

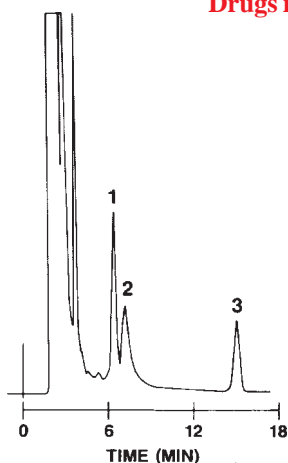
# Regis Semi-Permeable Surface (SPS) Columns

HYDROPHILIC  
OUTER PHASE/SURFACE  
[-O-CH<sub>2</sub>-CH<sub>2</sub>-O-]

HYDROPHOBIC  
INNER PHASE  
[CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>]

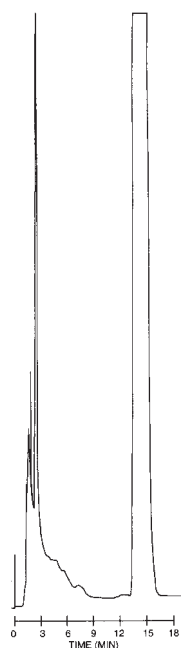


## Drugs in Serum



The 15cm SPS C8 separates theophylline (1) from serum very cleanly and could be used to separate it from caffeine (2) and

## Column Switching Application: SPS C8 1 x 1 to SPS C8 Analytical



**Column 1:** SPS-5PM-S5-100-C8 Column  
1cm x 10.0mm ID  
**Eluent:** 0.1M Potassium Dihydrogen Phosphate,  
pH 6.8/acetonitrile (90/10)  
**Flow rate:** 1.0mL/min.

**Column 2:** SPS-5PM-S5-100-C8 Column  
1cm x 10.0mm ID  
**Eluent:** 0.1M Potassium Dihydrogen Phosphate,  
pH 6.8/acetonitrile (77/23)  
**Flow rate:** 1.0mL/min.

**Load:** 1.0 mL  
**Detection:** UV 254nm  
**Sample:** Carbamazine, 10 µg/mL in Serum

In an effort to extend the applicability of the RAM (Restricted Access Media) direct injection columns, Regis, in conjunction with Dr. Fred Regnier and Dr. Carla Desilets at Purdue University, developed the Semi-Permeable Surface (SPS) phases.

## SPS Structure

The SPS phases consist of both hydrophilic outer and hydrophobic inner surfaces. The inner and outer surfaces of the SPS are bonded separately, allowing each to be varied independently. The SPS structure includes a hydrophobic inner phase such as ODS, and a hydrophilic outer phase of polyethylene glycol. The outer phase provides size exclusion and hydrophilic shielding, which repels large biomolecules. The various inner phases allow for separation of small analytes.

## SPS Column Advantage

The SPS offers the following advantages:

- Increased durability
- Increased selectivity
- Allows use of buffered, normal phase, and reversed phase systems

## SPS Selectivity

The primary advantage of SPS is that the inner surface of SPS may be varied independently of the outer, resulting in a wider scope of analysis opportunities. Available inner phases include the following:

- Nitrile
- Octyl (C8)
- ODS (C18)
- Phenyl

The retention mechanism of these SPS phases involves hydrogen bonding by the outer phase and hydrophobic interaction by the inner phase. Polar solutes interact primarily with the outer phase and show little discrimination among the various inner phases. Conversely, the nonpolar solutes interact primarily with the inner phase.

The SPS phases allow use of buffered, normal phase, and reversed phase eluents. The actual composition is limited only by the pH and organic modifier parameters dictated by the proteins contaminated within the sample.

## SPS Columns (4.6mm ID, 5µm, 100Å)

| Inner Phase | 5cm    | 15cm   | 25cm   | Guard Kit | Guard Repl. |
|-------------|--------|--------|--------|-----------|-------------|
| Octyl       | 785008 | 785108 | 785208 | 785408    | 785508      |
| ODS         | 785018 | 785118 | 785218 | 785418    | 785518      |
| Nitrile     | –      | 785105 | 785205 | 785405    | 785505      |
| Phenyl      | –      | 785107 | 785207 | 785407    | 785507      |

Column fittings are internal nut. Guard kits include a holder, coupler and 2 cartridges. Guard replacements are 3 cartridges.

