

Dr. Maisch GmbH

Beim Brückle 14
D-72119 Ammerbuch

Fon +49(0)7073 50 35 7
Fax +49(0)7073 42 16

e-Mail: maisch@reprosil.com
i-Net: www.reprosil.com

Reprosil Chiral Stationary Phases

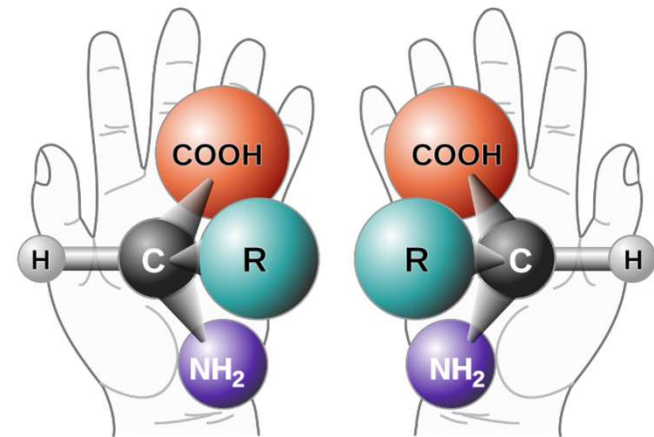
Dr. Maisch GmbH

Any Column, Any Size, Any Media

Chirality - Definition

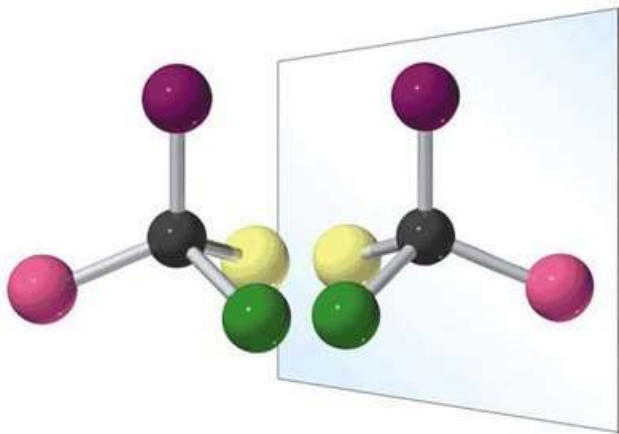
Chirality [/kai'raeɪti:/](#) is a property of [asymmetry](#) important in several branches of science. The word *chirality* is derived from the [Greek](#) $\chi\epsilon\iota\rho$ (*kheir*), "hand," a familiar chiral object. An object or a system is *chiral* if it is distinguishable from its [mirror image](#); that is, it cannot be [superimposed](#) onto it.

(source: Wikipedia)



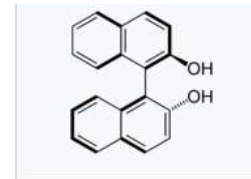
Reasons for Chirality

**Chiral (stereogenic) centre:
Asymmetric carbon atom:**

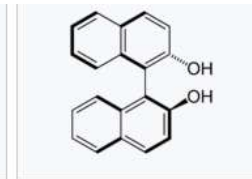


But not always:

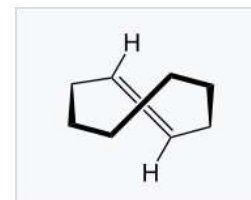
**Examples of molecules without
chiral centre →
Axial, planar and helical
chirality**



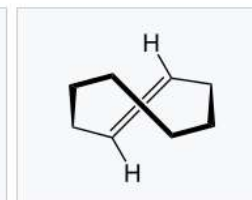
(*R*)-BINOL: Die Chiralitätsachse
entspricht der Bindung
zwischen den beiden
Naphthylsystemen



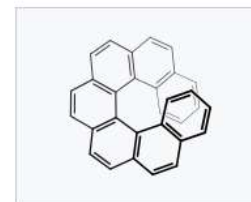
(*S*)-BINOL



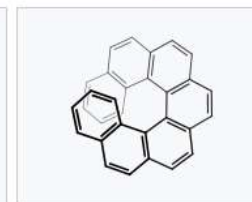
(*R*)-(-)- *trans*-Cycloocten
Andere Namen:
(*P*)-(-)-*trans*-Cycloocten und
(-)-(*E*)-Cycloocten



(*S*)-(+)- *trans*-Cycloocten
Andere Namen:
(*M*)-(+)-*trans*-Cycloocten und
(+)-(*E*)-Cycloocten



(*P*)-Helizität



(*M*)-Helizität



(*P*)-Helizität einer Wendeltreppe
im Vatikan-Museum

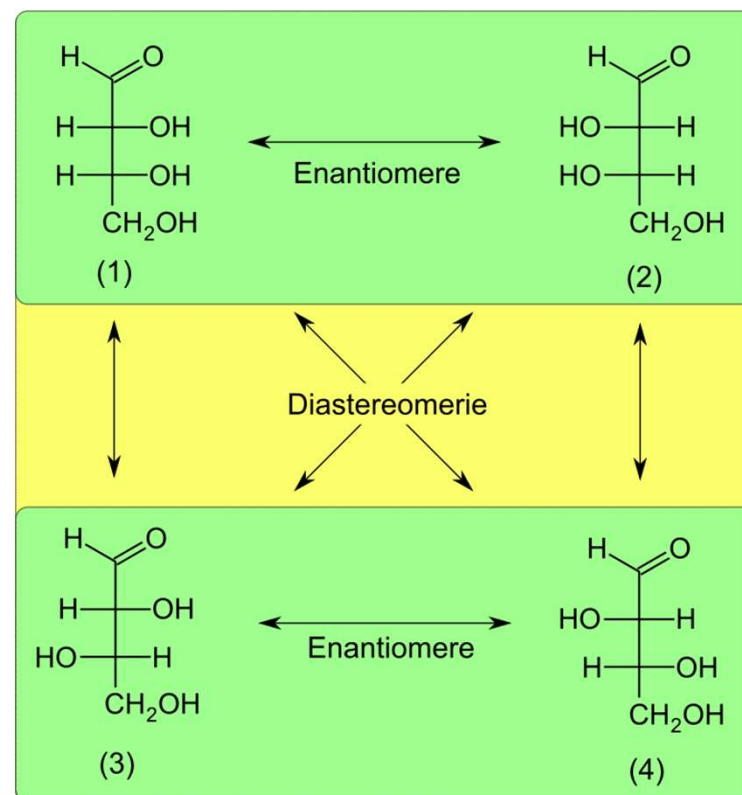


(*M*)-Helizität eines Spiegelbilds
des Originals der Wendeltreppe
im Vatikan-Museum

Multiple Chiral Centres – Enantiomers vs. Diastereomers

For compounds with more than one, namely „n“ chiral centres, there exist 2^n stereoisomers, consisting of 2^{n-1} pairs of enantiomers.

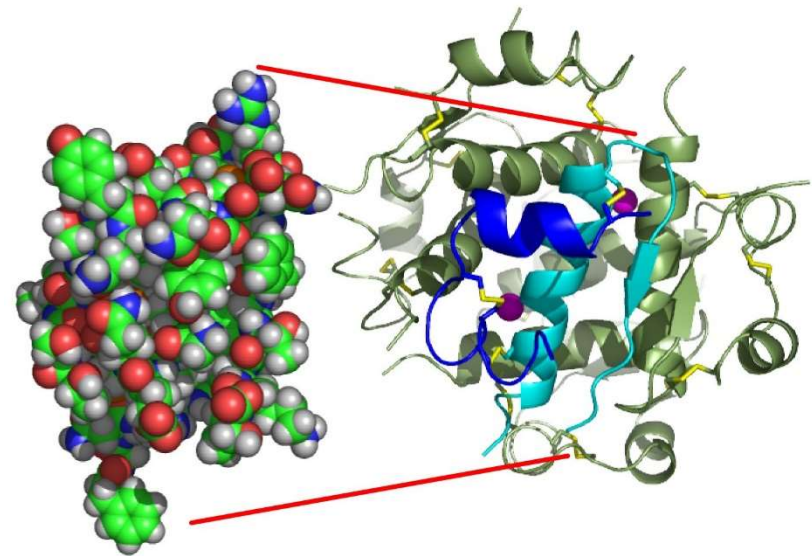
Stereoisomers which are not enantiomers are called diastereomers.



