

# Utility and Stability of A New High Enantioselective Dinitrophenyl Substituted Cyclodextrin LC Stationary Phase

Brian L. He<sup>†</sup>, Thomas E. Beesley<sup>†</sup>,

Qiqing Zhong<sup>‡</sup>, Walter S. Trahanovsky<sup>‡</sup>

<sup>†</sup>Advanced Separation Technologies Inc. (astec), 37 Leslie Court, P.O.  
Box 297, Whippany, NJ 07981, USA

<sup>‡</sup>Iowa State University, Department of Chemistry, Gilman Hall, Ames,  
IA 50011, USA

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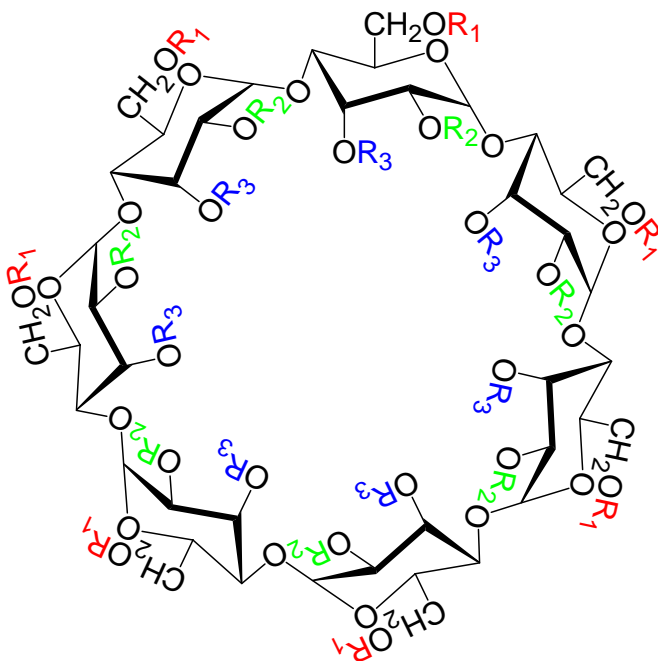
# Abstract

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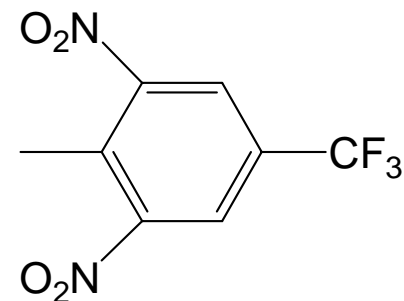
A stable dinitrophenyl substituted  $\beta$ -cyclodextrin derivative has been successfully synthesized and evaluated as LC chiral stationary phase (CSP). After covalently bonding through an ether linkage to 5 $\mu$ m spherical silica particles, the new CSP showed broad enantio-selectivity towards a variety of chiral compounds specifically with aromatic groups. Nearly 60% of 230 tested chiral molecules were resolved in reversed phase, polar organic or normal phase conditions. Reversed phase mode proved to be the most useful mobile phase mode. The bonded dinitrophenyl substituted  $\beta$ -cyclodextrin CSP, which is designated as CYCLOBOND I 2000 DNP, exhibited exceptional stability under all experimental conditions. No degradation of column performance was observed after more than 1000 injections.

This poster will focus on the applications of this new CYCLOBOND I 2000 DNP. Its chromatographic behavior and strategies to optimize a chiral separation will also be discussed.

