




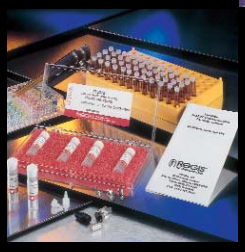


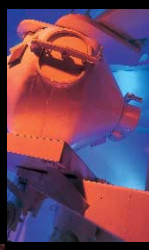

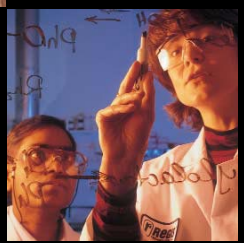

Chromatography Catalog

Greyhound CHROMATOGRAPHY AND ALLIED CHEMICALS

REGIS® TECHNOLOGIES, INC.

Greyhound CHROMATOGRAPHY AND ALLIED CHEMICALS

Supplied throughout Europe and the Middle East by Greyhound Chromatography

Greyhound Chromatography and Allied Chemicals
6 Kelvin Park, Birkenhead, Merseyside CH41 1LT United Kingdom

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- Fax: (+44)-0-151 649 4001
- www.greyhoundchrom.com

REGIS® TECHNOLOGIES, INC.

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Regis Technologies, Inc.

Growth and Leadership

Regis Technologies, Inc. is proud of its continued growth and leadership role in its three areas of business: technically advanced chromatography products, custom bulk pharmaceuticals and other fine organic chemicals chromatographic separations.

Expanded Manufacturing Facility

Regis management continuously reinvests in the company. Between 1995 and 2006, Regis expanded and improved every lab and manufacturing area. This included a new production facility with six dedicated reactor suites, individual kilo lab suites, an expanded quality control department and the installation of a cryogenic reactor. Plans are underway for increasing capacity with additional production labs, individual kilo lab suites and reactor suites. The net result is loyal and satisfied customers and employees.

Chromatography

Regis Technologies, Inc. has a long tradition of serving the analytical needs of scientists and researchers worldwide with its vast array of chromatography products and technical assistance. Regis manufactures an extensive line of chromatography stationary phases and high purity GC derivatization reagents. Regis is the exclusive manufacturer of Pirkle-Type Chiral Stationary Phases for analytical, semi-preparative and preparative applications and separations. Regis is also the exclusive manufacturer of many other specialty HPLC phases. Research and development are essential parts of our chromatography business that allows us to introduce new and innovative products that meet the needs of our customers.

Custom Synthesis

In addition to chromatography products, Regis manufactures Active Pharmaceutical Ingredients (APIs) and intermediates on a custom basis. We are committed to meeting our customers' needs through initial process development, scale-up and ongoing production. Our facility is run according to current Good Manufacturing Practices (cGMP) and is routinely inspected by the FDA. All pharmaceutical intermediates and final products are manufactured and tested to customer specifications. All technology developed for the customer is owned by the customer and all batch records are provided to the customer as well. In addition to APIs, Regis also produces and catalogs a number of fine organic chemicals. For a complete list, check our Web site at www.registech.com/portfolio.html.

Preparative Chromatography

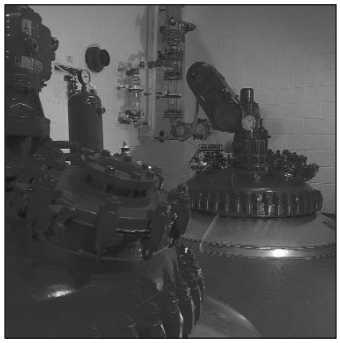
Regis' production department includes a pilot plant dedicated to cGMP chromatography. Glass gravity columns are available in diameters of 4", 6", 9" and 12", with capacities of up to 50 kg of packing material.

Regis Technologies, Inc.

Quality Products and Services

Our high purity reagents and HPLC columns are manufactured on-site according to controlled manufacturing procedures, and must meet strict quality control specifications before release. A full customer service and sales staff is available to answer questions and take orders. Regis also has a complete applications laboratory and knowledgeable technical support staff. With years of chromatography experience, our support staff is dedicated to assisting customers with method development and column or reagent selection.

We are committed to our customer. With this in mind, you can expect Regis Technologies to provide the highest quality products, services and technical support as we continue to grow and meet the challenges of the future.



Chiral Stationary Phases

Chiral Stationary Phases

Chiral Chromatography

Chirality has become vitally important in the pharmaceutical, chemical and agricultural industries.

The differences that make compounds chiral can produce critically different pharmacological effects in biological systems. As a result, demand for stereoselective separation techniques and analytical assays to evaluate the enantiomeric purity of chiral compounds has increased.

Chiral chromatography has become a necessary tool—not only for the analytical determination of enantiomeric purity, but also for the isolation of pure enantiomers.

Regis Technologies is proud to be a leader in chiral separations that serves both the analytical and preparative needs of chromatographers and researchers worldwide. Regis offers three different classes of Chiral Stationary Phases (CSPs):

- Pirkle-Concept
- ChiroSil®, Crown Ether
- Protein-based

Regis manufactures a complete line of Pirkle Chiral Stationary Phases and columns at its pharmaceutical manufacturing facility. Columns range from analytical to preparative in size. A line of protein-based chiral stationary phases is also available. All products meet rigorous manufacturing and quality control specifications before release.

Pirkle Stationary Phases

In 1980, Regis Technologies along with Professor William Pirkle of the University of Illinois, introduced the Pirkle Chiral Stationary Phases. These Chiral Stationary Phases offer many advantages:

- Enantiomer separation on a wide variety of compound groups
- Column durability resulting from covalent phase bonding
- Ability to invert elution order
- Availability of analytical- to preparative-sized columns and bulk packing material
- Universal solvent compatibility

Enantiomer Separation

Regis manufactures 9 Pirkle CSPs. These can separate a wide variety of enantiomers in numerous compound groups. Examples include:

- Aryl Propionic Acid Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)
- Agricultural Compounds
- Natural Products
- ß-Blockers
- Many Pharmaceuticals

Additional examples of enantiomer separations can be found in the Regis Chiral Application Guide or on our Web site at www.registech.com/chiral. Our Web site is updated monthly with new applications and current chiral events.

Chiral Stationary Phases

Chiral Chromatography

Column Durability

The Pirkle CSPs are covalently bonded to the silica, providing excellent column durability. Covalently bonded phases assure long-lasting columns and offer added benefits for preparative columns. Our covalently bonded preparative columns are longer lasting than their coated preparative column counterparts because noncovalent coatings can leach off. Additional benefits include the columns' capacity to tolerate sample overload.

Ability to Invert Elution Order

An important advantage of the Pirkle CSPs is the ability to invert elution order by using the same type of CSP, but with the opposite absolute configuration. As a result, it is possible to have the trace enantiomer elute before the major—a desirable feature for enantiomeric purity determinations. For preparative separations it is beneficial to elute the desired component first.

Analytical and Preparative-Sized Columns

All of Regis' Pirkle HPLC columns are available in both analytical and preparative sizes. Since all chiral stationary phases are manufactured on-site, Regis can pack special or custom-sized columns quickly and easily.

Universal Solvent Compatibility

Choice of mobile phase is not a limitation with the Pirkle HPLC columns. They are compatible with most mobile phases. The pH of the mobile phase, however, must be between 2.5 and 7.5. Both normal-phase and reversed-phase modes can be used, although normal-phase is most common. For normal-phase separations, the classic mobile phase is a binary or ternary mixture of a hydrocarbon and a modifier, usually an aliphatic alcohol.

Typical uncharged organic modifers include ethanol, isopropanol and butanol. Under reversed-phase conditions, water-alcohol mixtures, or aqueous phosphate buffers with charged organic modifiers are also employed.

Supercritical Fluid Chromatography (SFC) utilizing carbon dioxide is now a proven technique for the separation of enantiomers using Pirkle CSPs.

Chiral Stationary Phases

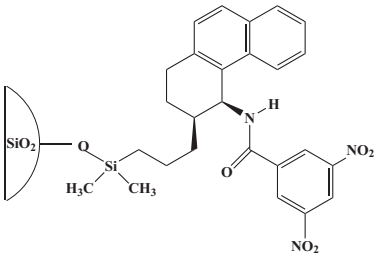
Pirkle Chiral HPLC Columns

Whelk-O® 1

Analytical to Preparative Columns

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 π-electron acceptor/π-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. Other advantages include column durability, excellent efficiency, ability to invert elution order and excellent preparative capacity.