Our World Is Chiral

Many natural compounds are chiral and are only found in nature in one enantiomeric form only:

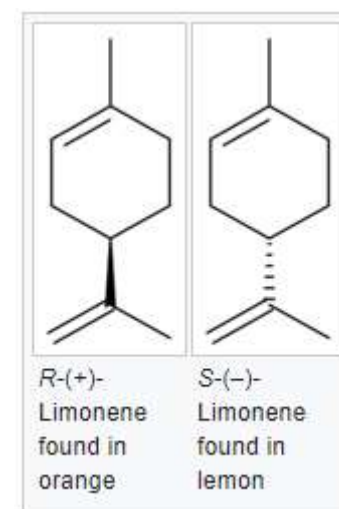
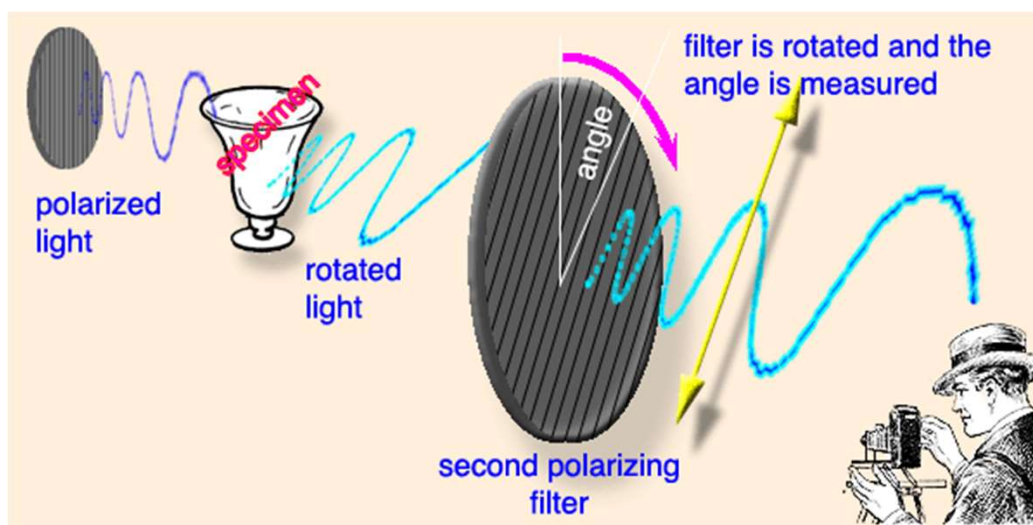
- Amino acids → peptides, proteins
- Monosaccharides → oligosaccharides (cellulose, amylose ...)
- Nucleic acids → oligonucleotides (DNA, RNA...)
- Steroids



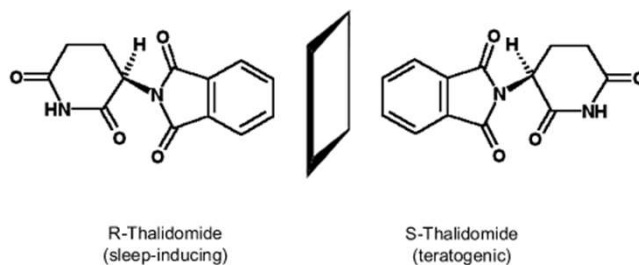
Properties of Stereoisomers

Distereomers have different chemical and physical properties.

Enantiomers have the same chemical and physical properties and can only be distinguished in a chiral environment, e. g. by their ability to rotate the axis of polarised light or by their flavour or their effect on the (human) body.



Relevance of Chirality in the Pharmaceutical Industry



The Contergan Case: When taken during pregnancy, Thalidomide, the API in Contergan, prevented the proper growth of the fetus resulting in birth defects of thousands of children

Relevance of Chiral Chromatography

- Test raw materials for chiral purity
- Monitor enantiomeric excess of chemical synthesis
- Control chiral purity of final API
- Test for chiral stability of pharmaceuticals (racemisation)
- Preparative separation of enantiomers

Separation of Enantiomers

Enantiomers can only be separated in a chiral environment:

1. Indirect methods

- Chiral derivatization (transforming enantiomers into diastereomers by introducing a second chiral centre)
- Use of chiral mobile phase additives

2. Direct methods

- Chiral Stationary Phases („CSP“)

Type of CSPs Used in HPLC

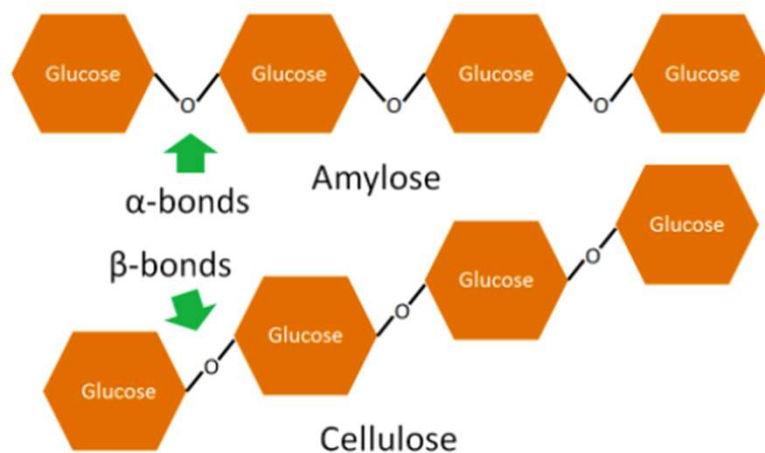
- **Polysaccharide Phases**
- Ligand Exchange
- Protein Phases
- Chiral Crown Ether Phases
- Cyclodextrine Phases
- Pirkle-Type (Brush-Type) Phases
- Macrocyclic Glycopeptide Phases
- Cinchona Alkaloid Phases

Reprosil Chiral Polysaccharide Phases

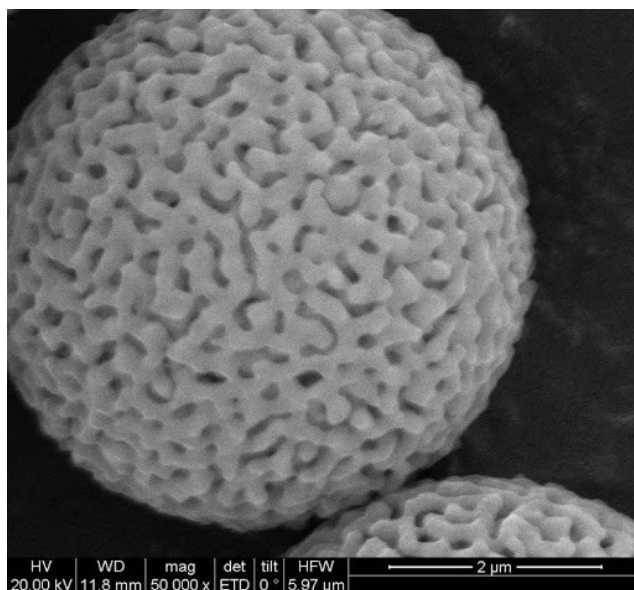
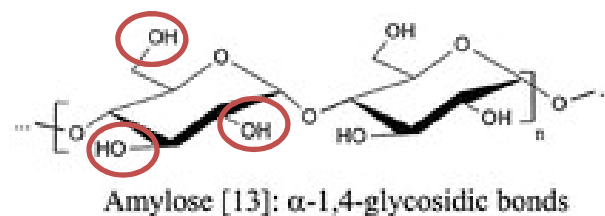
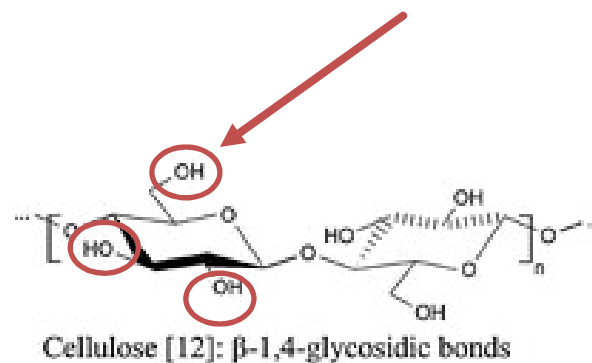
Advantages of Polysaccharide Based CSPs:

- Wide chiral recognition
- Multiple chiral selectors available
- High loading capacity and efficiency
- Suitable for various modes of separation
- High pressure resistance (silica based)
- Scalability to prep (and bulk media available)

Reprosil Chiral Polysaccharide Phases – General Structure



Chiral Selectors attached to Hydroxyl Groups



Reprosil Chiral Polysaccharide Phases – Mechanism of Chiral Recognition

In order for a chiral recognition to work, the necessity of a 3-point interaction has been postulated.



Is chiral recognition a three-point process?

Tristan D. Booth, Daphne Wahnou, Irving W. Wainer

Pages: 96-98 | First Published: 07 December 1998

[PDF](#) | [References](#) | [Request permissions](#)

The nature of chiral recognition: Is it a three-point interaction?

Vadim A. Davankov

Pages: 99-102 | First Published: 07 December 1998

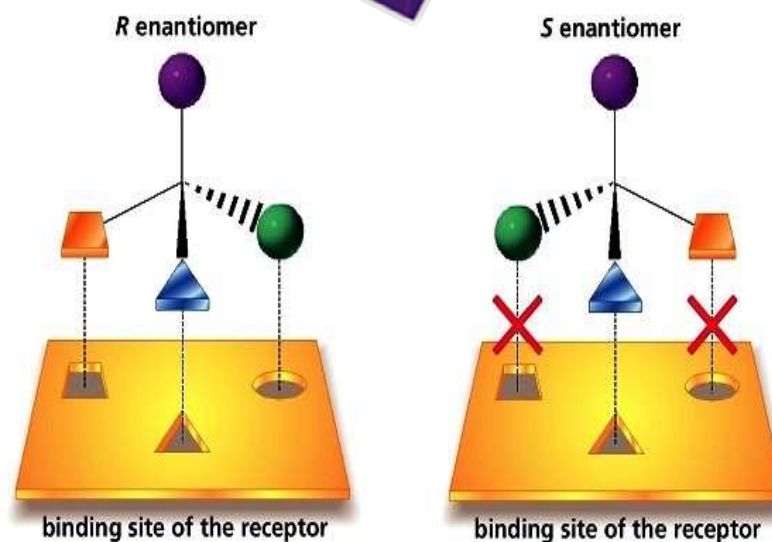
[Abstract](#) | [PDF](#) | [References](#) | [Request permissions](#)

On the minimum requirements for chiral recognition

William H. Pirkle

Pages: 103 | First Published: 07 December 1998

[PDF](#) | [Request permissions](#)

