CHROMATOGRAPHY

REGIS® CHROMATOGRAPHY

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Riki Global Trading Pvt. Ltd.

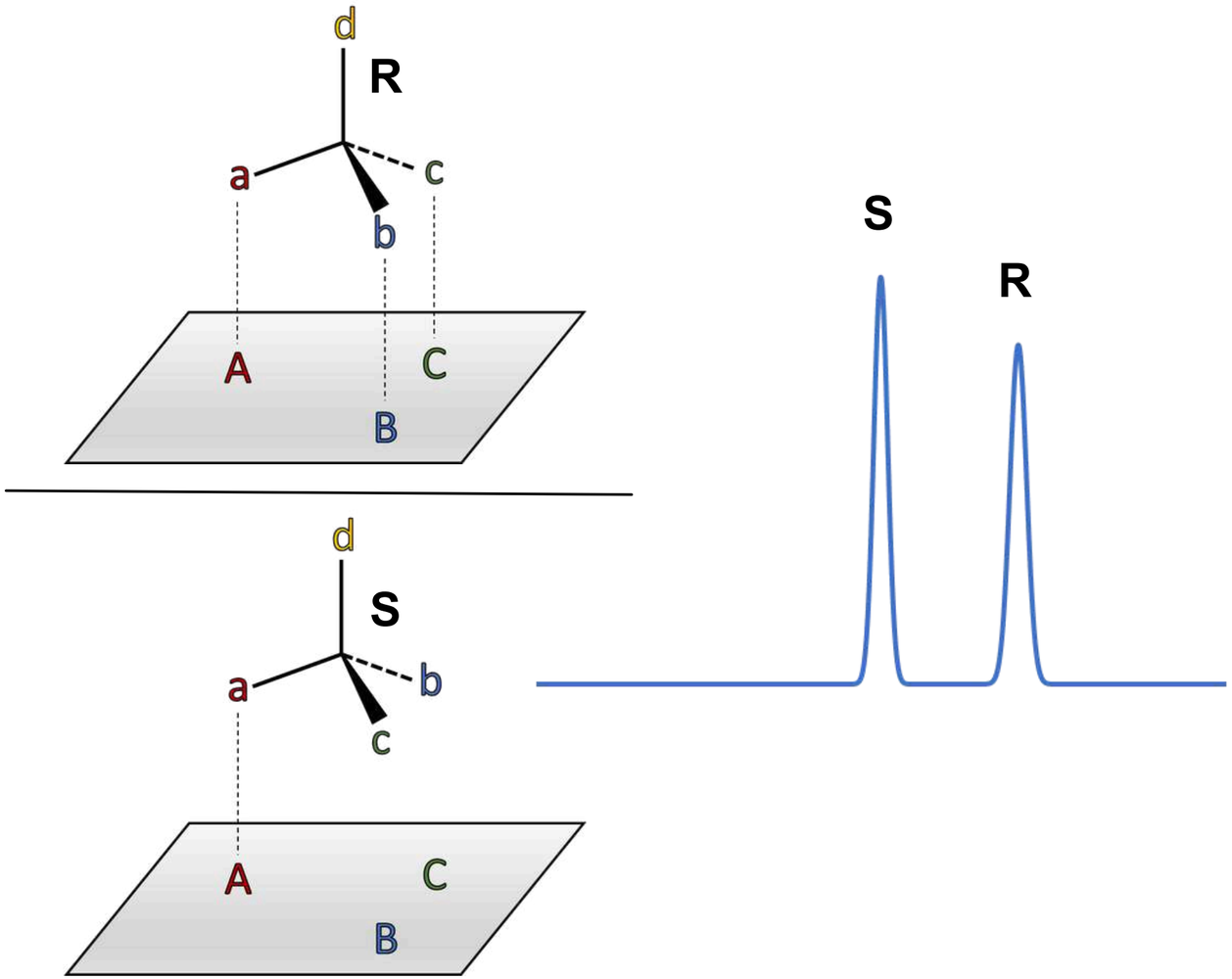
RIKI GLOBAL

“Unveiling new chiral chemistries to address industry challenges”

Outline

- Chiral screening approaches and method development
- Polysaccharide-based phases
- Pirkle-type phases
  - Whelk-O1
- CSPs on core-shell particles
  - Whelk-O1
  - AZYP
- Conclusion

Mechanisms of chiral distinction



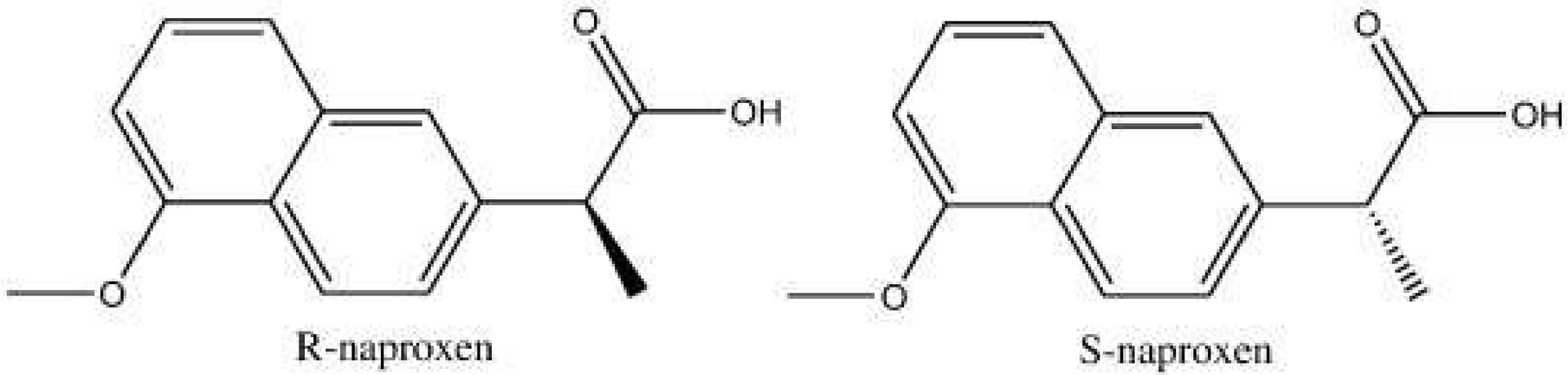
Interaction	Strength
Coulombic	Very strong
Hydrogen bonding	Very strong
Steric hinderance	Weak – Very Strong
π–π interactions	Strong
Ion-dipole	Strong
Dipole-dipole	Intermediate
Dipole-induced dipole	Weak
Dispersion forces	Very weak