Product	Particle Size	Column Dimensions	Catalog #
Spherical Silica			
(S,S)-Whelk-O 1	5 µm, 100Å	25 cm x 4.6 mm i.d.	786101
(S,S)-Whelk-O 1	5 µm, 100Å	25 cm x 10.0 mm i.d.	786102
(S,S)-Whelk-O 1	5 µm, 100Å	25 cm x 30.0 mm i.d.	786105
(R,R)-Whelk-O 1	5 µm, 100Å	25 cm x 4.6 mm i.d.	786201
(R,R)-Whelk-O 1	5 µm, 100Å	25 cm x 10.0 mm i.d.	786202
(R,R)-Whelk-O 1	5 µm, 100Å	25 cm x 30.0 mm i.d.	786205
Spherical Kromasil® Silica			
(S,S)-Whelk-O 1	5 µm, 100Å	25 cm x 4.6 mm i.d.	780101
(S,S)-Whelk-O 1	5 µm, 100Å	25 cm x 10.0 mm i.d.	780102
(S,S)-Whelk-O 1	5 µm, 100Å	25 cm x 30.0 mm i.d.	780103
(S,S)-Whelk-O 1	5 µm, 100Å	25 cm x 50.0 mm i.d.	780104
(R,R)-Whelk-O 1	5 µm, 100Å	25 cm x 4.6 mm i.d.	780201
(R,R)-Whelk-O 1	5 µm, 100Å	25 cm x 10.0 mm i.d.	780202
(R,R)-Whelk-O 1	5 µm, 100Å	25 cm x 30.0 mm i.d.	780203
(R,R)-Whelk-O 1	5 µm, 100Å	25 cm x 50.0 mm i.d.	780204
(S,S)-Whelk-O 1	10 µm, 100Å	25 cm x 4.6 mm i.d.	786615
(S,S)-Whelk-O 1	10 µm, 100Å	25 cm x 10.0 mm i.d.	786625
(S,S)-Whelk-O 1	10 µm, 100Å	25 cm x 21.1 mm i.d.	786635
(S,S)-Whelk-O 1	10 µm, 100Å	50 cm x 21.1mm i.d.	786645
(S,S)-Whelk-O 1	10 µm, 100Å	25 cm x 30.0 mm i.d.	786702
(S,S)-Whelk-O 1	10 µm, 100Å	25 cm x 50.0 mm i.d.	786703
(S,S)-Whelk-O 1	10 µm, 100Å	50 cm x 50.0 mm i.d.	786704
(S,S)-Whelk-O 1	10 µm, 100Å	50 cm x 30.0 mm i.d.	786716
(R,R)-Whelk-O 1	10 µm, 100Å	25 cm x 4.6 mm i.d.	786515
(R,R)-Whelk-O 1	10 µm, 100Å	25 cm x 10.0 mm i.d.	786525
(R,R)-Whelk-O 1	10 µm, 100Å	25 cm x 21.1 mm i.d.	786535
(R,R)-Whelk-O 1	10 µm, 100Å	50 cm x 21.1 mm i.d.	786545
(R,R)-Whelk-O 1	10 µm, 100Å	25 cm x 30.0 mm i.d.	786708
(R,R)-Whelk-O 1	10 µm, 100Å	25 cm x 50.0 mm i.d.	786709
(R,R)-Whelk-O 1	10 µm, 100Å	50 cm x 50.0 mm i.d.	786710
(R,R)-Whelk-O 1	10 µm, 100Å	50 cm x 30.0 mm i.d.	786713

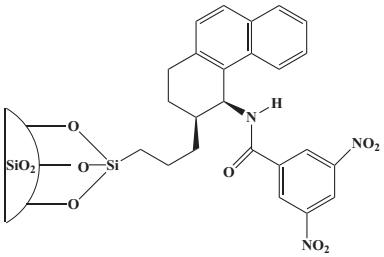
Chiral Stationary Phases

Pirkle Chiral HPLC Columns

Whelk-O® 2

Analytical to Preparative Columns

Our newest addition to the Whelk-O line of chiral stationary phases is the Whelk-O 2. The Whelk-O 2 is the 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. The Whelk-O 2 is ideal for preparative separations since the material is bonded on 10 µm, 100Å spherical Kromasil silica. This allows the preparative chromatographer to perform method development on an analytical column and immediately scale up to larger diameter columns.

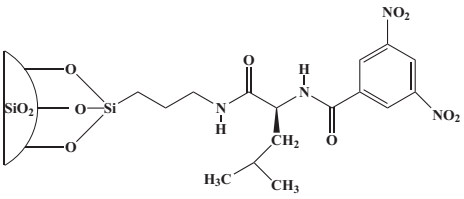


Product	Particle Size	Column Dimensions	Catalog #
Spherical Kromasil® Silica			
(S,S)-Whelk-O 2	10 µm, 100Å	25 cm x 4.6 mm i.d.	786415
(S,S)-Whelk-O 2	10 µm, 100Å	25 cm x 10.0 mm i.d.	786425
(S,S)-Whelk-O 2	10 µm, 100Å	25 cm x 21.1 mm i.d.	786435
(S,S)-Whelk-O 2	10 µm, 100Å	50 cm x 21.1 mm i.d.	786445
(S,S)-Whelk-O 2	10 µm, 100Å	25 cm x 30.0 mm i.d.	786721
(S,S)-Whelk-O 2	10 µm, 100Å	25 cm x 50.0 mm i.d.	786722
(S,S)-Whelk-O 2	10 µm, 100Å	50 cm x 50.0 mm i.d.	786723
(S,S)-Whelk-O 2	10 µm, 100Å	50 cm x 30.0 mm i.d.	786736
(R,R)-Whelk-O 2	10 µm, 100Å	25 cm x 4.6 mm i.d.	786315
(R,R)-Whelk-O 2	10 µm, 100Å	25 cm x 10.0 mm i.d.	786325
(R,R)-Whelk-O 2	10 µm, 100Å	25 cm x 21.1 mm i.d.	786335
(R,R)-Whelk-O 2	10 µm, 100Å	50 cm x 21.1 mm i.d.	786345
(R,R)-Whelk-O 2	10 µm, 100Å	25 cm x 30.0 mm i.d.	786727
(R,R)-Whelk-O 2	10 µm, 100Å	25 cm x 50.0 mm i.d.	786728
(R,R)-Whelk-O 2	10 µm, 100Å	50 cm x 50.0 mm i.d.	786729
(R,R)-Whelk-O 2	10 µm, 100Å	50 cm x 30.0 mm i.d.	786732

Leucine

Analytical and Semi-Preparative Columns

The π-acceptor leucine CSP is based on 3,5-dinitrobenzoyl leucine, covalently bonded to 5 µm aminopropyl silica. Columns derived from either L- or D-leucine are available. This phase demonstrates enhanced enantioselectivities for several classes of compounds, including benzodiazapines.



Product	Particle Size	Column Dimensions	Catalog #
Spherical Silica:			
D-Leucine	5 µm, 100Å	25 cm x 4.6 mm i.d.	731054
D-Leucine	5 µm, 100Å	25 cm x 10.0 mm i.d.	731254
D-Leucine	5 µm, 100Å	25 cm x 21.1 mm i.d.	731354
L-Leucine	5 µm, 100Å	25 cm x 4.6 mm i.d.	731041
L-Leucine	5 µm, 100Å	25 cm x 10.0 mm i.d.	731241
L-Leucine	5 µm, 100Å	25 cm x 21.1 mm i.d.	731341

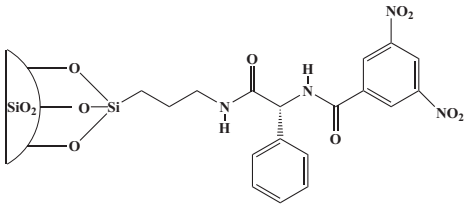
Chiral Stationary Phases

Pirkle Chiral HPLC Columns

Phenylglycine

Analytical and Semi-Preparative Columns

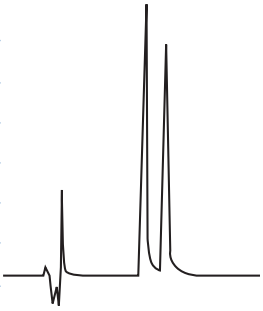
Phenylglycine, a π-acceptor chiral phase, is based on 3,5-dinitrobenzoyl phenylglycine, covalently bonded to 5 µm aminopropyl silica. Phenylglycine columns are available in both L- and D-configurations. This CSP resolves a wide variety of compounds containing π-basic groups, including: aryl-substituted cyclic sulfoxides, bi-β-naphthol and its analogs, α-indanol and α-tetralol analogs, and aryl-substituted hydantoins.



