



HICHROM

Chromatography Columns and Supplies

LC COLUMNS
Regis
Chiral Phases

Catalogue 9

Hichrom Limited

1 The Markham Centre, Station Road
Theale, Reading, Berks, RG7 4PE, UK

Tel: +44 (0)118 930 3660 Fax: +44 (0)118 932 3484

Email: sales@hichrom.co.uk www.hichrom.co.uk

- Wide range of brush-type chiral phases
- Polysaccharide based phases
- Free chiral screening service
- Chiral application guide
- IAM and RAM phases
- GC derivatisation reagents
- Ion pairing reagents

Regis® Technologies manufactures a wide range of chiral and speciality HPLC and SFC columns for analytical to preparative applications and supplies the ChiroSil® range of crown ether type chiral columns manufactured by RStech Corporation, Korea (see page 208). RegisCell®, RegisPack® and RegisPack CLA-1™ are polysaccharide phases, described on page 206.

Pirkle Chiral Phases

- Column durability due to covalent bonding
- Ability to invert elution order
- Excellent chromatographic efficiency
- Universal solvent compatibility
- Analytical to preparative dimensions
- Enantiomer separation of wide variety of compound types
- Applicable for HPLC and SFC methods

Regis Technologies is the leading manufacturer of 'brush-type' or Pirkle concept chiral stationary phases, for use in both normal-phase and reversed-phase modes. For each phase, the optically active ligand is covalently bonded to 5µm, 100Å spherical silica.

Regis Pirkle Chiral Phases

| Phase | Bonding | Class | Typical Applications |
|----------------------|--|---------------------------|---|
| α-Burke 2 | N-3,5-dinitrobenzoyl-α-amino-2,2-dimethyl-4-pentenyl phosphonate | π-electron acceptor | β-Blockers, amino alcohols |
| β-Gem 1 | N-3,5-dinitrobenzoyl-3-amino-3-phenyl-2-(1,1-dimethylethyl)-propanoate | π-electron acceptor | Anilide derivatives of wide range of carboxylic acids |
| DACH-DNB | 3,5-Dinitrobenzoyl derivative of 1,2-diaminocyclohexane | π-electron acceptor/donor | Broad range |
| Leucine | 3,5-Dinitrobenzoylleucine | π-electron acceptor | Benzodiazepines |
| Phenylglycine | 3,5-Dinitrobenzoylphenylglycine | π-electron acceptor | Wide variety of compounds containing π-basic groups |
| Pirkle 1-J | 3-(3,5-Dinitrobenzamido)-4-phenyl-β-lactam | π-electron acceptor | Underivatised β-blockers, arylpropionic acids |
| ULMO | 3,5-Dinitrobenzoyl derivative of diphenylethylene-diamine | π-electron acceptor/donor | Wide range, particularly aryl carbinols |
| Whelk-01 Whelk-02 | 1-(3,5-dinitrobenzamido)-tetrahydrophenanthrene | π-electron acceptor/donor | Broad range of compounds |

Features of Pirkle Phases

1) Inversion of elution order

An important advantage of the Pirkle chiral stationary phases (CSP) is the ability to invert elution order by using the same type of CSP, but with the opposite absolute configuration. As a result, by selecting the phase with the optimum configuration, it is possible to have the trace enantiomer elute before the major, which is beneficial for enantiomeric purity determinations and for preparative separations.

2) Analytical and preparative columns

All of Regis' Pirkle columns are available in both analytical and preparative sizes. The high loading factors offered by these phases make them particularly suited for scaling up.

3) Enantiomeric purity determination

Pirkle CSPs are particularly useful for the accurate determination of enantiomeric purity, especially in trace analysis because they provide excellent resolution. Such determinations are fast, accurate and sensitive.

4) Universal solvent compatibility

Pirkle HPLC columns are compatible with most HPLC eluents with pH between 2.5 and 7.5. Although superior resolution is typically observed with normal-phase elution, many racemates can be separated in either normal- or reversed-phase mode. The separation of enantiomers using SFC with Pirkle columns is now well proven.