## SPS Columns (2.1mm ID)

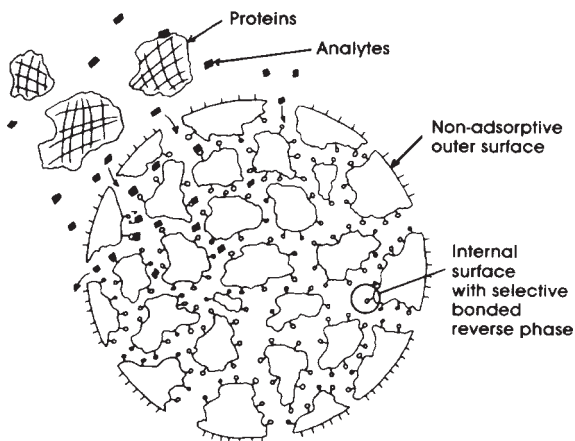
| Cat. No. | Description        |
|----------|--------------------|
| 785308   | Octyl, 2.1mm x 5cm |
| 785318   | ODS, 2.1mm x 5cm   |

## SPS Cartridges Columns

|        |                                     |
|--------|-------------------------------------|
| 785608 | Octyl kit, 5µm, 100Å, 1cm x 10mm ID |
| 785708 | Octyl replacement cartridges, 3/pk  |
| 785618 | ODS kit, 5µm, 100Å, 1cm x 10mm ID   |
| 785718 | ODS replacement cartridges, 3/pk    |

Kits include a holder and 2 cartridges.

# Regis ISRP (Internal Surface Reversed Phase)



Demonstrates the inner and outer layers of a typical ISRP phase.

Developed by Dr. Thomas Pinkerton, this material was created specifically for the direct analysis of drugs in serum without extensive sample preparation. The result was a new phase that allows for chromatographic separations without interference by protein adsorption.

## GFF I

The GFF name is derived from the glycine-L-phenylalanine-L-phenylalanine tripeptide bonded within the silica pores. GFF was chosen from many available peptides for its selectivity towards positively charged aromatic analytes and a variety of neutral molecules. The hydrophilic outer surface, created from the cleavage of the original tripeptide, is comprised of the single amino acid, glycine.

## GFF II

Continuing product improvement efforts resulted in the development of the ISRP GFFII, a second generation phase with an improved bonding process—bonding the GFF peptide to the silica surface through a monofunctional glycidoxypopyl linkage rather than the original trifunctional linkage. This resulted in the following improvements:

- Increased sample retention
- Higher column efficiency
- Greater batch-to-batch reproducibility

## GFF I

| Cat. No. | Description  |
|----------|--|
| 731449   | GFF I, 2.1mm x 5cm   |
| 731450   | GFF I, 4.6mm x 5cm   |
| 731451   | GFF I, 4.6mm x 15cm  |
| 731452   | GFF I, 4.6mm x 25cm  |
| 731440   | GFF I, Guard cartridge starter kit<br>(Holder, 2 cartridges and 1 connector) |
| 731444   | 3 GFF I, Guard cartridges  |
| 731441   | Guard cartridge holder   |

## GFF II

|        |   |
|--------|---|
| 731469 | GFF II, 2.1mm x 5cm   |
| 731470 | GFF II, 4.6mm x 5cm   |
| 731471 | GFF II, 4.6mm x 15cm  |
| 731472 | GFF II, 4.6mm x 25cm  |
| 731475 | GFF II, Guard cartridge starter kit<br>(Holder, 2 cartridges and 1 connector) |
| 731474 | 3 GFF II, Guard Cartridges  |

## ISRP Selectivity

Many variables can affect the selectivity of the ISRP phase, including:

## Mobile Phase Composition

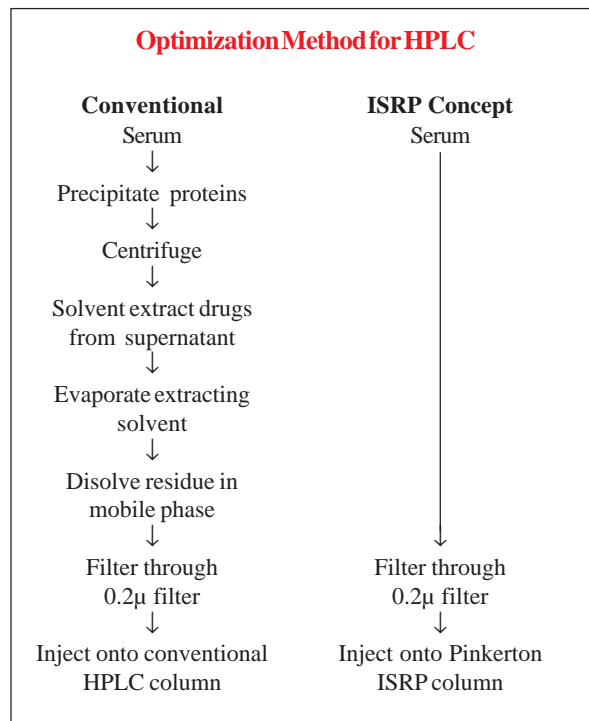
The nature of ISRP analytes requires that the mobile phases consist of a buffer with varying degrees of modification. Modifiers can include acetonitrile, methanol, isopropanol, and tetrahydrofuran. Caution: too much modifier can result in matrix precipitation.

## pH:

The pH of the mobile phase can be controlled to avoid protein denaturing and to enhance selectivity. The pH range of the column is between 2.5 and 7.5; however, within the optimal pH range of 6.0 to 7.5, both the proteins and the glycine outer surface take on a negative charge. As a result, negatively charged proteins are repulsed by the outer phase, and pass quickly through the column.

## Temperature:

Separations can also be optimized by varying column temperature. Lower temperatures have been shown to result in increased retention and selectivity.



# Regis Pirkle Chiral HPLC Columns

## Pirkle Chiral HPLC Columns

- Enantiomer separations
- Analytical to preparative scale

### Whelk-O 1

The Whelk-O 1 is useful for the separation of underivatized enantiomers in a number of families including amides, epoxides, esters, ureas, carbamates, ethers, aziridines, phosphonates, aldehydes, ketones, carboxylic acids, alcohols and non-steroidal anti-inflammatory drugs (NSAIDs).

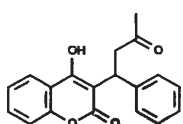
This  $\pi$ -electron acceptor/ $\pi$ -electron donor phase exhibits an extraordinary degree of generality. The broad versatility observed on the Whelk-O 1 column compares favorably with polysaccharide-derived chiral stationary phases. In addition, because Whelk-O 1 is covalently bonded to the support, the phase is compatible with all commonly used mobile phases, including aqueous systems – a distinct advantage over polysaccharide-derived chiral stationary phases.

### Whelk-O 2

The Whelk-O 2 is a covalent trifunctional version of the Whelk-O 1. The Whelk-O 2 retains the same chiral selector but incorporates a trifunctional linkage to the silica support. In most cases, the enantioselectivity remains the same as that obtained with the Whelk-O 1. Whelk-O 2 was designed to improve the resistance of the stationary phase to hydrolysis while using strong organic modifiers such as trifluoroacetic acid.

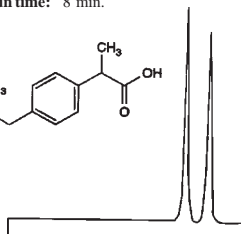
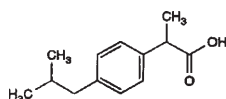
### Warfarin (reversed phase)

**Column:** Whelk-O 1,  
25cm x 4.6mm ID  
**Mobile phase:** 75/25 methanol/water  
**Flow rate:** 1.0mL/min.  
**Detection:** UV at 254nm  
**Run time:** 13 min.



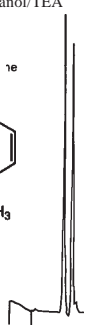
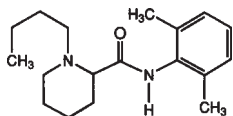
### Ibuprofen

**Column:** Whelk-O 1,  
25cm x 4.6mm ID  
**Mobile phase:** 98/2/0.05  
hexane/isopropanol/  
acetic acid  
**Flow rate:** 0.9mL/min.  
**Load:** 20 $\mu$ L  
**Detection:** UV at 254nm  
**Run time:** 8 min.