Product	Particle Size	Column Dimensions	Catalog #
Spherical Silica:			
D-Phenylglycine	5 µm, 100 Å	25 cm x 4.6 mm i.d.	731021
D-Phenylglycine	5 µm, 100 Å	25 cm x 10.0 mm i.d.	731221
D-Phenylglycine	5 µm, 100 Å	25 cm x 21.1 mm i.d.	731331
L-Phenylglycine	5 µm, 100 Å	25 cm x 4.6 mm i.d.	731024
L-Phenylglycine	5 µm, 100 Å	25 cm x 10.0 mm i.d.	731224
L-Phenylglycine	5 µm, 100 Å	25 cm x 21.1 mm i.d.	731334

1-Naphthylureaphenethylamine

Column:	D-Phenyglycine 4.6 mm x 25 cm
Mobile Phase:	30% EtOH/Hexane
Flow Rate:	1 ml/min
Detection:	UV 254 nm
Run Time:	10 min
k'_1	2.37
α	1.22

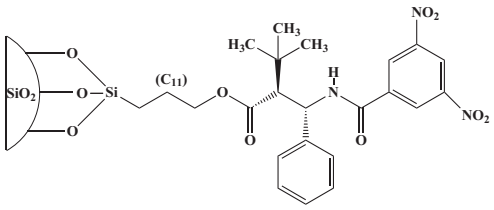


β-Gem 1

Analytical and Semi-Preparative Columns

β-Gem1 is a π-acceptor chiral stationary phase and is prepared by covalently bonding N-3,5-dinitrobenzoyl-3-amino-3-phenyl-2-(1,1-dimethylethyl)-propanoate, to 5 µm silica through an ester linkage.

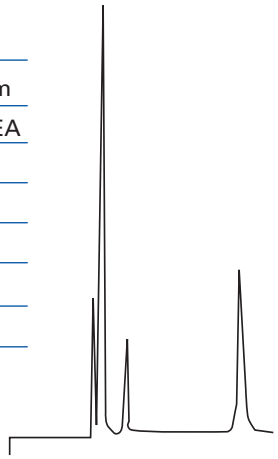
In many cases, this chiral phase considerably outperforms its widely used analog, phenylglycine. It can separate anilide derivatives of chiral carboxylic acids, including nonsteroidal anti-inflammatory agents.



Product	Particle Size	Column Dimensions	Catalog #
Spherical Silica:			
(R,R)-β-GEM 1	5 µm, 100 Å	25 cm x 4.6 mm i.d.	731043
(R,R)-β-GEM 1	5 µm, 100 Å	25 cm x 10.0 mm i.d.	731243
(R,R)-β-GEM 1	5 µm, 100 Å	25 cm x 21.1 mm i.d.	731343
(S,S)-β-GEM 1	5 µm, 100 Å	25 cm x 4.6 mm i.d.	731029
(S,S)-β-GEM 1	5 µm, 100 Å	25 cm x 10.0 mm i.d.	731229
(S,S)-β-GEM 1	5 µm, 100 Å	25 cm x 21.1 mm i.d.	731329

Tofisopam and it's Conformers

Column:	(R,R)-β-Gem 1 25 cm x 4.6 mm
Mobile Phase:	(70/30) Hexane/Ethanol + 0.1% TEA
Flow Rate:	1.0 mL/min
Detection:	UV 254 nm
Run Time	25.0 min
k'_1	2.66
α	3.13



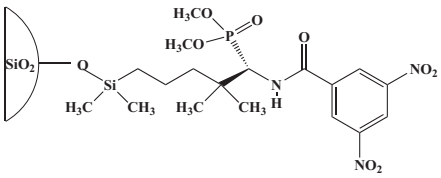
Chiral Stationary Phases

Pirkle Chiral HPLC Columns

α-Burke 2

Analytical and Semi-Preparative Columns

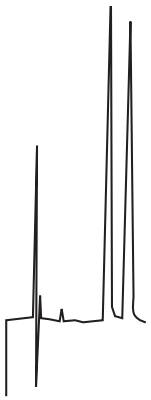
The α-Burke 2 phase is derived from dimethyl N-3,5-dinitro-benzoyl--amino-2,2-dimethyl- 4-pentenyl phosphonate covalently bound to 5 µm silica. This π-acceptor chiral stationary phase is particularly valuable in the HPLC separation of β-blocker enantiomers, an important class of cardiovascular drugs whose enantiomers often exhibit differing pharmacological activities. The α-Burke 2 has been specifically designed to separate the enantiomers of β-blockers without chemical derivatization. In addition, it also resolves the enantiomers of many compounds separated on π-acceptor Pirkle type chiral stationary phases.



Product	Particle Size	Column Dimensions	Catalog #
Spherical Silica:			
(S)-α-Burke 2	5 µm, 100 Å	25 cm x 4.6 mm i.d.	735037
(S)-α-Burke 2	5 µm, 100 Å	25 cm x 10.0 mm i.d.	735237
(S)-α-Burke 2	5 µm, 100 Å	25 cm x 21.1 mm i.d.	735238
(R)-α-Burke 2	5 µm, 100 Å	25 cm x 4.6 mm i.d.	735035
(R)-α-Burke 2	5 µm, 100 Å	25 cm x 10.0 mm i.d.	735235
(R)-α-Burke 2	5 µm, 100 Å	25 cm x 21.1 mm i.d.	735236

Metroprolol

Column:	α-Burke 2 25 cm x 4.6 mm
Mobile Phase:	(85/10/5) CH_2Cl_2/EtOH/MeOH
Flow Rate:	1.0 mL/min
Detection:	UV 254 nm
Run Time	13.0 min
k'_1	2.66
α	1.28

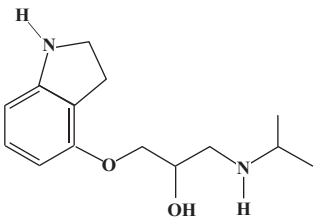


Pirkle 1-J

Analytical and Semi-Preparative Columns

The Pirkle 1-J column is the latest in a series of CSPs from the research laboratories of Professor Pirkle. This new CSP contains an unusual β-lactam structure which significantly alters its molecular recognition properties. The Pirkle 1-J is useful for the direct separation of underivatized β-blocker enantiomers.

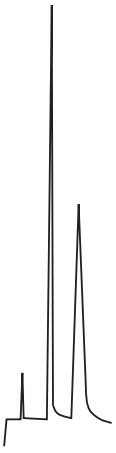
It can also be used for the separation of the enantiomers of arylpropionic acid NSAIDs, as well as other drugs.



Product	Particle Size	Column Dimensions	Catalog #
Spherical Silica:			
(3R,4S)-Pirkle 1-J	5 µm, 100 Å	25 cm x 4.6 mm i.d.	731044
(3R,4S)-Pirkle 1-J	5 µm, 100 Å	25 cm x 10.0 mm i.d.	731244
(3R,4S)-Pirkle 1-J	5 µm, 100 Å	25 cm x 21.1 mm i.d.	731344
(3S,4R)-Pirkle 1-J	5 µm, 100 Å	25 cm x 4.6 mm i.d.	731045
(3S,4R)-Pirkle 1-J	5 µm, 100 Å	25 cm x 10.0 mm i.d.	731245
(3S,4R)-Pirkle 1-J	5 µm, 100 Å	25 cm x 21.1 mm i.d.	731345

Prindolol

Column:	(3R, 4S)-Pirkle 1-J 25 cm x 4.6 mm
Mobile Phase:	(80/20) CH_2Cl_2/EtOH+0.04M Ammonium Acetate
Flow Rate:	1.0 mL/min
Detection:	UV 254 nm
Run Time	11.0 min
k'_1	1.56
α	2.06



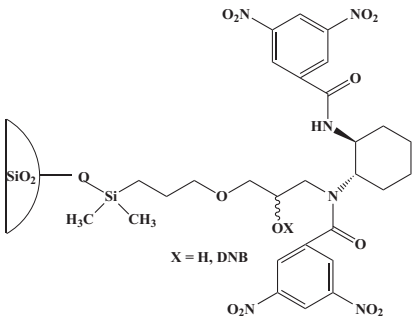
Chiral Stationary Phases

Pirkle Chiral HPLC Columns

DACH-DNB

Analytical to Preparative Columns

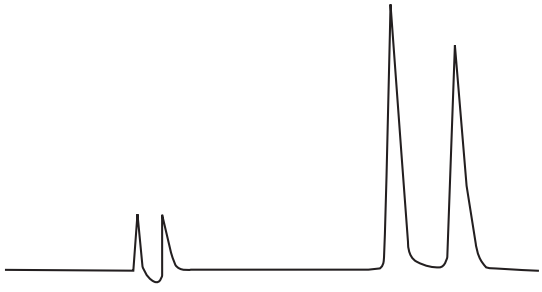
The innovative DACH-DNB CSP was designed by Italian chemist, Professor Francesco Gasparrini with Dr. Villani and Dr. Misitiat, Rome University "La Sapienza." The DACH-DNB CSP, which contains the 3,5-dinitrobenzoyl derivative of 1,2-diaminocyclohexane, has been found to resolve a broad range of racemate classes including amides, alcohols, esters, ketones, acids, sulfoxides, phosphine oxides, selenoxides, phosphonates, thiophosphineoxide, phosphineselenide, phosphine-borane, beta-lactams, organometallics, atropisomers and heterocycles.