Possible interactions between stationary phase and analytes

3-point interaction model: formation of transient diastereomers

Analytical Target Profile

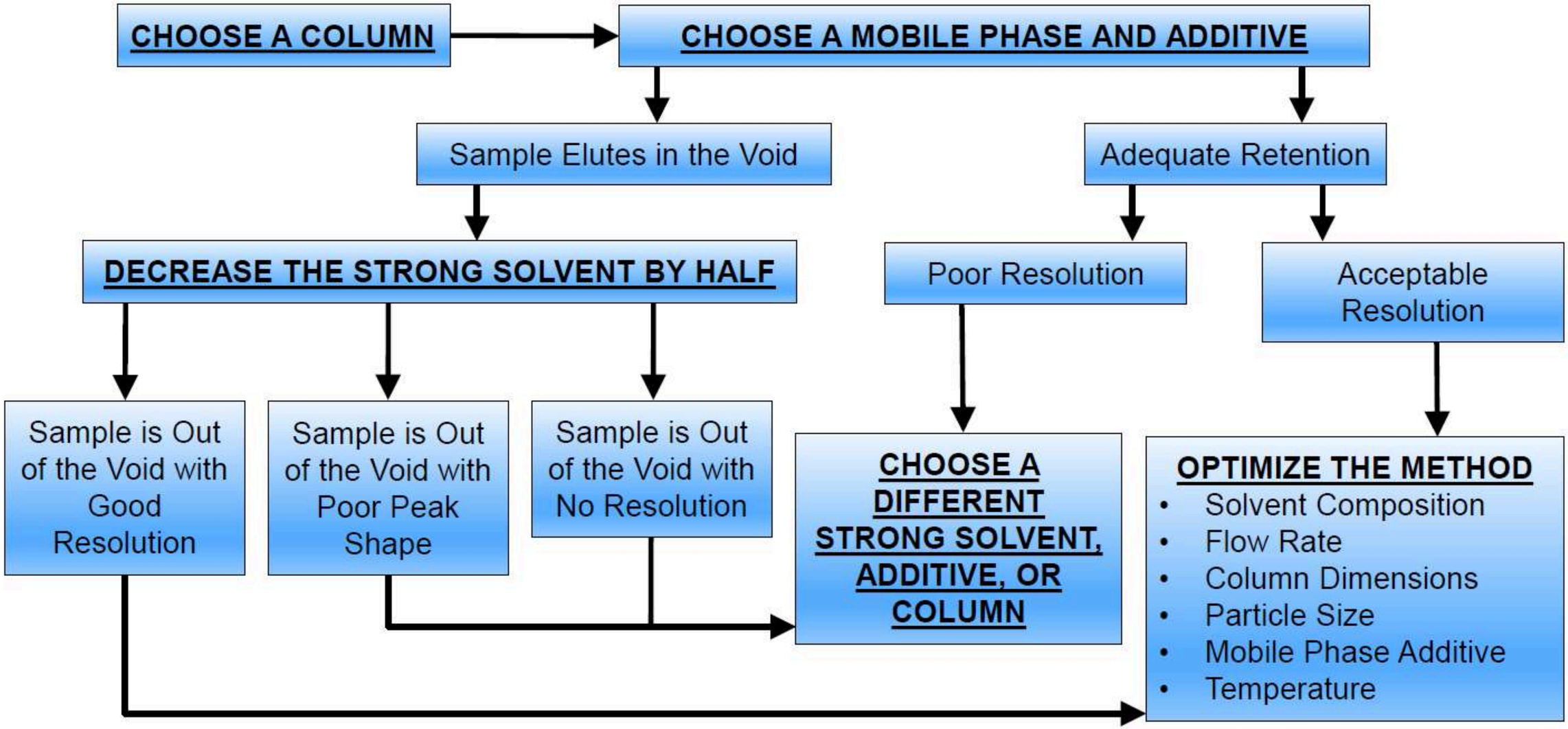
Naproxen
2-(6-methoxynaphthalen-2-yl)propanoic acid



a non-steroidal anti-inflammatory drug, which is administered as a single enantiomer

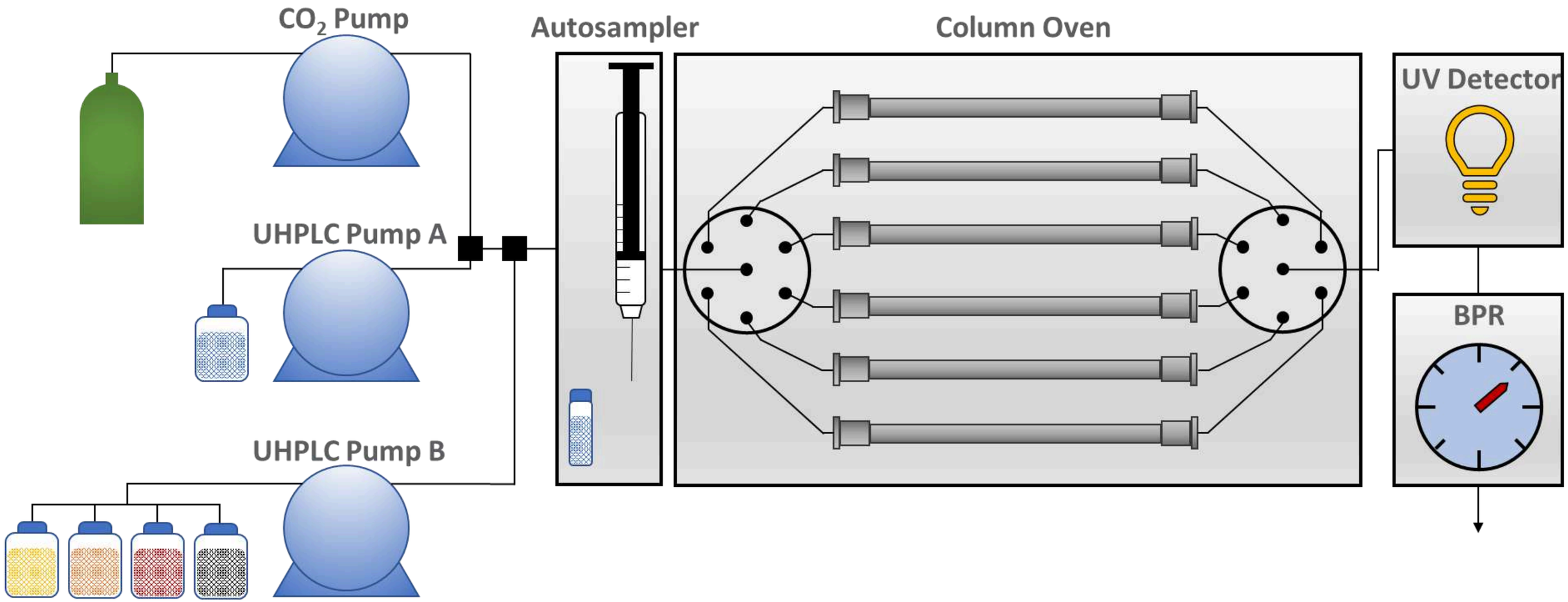
- Develop a purification method which should be simple, fast and effective method