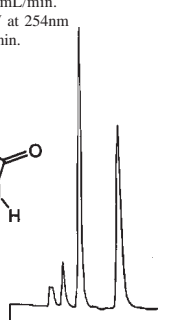
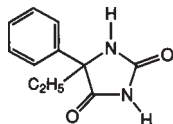
### Bupivacaine

**Column:** Whelk-O 1,  
25cm x 4.6mm ID  
**Mobile phase:** 80/20/0.1  
hexane/isopropanol/TEA  
**Flow rate:** 1.0mL/min.  
**Detection:** UV at 254nm  
**Run time:** 7-8 min.



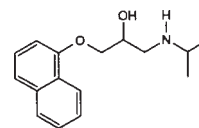
### Nirvanol

**Column:** (R,R)-Whelk-O 1,  
25cm x 4.6mm ID  
**Mobile phase:** 80/20 hexane/isopropanol  
**Flow rate:** 1.0mL/min.  
**Detection:** UV at 254nm  
**Run time:** 8 min.



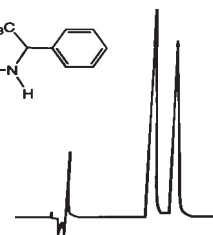
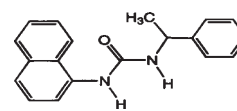
### Propranolol

**Column:**  $\alpha$ -Burke  
**Eluent:** 4/1.2/15mM  
CH<sub>3</sub>CN/EtOH/CHCO<sub>2</sub>NH<sub>4</sub>  
**Flow:** 1.0mL/min.  
**Temperature:** Ambient  
**Detection:** UV at 254nm



### N-(1-Naphthyl)-N'-(1-methylbenzyl) urea

**Column:** D-Phenylglycine,  
25cm x 4.6mm ID  
**Mobile phase:** 70/30 hexane/ethanol  
**Flow rate:** 1.0mL/min.  
**Load:** 20 $\mu$ L  
**Detection:** UV at 254nm  
**Run time:** 10 min.



### Phenylglycine

This  $\pi$ -acceptor chiral phase is based on 3,5-dinitrobenzoyl phenylglycine, covalently bonded to 5 $\mu$ m aminopropyl silica. This column resolves a wide variety of compounds containing  $\pi$ -basic groups, including: aryl substituted cyclic sulfoxides, bi- $\beta$ -naphthol and its analogs,  $\alpha$ -indanol and  $\alpha$ -tetralol analogs, and aryl-substituted hydantoins.

### Leucine

This  $\pi$ -acceptor chiral phase is based on 3,5-dinitrobenzoyl leucine, covalently bonded to 5 $\mu$ m aminopropyl silica. This phase demonstrates enhanced enantioselectivities for several classes of compounds, including benzodiazepines.

### $\beta$ -Gem 1

This  $\pi$ -acceptor chiral phase is prepared by covalently bonding N-3, 5-dinitrobenzoyl-3-amino-3-phenyl-2-(1,1-dimethyl)-propanoate, to 5 $\mu$ m silica through an ester linkage. In many cases, this chiral phase considerably outperforms its widely used analog, phenylglycine. It can separate anilide derivatives of chiral carboxylic acids, including nonsteroidal anti-inflammatory agents.

### $\alpha$ -Burke 2 Chiral Columns

This  $\pi$ -electron accepting chiral stationary phase is specifically designed for the enantio separation of underivatized  $\beta$ -blockers.

### Pirkle 1-J

This phase contains an unusual  $\beta$ -lactam structure which significantly alters its molecular recognition properties. The Pirkle 1-J is useful for the direct separation of underivatized  $\beta$ -blocker enantiomers. It can also be used for the separation of the enantiomers of arylpropionic acid NSAIDs, as well as other drugs.