# Structure of CYCLOBOND I 2000 DNP



$R_1, R_2, R_3 = \text{H, or}$



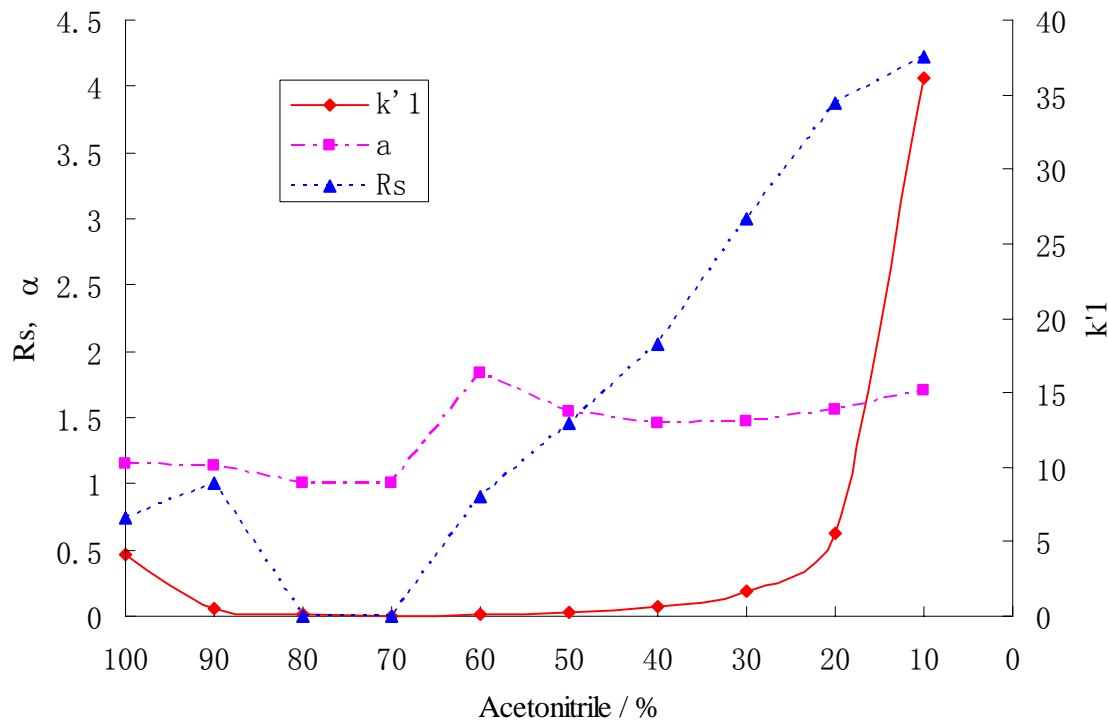


# Advantages of CYCLOBOND I *2000* DNP

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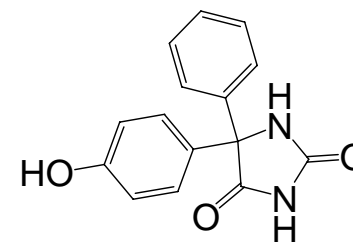
- Enhanced enantio-selectivity over typical CYCLOBOND chiral stationary phases
- Stable  $\pi$ -electron sharing derivative > 1000 injections
- Separations obtained in the reversed phase, polar organic and normal phase modes
- Highest selectivity observed in the reversed phase mode

# % ACN Effect on Retention, Selectivity and Resolution



Compound:

DL-5-(4-hydroxyphenyl)-  
5-phenylhydantoin



Column:

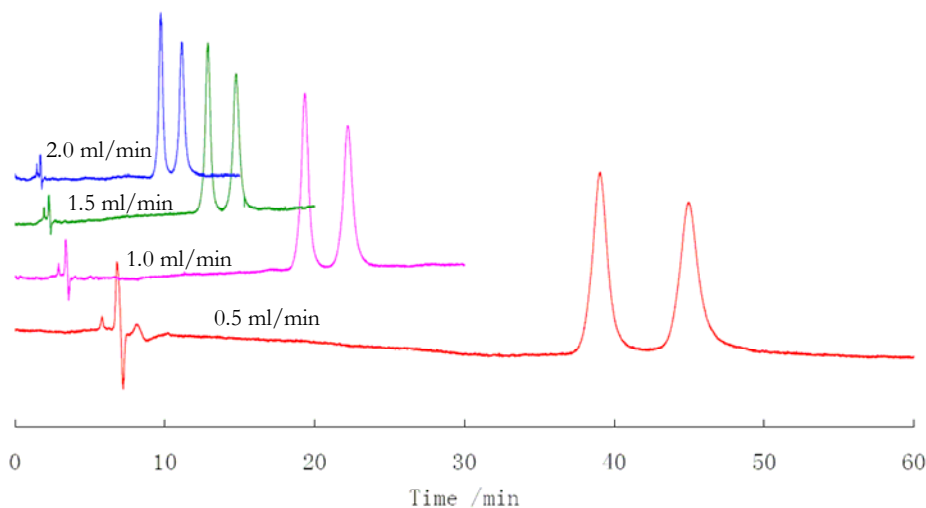
CYCLOBOND I 2000 DNP

Buffer:

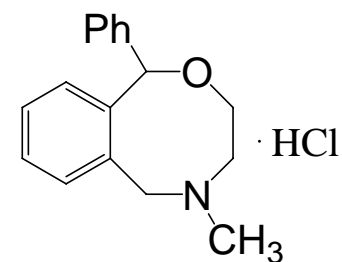
TEAA 0.1%, pH4.1

UV@254nm; Ambient

# Flow Rate Effects



Compound:  
Nefopam hydrochloride

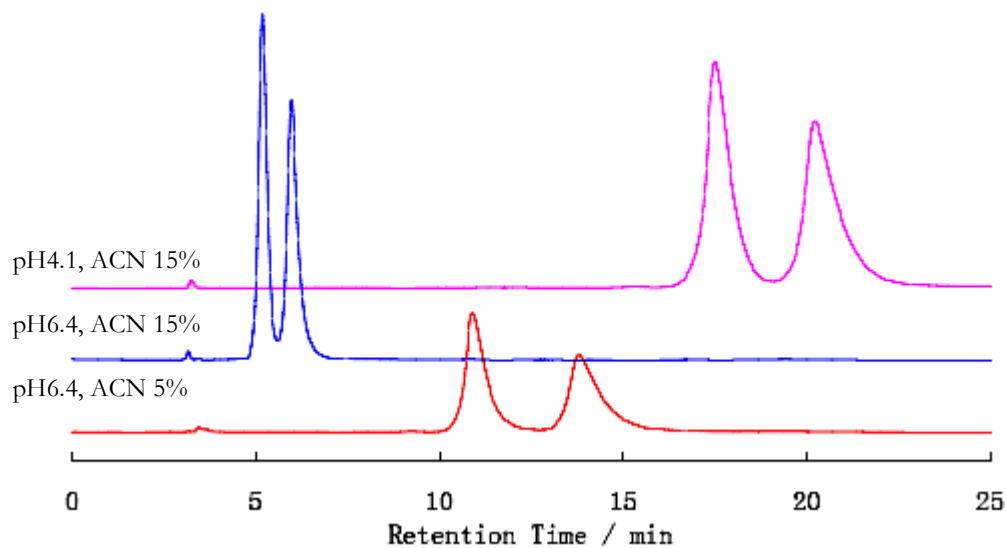


Column:  
CYCLOBONDI 2000 DNP

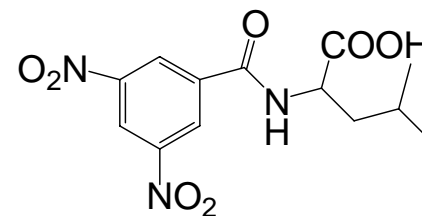
Mobile Phase: 80/20/0.1:  
ACN/H<sub>2</sub>O/TEAA pH4.1  
UV@264nm

Flow rate ml/min	$k'_1$	$\alpha$	$R_s$	$N$
0.5	5.66	1.18	2.70	6237
1.0	5.67	1.17	2.66	6183
1.5	5.67	1.17	2.51	5811
2.0	5.64	1.17	2.38	5779