HPLC chiral method development

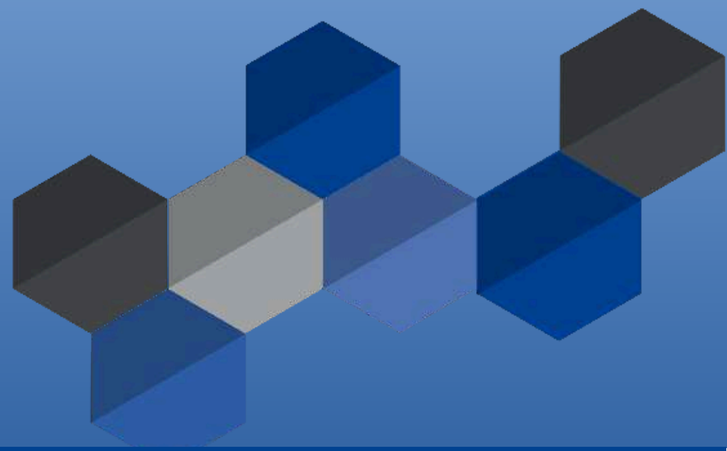


Automated chiral screening

Shimadzu Nexera UC – HPLC & SFC



Polysaccharide Chiral Stationary Phases (CSPs)



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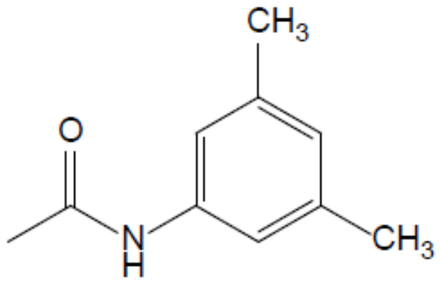
Polysaccharide-based CSPs

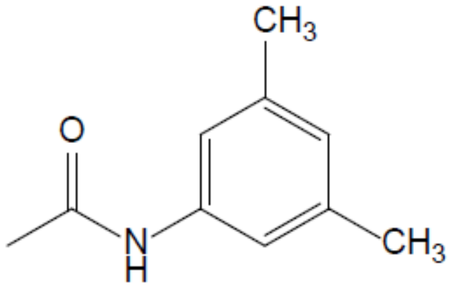
R =

C-Amylose A and C-Cellulose B



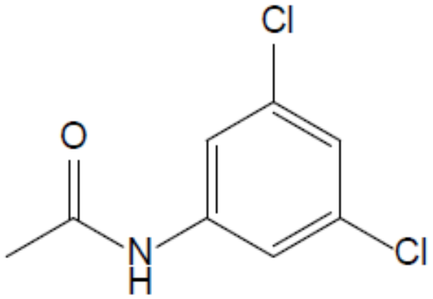
tris (3,5-dimethyl phenylcarbamate)

I-Amylose A and I-Cellulose B



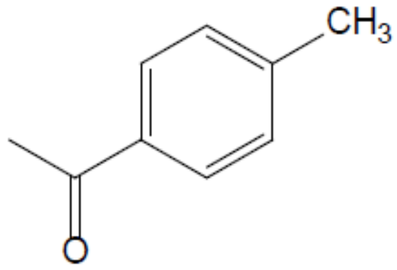
tris (3,5-dimethyl phenylcarbamate)

I-Cellulose C



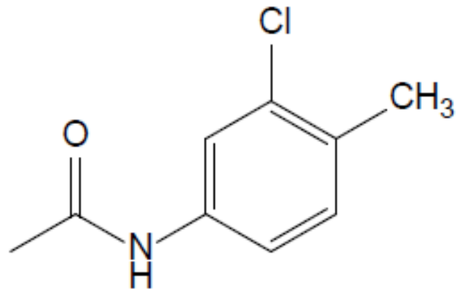
tris (3,5-chloro phenylcarbamate)

I-Cellulose J

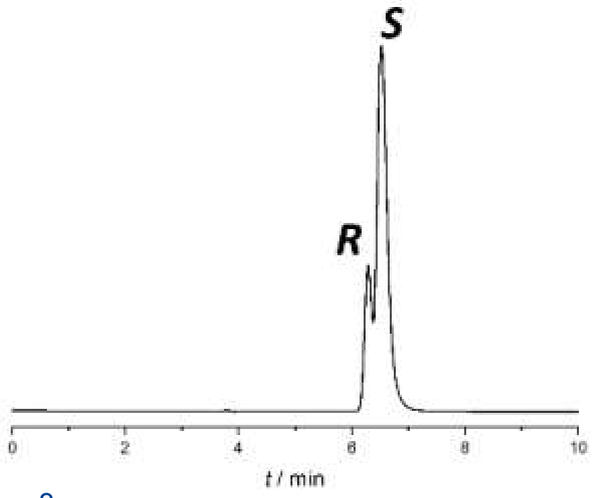


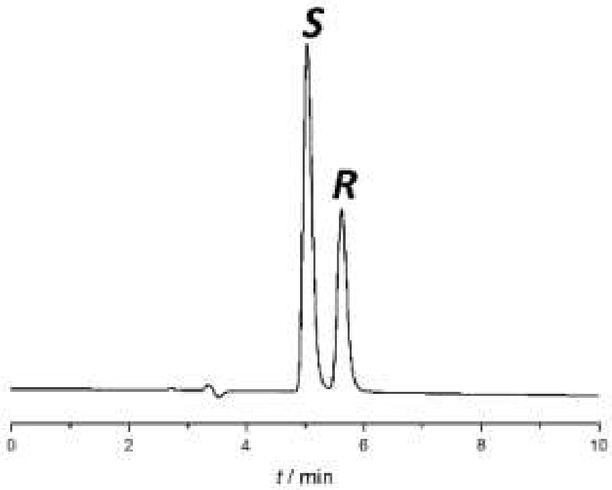
tris (4-methyl benzoate)