Pirkle Chiral Phases (continued)

Whelk-0[®]1 and Whelk-0[®]2

Whelk-0[®]1 is the most widely applicable chiral phase, due to the incorporation of both π -acceptor and π -donor characteristics. It was originally designed for the separation of underivatized non-steroidal anti-inflammatory drugs, but shows versatility in the analysis of a wide range of compounds, including amides, epoxides, esters, ureas, carbamates, ethers, aldehydes, ketones, carboxylic acids and alcohols.

The newer **Whelk-01** phase, based on 5 μ m Kromasil silica, shows higher efficiencies and greater resolving power in both HPLC and SFC modes than the original 5 μ m Whelk-01 phase. Although the original material is still offered for the continuation of validated methods, for all other methods it is recommended that the newer Whelk-01 product is used. Figures 1 and 2 show comparisons of the HPLC and SFC performances of these Whelk-01 phases for modafinil and chlormezanone respectively.

Columns: Whelk-01 5 μ m (Exsil) – blue trace
Whelk-01 5 μ m (Kromasil) – red trace

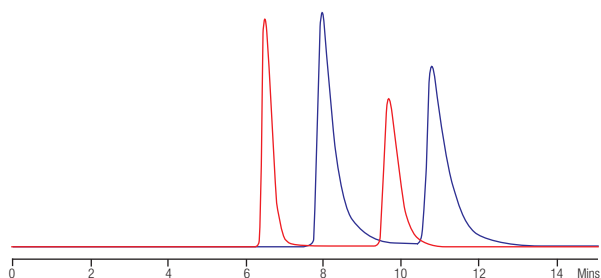


Figure 1. HPLC separation of modafinil

Columns: Whelk-01 5 μ m (Exsil) – blue trace
Whelk-01 5 μ m (Kromasil) – red trace
Eluent: CO₂ – EtOH (75:25)
Flow rate: 2.25ml/min

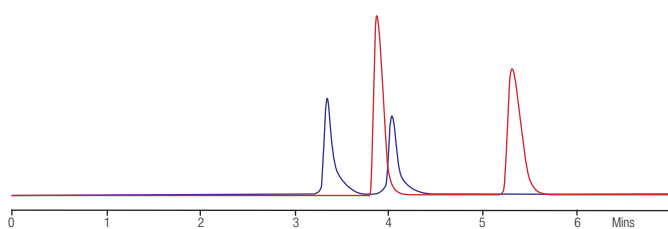
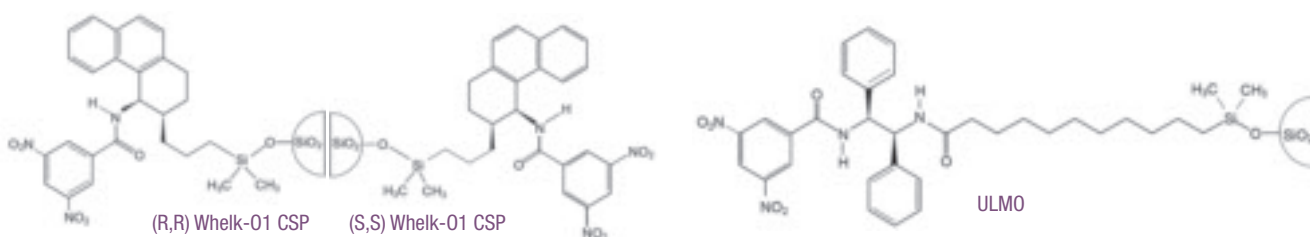


Figure 2. SFC separation of chlormezanone

Whelk-0[®]2 is the covalent trifunctional version of the Whelk-01. It shows similar enantioselectivity as the Whelk-01, but enhanced stability with strong organic modifiers.