# pH Effects



Compound:  
**N-(3,5-dinitrobenzoyl)-  
D,L-Leucine**

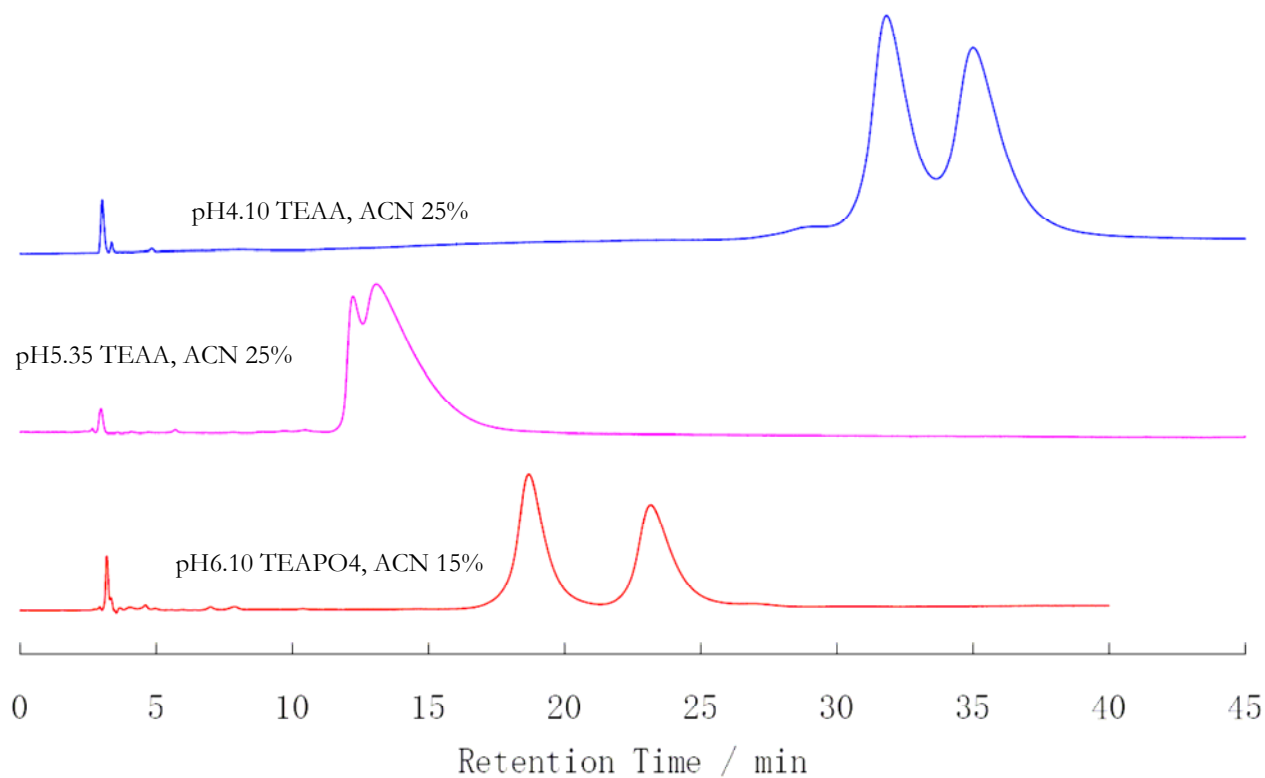


Column:  
CYCLOBONDI 2000 DNP

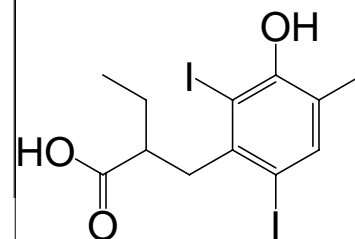
Buffer: TEAA 0.1%

UV@254nm; Ambient

# Buffer: TEAA vs. TEAPO<sub>4</sub>



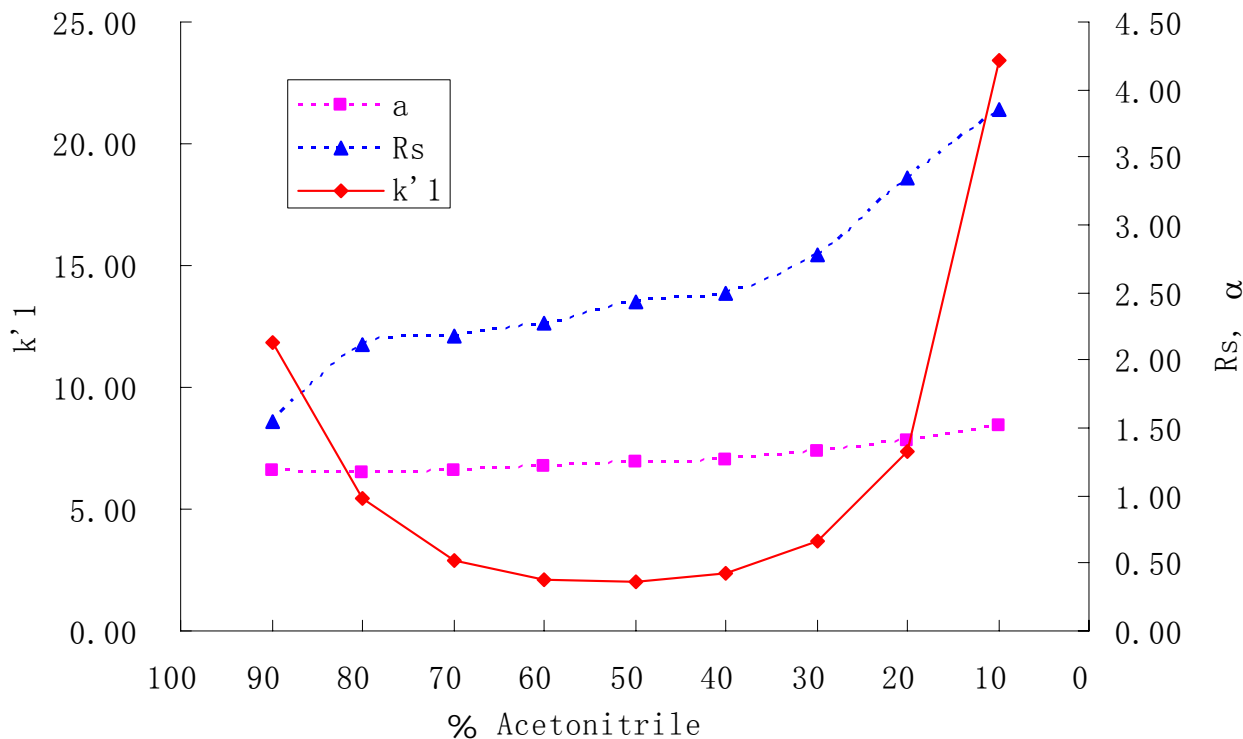
Compound:  
Iophenoxic acid



Column:  
CYCLOBOND I 2000 DNP

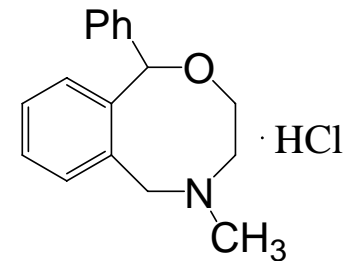
UV@254nm; Ambient