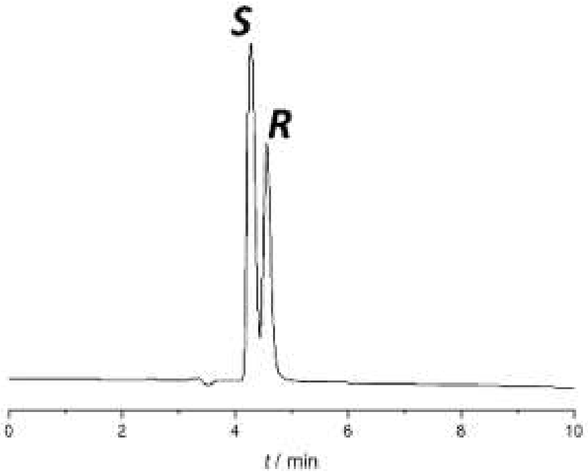
I-Cellulose Z

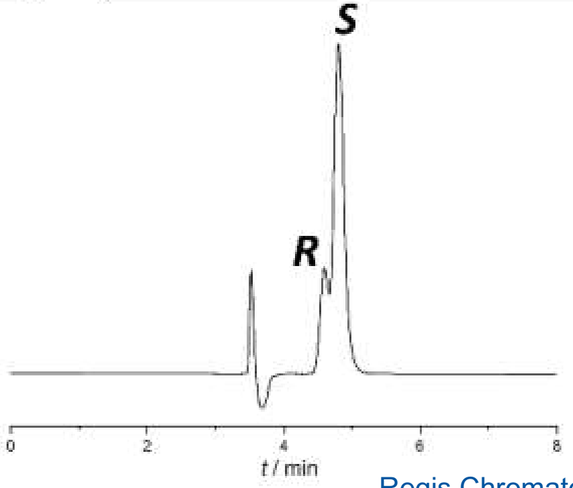


tris (3-chloro-4-methyl phenylcarbamate)

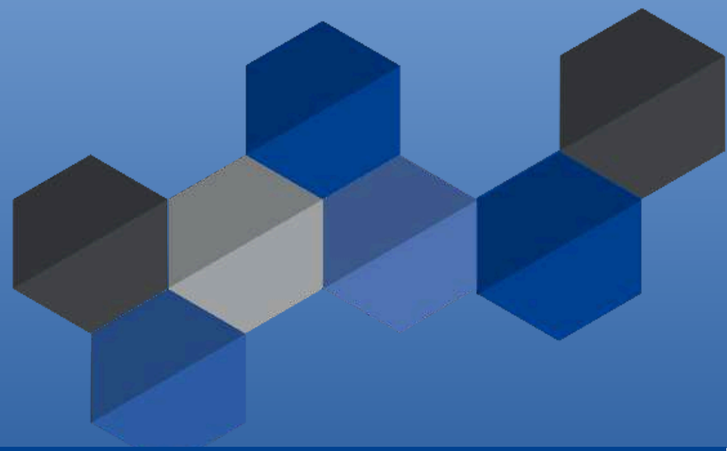








Pirkle-Type CSPs



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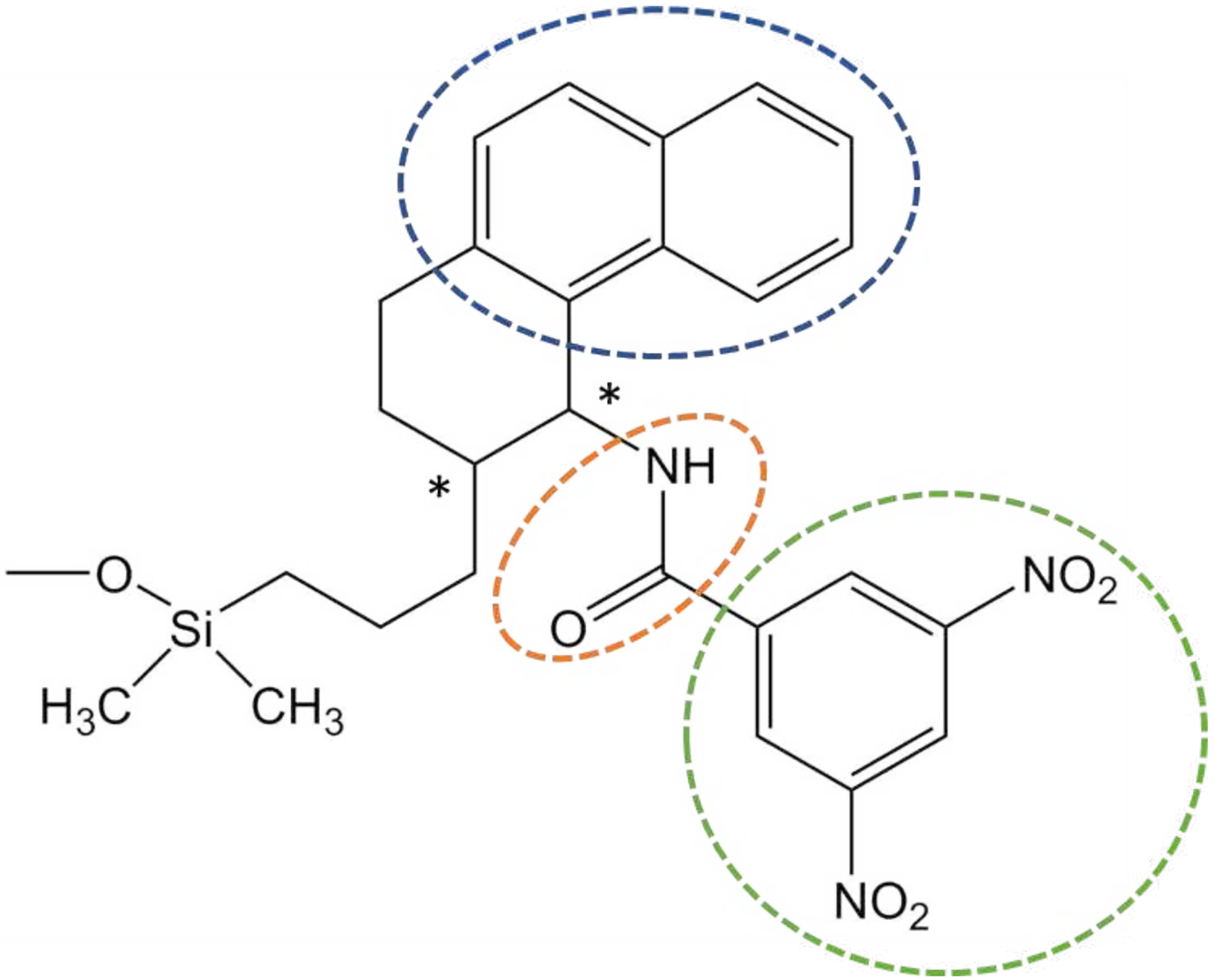
Pirkle-type CSPs