Product	Particle Size	Column Dimensions	Catalog #
Spherical Silica:			
(S,S)-DACH-DNB	5 µm, 100 Å	25 cm x 4.6 mm i.d.	788201
(S,S)-DACH-DNB	5 µm, 100 Å	25 cm x 10.0 mm i.d.	788202
(S,S)-DACH-DNB	5 µm, 100 Å	25 cm x 30.0 mm i.d.	788204
(R,R)-DACH-DNB	5 µm, 100 Å	25 cm x 4.6 mm i.d.	788101
(R,R)-DACH-DNB	5 µm, 100 Å	25 cm x 10.0 mm i.d.	788102
(R,R)-DACH-DNB	5 µm, 100 Å	25 cm x 30.0 mm i.d.	788104
Spherical Kromasil® Silica			
(S,S)-DACH-DNB	10 µm, 100 Å	25 cm x 4.6 mm i.d.	788301
(S,S)-DACH-DNB	10 µm, 100 Å	25 cm x 10.0 mm i.d.	788302
(S,S)-DACH-DNB	10 µm, 100 Å	25 cm x 21.1 mm i.d.	788203
(S,S)-DACH-DNB	10 µm, 100 Å	25 cm x 30.0 mm i.d.	788701
(S,S)-DACH-DNB	10 µm, 100 Å	25 cm x 50.0 mm i.d.	788702
(S,S)-DACH-DNB	10 µm, 100 Å	50 cm x 50.0 mm i.d.	788705
(S,S)-DACH-DNB	10 µm, 100 Å	50 cm x 30.0 mm i.d.	788715
(R,R)-DACH-DNB	10 µm, 100 Å	25 cm x 4.6 mm i.d.	788401
(R,R)-DACH-DNB	10 µm, 100 Å	25 cm x 10.0 mm i.d.	788402
(R,R)-DACH-DNB	10 µm, 100 Å	25 cm x 21.1 mm i.d.	788103
(R,R)-DACH-DNB	10 µm, 100 Å	25 cm x 30.0 mm i.d.	788707
(R,R)-DACH-DNB	10 µm, 100 Å	25 cm x 50.0 mm i.d.	788708
(R,R)-DACH-DNB	10 µm, 100 Å	50 cm x 50.0 mm i.d.	788709
(R,R)-DACH-DNB	10 µm, 100 Å	50 cm x 30.0 mm i.d.	788712

Fluazifop-butyl

Column:	(S,S)-DACH-DNB 25 cm x 4.6 mm
Mobile Phase:	(95/5) Hexane/IPA
Temperature:	20° C
Flow Rate:	1.0 mL/min
Detection:	UV 254 nm
Run Time	18.0 min
k'_1	4.70
α	1.15



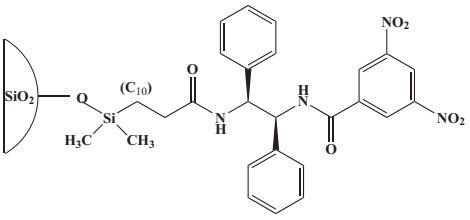
Chiral Stationary Phases

Pirkle Chiral HPLC Columns

ULMO

Analytical to Preparative Columns

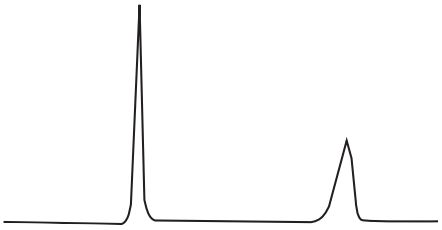
The ULMO chiral stationary phase was developed by Austrian researchers Dr. Georg Uray, Dr. Wolfgang Linder and Dr. Nobert Maier. The ULMO CSP is based on a 3,5-dintrobenzoyl derivative of diphenylethylenediamine. This CSP has a general ability to separate the enantiomers of many racemate classes and is particularly good at separating the enantiomers of aryl carbinols.



Product	Particle Size	Column Dimensions	Catalog #
Spherical Silica:			
(S,S)-ULMO	5 µm, 100 Å	25 cm x 4.6 mm i.d.	787100
(S,S)-ULMO	5 µm, 100 Å	25 cm x 10.0 mm i.d.	787101
(S,S)-ULMO	5 µm, 100 Å	25 cm x 30.0 mm i.d.	787103
(R,R)-ULMO	5 µm, 100 Å	25 cm x 4.6 mm i.d.	787200
(R,R)-ULMO	5 µm, 100 Å	25 cm x 10.0 mm i.d.	787201
(R,R)-ULMO	5 µm, 100 Å	25 cm x 30.0 mm i.d.	787203
Spherical Kromasil® Silica			
(S,S)-ULMO	10 µm, 100 Å	25 cm x 4.6 mm i.d.	787300
(S,S)-ULMO	10 µm, 100 Å	25 cm x 10.0 mm i.d.	787301
(S,S)-ULMO	10 µm, 100 Å	25 cm x 21.1 mm i.d.	787102
(S,S)-ULMO	10 µm, 100 Å	25 cm x 30.0 mm i.d.	787701
(S,S)-ULMO	10 µm, 100 Å	25 cm x 50.0 mm i.d.	787702
(S,S)-ULMO	10 µm, 100 Å	50 cm x 50.0 mm i.d.	787703
(S,S)-ULMO	10 µm, 100 Å	50 cm x 30.0 mm i.d.	787715
(R,R)-ULMO	10 µm, 100 Å	25 cm x 4.6 mm i.d.	787400
(R,R)-ULMO	10 µm, 100 Å	25 cm x 10.0 mm i.d.	787401
(R,R)-ULMO	10 µm, 100 Å	25 cm x 21.1 mm i.d.	787202
(R,R)-ULMO	10 µm, 100 Å	25 cm x 30.0 mm i.d.	787707
(R,R)-ULMO	10 µm, 100 Å	25 cm x 50.0 mm i.d.	787708
(R,R)-ULMO	10 µm, 100 Å	50 cm x 50.0 mm i.d.	787709
(R,R)-ULMO	10 µm, 100 Å	50 cm x 30.0 mm i.d.	787712

Vapol

Column:	(R,R)-ULMO 25 cm x 4.6 mm
Mobile Phase:	100% Methanol
Flow Rate:	1.5 mL/min
Detection:	UV 254 nm
Run Time	13.0 min
k'_1	1.74
α	3.37



Chiral Stationary Phases

Protein-Based Chiral Stationary Phases

Protein-Based Chiral Stationary Phases

Regis carries a line of protein-based chiral columns manufactured by ChromTech LTD. These include:

- Chiral AGP-(α-glycoprotein)
- Chiral CBH-(cellobiohydrolase)
- Chiral HSA-(human serum albumin)

For additional product information and a Protein-Based Stationary Phase Application Guide, please contact Regis at sales@registech.com.

Chiral AGP

Micro, Analytical and Semi-Preparative Columns

Chiral AGP is the second generation chiral selector based on the α_1-acid glycoprotein (α_1-AGP) as the chiral stationary phase. The AGP has been immobilized on spherical, 5 µm particles. The Chiral AGP column is typically used in the reversed-phase mode, where it can be used for the resolution of an extremely broad range of chiral compounds, such as amines, (primary, secondary, tertiary and quaternary ammonium), acids, esters, sulphoxides, amides, and alcohols. The enantioselectivity and the retention can easily be regulated by the pH of the mobile phase, the buffer concentration and the nature and concentration of the organic modifier.

Product	Particle Size	Column Dimensions	Catalog #
Spherical Silica:			
Chiral AGP	5 µm	10 cm x 2.0 mm i.d.	732196
Chiral AGP	5 µm	15 cm x 2.0 mm i.d.	732197
Chiral AGP	5 µm	5 cm x 4.0 mm i.d.	732198
Chiral AGP	5 µm	15 cm x 4.0 mm i.d.	732199
Chiral AGP	5 µm	10 cm x 4.0 mm i.d.	732200
Chiral AGP	5 µm	5 cm x 2.0 mm i.d.	732201
Chiral AGP	5 µm	5 cm x 3.0 mm i.d.	732203
Chiral AGP	5 µm	10 cm x 10.0 mm i.d.	732301
Chiral AGP	5 µm	15 cm x 10.0 mm i.d.	732302
Chiral AGP	5 µm	1 cm x 3.0 mm i.d. guard	732300

Chiral Stationary Phases

Protein-Based Chiral Stationary Phases

Chiral CBH

Micro, Analytical and Semi-Preparative Columns

Cellobiohydrolase (CBH) is a stable enzyme which has been immobilized onto 5 µm spherical silica particles. The column is used in reversed-phase mode and is effective for the separation of enantiomers of basic drugs from many compound classes. The retention and the enantioselectivity can be regulated by changes in pH, buffer concentration and the nature and concentration of organic modifer.