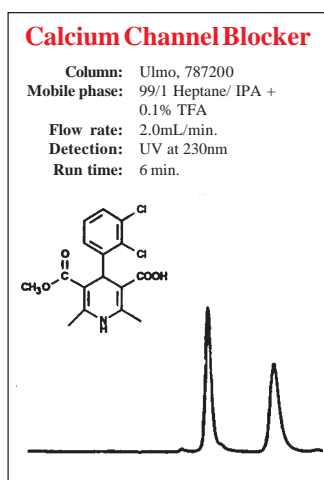
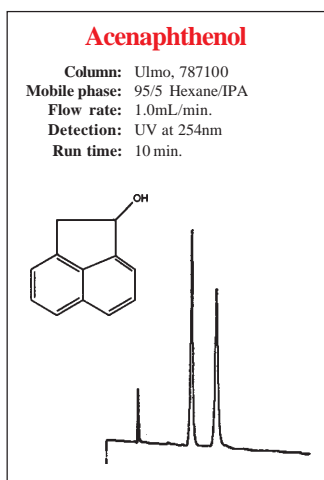
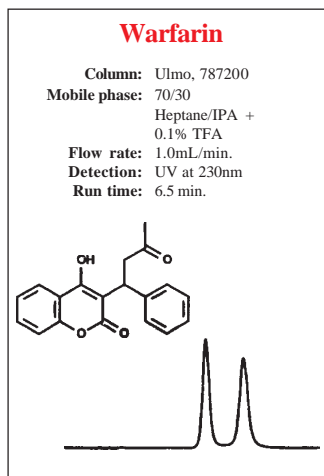
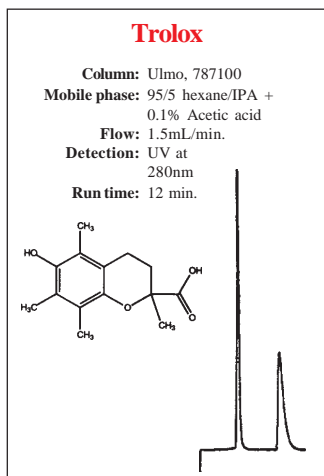
### Naphthylleucine

This  $\pi$ -electron donor is based on N-(1-naphthyl) leucine, covalently bonded to 5 $\mu$ m silica through an ester linkage. This phase resolves DNB derivatives of amino acids as the free acid when used in reversed phase mode. In the normal phase, this can resolve the amides and esters of DNB amines, alcohols and amino acids.

### ULMO

The ULMO chiral stationary phase was developed by Austrian researchers, Uray, Lindner, and Maier. This CSP has a general ability to separate the enantiomers of many racemate classes and is particularly good at separating the enantiomers of aryl carbinols.

# Regis Pirkle and Davankov Chiral HPLC Columns



## Pirkle Chiral HPLC Columns Ordering Information (Cont.)

| Cat. No. | Description                                |
|----------|--|
| 731044   | (3R, 4S)-Pirkle 1-J, 5µm, 25cm x 4.6mm ID  |
| 731244   | (3R, 4S)-Pirkle 1-J, 5µm, 25cm x 10.0mm ID |
| 731045   | (3S, 4R)-Pirkle 1-J, 5µm, 25cm x 4.6mm ID  |
| 731245   | (3S, 4R)-Pirkle 1-J, 5µm, 25cm x 10.0mm ID |
| 731034   | L-Naphthylleucine, 5µm, 25cm x 4.6mm ID    |
| 731234   | L-Naphthylleucine, 5µm, 25cm x 10.0mm ID   |
| 787100   | (S,S)-ULMO, 25cm x 4.6mm, 5µm              |
| 787101   | (S,S)-ULMO, 25cm x 10mm, 5µm               |
| 787200   | (R,R)-ULMO, 25cm x 4.6mm, 5µm              |
| 787201   | (R,R)-ULMO, 25cm x 10mm, 5µm               |

## Davankov Ligand Exchange

### • Separation of underivatized amino acid enantiomers

The Davankov chiral stationary phase is useful for the separation of underivatized amino acid enantiomers. This phase operates according to the principles of ligand-exchange chromatography (LEC), a technique pioneered by Professor V. Davankov.

