

**gatec**

# CHIRAL METHOD DEVELOPMENT SCREEN IN DRUG DISCOVERY

**1.**

## COLUMN INSTALLATION

CHIROBIOTIC™ columns are shipped in methanol. Before starting to use a new column, wash with 20 mL HPLC grade methanol at 1 mL/min. The column test standard, 5-methyl-5-phenylhydantoin, can be injected at this stage.

CYCLOBOND™ columns are shipped in IPA and should be washed with 30 mL HPLC grade water at 0.8 mL/min before starting the method development screen.

**2.**

## MOBILE PHASE CHOICE

No.	Mobile Phase	Composition (% v)
<b>REVERSED PHASE MODE:</b>		
1	MeOH/20mM NH <sub>4</sub> OAc, pH 4.0	20/80
2	MeOH/20mM NH <sub>4</sub> OAc, pH 6.0	25/75
3	ACN/20mM NH <sub>4</sub> OAc, pH 4.0	20/80
4	ACN/20mM NH <sub>4</sub> OAc, pH 6.0	25/75
<b>POLAR IONIC MODE®:</b>		
5	MeOH/HOAc/TEA*	100/0.2/0.1
<b>POLAR ORGANIC MODE:</b>		
6	ACN/MeOH/HOAc/TEA	95/5/0.3/0.2
	If not progressing to normal phase, wash with MeOH at this stage to test and store the column.	100% MeOH
<b>NORMAL PHASE MODE:</b>		
7	EtOH/Hexane (or heptane, isohexane)**	20/80
8	Washing cycle	100% EtOH
9	Column storage	100% IPA or MeOH**

\* Use salts (NH<sub>4</sub>O<sub>2</sub>CCF<sub>3</sub> for bases, NH<sub>4</sub>OAc for acids) when developing methods for prep.

\*\* If stored in MeOH, wash with EtOH before proceeding to normal phase.

**3.**

## COLUMN CHOICE AND RUN TABLE

Select your choice of columns from the list below. For a 6-column switching system, we recommend CHIROBIOTIC V, T, R and CYCLOBOND I 2000, SN and RSP.

No.	Column Type (250x4.6mm)	1	2	3	4	5	6	7
I	CHIROBIOTIC V			y	y	y		y
II	CHIROBIOTIC T	y	y			y		y
III	CHIROBIOTIC R	y	y			y		y
IV	CHIROBIOTIC TAG	y	y			y		y
V	CYCLOBOND I 2000 SN	y		y			y	y
VI	CYCLOBOND I 2000	y		y			y	y
VII	CYCLOBOND I 2000 DMP	y		y			y	y
VIII	CYCLOBOND I 2000 RSP	y		y			y	
IX	CYCLOBOND I 2000 AC	y		y			y	

**4.**

## RUN CONDITIONS

Flow Rate: 1.0 mL/min.  
 Equilibration Time: 25 minutes  
 Run Time: 25 minutes  
 Temperature: Ambient  
 Detector: UV  
 Sample: 1 mg/mL in MeOH

## Notes

- The recommended protocol assumes the use of 250 x 4.6mm columns. For 100 x 4.6mm columns, use the same conditions at 0.5 mL/min.
- It is permissible to run straight from the reversed phase to the polar ionic mode®, and from the polar ionic mode to normal phase without an intermediate solvent wash.
- If any screening run results in a retention time less than 5 minutes, reduce the strength of the mobile phase and re-run. Aim for retention times from 10 to 20 minutes. In reversed phase mode reduce organic component, in polar ionic mode® or polar organic mode reduce acid/base concentration. Retention times can be later reduced in the optimization process.
- If a separation occurs in the polar ionic mode®, for a neutral molecule, change to 100% organic solvent (i.e. MeOH, EtOH or ACN).
- If the compound does not elute in reversed phase, increase the organic content to 40%. In the polar ionic mode®, increase the acid/base concentration up to 1.0/0.5. In the polar organic mode for the CYCLOBOND columns, increase the MeOH concentration up to 10%.

**5.**

## OPTIMIZATION PROCEDURES

Polar ionic mode® (CHIROBIOTIC phases only)	<ul style="list-style-type: none"> <li>● Test alternative acid/base ratios (generally higher acid for basic molecules, higher base for acidic molecules)</li> <li>● Change the acid/base to a salt (ammonium trifluoroacetate, formate or acetate at a concentration of 0.1%)</li> </ul>
Polar organic mode	<ul style="list-style-type: none"> <li>● Eliminate MeOH</li> <li>● Test alternative acid/base ratios</li> </ul>
Reversed phase mode	<ul style="list-style-type: none"> <li>● Test smaller pH changes</li> <li>● Change organic to THF, ACN, MeOH</li> <li>● Change buffer type and buffer concentration</li> <li>● Change temperature</li> </ul>
Normal phase mode	<ul style="list-style-type: none"> <li>● Change EtOH concentration</li> </ul>

**6.**

## OPTIMIZING FOR MS DETECTION

- Use salts, as in Step 5, when using the polar ionic or polar organic modes.
- Use ammonium acetate or formate when using reversed phase.

**7.**

## RETESTING YOUR METHOD DEVELOPMENT COLUMNS

To ensure the selectivity performance of CHIROBIOTIC columns, periodically test with 5-methyl-5-phenylhydantoin in 100% MeOH. For testing CYCLOBOND columns, please refer to your CYCLOBOND Handbook.

### ADVANCED SEPARATION TECHNOLOGIES

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