Product	Particle Size	Column Dimensions	Catalog #
Spherical Silica:			
Chiral CBH	5 µm	10 cm x 4.0 mm i.d.	732350
Chiral CBH	5 µm	15 cm x 4.0 mm i.d.	732351
Chiral CBH	5 µm	5 cm x 4.0 mm i.d.	732352
Chiral CBH	5 µm	10 cm x 2.0 mm i.d.	732353
Chiral CBH	5 µm	15 cm x 2.0 mm i.d.	732354
Chiral CBH	5 µm	10 cm x 10.0 mm i.d.	732355
Chiral CBH	5 µm	15 cm x 10.0 mm i.d.	732356
Chiral CBH	5 µm	1 cm x 3.0 mm i.d. guard	732358

Chiral HSA

Analytical and Semi-Preparative Columns

With the Chiral human serum albumin (HSA) column, the enantiomers of many carboxylic acids and amino acids can be resolved directly, without derivatization. Enantioselectivity and retention can be regulated by changing the mobile phase composition, pH, buffer concentration and/or nature of the organic modifier. HSA has been immobilized onto 5 µm spherical silica particles. The surface chemistry of the silica and the method of immobilization provide a stable chiral separation material.

Product	Particle Size	Column Dimensions	Catalog #
Spherical Silica:			
Chiral HSA	5 µm	10 cm x 2.0 mm i.d.	732202
Chiral HSA	5 µm	15 cm x 2.0 mm i.d.	732238
Chiral HSA	5 µm	15 cm x 4.0 mm i.d.	732239
Chiral HSA	5 µm	10 cm x 4.0 mm i.d.	732240
Chiral HSA	5 µm	5 cm x 4.0 mm i.d.	732241
Chiral HSA	5 µm	5 cm x 3.0 mm i.d.	732242
Chiral HSA	5 µm	5 cm x 2.0 mm i.d.	732243
Chiral HSA	5 µm	10 cm x 10.0 mm i.d.	732341
Chiral HSA	5 µm	15 cm x 10.0 mm i.d.	732342
Chiral HSA	5 µm	1 cm x 3.0 mm i.d. guard	732340

Chiral Stationary Phases

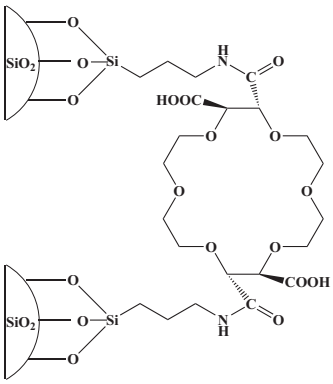
Crown Ether Phase

ChiroSil®

Analytical to Preparative Columns

The ChiroSil® RCA(+) and SCA(-) chiral stationary phases were developed by RStech Corporation in Daejeon, South Korea. This phase is prepared by a covalent trifunctional bonding of (+) or (-)-(18-Crown-6)-tetracarboxylic acid as the chiral selector. The covalent bonding ensures universal solvent compatibility and allows operation under ambient conditions. Columns are available in both enantiomeric forms to allow the scientist to invert elution order by simply switching columns.

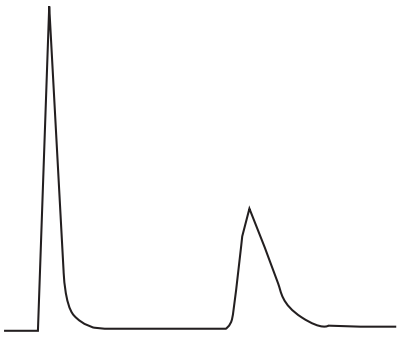
This chiral stationary phase is the choice for the separation of amino acids and compounds containing primary amines on the analytical or preparative scale.



Product	Particle Size	Column Dimensions	Catalog #
Spherical Silica:			
ChiroSil® SCA(-)	5 µm, 100Å	15 cm x 4.6 mm i.d.	799101
ChiroSil® SCA(-)	5 µm, 100Å	25 cm x 4.6 mm i.d.	799102
ChiroSil® SCA(-)	5 µm, 100Å	25 cm x 10.0 mm i.d.	799106
ChiroSil® SCA(-)	5 µm, 100Å	25 cm x 21.1 mm i.d.	799105
ChiroSil® SCA(-)	5 µm, 100Å	25 cm x 30.0 mm i.d.	799107
ChiroSil® RCA(+)	5 µm, 100Å	15 cm x 4.6 mm i.d.	799001
ChiroSil® RCA(+)	5 µm, 100Å	25 cm x 4.6 mm i.d.	799002
ChiroSil® RCA(+)	5 µm, 100Å	25 cm x 10.0 mm i.d.	799006
ChiroSil® RCA(+)	5 µm, 100Å	25 cm x 21.1 mm i.d.	799005
ChiroSil® RCA(+)	5 µm, 100Å	25 cm x 30.0 mm i.d.	799007

Phenylglycine

Column:	ChiroSil® RCA(+) or SCA(-) 15 cm x 4.6 mm
Mobile Phase:	(70/30) CH_3OH/H2O +10 mM H_2SO_4 and 0.1% TEA
Flow Rate:	1.0 mL/min
Detection:	UV 210 nm
Run Time	13.1 min
k'_1	3.14
α	2.60



RAM Chromatography

RAM Direct Injection

(Restricted Access Media)

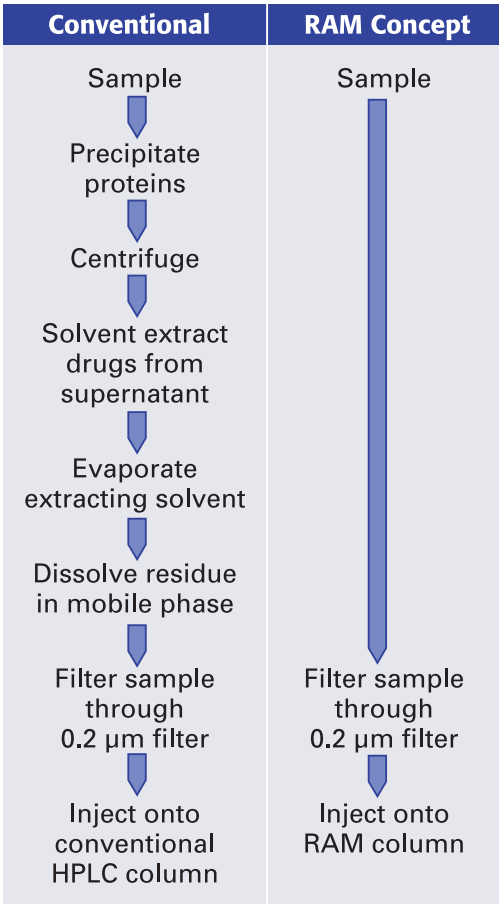


Figure 1. RAM Direct Injection eliminates the lengthy pretreatment steps needed in conventional methods.

A Tool for the Separation of Small Molecules in the Presence of Large Biomolecules

HPLC analysis of small molecules contained within a protein matrix can be a difficult and time consuming task. The analysis often involves multi-step pretreatment procedures including centrifugation, extraction and filtration. RAM Direct Injection allows for the chromatographic resolution of small molecules in the presence of much larger analytes without extensive sample pretreatment (figure 1). RAM Direct Injection HPLC columns eliminate prior sample clean-up making it possible to directly inject a variety of complex sample matrices for the separation and detection of drugs, drug metabolites, peptides, and other analytes.

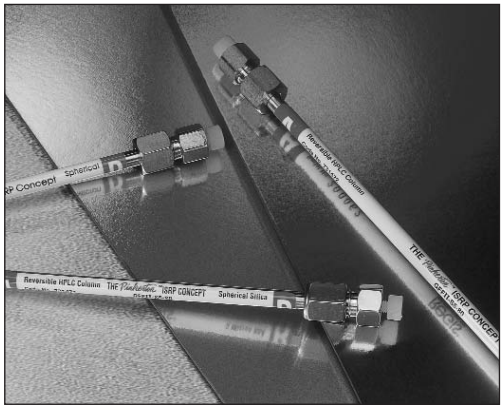
RAM Direct Injection Advantages

RAM Direct Injection technology:

- Eliminates multiple sample pretreatment steps
  The use of RAM Direct Injection HPLC columns eliminates the precipitation, centrifugation, solvent evaporation, and residue dissolution steps (figure 1) of typical procedures. Simply filter the sample and inject directly onto the column.
- Useful with a variety of sample matrices
  The RAM Direct Injection HPLC columns have demonstrated efficacy in the analysis of drugs, drug metabolites, peptides, and other analytes in matrices such as plasma, serum, whole blood, urine, plant and tissue extract, food and beverage, and environmental samples.
- Compatible with automated sample processing
  Simplified sample preparation and use of HPLC columns allows customers to employ automated systems.
- Reduces potentially dangerous sample handling
  With direct injection, sample handling is significantly reduced; therefore, potentially dangerous samples such as plasma, serum, urine and environmental samples do not pose as significant a threat to the worker.
- Reduces biohazardous waste
  Use of SPE (solid phase extraction) disks can create biohazardous waste. RAM Direct Injection columns limit the creation of unnecessary biohazardous waste.
- The lowest cost solution
  Because of the benefits described above, RAM Direct Injection often offers the lowest total cost solution.

