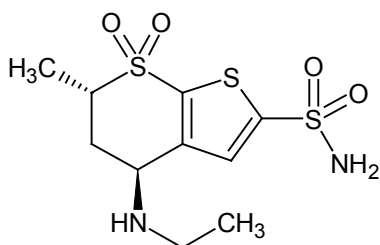


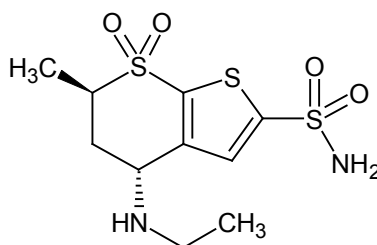
## HPLC Chiral Screening Report

Analyte Description:	Dorzolamide Hydrochloride (CAS: 130693-82-2, USP 1225281), Dorzolamide Hydrochloride Related Compound A (CAS: N/A, USP 1225292, [(4R, 6R)-4-(Ethylamino-5,6-dihydro-6-methyl-4H-thieno[2,3-b]thiopyran-2-sulfonamide 7,7-dioxide, monohydrochloride)])
Supelco Sample No.:	R&D Application Request #739; Notebook 1688-18
Quote No.:	
Report to:	Internal

The sample has been tested through our method development protocol employing 3 CHIROBIOTIC (V2, T, TAG) columns and 3 CYCLOBOND (B-CD, DNP, and HP-RSP) columns with a combination of mobile phases encompassing polar ionic (PI), reversed-phase (RP), polar organic (PO) and normal-phase (NP) chromatographic modes of operation.



Dorzolamide  
 Hydrochloride




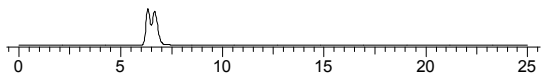

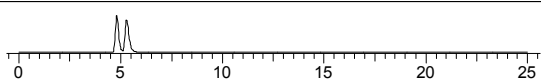
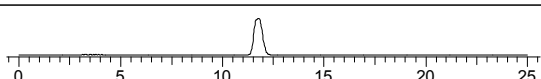
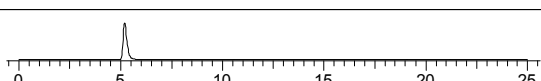
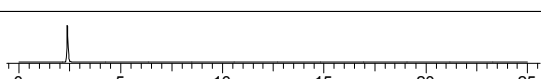
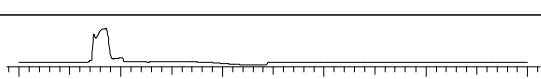
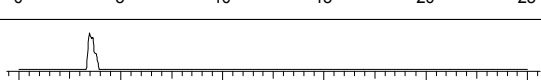

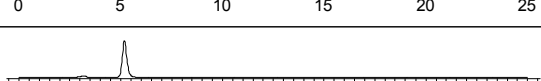
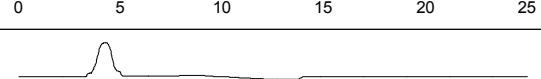
Dorzolamide Hydrochloride  
 Related Compound A

### Positive Results of Primary Screen:

The following combinations of stationary phase and chromatographic mode provided evidence of enantiomeric selectivity:

- CHIROBIOTIC V2: PI mode (best)
- CHIROBIOTIC V2: RP mode
- CHIROBIOTIC TAG: PI mode