Whelk-O 1



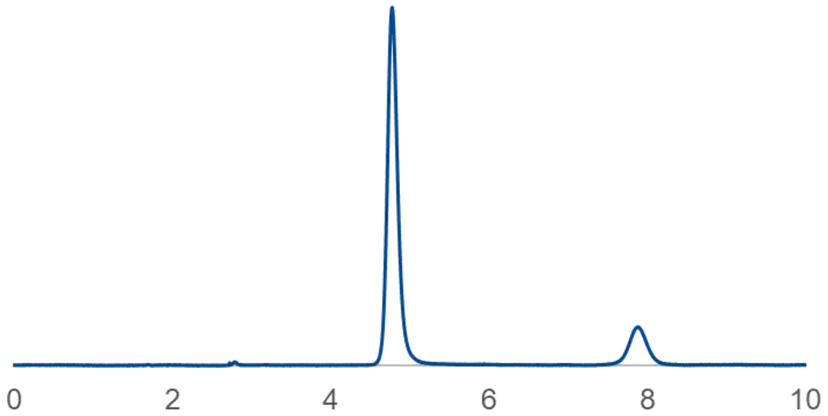
- Covalently-bonded, semi-rigid scaffold
  - π-electron donor (tetrahydrophenanthren)
  - π-electron acceptor (3,5-dinitrobenzoyl)
  - Amide hydrogen donor-acceptor
- Available in two absolute configurations – (R,R) and (S,S)
  - Invert elution order of enantiomers by switching columns

Elution order of enantiomers

Column: 25 cm x 4.6 mm, 5 µm **(R,R)** Whelk-O 1

MP: 50/50/0.1 Hexane/EtOH/TFA

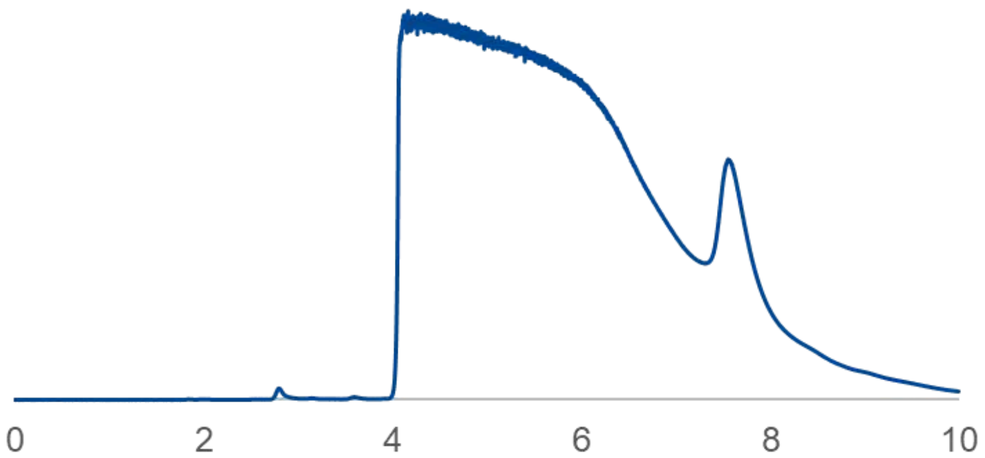
Flow: 1.5 mL/min



Time (minutes)

Column: 25 cm x 4.6 mm, 5 µm **(R,R)** Whelk-O 1

MP: 50/50/0.1 Hexane/EtOH/TFA



Time (minutes)

Comparison of methods for Naproxen and its enantiomer

Column	Mobile Phase	Separation Mode	Enantiomeric Quality Control	R_s
Lux Amylose-1	MeOH:H_2O:acetic acid mixture	RP	Yes (0.1% limit)	3.2 (optimized by FCCD)
Silica gel π-acceptor/π-donor for chiral separations	Hexane:IPA:ACN:acetic acid	NP	Yes (2.5% limit)	~3
Chiralcel OD	Hexane:IPA:acetic acid	NP	No	1.7
Chiralpak IC	Hexane:EtOH:TFA	NP	N	3.4
Chiralpak AS-3R	ACN:phosphate buffer	RP	Yes (not complete validation)	2.55
FLM Chiral NQ(2)-RH	ACN:formic acid in water	RP	Yes	2.38

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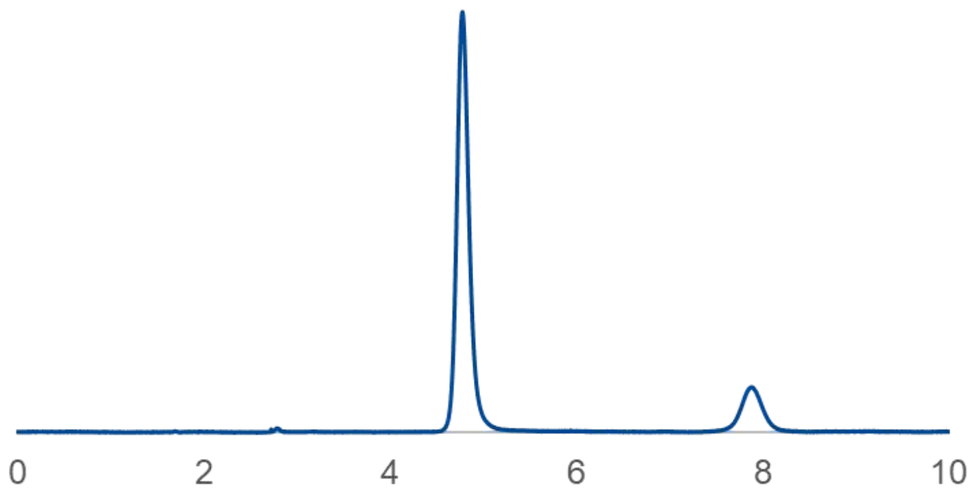
Reverse elution order of enantiomers

Column: 25 cm x 4.6 mm, 5 µm **(R,R)** Whelk-O 1

MP: 50/50/0.1 Hexane/EtOH/TFA

Flow: 1.5 mL/min

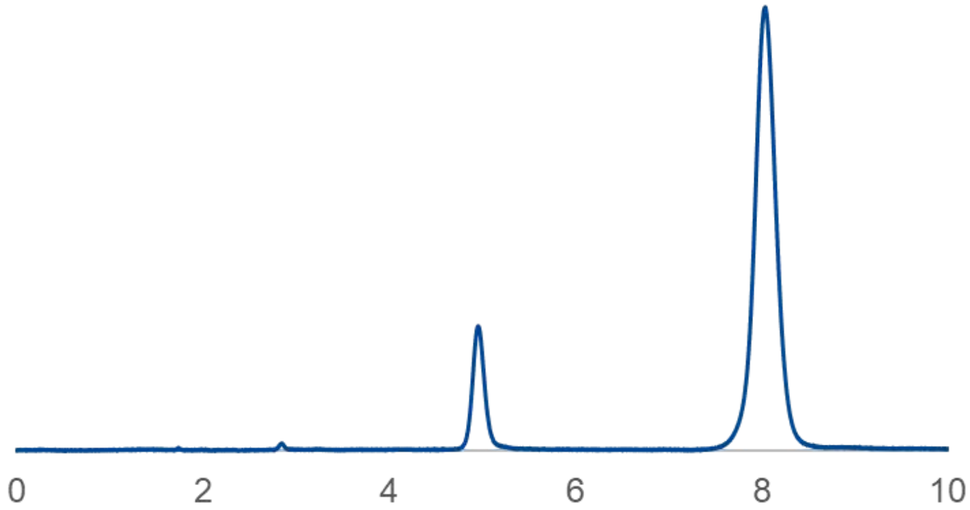


Time (minutes)