ULMO

The ULMO chiral stationary phase has the general capability of separating enantiomers of many racemate classes and is particularly good at separating enantiomers of aryl carbinols.

DACH-DNB

DACH-DNB contains the 3,5-dinitrobenzoyl derivative of trans 1,2-diaminocyclohexane. It can resolve a wide range of compound classes, including sulphoxides, phosphine oxides, selenoxides, organometallics and atropisomers.

Column: (S,S)-ULMO
(250 x 4.6mm)
Eluent: Heptane - IPA (99:1)
Flow rate: 1ml/min

Run time: 6 mins
 k_1' : 0.86
 α : 1.38

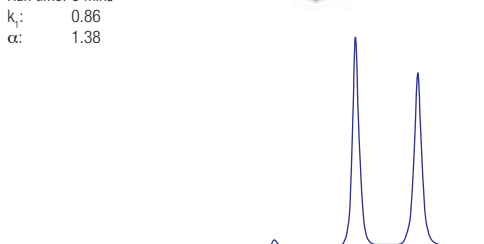
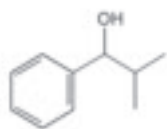


Figure 3. Separation on (S,S)-ULMO

Column: (S,S)-DACH-DNB
(250 x 4.6mm)
Eluent: CH₂Cl₂ - IPA (98:2)
Flow rate: 1ml/min

Run time: 17 mins
 k_1' : 3.33
 α : 1.63

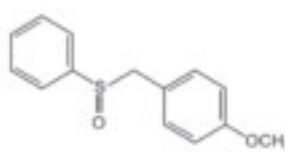


Figure 4. Separation on (S,S)-DACH-DNB

RegisCell®, RegisPack® and RegisPack CLA-1™

- Polysaccharide coated phases
- Excellent selectivity
- Fast equilibration times
- Applicable for HPLC and SFC methods

RegisCell® and RegisPack® chiral columns are polysaccharide based stationary phases. They are produced using a unique manufacturing process involving the coating of the proven chiral selectors tris-(3,5-dimethylphenyl) carbamoyl cellulose and tris-(3,5-dimethylphenyl) carbamoyl amylose respectively onto a high purity wide pore (1000Å) silica.

Columns are packed and tested at high pressures for use in both SFC and HPLC modes. The packing materials have a high pressure limit (450 bar), which enables faster flow rates and decreased equilibration times when switching from one compatible eluent to another.

RegisCell and RegisPack produce excellent performance and resolution in the separation of a wide range of compounds, by both HPLC and SFC. Figure 5 shows the separation of metalaxyl on RegisCell and Figure 6 shows the separation of methaqualone on RegisPack. Fast SFC analyses have been developed for a number of applications, using simple eluent conditions, such as shown in Figure 7 for the analysis of atenolol in less than one minute.

The newer RegisPack CLA-1, a coated chlorinated phase with the chiral selector tris-(5-chloro-2-methylphenyl) carbamoyl amylose, shows complementary selectivity to the other Regis phases. In cases where the proven chiral phases Whelk-O1, RegisPack and RegisCell give incomplete separation, RegisPack CLA-1 can be a useful addition to the screening process. Figure 8 shows the separation of the four diastereomers of cyclandelate by HPLC on a RegisPack CLA-1 column.