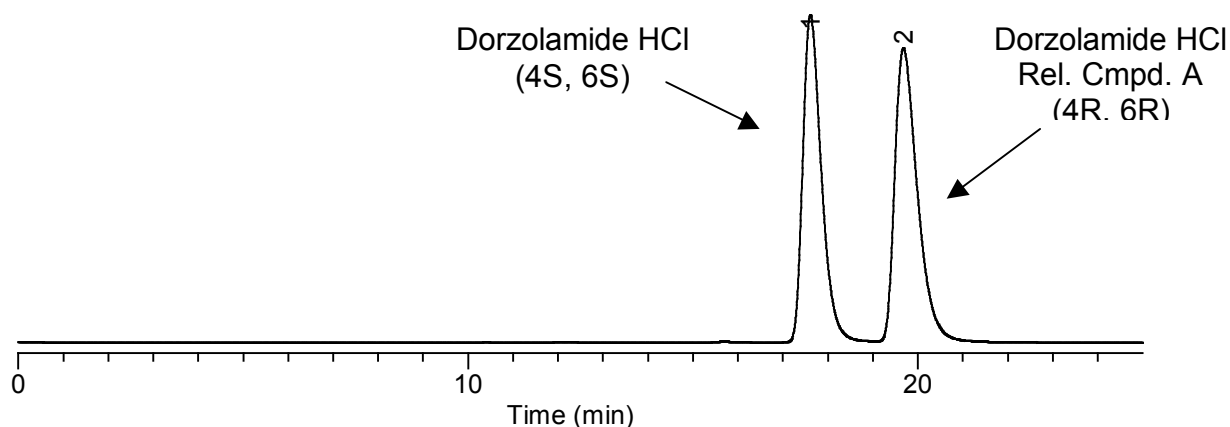
Summary of Primary Screen:

Spectrum	Column	mode	elution	File
	CHIROBIOTIC TAG	RP	No Retention	C:\asci\IAC D_Chrom1 373.cdf
	CHIROBIOTIC TAG	PIM	Separation	C:\asci\IAC D_Chrom1 384.cdf
	CHIROBIOTIC V2	RP	Separation	C:\asci\IAC D_Chrom1 397.cdf
	CHIROBIOTIC V2	PIM	Separation	C:\asci\IAC D_Chrom1 404.cdf
	CHIROBIOTIC T	RP	No Separation	C:\asci\IAC D_Chrom1 416.cdf
	CHIROBIOTIC T	PIM	No Separation	C:\asci\IAC D_Chrom1 423.cdf
	Cyclobond I 2000	RP	No Retention	C:\asci\IAC D_Chrom1 435.cdf
	Cyclobond I 2000	POM	Unknown	C:\asci\IAC D_Chrom1 442.cdf
	Cyclobond 2000 HP-RSP	RP	No Retention	C:\asci\IAC D_Chrom1 461.cdf
	Cyclobond 2000 HP-RSP	POM	Unknown	C:\asci\IAC D_Chrom1 485.cdf
	Cyclobond 2000 DNP	RP	No Separation	C:\asci\IAC D_Chrom1 497.cdf
	Cyclobond 2000 DNP	POM	Unknown	C:\asci\IAC D_Chrom1 504.cdf

Chromatographic Results:

1:1 Dorzolamide HCl:Dorzolamide Related Compound A:

UV



Conditions:

Column: CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)  
Mobile Phase: 5:100, H<sub>2</sub>O:0.05 % w/v Ammonium TFA in Methanol  
Temperature: 22 °C  
Flow Rate: 0.3 mL/min  
Detection: UV at 254 nm  
Injection Volume: 10 µL  
Sample: 1.0 mg/mL in MeOH

Peak 1 retention time (R<sub>t1</sub>): 17.61 min.

Peak 2 retention time (R<sub>t2</sub>): 19.68 min.

Recommendation for Optimization/Conclusions:

1. The best resolution of the chiral enantiomers Dorzolamide Hydrochloride and Dorzolamide Hydrochloride Related Compound A was observed on the CHIROBIOTIC V2. The aforementioned method was used for confirmation and optimization purposes.
2. The buffer salt ammonium trifluoroacetate was employed to sharpen the peaks, and thus, increase resolution.
3. The two enantiomers were successfully separated in under 25 minutes, and it was determined that Dorzolamide Hydrochloride (4S, 6S) elutes before Dorzolamide Hydrochloride Related Compound A under these conditions.