RAM Direct Injection

(Restricted Access Media)



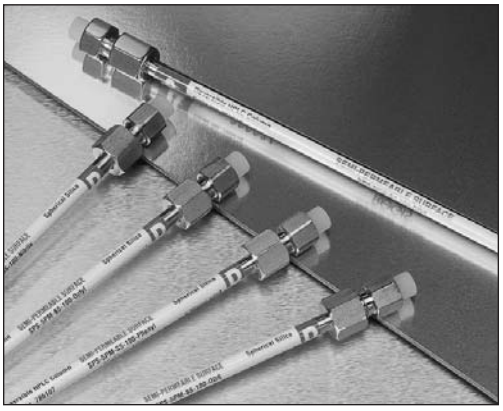
RAM Direct Injection Phases

RAM phases employ a porous silica support that consists of an external, directly accessible surface and internal pores accessible only to molecules with an approximate molecular weight of less than 12,000 Daltons. Most conventional HPLC phases have a homogenous stationary phase on both silica surfaces. In contrast, the RAM phases are prepared by unique bonding processes that result in distinct inner and outer surfaces.

A dual surface configuration is especially important because the pores provide the majority of the silica's surface area. This dual-phase system allows for the separation of analytes through a combination of size exclusion and conventional phase partitioning. The outer surface employs both size exclusion and hydrophilic interaction to prevent large biomolecules from accessing the inner layer. As a result, these compounds elute from the column at the void volume. Small molecules penetrate through to the inner surface where they are retained and separated by the underlying hydrophobic support.

There are two RAM Direct Injection Technologies:

1. ISRP (Internal Surface Reversed Phase)
   GFF II
2. SPS (Semi-Permeable Surface)
   Octyl (C8)
   ODS (C18)
   Phenyl



ISRP

(Internal Surface Reversed 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 II

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

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

ISRP Selectivity

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

Mobile Phase Composition:

The nature of ISRP analytes requires that 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.

Product	Particle Size	Column Dimensions	Catalog #
GFF II	5 µm, 80 Å	5 cm x 2.1 mm i.d.	731469
GFF II	5 µm, 80 Å	5 cm x 4.6 mm i.d.	731470
GFF II	5 µm, 80 Å	15 cm x 4.6 mm i.d.	731471
GFF II	5 µm, 80 Å	25 cm x 4.6 mm i.d.	731472
GFF II Guard Kit*	5 µm, 80 Å	1 cm x 3.0 mm i.d	731475
GFF II Guard Cartridges**	5 µm, 80 Å	1 cm x 3.0 mm i.d	731474

* Includes 1 holder and 2 guard cartridges
** Includes 3 guard cartridges

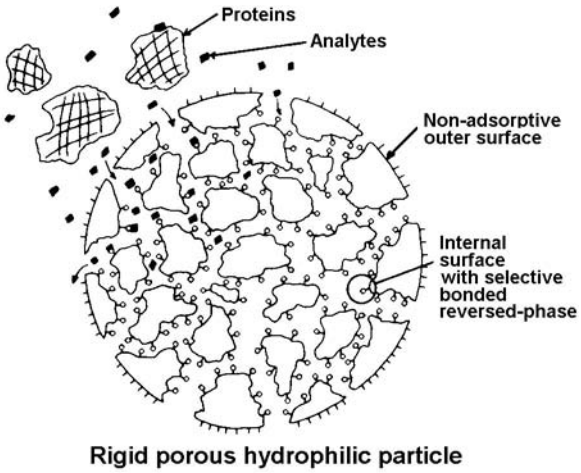


Figure 2. Demonstrates the inner and outer layers of a typical ISRP phase.

SPS

(Semi-Permeable Surface)

In an effort to extend the applicability of the RAM 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

Like the ISRP phase, the SPS phases consist of both hydrophilic outer and hydrophobic inner surfaces. The distinct difference is that 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 (figure 3). The outer phase provides size exclusion and hydrophilic shielding, which repels large biomolecules. The various inner phases allow for separation of small analytes.

Product	Particle Size	Column Dimensions	Catalog #
Phenyl	5 µm, 100 Å	15 cm x 4.6 mm i.d.	785107
Phenyl	5 µm, 100 Å	25 cm x 4.6 mm i.d.	785207
Octyl	5 µm, 100 Å	5 cm x 2.1 mm i.d.	785308
Octyl	5 µm, 100 Å	5 cm x 4.6 mm i.d.	785008
Octyl	5 µm, 100 Å	15 cm x 4.6 mm i.d.	785108
Octyl	5 µm, 100 Å	25 cm x 4.6 mm i.d.	785208
ODS	5 µm, 100 Å	5 cm x 2.1 mm i.d.	785318
ODS	5 µm, 100 Å	5 cm x 4.6 mm i.d.	785018
ODS	5 µm, 100 Å	15 cm x 4.6 mm i.d.	785118
ODS	5 µm, 100 Å	25 cm x 4.6 mm i.d.	785218

Product	Particle Size	Column Dimensions	Catalog #
Phenyl Guard Kit*	5 µm, 100 Å	1 cm x 3.0 mm i.d	785407
Phenyl Guard Cartridges**	5 µm, 100 Å	1 cm x 3.0 mm i.d	785507
Octyl Guard Kit*	5 µm, 100 Å	1 cm x 3.0 mm i.d	785408
Octyl Guard Cartridges**	5 µm, 100 Å	1 cm x 3.0 mm i.d	785508
ODS Guard Kit*	5 µm, 100 Å	1 cm x 3.0 mm i.d	785418
ODS Guard Cartridges**	5 µm, 100 Å	1 cm x 3.0 mm i.d	785518

* Includes 1 holder and 2 guard cartridges
** Includes 3 guard cartridges

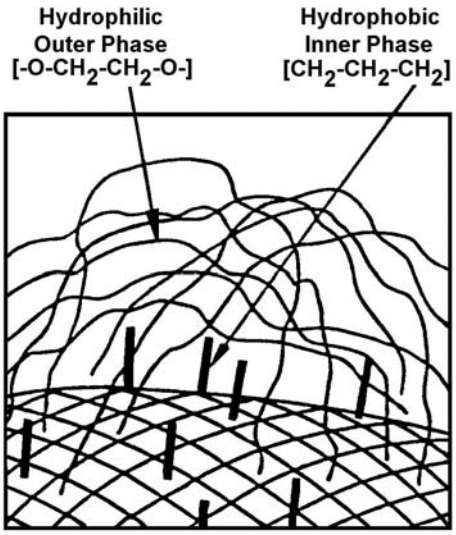


Figure 3. SPS structure highlighting the inner and outer phases.

SPS

(Semi-Permeable Surface)

SPS Column Advantages

The SPS offers the following advantages:

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

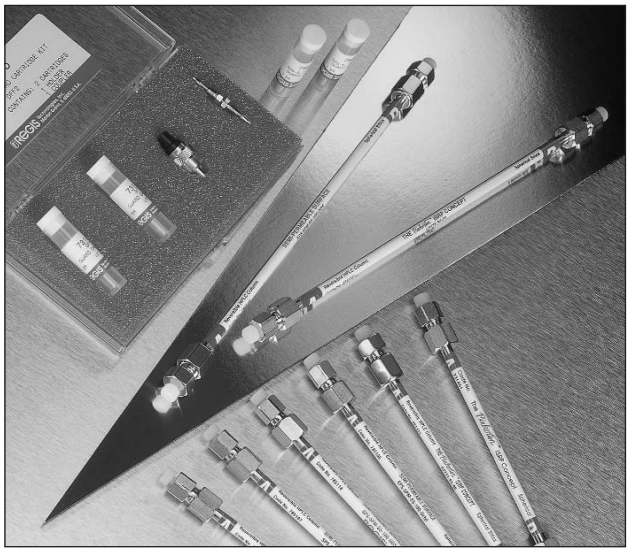
SPS Selectivity

The primary advantage of SPS over ISRP GFF II 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:

- 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 contained within the sample.

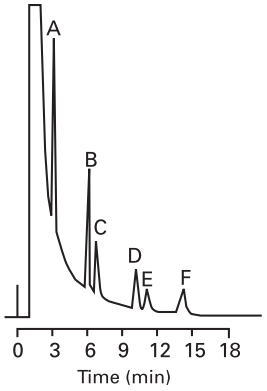


RAM Direct Injection Applications

RAM Direct Injection has been effective in numerous applications. Adjacent is a listing of some of the compounds analyzed by RAM Direct Injection. For additional applications, please contact Regis for the RAM Direct Injection Application Guide or download from Regis Web site at www.registech.com/ram/.