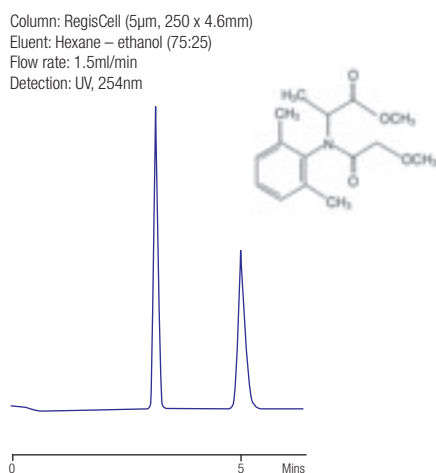


Figure 5. Separation of metalaxyl on RegisCell

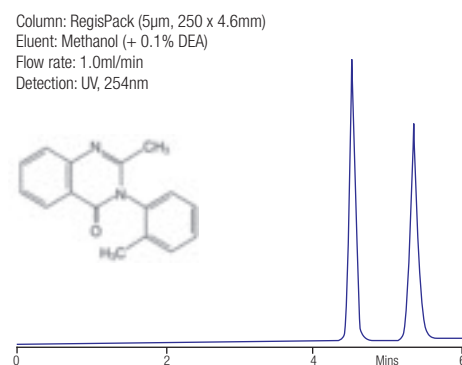


Figure 6. Separation of methaqualone on RegisPack

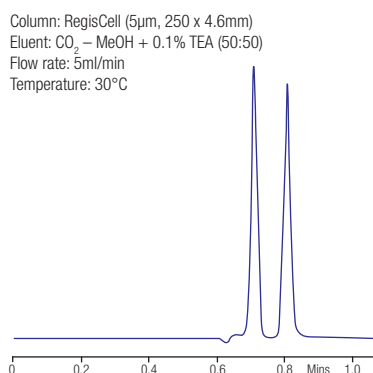


Figure 7. Fast SFC separation of atenolol

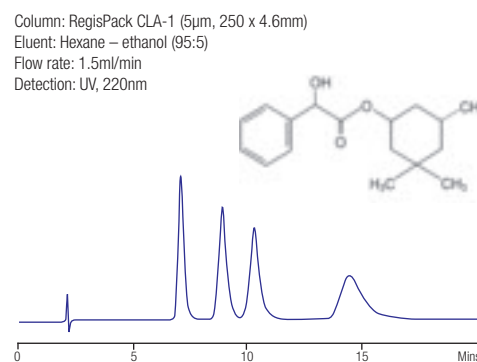


Figure 8. Cyclandelate on RegisPack CLA-1

Ordering Information – Regis Chiral Columns

| Regis Phase (5µm) ¹ | Column Dimensions ² (mm) | | | | | | | |
|------------------------------------|-------------------------------------|------|----------|--------|-----------|--------|----------|--------|
| | 50 x 2.1 | | 50 x 4.6 | | 250 x 4.6 | | 250 x 10 | |
| (R,R)-α-Burke 2 | 731602 | £902 | 731613 | £902 | 735035 | £1,694 | 735235 | £4,117 |
| (S,S)-α-Burke 2 | 731603 | £902 | 731614 | £902 | 735037 | £1,694 | 735237 | £4,117 |
| (R,R)-β-Gem 1 | 731600 | £799 | 731611 | £799 | 731043 | £1,694 | 731243 | £4,117 |
| (S,S)-β-Gem 1 | 731601 | £799 | 731612 | £799 | 731029 | £1,694 | 731229 | £4,117 |
| (R,R)-DACH-DNB | 788246 | £902 | 788248 | £902 | 788101 | £1,468 | 788102 | £3,603 |
| (S,S)-DACH-DNB | 788247 | £902 | 788249 | £902 | 788201 | £1,468 | 788202 | £3,603 |
| D-Leucine | 731607 | £799 | 731618 | £799 | 731054 | £802 | 731254 | £1,872 |
| L-Leucine | 731608 | £799 | 731619 | £799 | 731041 | £802 | 731241 | £1,872 |
| D-Phenylglycine | 731609 | £799 | 731620 | £799 | 731021 | £802 | 731221 | £1,872 |
| L-Phenylglycine | 731610 | £799 | 731621 | £799 | 731024 | £802 | 731224 | £1,872 |
| (3R,4S)-Pirkle 1-J | 731604 | £799 | 731615 | £799 | 731044 | £1,694 | 731244 | £4,117 |
| (3S,4R)-Pirkle 1-J | 731605 | £799 | 731616 | £799 | 731045 | £1,694 | 731245 | £4,117 |
| (R,R)-ULMO | 787648 | £902 | 787650 | £902 | 787200 | £1,468 | 787201 | £3,603 |
| (S,S)-ULMO | 787647 | £902 | 787649 | £902 | 787100 | £1,468 | 787101 | £3,603 |
| (R,R)-Whelk-01 ³ | 780255 | £902 | 780252 | £902 | 780201 | £1,468 | 780202 | £3,603 |
| (S,S)-Whelk-01 ³ | 780155 | £902 | 780152 | £902 | 780101 | £1,468 | 780102 | £3,603 |
| (R,R)-Whelk-01 ⁴ | 786901 | £902 | 786905 | £902 | 786201 | £1,468 | 786202 | £3,603 |
| (S,S)-Whelk-01 ⁴ | 786900 | £902 | 786904 | £902 | 786101 | £1,468 | 786102 | £3,603 |
| (R,R)-Whelk-01 ³ (10µm) | 786903 | £902 | 786907 | £902 | 786515 | £1,468 | 786525 | £3,603 |