# Resolution, Selectivity and Retention as a Function of %ACN



Compound:

**Nefopam HCl**



Column:

CYCLOBOND I 2000 DNP

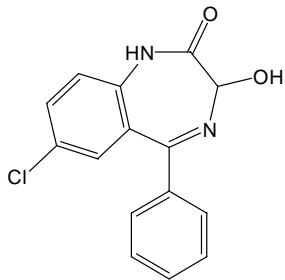
Buffer:

TEAA 0.1%, pH4.1

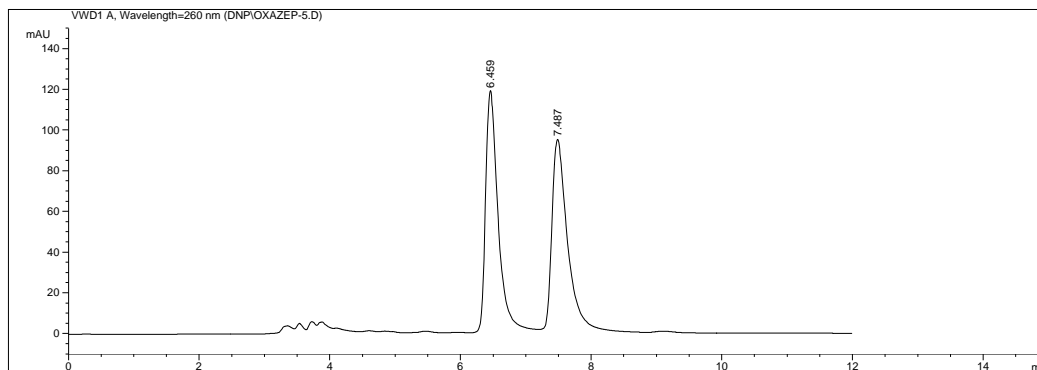
UV@264nm; Ambient

# Polar Organic Mode

Compound: Oxazepam

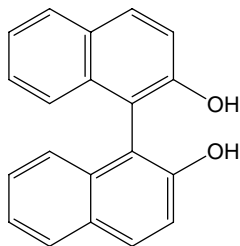


Mobile phase: ACN  
1.0ml/min; 25°C;  
UV@260 nm

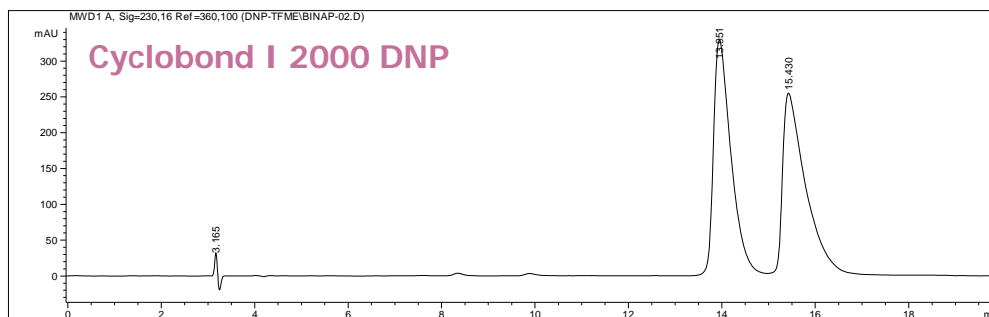
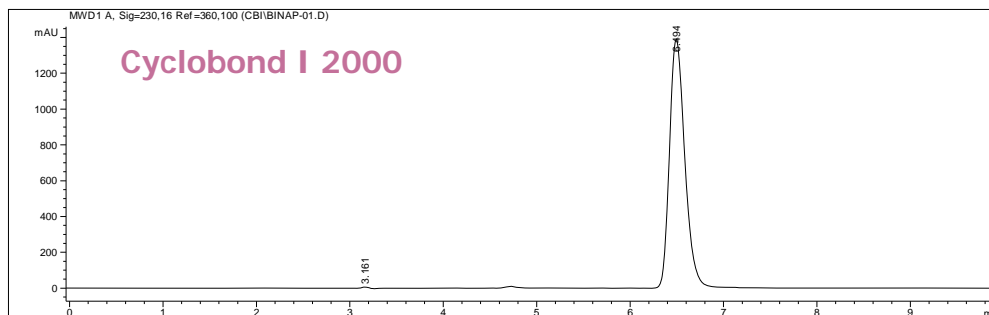