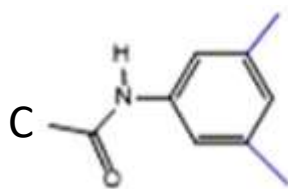


Reprosil Chiral Polysaccharide Phases – Analyte – CSP interaction

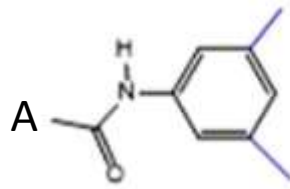
In case of Reprosil Chiral Polysaccharide Phases, the analyte-CSP three-point interaction is realized through various forces:

- ❖ Hydrogen bonding
- ❖ Dipole-dipole
- ❖ π - π interactions
- ❖ Van-der-Waals forces
- ❖ Steric interactions

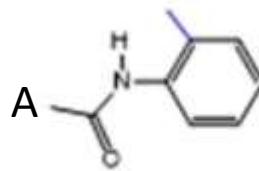
„Coated“ Reprosil Chiral Polysaccharide Phases



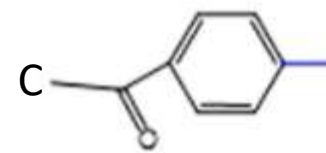
Reprosil Chiral OM
(Chiralcel OD, Lux Cellulose-1)



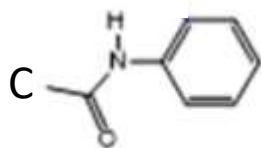
Reprosil Chiral AM
(Chiralpak AD, Lux Amylose-1)



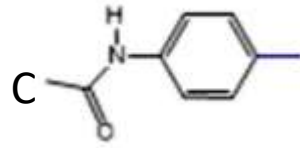
Reprosil Chiral AMS
(Chiralpak AS)



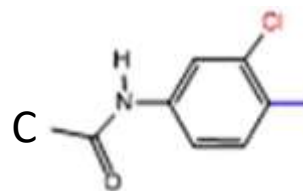
Reprosil Chiral JM
(Chiralcel OJ, Lux Cellulose-3)



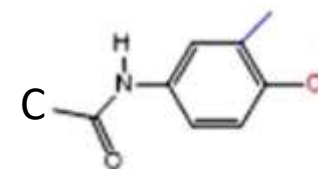
Reprosil Chiral CM
(Chiralcel OC)



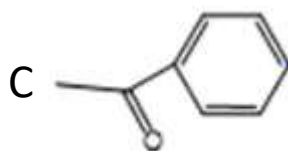
Reprosil Chiral GM
(Chiralcel OG)



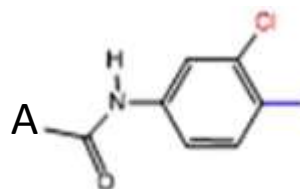
Reprosil Chiral ZM
(Chiralcel OZ, Lux Cellulose-2)



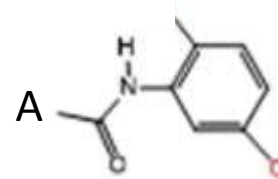
Reprosil Chiral XM
(Chiralcel OX, Lux Cellulose-4)



Reprosil Chiral BM
(Chiralcel OB)

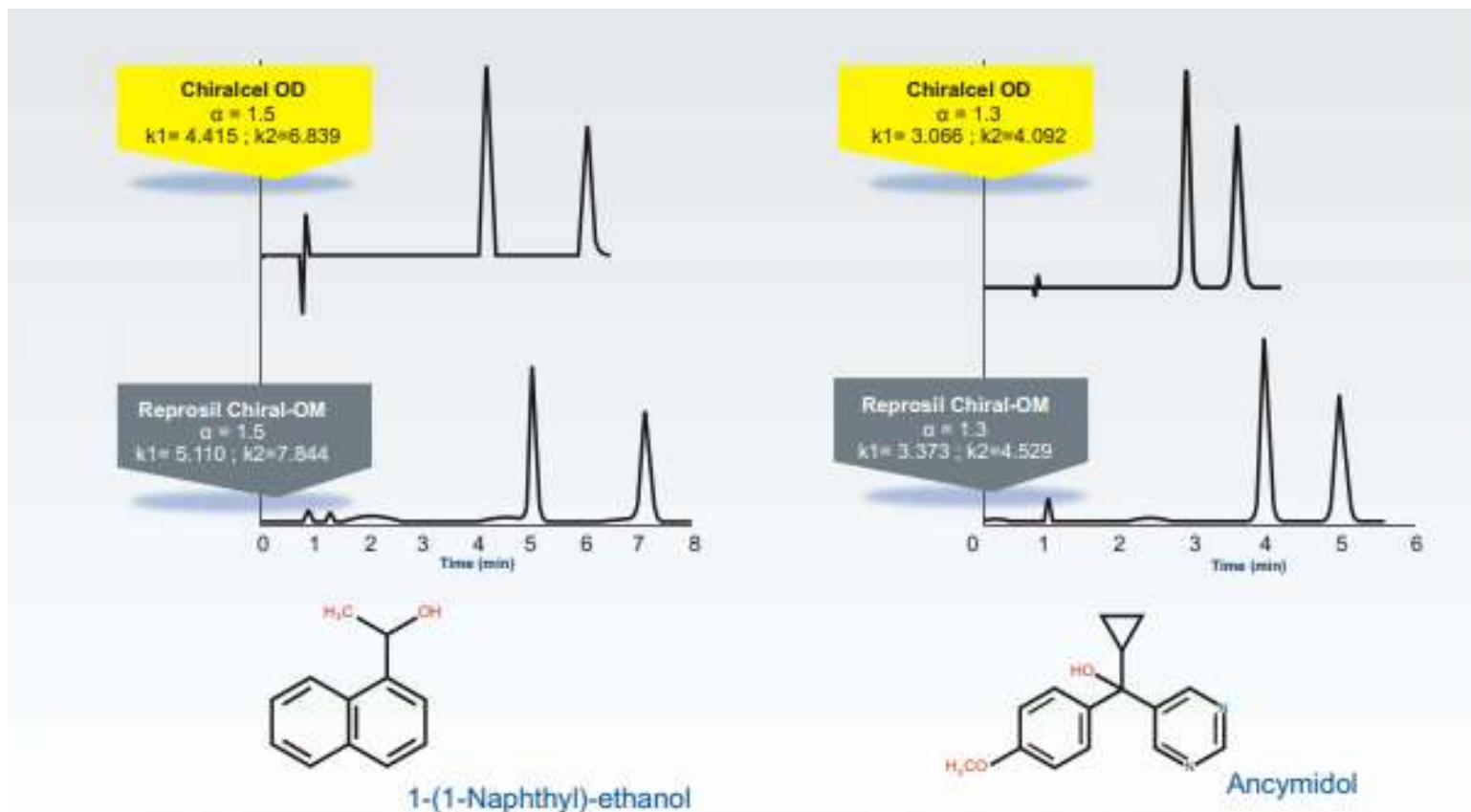


Reprosil Chiral ZA
(Chiralpak AZ)

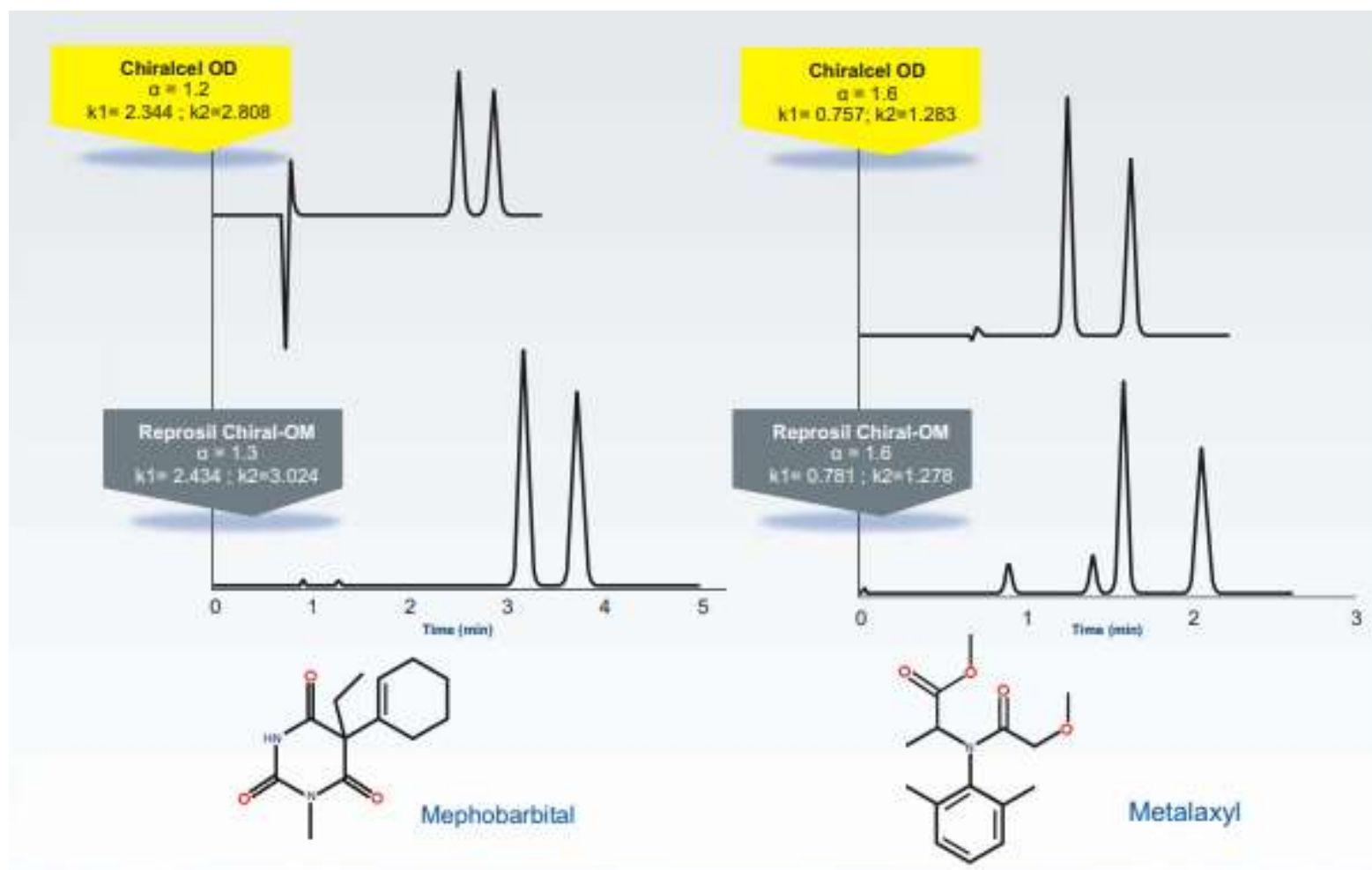


Reprosil Chiral YM
(Chiralpak AY, Lux Amylose-2)