| (S,S)-Whelk-01 ³ (10µm) | 786902 | £902 | 786906 | £902 | 786615 | £1,468 | 786625 | £3,603 |
| (R,R)-Whelk-02 ³ (10µm) | - | - | - | - | 786315 | £1,552 | 786325 | £3,603 |
| (S,S)-Whelk-02 ³ (10µm) | - | - | - | - | 786415 | £1,552 | 786425 | £3,603 |
| RegisCell | - | - | 784101 | £1,119 | 784104 | £1,306 | 784105 | £4,103 |
| RegisPack | - | - | 783101 | £1,119 | 783104 | £1,306 | 783105 | £4,103 |
| RegisPack CLA-1 | - | - | 793101 | £1,119 | 793104 | £1,306 | 793105 | £4,103 |
| RegisCell (10µm) | - | - | - | - | 784204 | £1,306 | 784205 | £4,103 |
| RegisPack (10µm) | - | - | 783201 | £1,119 | 783204 | £1,306 | 783205 | £4,103 |
| RegisPack CLA-1 (10µm) | - | - | 793201 | £1,119 | 793204 | £1,306 | 793205 | £4,103 |

¹ Bulk material and preparative columns available² Other dimension columns available³ Based on Kromasil silica⁴ Based on original Exsil silica

Free Chiral Screening Service

Regis offer a free chiral screening service, where a small amount (20mg) of customer supplied sample is screened on a range of the key Regis columns. If you are interested in this service, please contact Hichrom to obtain a submission form and Confidentiality Agreement. Screening in both HPLC and SFC modes is available. Advice can also be given on scaling up chiral separations to preparative dimensions.

Ion Pairing Reagents

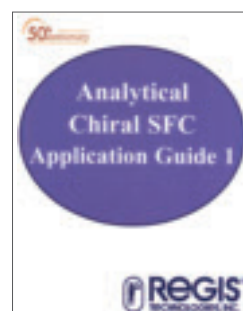
Ion pairing reagents can be used to selectively increase the retention of charged analytes on conventional hydrophobic HPLC phases. Regis manufactures both anionic sulphonate (S-Series) and cationic quaternary amine (Q-Series) ion pairing reagents with varying alkyl chain lengths. The S-Series reagents are available as 0.5M solutions of alkyl sulphonates (S5-S8, S12) or as bulk powder. The Q-Series consists of 0.5M solutions of quaternary alkyltriethylamines (Q5-Q8, Q12). Method development kits are also available.

GC Derivatization Reagents

Regis manufactures a wide range of high purity derivatization reagents for GC. These include reagents for silylation, alkylation and acylation reactions. Please see page 294 for further details and ordering information.

Regis Chiral Application Guides

A wide range of examples of enantiomer separations can be found in the Regis Chiral HPLC Application Guide and Chiral SFC Application Guide. Please contact us for a free copy.