Column: 25 cm x 4.6 mm, 5 µm **(S,S)** Whelk-O 1

MP: 50/50/0.1 Hexane/EtOH/TFA

Flow: 1.5 mL/min



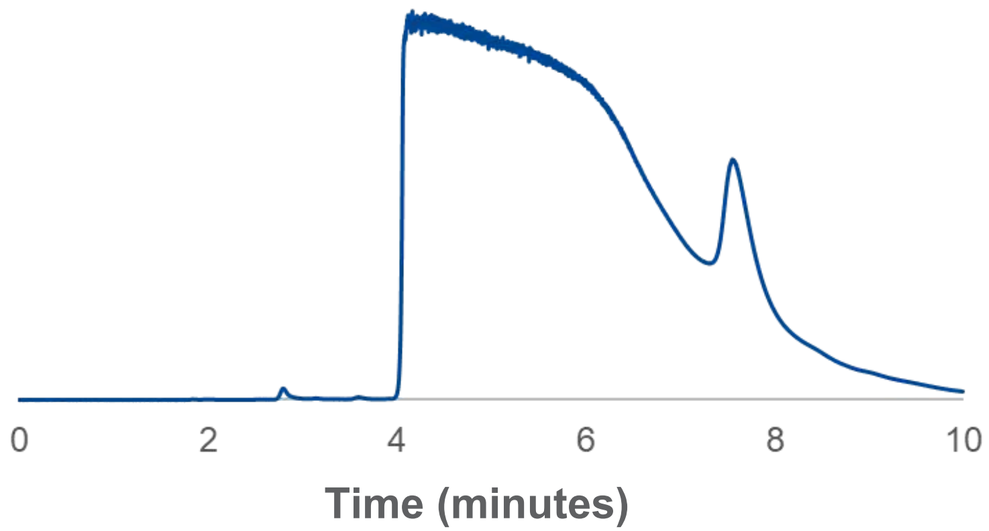
Time (minutes)

Reverse elution order of enantiomers

Column: 25 cm x 4.6 mm, 5 µm **(R,R)** Whelk-O 1

MP: 50/50/0.1 Hexane/EtOH/TFA

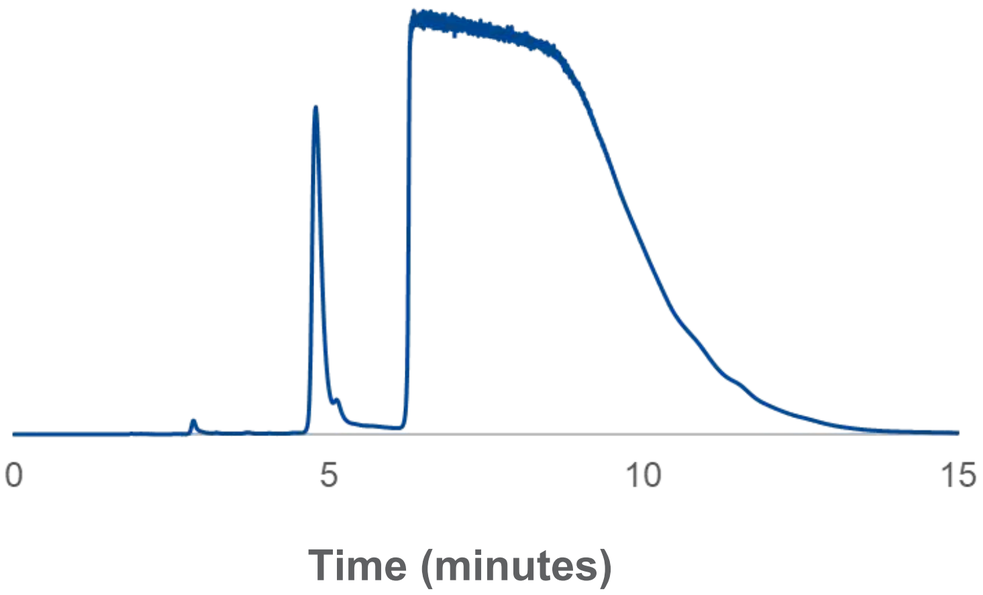
Flow: 1.5 mL/min



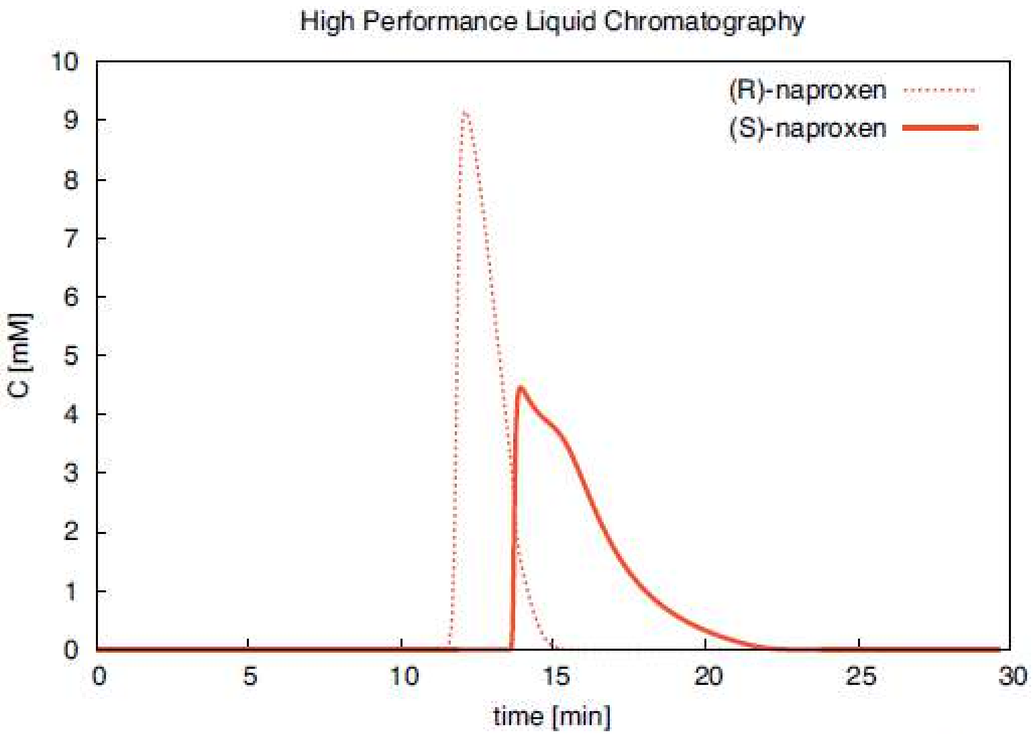
Column: 25 cm x 4.6 mm, 5 µm **(S,S)** Whelk-O 1