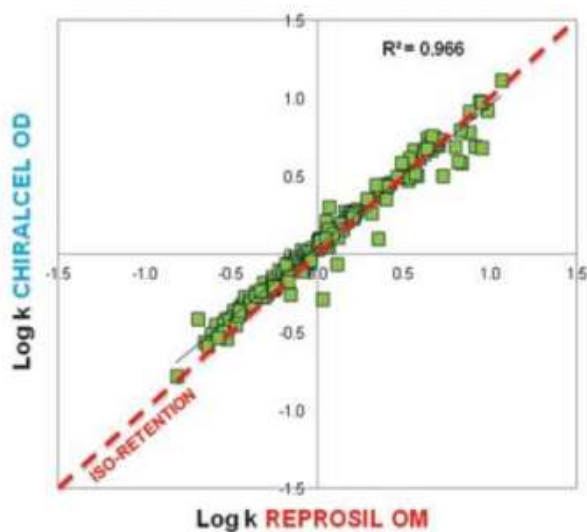
Reprosil Chiral OM vs. Chiralcel OD



Reprosil Chiral OM vs. Chiralcel OD



Reprosil Chiral OM vs. Chiralcel OD



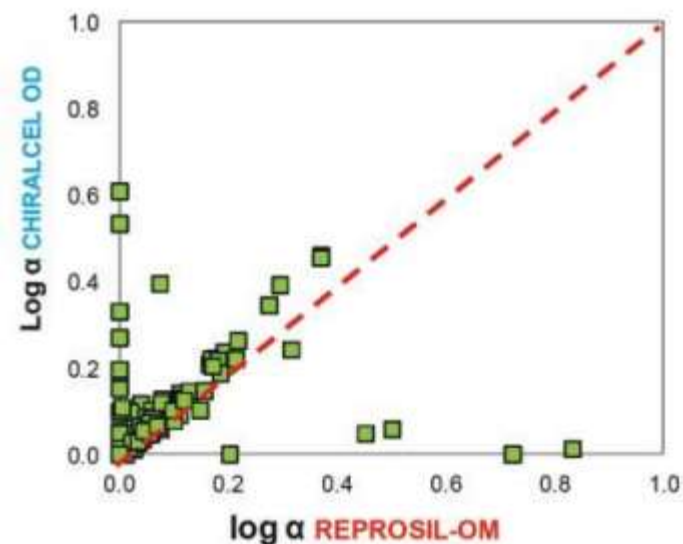
The investigation on non-specific interactions that control retention is based on the analysis of 230 achiral compounds.

The κ - κ plot on the left compares the logarithms of retention factors of 168 achiral species on Chiralcel OD vs. Reprosil Chiral-OM. The phases are expected to be similar since they possess the same chiral selector ($R^2 = 0.966$). They would provide similar non-specific interactions.

The α - α plot below compares the logarithm of separation factors measured for 130 racemates on Reprosil Chiral-OM vs. Chiralcel OD.

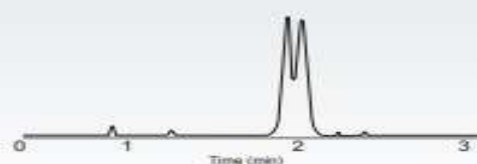
The major part of the compounds is located on the dotted line, indicating similar separation behaviour of the two columns.

Chiralcel OD provides a higher number of unique hits. Indeed, 81% of the tested chiral species are resolved on Reprosil Chiral-OM against 86% on Chiralcel OD. However, some racemates are well separated on Reprosil chiral-OM with little or no separation on Chiralcel OD.



Reprosil Chiral vs. Other USP-L40 Columns

> SEPARATION

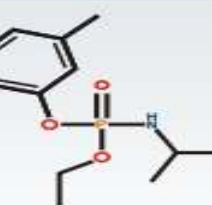


Reprosil Chiral-OM

> NO SEPARATION



RegisCell



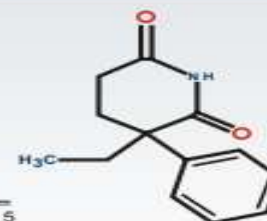
Fenamiphos



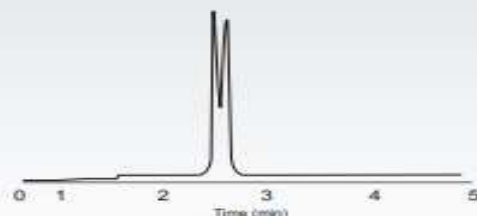
Reprosil Chiral-OM



Lux Cellulose-1



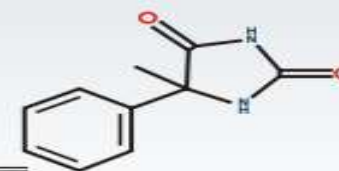
Gluthetimide



Reprosil Chiral-OM

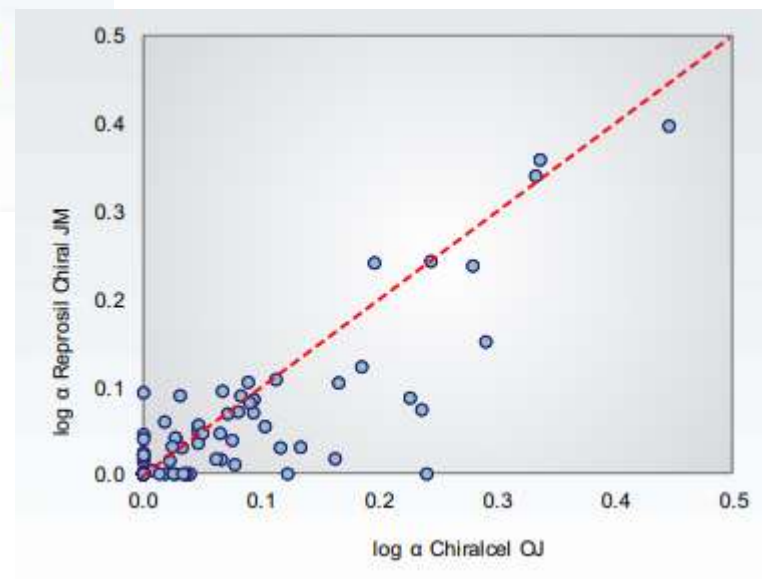
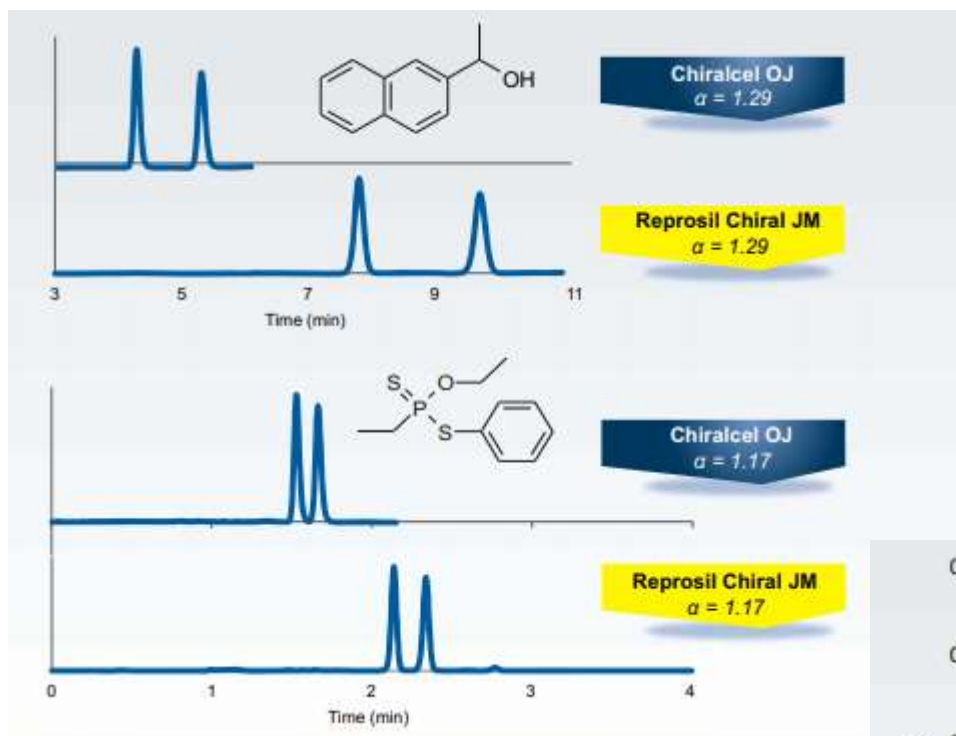


CelluCoat



5-Methyl-5-phenylhydantoin

Reprosil Chiral JM vs. Chiralcel OJ



„Coated“ Reprosil Chiral Polysaccharide Phases

Chromatography Modes

All coated Reprosil Chiral phases are amenable to multiple chromatography modes and may show different and orthogonal selectivities under different modes.