Some Compounds Analyzed by RAM Methods			
Acetazolamide	Cefaclor	Oxyphenbutazone	Sulfinpyrazone
Acetaminophen	Cefpiramide	Pentobarbital	Tamoxifen
Acetylsalicylic acid	3,4-Diaminopyridine	Phenelzine	Theophylline
4-Aminopyridine	Furosemide	Phenobarbital	Trazodone
Amobarbital	Heparin	Phenylalanine	Trimethoprim
Aprotinin	Hydroxyzine	Phenylbutazone	Trimipramine
Barbital	Imipramine	Phenytoin	Tryptophan
Butabarbital	Imirestat	Propranolol	Tyrosine
Caffeine	Methyl salicylate	Salicylic Acid	Verapamil
Carbamazepine	Norverapamil	Secobarbital	Warfarin

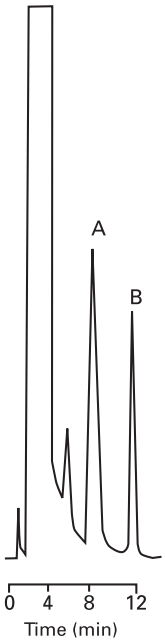
ISRP



Separation of Barbiturates in Human Serum	
Column:	ISRP GFF II 5 µm 80 Å 15 cm x 4.6 mm i.d.
Mobile Phase:	(95/5) 0.1 M potassium phosphate buffer, pH 7.5/methanol
Flow Rate:	1.0 mL/min
Load:	10 µL
Detection:	UV 240 nm

Sample Composition in Human Serum:
A. Barbital
B. Phenobarbital
C. Butabarbital
D. Amobarbital
E. Pentobarbital
F. Secobarbital

SPS



Determination of Antipyrine and Acetaminophen	
Column:	SPS C8 5 µm 100 Å 25 cm x 4.6 mm i.d.
Mobile Phase:	(99/0.5/0.5) 0.1 M potassium phosphate buffer, pH 7.4/acetonitrile/tetrahydrofuran
Flow Rate:	1.0 mL/min, 37° C
Load:	25 µL
Detection:	UV 244 nm

Sample Composition in Human Serum:
A. Antipyrine
B. Acetaminophen

Reference: Gurley, B.J.: et al.; Determination of Antipyrine in Human Serum by Direct Injection Restricted Access Media Liquid Chromatography; J. Pharm. Biomed Anal. 1994, 12 (12), 1591–1595.

RAM Direct Injection Applications

Column Switching with RAM Columns

For Improved Sensitivity

There has been growth in the use of column switching to process a large number of samples and achieve high sensitivity. The RAM Direct Injection column can be used in a column switching application to retain the small nonpolar analytes while allowing the matrix to pass through to waste. A less polar organic mobile phase is then used to elute the accumulated analytes onto an analytical column for subsequent chromatography.

Recent column switching work involves the use of short RAM guard columns. The guard column is used to separate the analytes from the matrix before switching to an analytical column. The low cost of the guard column allows it to be discarded after 60 to100 samples. The RAM guard column is an in expensive and simpler alternative to Solid Phase Extraction.

Figure 4 depicts a typical column switching system. In this procedure, the prefiltered but otherwise untreated sample is injected directly onto a RAM column. In the RAM column the smaller molecules are retained and concentrated, while most of the larger molecules are passed to waste. A stronger mobile phase is then used to elute the analytes onto a second column—often octadecylsilyl (ODS)—where they are separated and analyzed.

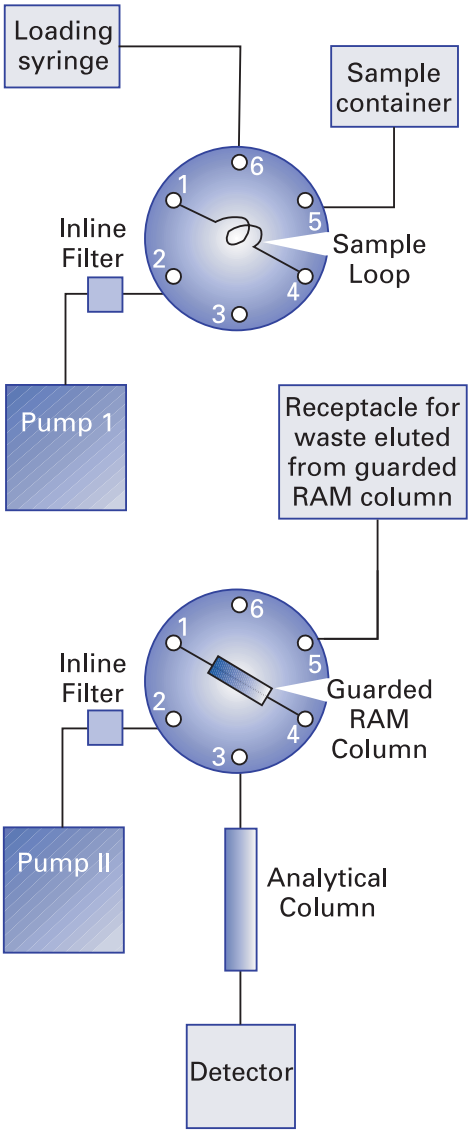


Figure 4. Column switching system.

IAM Chromatography

IAM Chromatography

Immobilized Artificial Membrane (IAM) technology is an innovative approach to chromatography in which the chromatographic surface emulates the lipid environment of the cell membrane.[1,2]

HPLC Separation Tools for Membrane Protein Purification and Drug Membrane Permeability Prediction

Phosphatidylcholine (PC) is the major phospholipid found in cell membranes. IAM chromatography phases prepared from PC analogs closely mimic the surface of a biological cell membrane. Consequently, IAM phases display a high affinity for membrane proteins and are useful in membrane protein purification and in the study of drug-membrane interactions. The IAM surface is formed by covalently bonding the membrane-forming phospholipids to silica. Several different types of IAM columns are used for various applications:

Membrane Protein Purification

IAM.PC
IAM.PC.MG

Drug Discovery

IAM.PC.DD2

- Drug membrane permeability prediction
- Hydrophobic in nature

IAM Fast-Screen Mini Columns

- High throughput estimation of drug permeability

Membrane Protein Purification

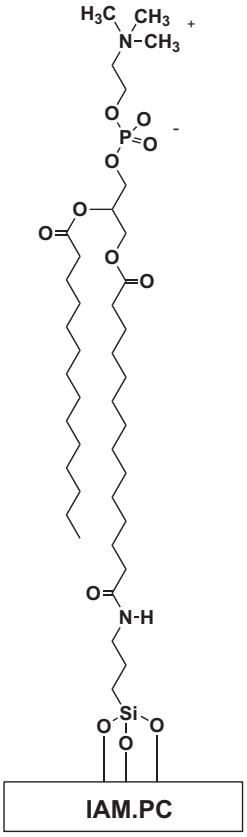


Figure 1. The Phosphatidylcholine is covalently bound to propylamine groups, which are in turn bound to silica. Because the bulky PC groups limit access to the unbonded amine groups, these may or may not affect the separation of a given protein.

IAM.PC

The IAM.PC phase, developed by Dr. Charles Pidgeon of Purdue University, was the first in a line of IAM phases to be manufactured by Regis. Use of this phase has simplified the inherent difficulties of protein isolation and purification,[3-9] allowing for rapid purification of membrane proteins while maintaining biological activity. The IAM.PC phase is an important tool for the pharmaceutical industry and academia alike.

The first IAM stationary phase was based on the prevalent membrane lipid, phosphatidylcholine (PC), and consists of monolayers of amphiphilic phospholipids covalently bonded to aminopropyl silica particles through a terminal amide linkage. As a result, the bulky phosphatidylcholine groups shield many of the amine binding sites on the silica surface, preventing amine interaction with the protein molecules.

The membrane nature of the IAM phase imparts surface characteristics which are useful in the chromatography of membrane proteins. These include: high protein loading, increased protein recovery, recovery of functional activity, and selectivity for membrane proteins.

Large membrane proteins can interact with any combination of polar headgroup, hydrophobic chain, or inner amine groups. The subsurface has been shown to interact with certain solutes, and may or may not contribute to the separation of a given biomolecule. The residual amines can be left unaltered on the subsurface or deactivated through an endcapping procedure, which results in increased stability of the bonded phase. The methyl glycolate endcapping, for example, converts residual amines to neutral amides and introduces a hydroxyl group (IAM.PC.MG).