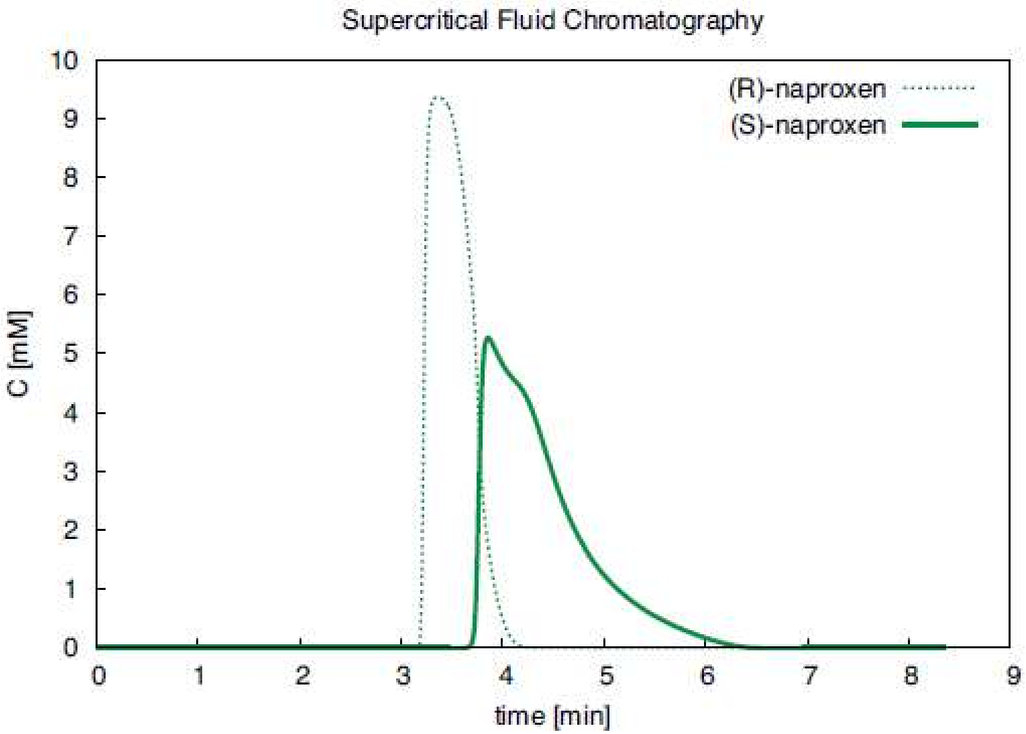
MP: 50/50/0.1 Hexane/EtOH/TFA

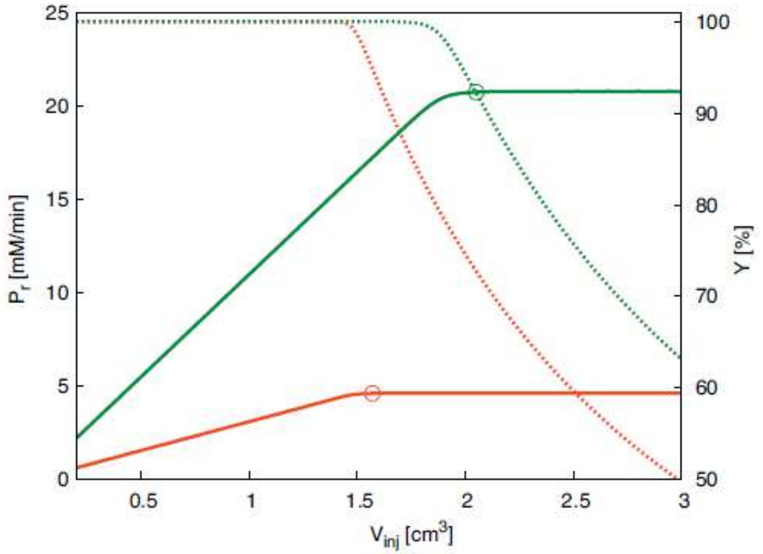
Flow: 1.5 mL/min



HPLC Vs SFC







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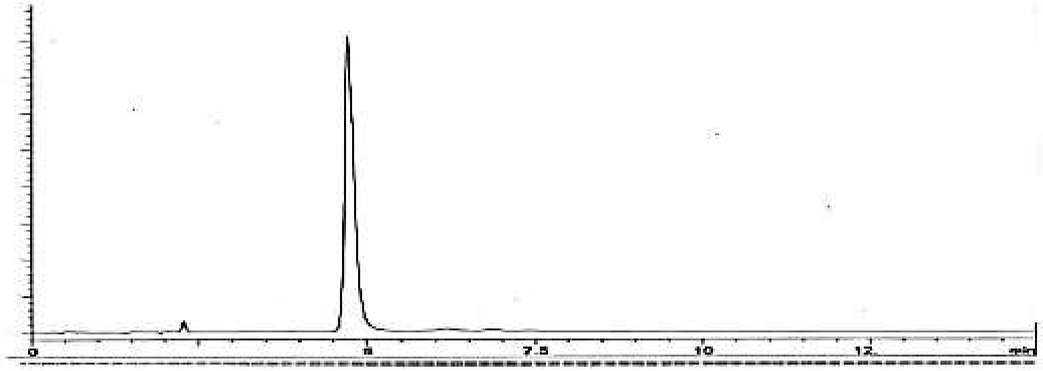
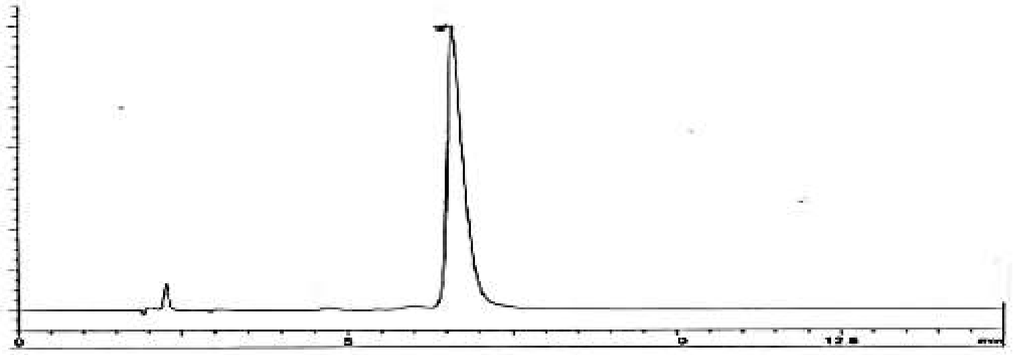
Post-Preparative Purification Purity Determination