- Normal Phase
 - Mixture of alkane and alcohol
- Polar Organic
 - Mixture of acetonitrile and alcohol
- Reversed Phase
 - Mixture of water and alcohol or acetonitrile
 - We supply columns shipped under RP solvent
 - It is recommended to dedicate a column to reversed phase conditions because solvent switch to other modes is tedious.
- Supercritical Fluid Chromatography
 - Mixture of carbon dioxide and organic

„Coated“ Reprosil Chiral Polysaccharide Phases Solvent Compatibility

As the polysaccharide layer is not chemically bonded to the silica support but physically adsorbed, strong solvents have the ability to strip the polymer off the silica support and have therefore to be avoided even in trace amounts or as an injection solvent. Such strong solvents include:

- Ethers incl. THF
- Acetone
- Chlorinated solvents
- Ethyl acetate
- DMSO
- DMF
- Toluene
- Ketones
- Dimethylacetamid
- IPA > 50%

Recommended temperature range: 0 – 40°C

Recommended max. pressure: 150 bar

„Immobilised“ Reprosil Chiral Polysaccharide Phases

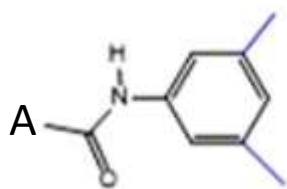
Solvent Compatibility

Immobilised Reprosil Chiral CSPs can be used in the same chromatography modes as the coated phases.

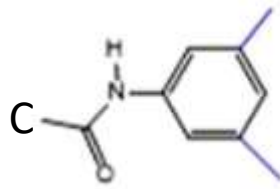
Through a proprietary process the polysaccharide layer is chemically immobilised enabling the use of an extended range of solvents as an eluent and / or injection solvent.

- Injection of poorly soluble compounds in a stronger solvent
- Use of strong solvents in the mobile phase
- Inertness against accidental use of a strong solvent

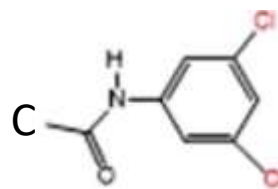
„Immobilised“ Reprosil Chiral Polysaccharide Phases



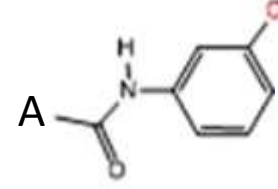
Reprosil Chiral MIA
(Chiralpak IA)



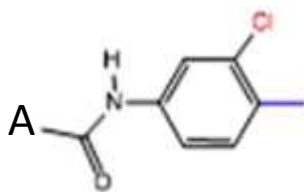
Reprosil Chiral MIB
(Chiralpak IB)



Reprosil Chiral MIC
(Chiralpak IC)

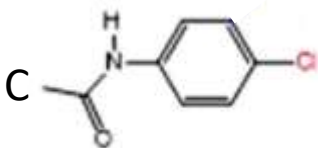


Reprosil Chiral MID
(Chiralpak ID)

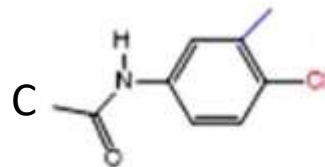


Reprosil Chiral MIF
(Chiralpak IF)

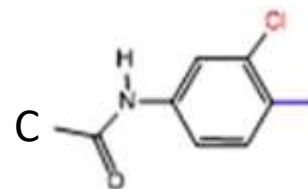
CSPs first introduced to the market by Dr. Maisch HPLC



Reprosil Chiral MOF



Reprosil Chiral MIX



Reprosil Chiral MIZ

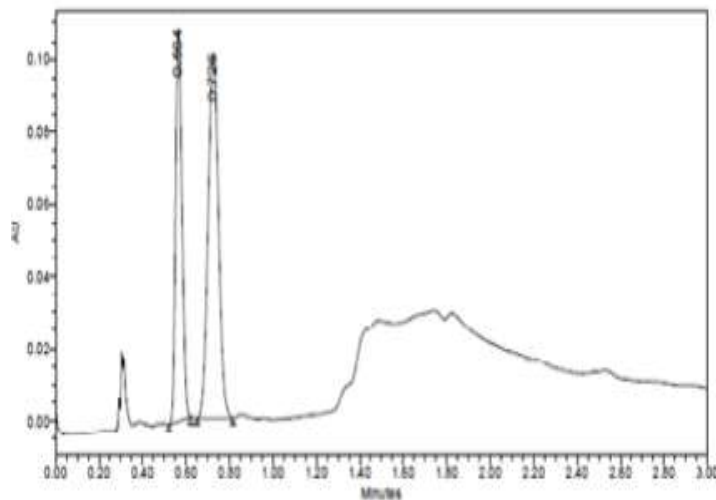
Reprosil Chiral – unique immobilised phases

Sample: customer sample

Eluent: CO₂/ 0 - 20% MeOH (0.1% DEA) in 2 min

Reprosil Chiral MIX

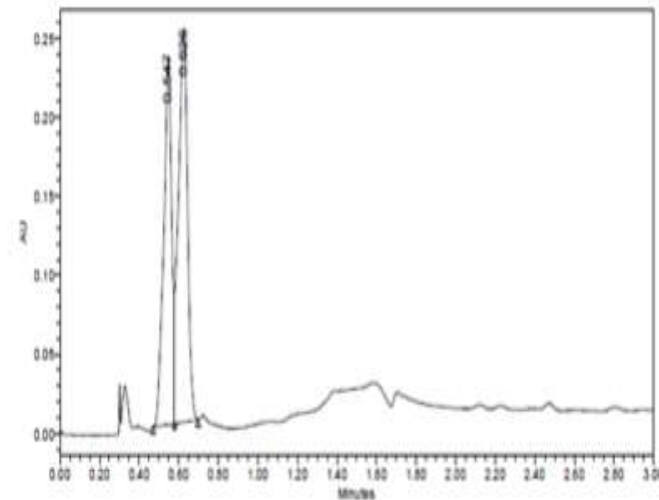
5 µm, 100 x 3.0 mm



	RT	Area	% Area	Resolution	USP Tailing
1	0.564	220320	39.72		1.20
2	0.726	334417	60.28	2.26	1.04

Lux Cellulose-4

3 µm 100 x 4.6 mm



	RT	Area	% Area	Resolution
1	0.547	847055	44.58	
2	0.626	804384	55.42	0.97

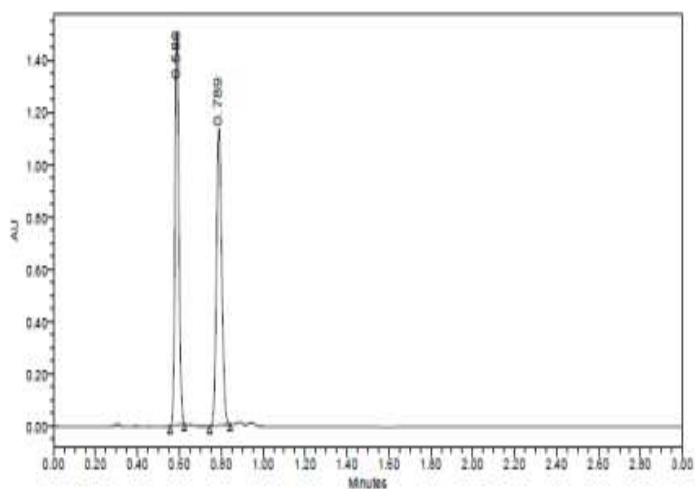
Reprosil Chiral – unique immobilised phases

Sample: TSO

Eluent: CO₂/ 10% MeOH (0.1% DEA)

Reprosil Chiral MIX

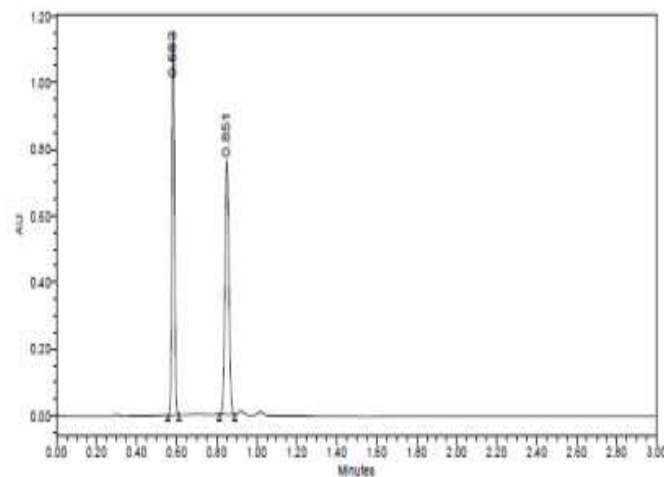
5 µm, 100 x 3.0 mm



	RT	Area	% Area	Resolution	USP Tailing
1	0.568	1970819	49.64		1.13
2	0.789	1999423	50.36	4.97	1.11