# Cyclobond I 2000 DNP vs. Cyclobond I 2000

Compound: **1,1'-Binaphthol**

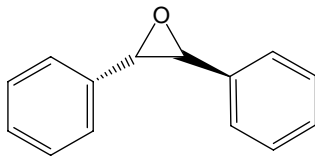


Conditions: 25/75  
ACN/0.1%TEAA, pH4.1;  
1.0ml/min; 25°C;  
UV@230 nm

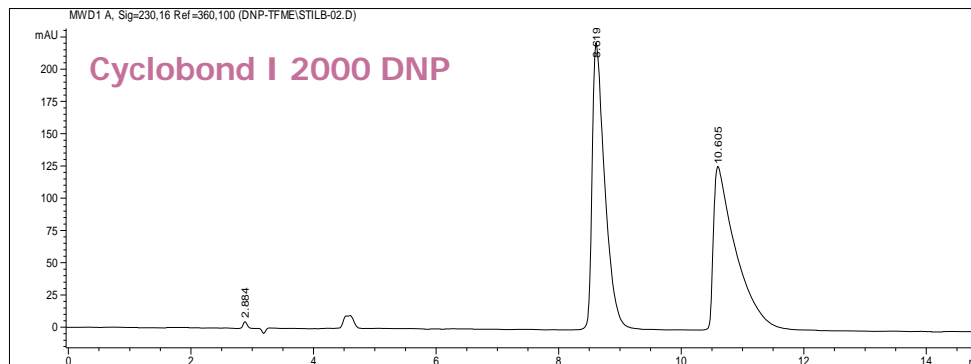
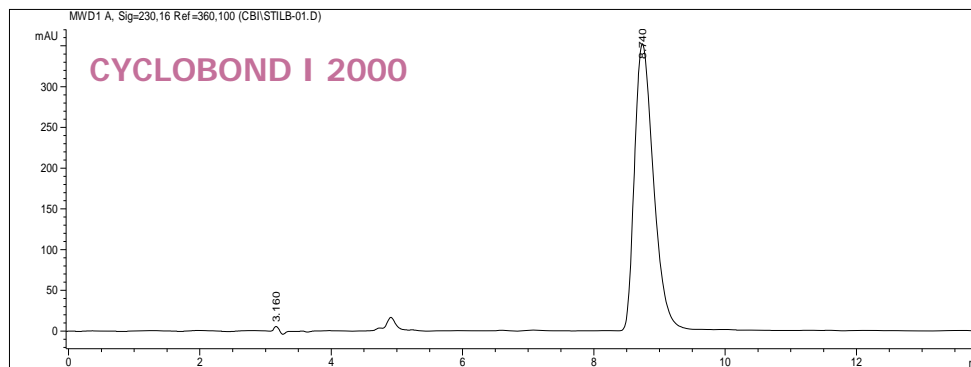


# Cyclobond I 2000 DNP vs. Cyclobond I 2000

Compound: **trans-Stilbene oxide**  
Conditions: 25/75 ACN/0.1%TEAA,  
pH4.1, 1.0ml/min; 25°C; UV@230 nm