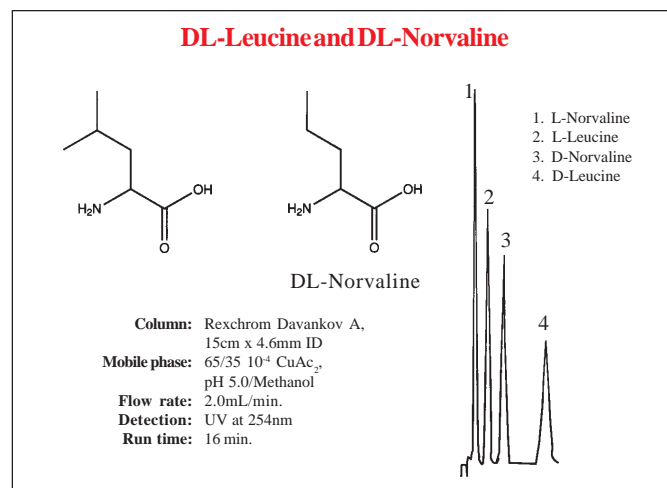
The Davankov column, requires a mobile phase of aqueous methanol containing copper(II) acetate. Enantioselectivity is extremely high with alphas up to 16 being reported.

Regis provides either a Davankov HPLC column or a kit which allows the user to convert a standard ODS column into a Davankov Chiral Stationary phase. The Davankov pre-converted column comes complete with care and use guide, column test conditions and performance results. The Davankov Reagent A kit contains Davankov Reagent A, a hydroxyproline derivative, and copper(II) acetate (sufficient quantities to coat one 15cm column and prepare mobile phase). The column coating procedure involves dissolving the Davankov Reagent A into methanol/water (80/20) and pumping this mixture through the column. This is followed by a wash with a concentrated solution of Cu(OAc)<sub>2</sub> in methanol/water (15/85). Detailed coating procedures are included with the kit.

Both of these Davankov products maintain a stable coating compatible with those mobile phases generally used in amino acid separations.

## Pirkle Chiral HPLC Columns Ordering Information

| Cat. No. | Description                            |
|----------|--|
| 786201   | Whelk-O 1(R,R), 25cm x 4.6mm, 5µm      |
| 786202   | Whelk-O 1(R,R), 25cm x 10mm, 5µm       |
| 786101   | Whelk-O 1(S,S), 25cm x 4.6mm, 5µm      |
| 786102   | Whelk-O 1(S,S), 25cm x 10mm, 5µm       |
| 786315   | Whelk-O 2 (R,R), 25cm x 4.6mm, 10µm    |
| 786325   | Whelk-O 2 (R,R), 25cm x 10mm, 10µm     |
| 786415   | Whelk-O 2 (S,S), 25cm x 4.6mm, 10µm    |
| 786425   | Whelk-O 2 (S,S), 25cm x 10mm, 10µm     |
| 731021   | D-Phenylglycine, 5µm, 25cm x 4.6mm ID  |
| 731221   | D-Phenylglycine, 5µm, 25cm x 10.0mm ID |
| 731024   | L-Phenylglycine, 5µm, 25cm x 4.6mm ID  |
| 731224   | L-Phenylglycine, 5µm, 25cm x 10.0mm ID |
| 731041   | L-Leucine, 5µm, 25cm x 4.6mm ID        |
| 731241   | L-Leucine, 5µm, 25cm x 10.0mm ID       |
| 731054   | D-Leucine, 5µm, 25cm x 4.6mm ID        |
| 731254   | D-Leucine, 5µm, 25cm x 10.0mm ID       |
| 731043   | (R,R)-β-Gem 1, 5µm, 25cm x 4.6mm ID    |
| 731243   | (R,R)-β-Gem 1, 5µm, 25cm x 10.0mm ID   |
| 731029   | (S,S)-β-Gem 1, 5µm, 25cm x 4.6mm ID    |
| 731229   | (S,S)-β-Gem 1, 5µm, 25cm x 10.0mm ID   |
| 735035   | (R)-α-Burke 2, 5µm, 25cm x 4.6mm ID    |
| 735235   | (R)-α-Burke 2, 5µm, 25cm x 10.0mm ID   |
| 735037   | (S)-α-Burke 2, 5µm, 25cm x 4.6mm ID    |
| 735237   | (S)-α-Burke 2, 5µm, 25cm x 10.0mm ID   |



| Cat. No. | Description                            | Price |
|----------|--|-------|
| 731653   | Davankov column, 5µm, 15cm x 4.6mm     |       |
| 731650   | Davankov reagent A kit                 |       |
| 728118   | Rexchrom ODS column, 5µm, 15cm x 4.6mm |       |