Lux Cellulose-4

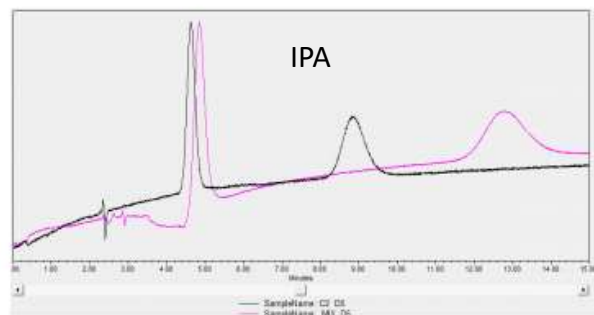
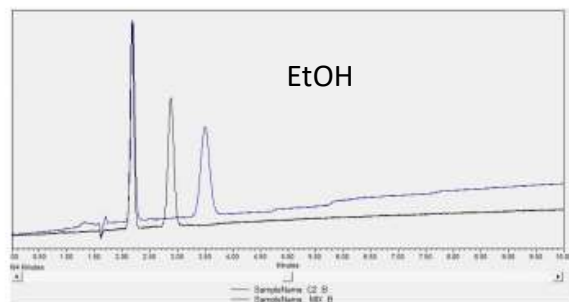
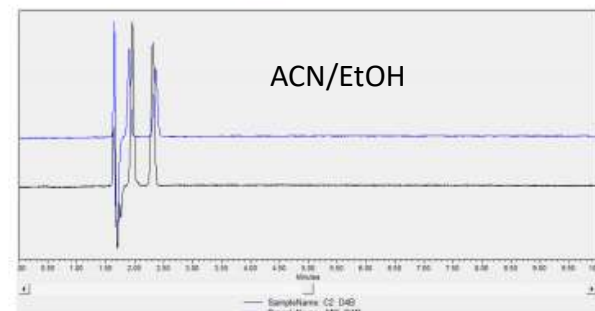
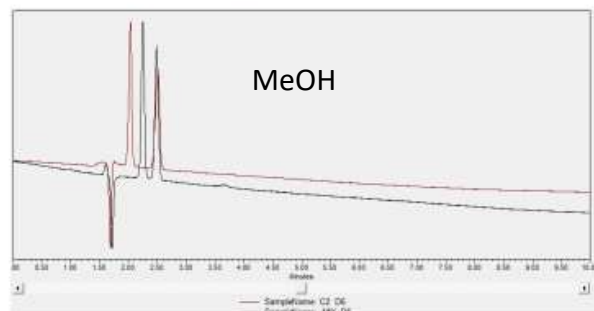
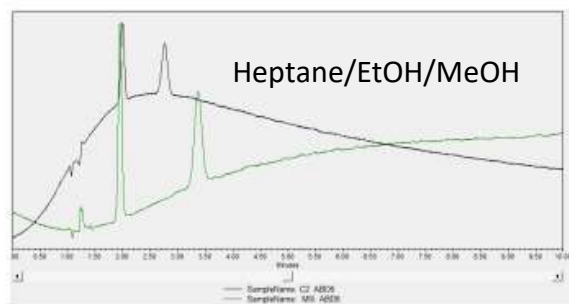
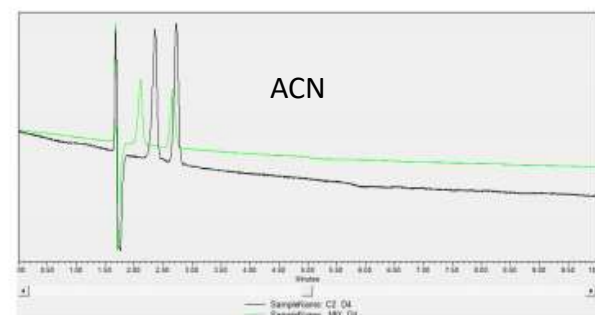
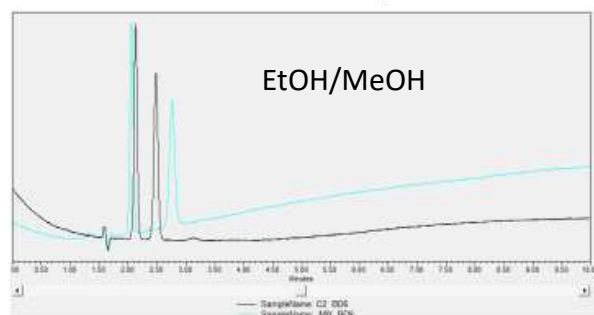
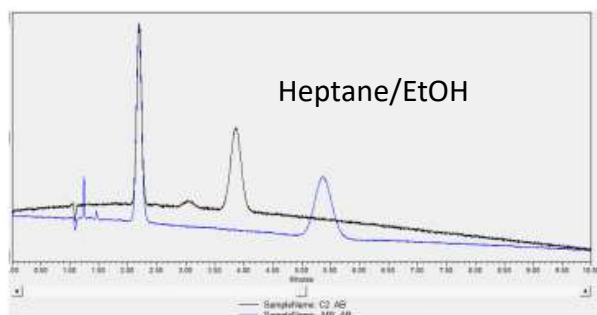
3 µm 100 x 4.6 mm



	RT	Area	% Area	Resolution	USP Tailing
1	0.563	1038300	49.91		1.05
2	0.851	1041945	50.09	8.89	1.04

Reprosil Chiral – unique immobilised phases

Reprosil Chiral MIX vs. Lux Cellulose-4 under NP/PO conditions



Customer sample
No additives used
Black: Lux Cellulose 4
Colour: Reprosil Chiral MIX

Reprosil Chiral – unique immobilised phases

Sample: customer sample

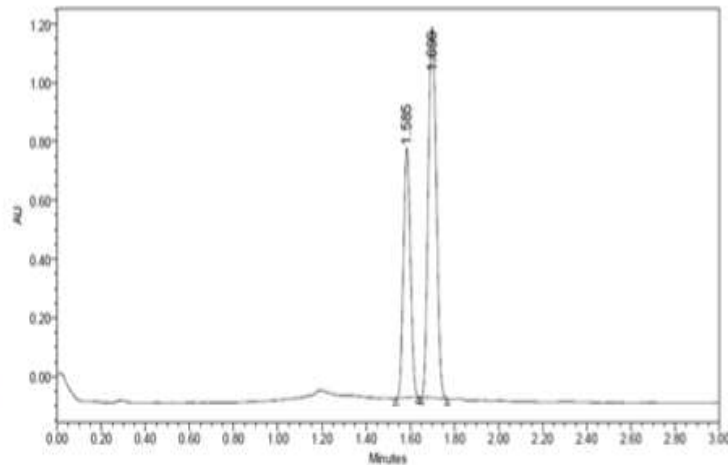
Eluent: CO₂/ 10-50% MeOH (0.1% DEA) in 2 min, hold until 5 min

Reprosil Chiral MIZ

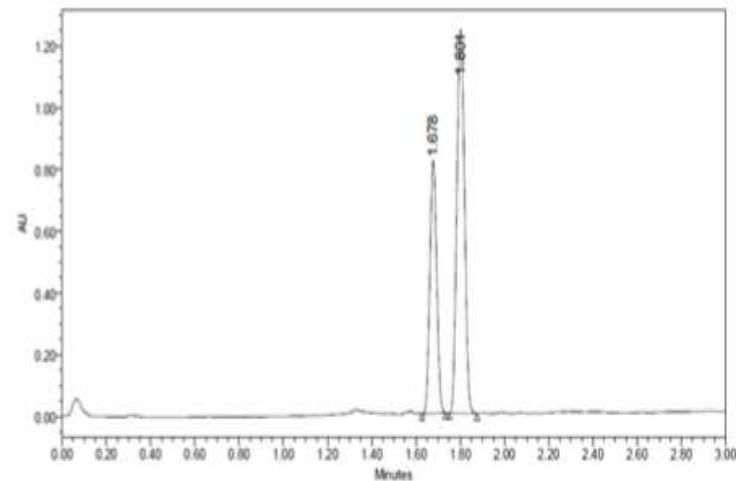
3 µm, 100 x 3.0 mm

Lux Cellulose-2

3 µm 50 x 4.6 mm



	RT	Area	% Area	Resolution	USP Tailing
1	1.585	1872263	36.75		1.06
2	1.699	3221889	63.25	1.77	1.09



	RT	Area	% Area	Resolution	USP Tailing
1	1.678	1757077	36.89		1.05
2	1.801	3005764	63.11	2.00	1.06

Reprosil Chiral – column screening

- It is not possible to predict the most successful CSP from the structure of the analyte
- Start in preferred mode
 - All modes have similar success rates, so the speed to success does rarely depend on where to start
 - NP most popular
- Screen multiple columns (Column Selector)
- Screen multiple mobile phase conditions
 - In parallel (multiple systems and columns)
 - In series (solvent switching and column equilibration)

Reprosil Chiral – typical mobile phase conditions

Normal Phase

- Mixtures of hexane or heptane with alcohols (EtOH, IPA) = 80:20 (vary % alcohol to adjust retention time and selectivity)
- Add 0.1 – 0.5% DEA or TEA for basic analytes and 0.1 – 0.5 % TFA or AcOH for acidic analytes

Polar Organic Phase

- Mixtures of ACN / IPA (95/5) or MeOH / IPA (90/10) or neat ACN
- Add 0.1 – 0.5% DEA or TEA for basic analytes and 0.1 – 0.5 % TFA or AcOH for acidic analytes

Reversed Phase

- ACN or MeOH or EtOH / water mixtures
- Water content must be < 85%
- Add 0.5 – 1 N perchlorate or 0.1% TFA for basic compounds and HClO₄/NaClO₄ buffer for acidic compounds together with ACN
- Use dedicated column for reversed phase conditions because solvent switch is tedious