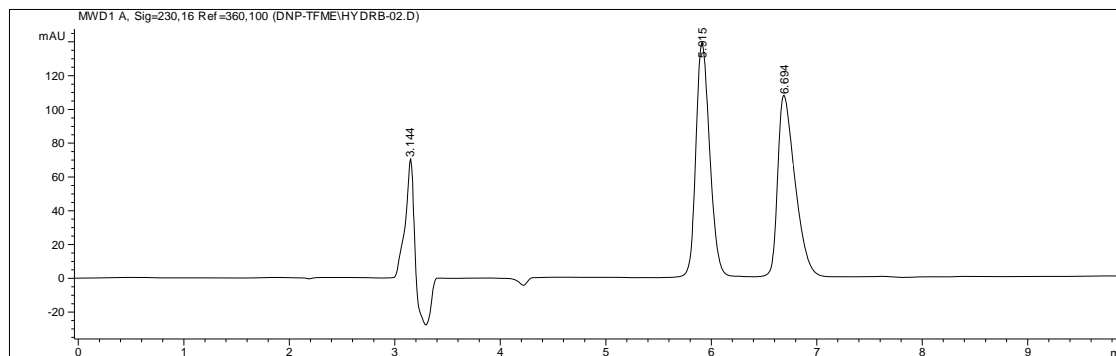
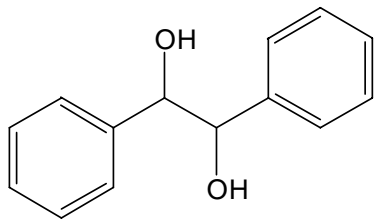


Conditions: 40/60:ACN/0.1%TEAA,  
pH4.1, 1.0ml/min; 25°C; UV@230 nm



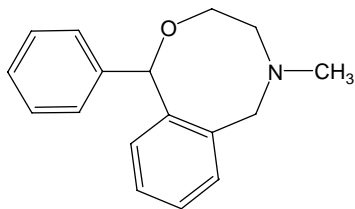
# QC Test Chromatogram

Compound: **Hydrobenzoin**

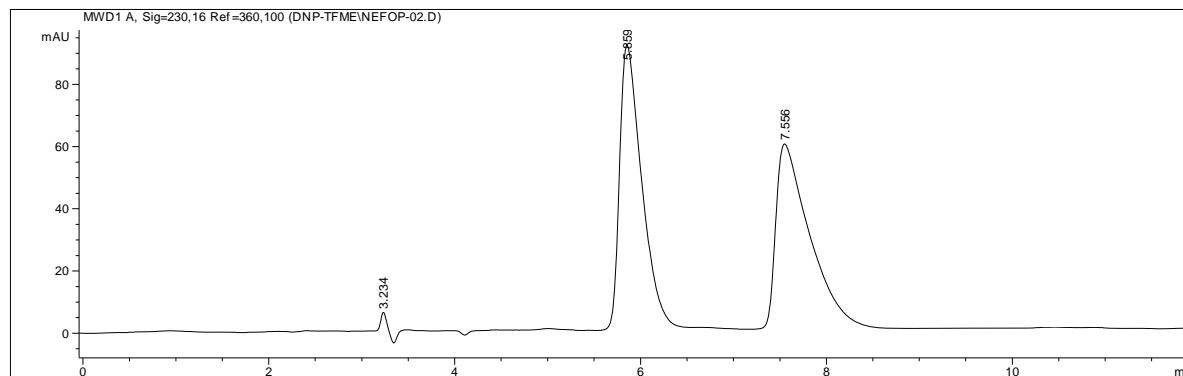


Conditions: 25/75:  
ACN/0.1%TEAA, pH4.1,  
1.0ml/min; 25°C; UV@230 nm

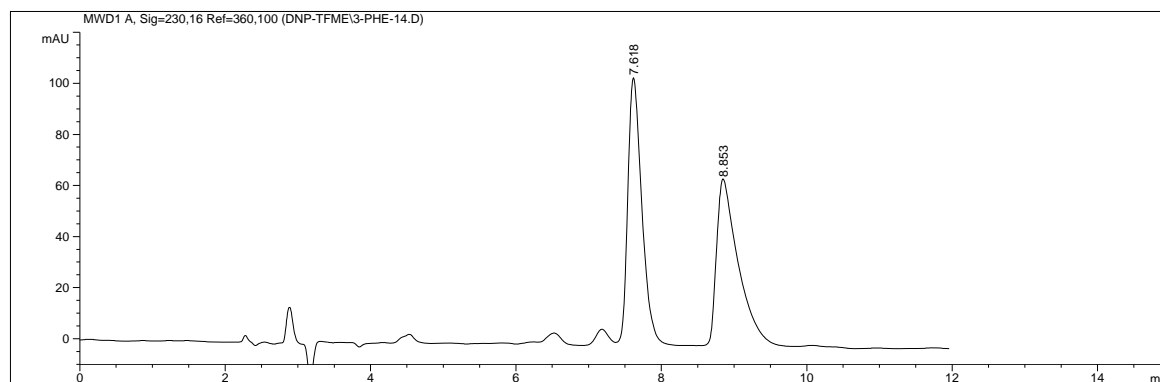
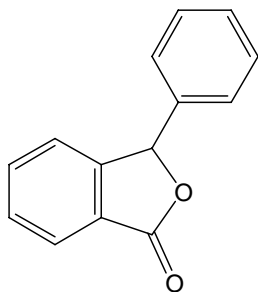
# Separation of Nefopam