CHIROSil®

- Efficient analysis of amino acids and primary amines
- Excellent durability due to covalent bonding
- Ability to invert elution order
- Robust crown ether phase for HPLC

The ChiroSil® RCA(+) and SCA(-) crown ether chiral stationary phases were developed by RStech Corporation in South Korea. These specialised phases are effective in separating various natural and unnatural amino acids, as well as compounds containing a primary amino group near the chiral centre. They are also successfully used for chiral resolution of chiral amino alcohols including therapeutically active compounds such as amphetamine, phenylethanolamine, octopamine and norepinephrine.

These phases are prepared by a covalent trifunctional bonding (+) or (-)-(18-crown-6)-tetracarboxylic acid chiral selector to aminopropyl silica (see Figure 9). This results in a material which shows excellent durability and reproducibility. The high resolution capability of the phases enables applications to be scaled up from analytical to preparative dimensions. The availability of both enantiomeric forms of the phase enables the elution order to be inverted so that a trace enantiomer can be eluted first.

ChiroSil RCA(+) and SCA(-) phases

| | |
|--|---------|
| Particle Size (µm) | 5, 10 |
| Pore Size (Å) | 100 |
| Maximum Recommended Operating Temperature (°C) | 50 |
| Recommended pH Range | 2 – 7.5 |

Figures 10 and 11 show the enantiomeric separation of glutamic acid and 1,2,3,4-tetrahydro-1-naphthylamine respectively using ChiroSil SCA(-).

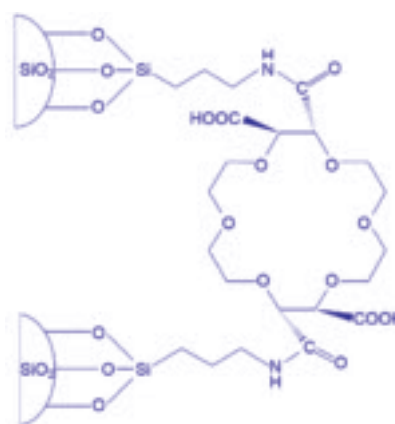
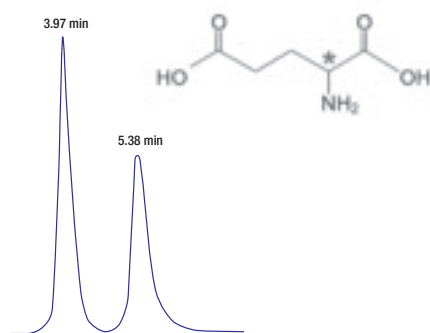
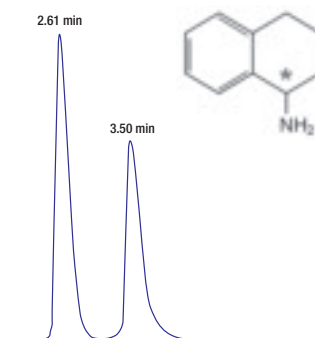


Figure 9. Bonding of ChiroSil phase



Eluent: CH₃OH - H₂O (84:16) in 5mM HClO₄
Flow rate: 0.8ml/min

Figure 10. Analysis of glutamic acid



Eluent: CH₃OH - H₂O (84:16) in 10mM H₂SO₄ + 1ml Et₃N
Flow rate: 1ml/min

Figure 11. Analysis of 1,2,3,4-tetrahydro-1-naphthylamine

Ordering Information – ChiroSil Phases

| ChiroSil Phase (5µm) | Column Dimensions (mm) | | | Guard Cartridge ¹ (for 4.6mm i.d. columns) |
|----------------------|------------------------|-----------|-----------|--|
| | 150 x 2.1 | 150 x 4.6 | 250 x 4.6 | |
| | £1,763 | £1,763 | £1,923 | £192 |
| RCA(+) | 799003 | 799001 | 799002 | 799200 |
| SCA(-) | 799103 | 799101 | 799102 | 799100 |

¹ Use with guard cartridge holder 731441 (£144)