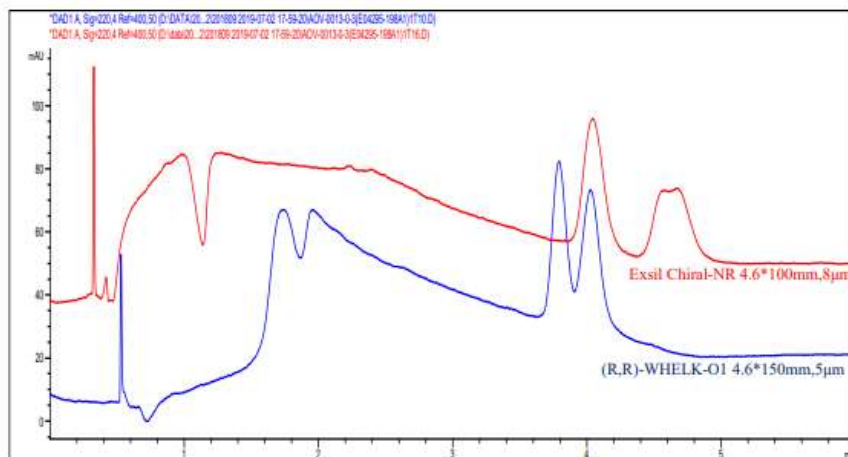
Reprosil Chiral NR – unique chemically bonded phase

- ❖ Immobilised brush-type phase
- ❖ Dinitro compound, π -electron and donor phase
- ❖ Particularly useful for aromatic compounds with O or N near chiral centre
- ❖ NR, RP, SFC mode
- ❖ Both antipodes of chiral selector available → elution order reversible

CHIRAL PREPARATIVE SEPARATIONS WITH HPLC
BY HOFFMANN-LA ROCHE, BASEL IN 2014.

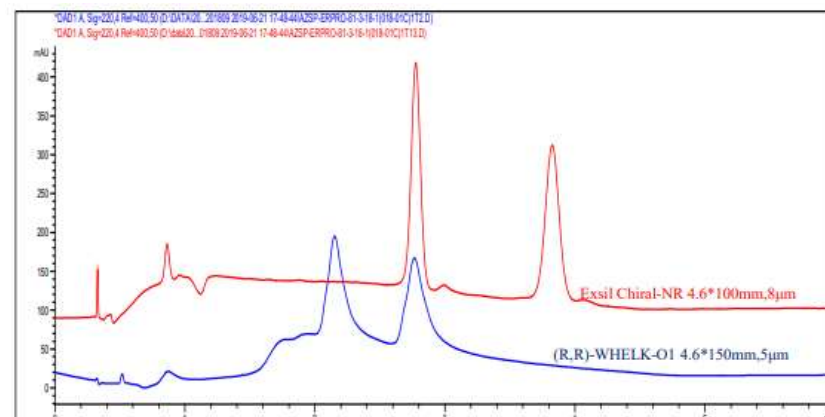
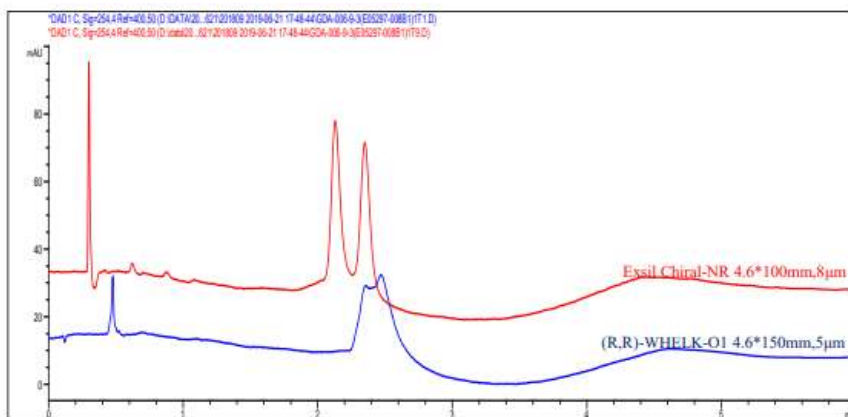


Reprosil Chiral NR vs. Whelk-O1



Co-Solvent: MeOH(0.1%DEA)
 Gradient (B%) : 10% to 50% in 2.0min, hold 1.0min at 50%
 Temperature : 35
 Flow (ml/min) : 4
 Back Pressure(psi): 1500.000
 Detector: 220nm

8 μ m Reprosil Chiral NR vs.
 5 μ m (R,R)-WHELK-O1

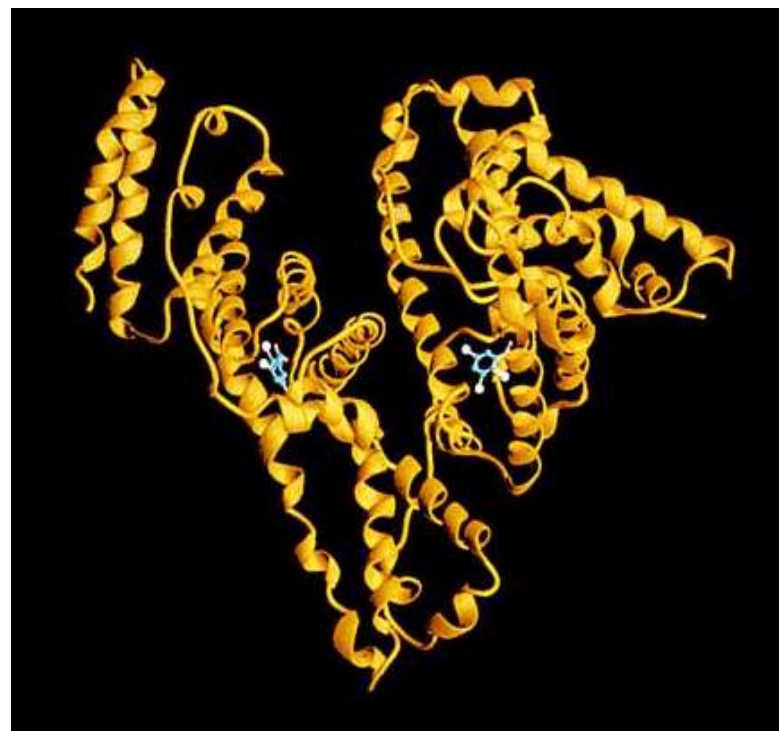


Other Specialty Reprosil Chiral CSPs Protein Phases

Reprosil Chiral HSA

Reprosil Chiral AGP

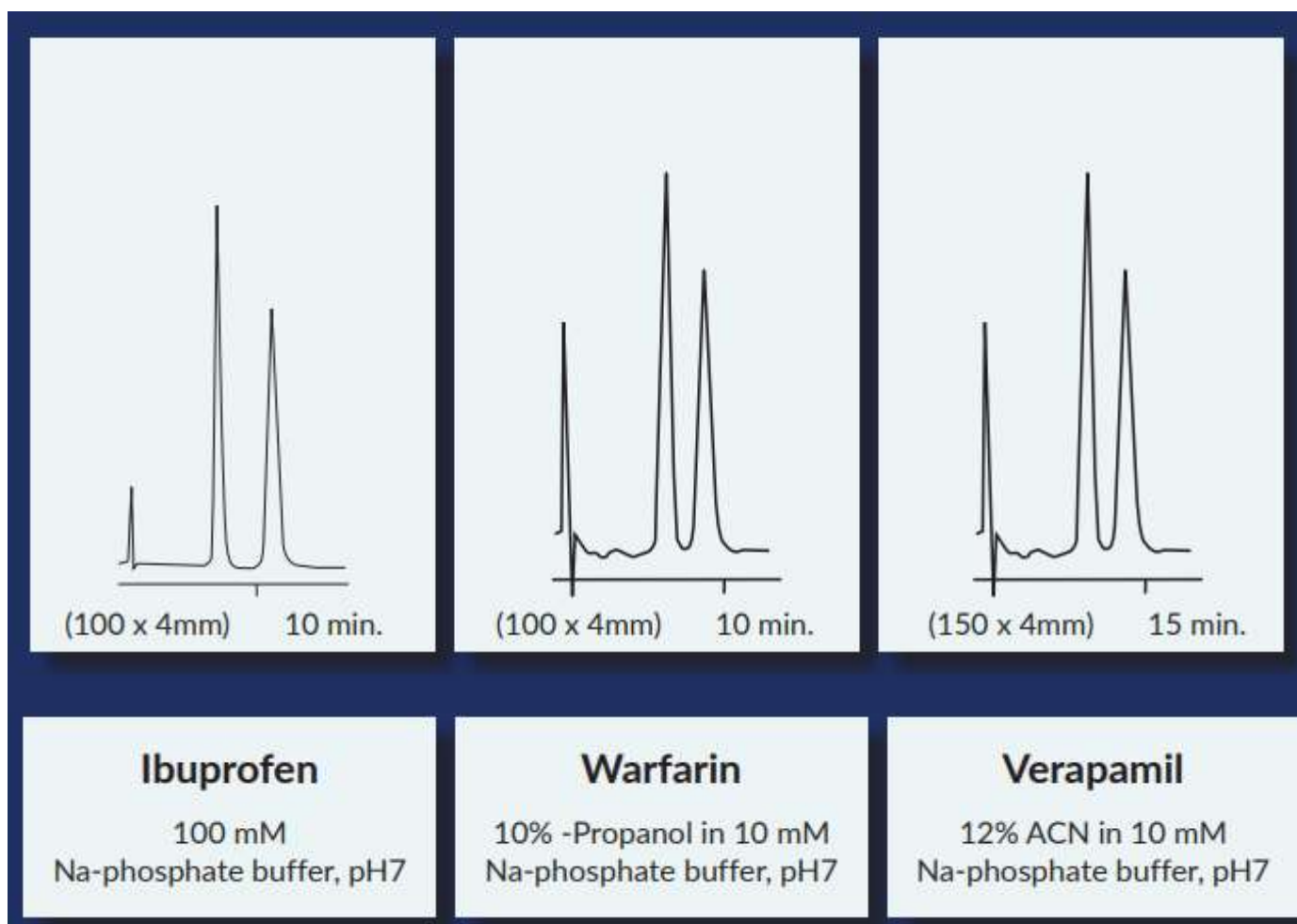
- Protein on 300 Å silica gel
- Very versatile in RP mode
- Complex mechanism of interaction
- Solvent limitations
- Low loading capacity