Conditions: 15/85:  
ACN/0.1%TEAA, pH4.1,  
1.0ml/min; 25°C; UV@230 nm



# Separation of 3-Phenylphthalide



Conditions: 40/60:  
ACN/0.1%TEAA, pH4.1,  
1.0ml/min; 25°C; UV@230 nm

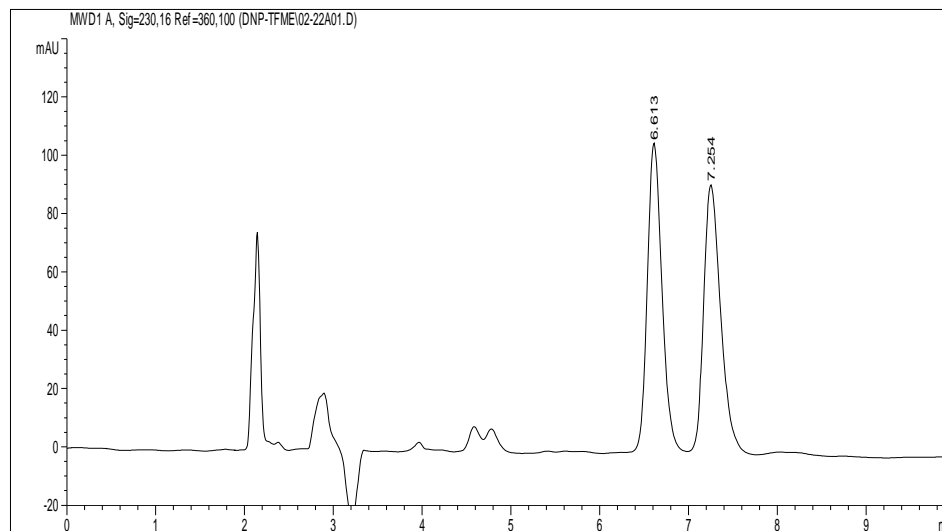
# New Separations on CYCLOBOND I 2000 DNP

Compound: **Customer sample**

Conditions: 40/60:

ACN/0.1%TEAA, pH4.1,

1.0ml/min; 25 °C; UV@230 nm



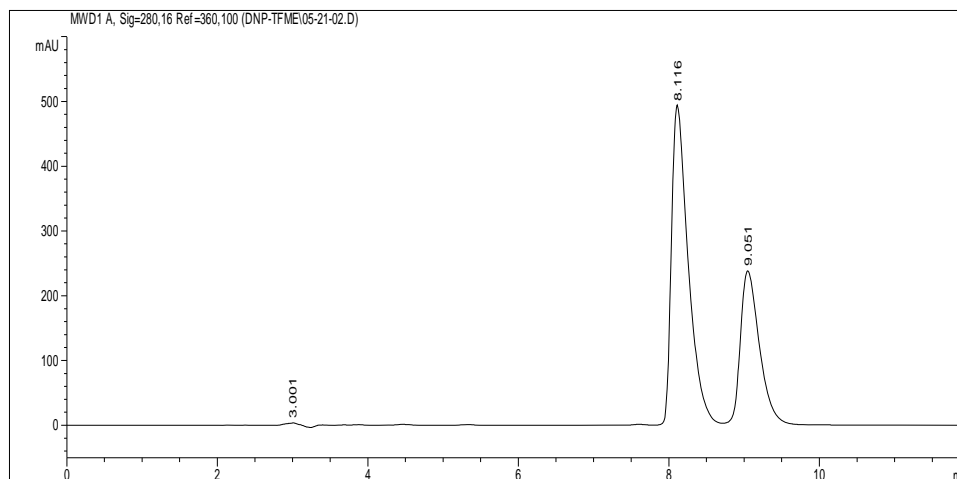
**Note: No separation on any other ASTEC column.**



# New Separations on CYCLOBOND I 2000 DNP

Compound: **Customer sample**  
(mixture of two enantiomers)

ACN/0.1%TEAA, pH4.1,  
1.0ml/min; 25°C; UV@230 nm



**Note: No separation on any other ASTEC columns.**





# Conclusions

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- The new dinitrophenoxy CD-based CSP is effective for enantiomeric separations.
- The best mobile phase mode for CYCLOBOND I 2000 DNP is reversed phase.
- Separations are fast and efficient.
- For charged analytes, appropriate buffer can shorten the retention time and improve separations.