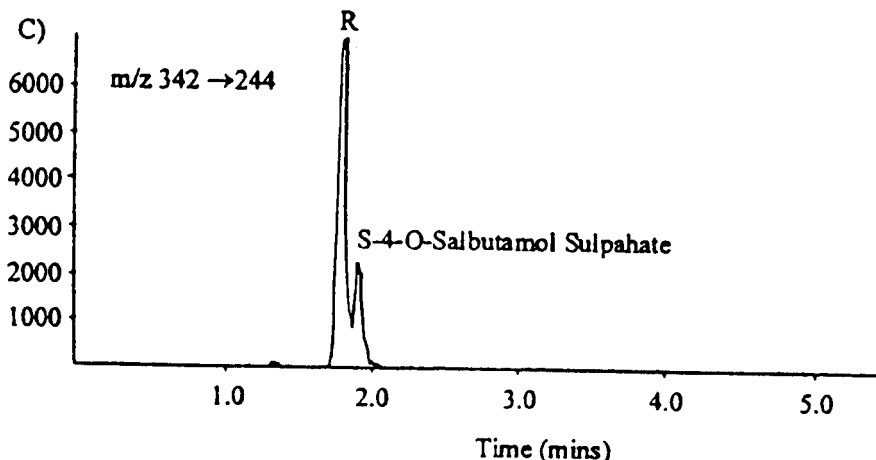
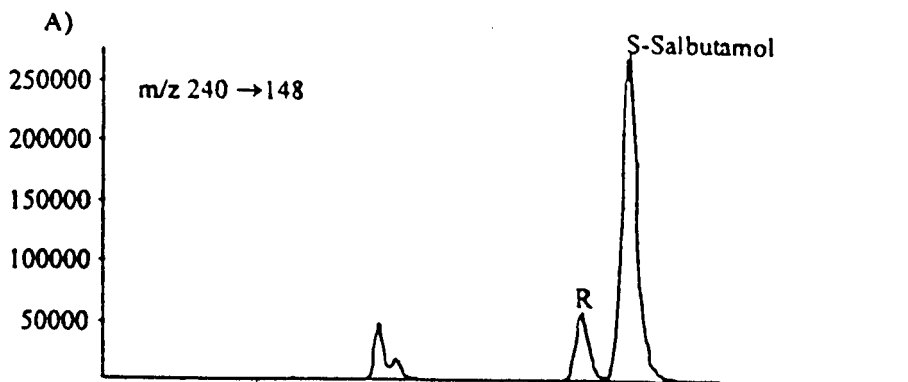
Assay Time: Complete chiral resolution of all four pairs of enantiomers was achieved in ~ 12 minutes.



# Application for Chiral LC/APCI/MS/MS Pharmacokinetics

## Salbutamol and its 4-O-sulphate metabolite by LC/MS/MS\*

- CHIROBIOTIC T (Teicoplanin), 250 x 4.6mm
- MeOH/HOAc/NH<sub>4</sub>OH: 100/0.5/0.1 @ 2 mL/min.
- 3 minute assay/96 well SPE
- 100 pg/mL LOQ for parent compound, 5 ng/mL for sulphate metabolism; 25 pg/mL LOQ for 80 uL injection
- 4000 sample clinical study

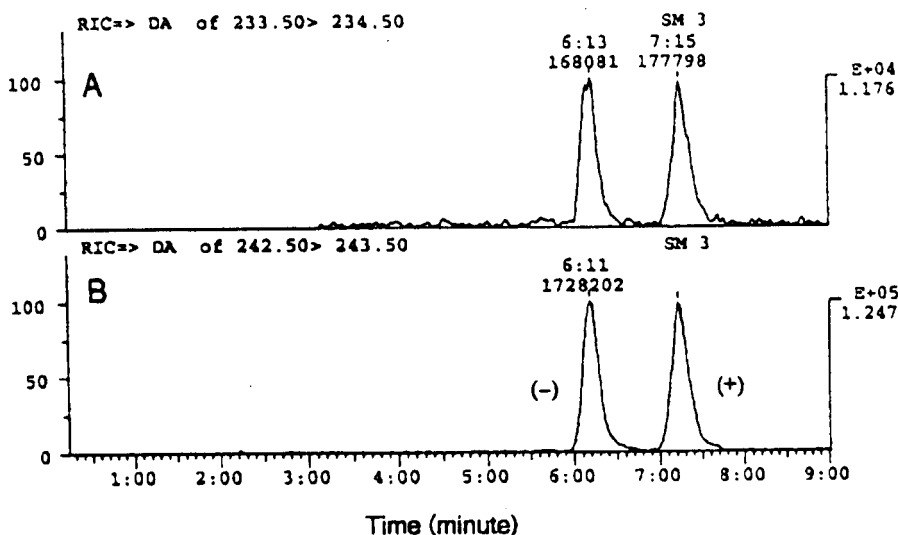


\* Karina B. Joyce, Anne E. Jones, Rebecca J. Scott, Robert A. Biddlecombe, Stephen Pleasance, Drug Metabolism and Bioanalysis, Glaxo Wellcome R&D, Ware, UK, Rapid Communications MS, 12, 1899-1910 (1998)

# Application for Chiral LC/APCI/MS/MS in Pharmacokinetics

## Ritalin (Methylphenidate) Drug Metabolism Study\*

- CHIROBIOTIC V (Vancomycin), 150 x 4.6mm
- MeOH/ammonium trifluoroacetate: 100/0.05% by weight
- Resolution of 1.6
- 7.5 minutes assay time/96 well plate SPE
- 87 pg/mL LOQ via 500  $\mu$ L extraction, 30  $\mu$ L injection
- 2500 samples injected without column deterioration



\* R. Bakhtiar et al, Drug Metabolism and Pharmacokinetics, Novartis, NJ, Rapid Communications MS, 13, 2054-2062 (1999).

# Conclusions

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- Macrocyclic glycopeptides CSPs offer unique opportunities for the powerful LC/MS/MS technique.
  - A wide variety of solvent choices compatible with APCI and ES can be used with these CSP's.
  - The simple mobile phase methanol/ammonium trifluoroacetate offers wide selectivity and versatility.
  - Compatible reversed phase systems are possible on these same CSPs in high organic and ammonium acetate buffers.
  - Column coupling three CHIROBIOTIC phases broadly extends the variety of racemates potentially resolved on these CSPs.
  - Biological samples as well as routine analytes can be easily processed in this system.
-