HSA Protein

Other Specialty Reprosil Chiral CSPs Protein Phases

Reprosil Chiral-AGP, 5 μm



Other Specialty Reprosil Chiral CSPs

Cyclodextrin Phases (developed at Univ. Tübingen, Institute of Prof. Schurig)

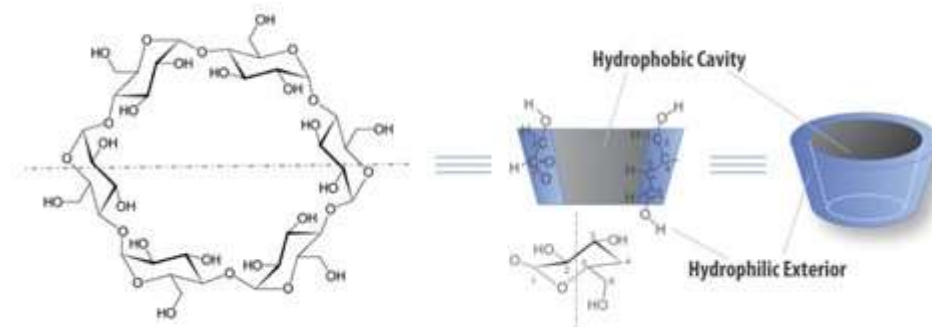
Reprosil Chiral Beta-CD

Native chiral beta-cyclodextrin phase covalently bonded to 100 Å silica

Reprosil Chiral Beta-PM

Permethylated beta-cyclodextrin phase covalently bonded to 100 Å silica.

Applications: dansyl amino acids, barbiturates, propranolol, sulfonamides, prostaglandins, chlorthalidone

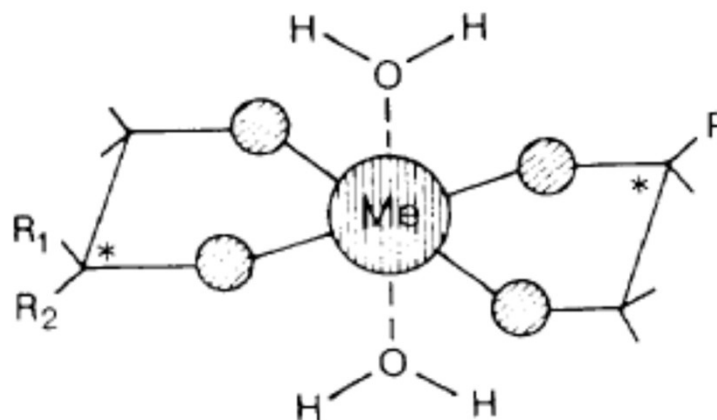


Other Specialty Reprosil Chiral CSPs Ligand Exchange Phases

Reprosil Chiral L-Prolin

Reprosil Chiral L-Hydroxy Prolin

- Amino acid chemically bonded to silica gel support
- Elution with 2-10 mM copper sulfate
- Excellent for the analysis of underivatized amino acids



Scale-up to preparative chromatography

ONGLIFE® Preparative Chromatography Column Hardware

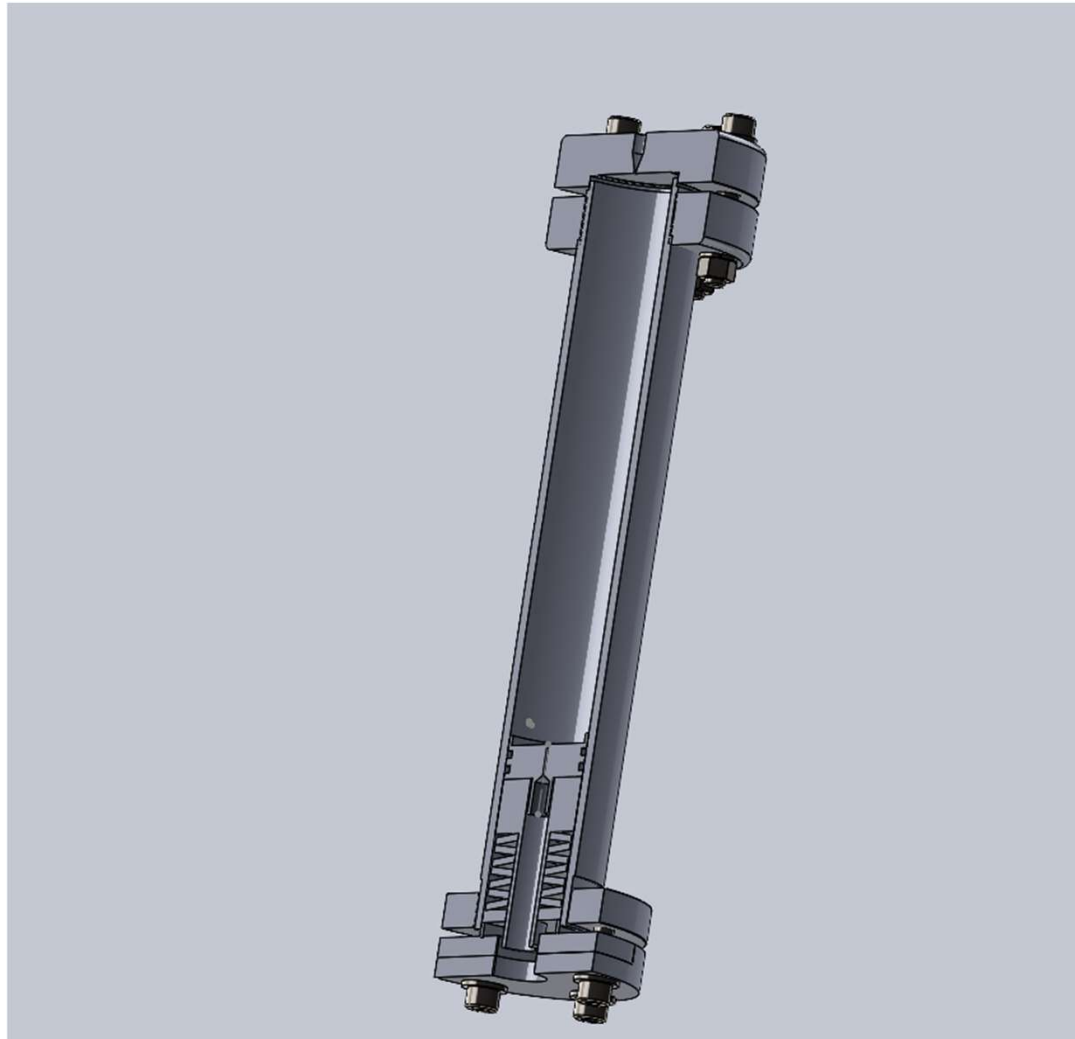
Technology patented by
Dr. Maisch HPLC

ONGLIFE















**THE ONLY PREPACKED COLUMN ON THE MARKET
WITH INTEGRATED DYNAMIC AXIAL
COMPRESSION MECHANISM**

Longlife hardware



Longlife: Benefits

	Longlife (Dr. Maisch)			Axia (Phenomenex)			OBD (Waters)		
Packed by piston									
Flexible bed length									
DAC <i>and</i> SAC mechanism									
Packing and <i>repacking</i> service									
Available column IDs	25, 30, 40, 50, 70			21.2, 30, 50			19, 30, 50		

Seamless Scale-up Without Loss of Performance

ReproSil Chiral-NR, 8 μ m upscaling

250 x 4,6

Flow: 1 ml/min



TEST CONDITIONS

Mobile Phase:
Heptane/IPA 85/15
Temperature: Ambient
Pressure: 15 bar
Detector: UV @ 229 nm
Sensitivity: 0,1 mV

250 x 20

Flow: 15 ml/min



Mobile Phase:
Heptane/IPA 85/15
Temperature: Ambient
Pressure: 9 bar
Detector: UV @ 254 nm
Sensitivity: 0,0 mV

250 x 30

Flow: 30 ml/min



Mobile Phase:
Heptane/IPA 85/15
Temperature: Ambient
Pressure: 11 bar
Detector: UV @ 254 nm
Sensitivity: -0,8 mV

250 x 50

Flow: 60 ml/min



Mobile Phase:
Heptane/IPA 85/15
Temperature: Ambient
Pressure: 12 bar
Detector: UV @ 254 nm
Sensitivity: -0,3 mV

Summary

- Reprosil Chiral Polysaccharide phases are available with multiple chiral selectors and are amenable to NP, PO, RP and SFC conditions with complementary selectivity
- Immobilised phases are available which tolerate a wider selection of solvents
- 3 unique immobilized phases available from Dr. Maisch
- Unique Reprosil Chiral NR phase
- Economically priced
- Wide selection of specialty phases (ligand exchange, cyclodextrine, amino acid phases ...)

THANK YOU

Dr. Maisch GmbH

Any Column, Any Size, Any Media

Beim Brückle 14
72119 Ammerbuch
Germany

Tel. +49 (0)7073 50357
info@reprosil.com
www.dr-maisch.com
www.modcol.eu