- Peak fractions were analyzed to determine the enantiomeric excess (EE)
- Peaks 1 and 2 had a %EE of greater than 99

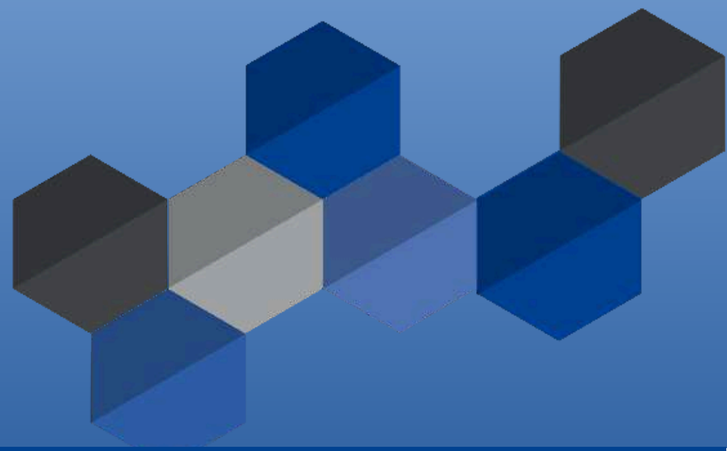
Peak 1
Chiral Purity: > 99 % EE



Peak 2
Chiral Purity: > 99 % EE



CSPs on Core-Shell Particles



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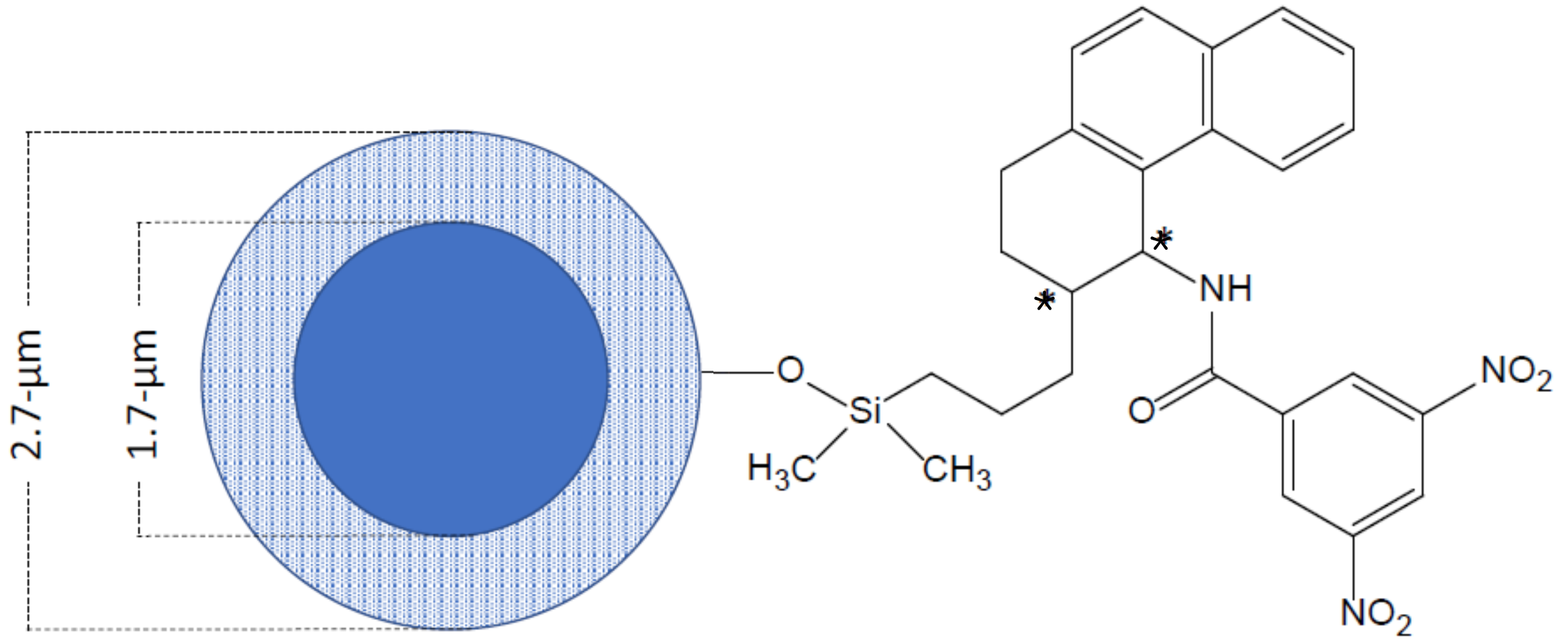
Whelk-O1 Core



Whelk-O1 CSP

Benefits of SPPs

- Reduced B-term contribution to van Deemter equation
- Improved radially homogeneity improves velocity-dependent A-term
- “UHPLC performance at HPLC pressures”

AZYP CSPs on Core-Shell Particles

AZYP Phases	Chiral Selector	Key Applications
CDShell-RSP	hydroxypropyl beta-cyclodextrin	stimulants, fungicides,t-boc amino acids, neutral & charges aromatics compounds, amides, pesticides, atropisomers
TeicoShell	teicoplanin	beta blockers, hydroxy acids, N-blocked amino acids, profens, hydantoins, benzodiazepines
NicoShell	modified macrocyclic glycopeptide	azoles, nicotine analogs, stimulants, beta blockers, amines
VancoShell	vancomycin	amines, profens, amino alcohols, B-blockers
LarihcShell-P	alkyl-derivatized cyclofructan CF-6	primary amines (amino alcohols, amino esters, amino amides)
QShell	quinine-based	N-blocked amino acids, carboxylates

Modes

NP, RP, POM, PIM, HILIC, SFC

Conclusion

- Elution order of major and minor enantiomers can be reversed depending on the RR or SS Whelk-O1 column used.
- SFC offers high sample load capability.
- UPLC, Prep-LC, and SFC dimensions are available.
- Special Core-shell technologies are available.

Additional Resources for Chiral Separations

Resources available on the Regis Technologies website

- Chiral Application Database
- The Chiral Handbook
- Chiral HPLC and SFC Method Development Posters
- Technical Posters
- Journal Articles
- Column Care and Use Guides