IAM.PC Applications

Numerous applications have been developed using IAM.PC columns:

- Purification of Cytochrome P450
- Isolation of membrane proteins
- Prediction of solute transport across human skin
- Prediction of amino acid transport across the blood-brain barrier
- Binding of solutes to liposome membranes
- Immobilization of Trypsin and α-chymotrypsin for the determination of their inhibitor and substrate activity

For additional information on IAM.PC applications please contact Regis' technical support staff.

Product	Particle Size	Column Dimensions	Catalog #
IAM.PC	10 µm, 300Å	3 cm x 4.6 mm i.d.	770007
IAM.PC	10 µm, 300Å	15 cm x 4.6 mm i.d.	770001
IAM.PC.MG	10 µm, 300Å	3 cm x 4.6 mm i.d.	772007
IAM.PC.MG	10 µm, 300Å	15 cm x 4.6 mm i.d.	772001
IAM.PC Guard Kit*	10 µm, 300Å	1 cm x 3.0 mm i.d	771001
IAM.PC Guard Cartridges**	10 µm, 300Å	1 cm x 3.0 mm i.d	774001
IAM.PC.MG Guard Kit*	10 µm, 300Å	1 cm x 3.0 mm i.d	773001
IAM.PC.MG Guard Cartridges**	10 µm, 300Å	1 cm x 3.0 mm i.d	775001

* Includes 1 holder and 2 guard cartridges
** Includes 3 guard cartridges

Drug Discovery-Predicting Drug Membrane Permeability

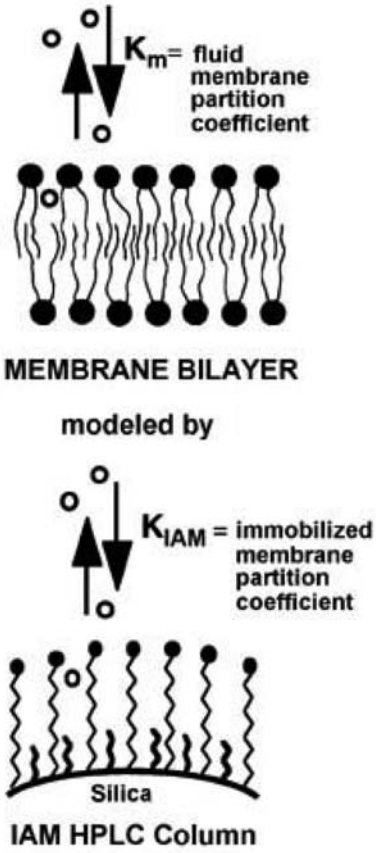


Figure 2. Fluid membrane bilayer can be modeled by IAM column.

IAM.PC.DD2
IAM Fast-Screen Mini Column

IAM chromatography has recently gained acceptance among drug discovery chemists for estimating the membrane permeability of small molecule drugs.

Figure 2 illustrates that the interaction between membrane bilayer and drug can be modeled by the IAM column/drug system.

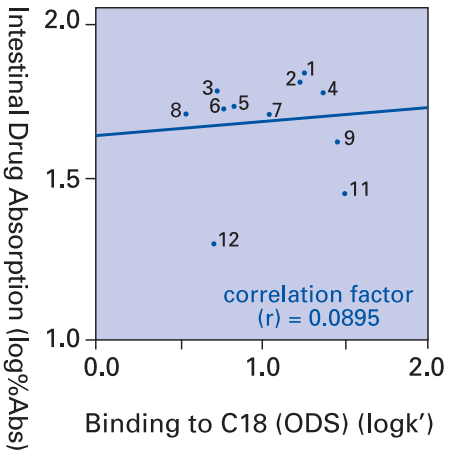
K_{IAM}, the equilibrium constant describing the relative concentrations of drug in the membrane and in the external fluid, is analogous to the k'_{IAM}.

This IAM technique provides superior correlation with experimentally determined drug permeability when compared to other chromatographic methods. ODS silica, for example, retains analytes solely on the basis of hydrophobicity. IAM more closely mimics the interaction of analytes with biological membranes, where a combination of hydrophobic, ion pairing, and hydrogen bonding interactions are possible. This combination of interactions measured by the IAM column is known as phospholipophilicity.

These advances have led to the development of several new IAM phases used for predicting drug membrane permeability:

- IAM.PC.DD2
- IAM Fast-Screen Mini Column

ODS Exhibits Poor Correlation with Intestinal Drug Absorption	
Column:	C18 (ODS)
Mobile Phase:	3 cm x 4.6 mm i.d.
Flow Rate:	1.0 mL/min
Load:	10 µL
Detection:	UV 220 nm



1. *m*-Nitroaniline
2. *p*-Nitroaniline
3. Salicylic acid
4. *p*-Toluidine
5. Aniline
6. *m*-Nitrobenzoic acid
7. Phenol
8. Benzoic acid
9. Acetanilide
10. Antipyrine
11. Theophylline
12. Acetylsalicylic acid

Figure 3. Drug partitioning into ODS does not correlate with intestnal drug absorption.

IAM .PC.DD2

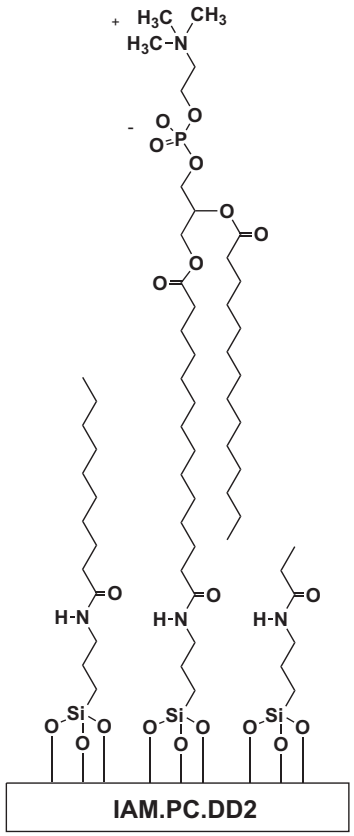
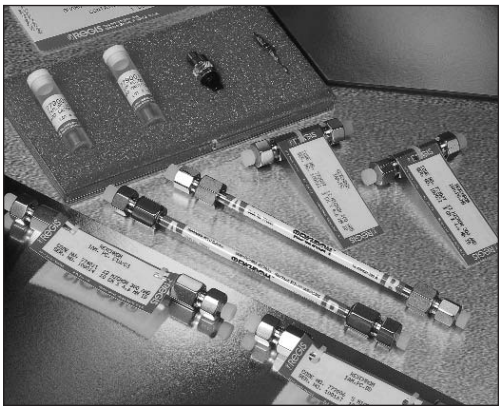


Figure 4. IAM.PC.DD2 is used to predict drug membrane permeability.



Intestinal Drug Permeability

The retention factors measured on reversed phase C18 (ODS) columns (a commonly used model to determine drug partitioning) show extremely poor correlation with intestinal drug absorption (figure 3). For this group of compounds, hydrophobicity alone, as measured by the reversed-phase C18 column, is a poor predictor of drug absorption. Since IAM.PC Drug Discovery columns measure both hydrophilic and hydrophobic interactions between drugs and membranes, the IAM.PC Drug Discovery Column is better suited to the prediction of intestinal drug absorption.

Like the first generation IAM.PC.DD material, the IAM.PC.DD2 is used to predict drug membrane permeability. The ester bonding of the DD2 packing offers more hydrophobicity than the first generation DD phase. This material is a diacylated or double chain ester PC ligand and is endcapped with C10/C3 alkyl chains as illustrated in figure 4.

Column Advantages

The IAM.PC.DD2 material offers the following advantages:

- Hydrophobic nature
- Greater stability
- Excellent correlation to traditional methods

Hydrophobic Nature

The IAM.PC.DD2 offers more hydrophobicity than the first generation IAM.PC.DD material. This hydrophobic nature allows for longer retention times to compounds not well retained on the IAM.PC.DD material.

Greater Stability

Another distinct advantage of the IAM.PC.DD2 material is its ability to tolerate mobile phases between pH's 7.0 to 7.5, thus resulting in longer column life under these conditions.

Excellent Correlation to Traditional Methods

The traditional means of predicting membrane permeability include the use of Caco-2 cell line cultures, intestinal tissue or liposome assays. These methods are laborious and costly to perform.

IAM.PC.DD2

Sample	% Absorption of Inverted Rat Intestine	(k') IAM.PC.DD2
m-nitroaniline	77	10.838
p-nitroaniline	68	16.086
salicylic acid	60	6.963
p-toluidine	59	4.546
aniline	54	2.069
m-nitrobenzoic acid	53	4.403
phenol	51	6.544
benzoic acid	51	2.088
acetanilide	42	5.096
antipyrine	32	3.350
theophylline	29	1.478
acetylsalcylic acid	20	0.931
r (correlation factor)*		**0.8025**

*r is calculated by plotting log k' vs. log % absorption ofinverted rat intestine.

Table 1. Correlating Drug Partitioning into IAM with rat intestinal drug absorption.

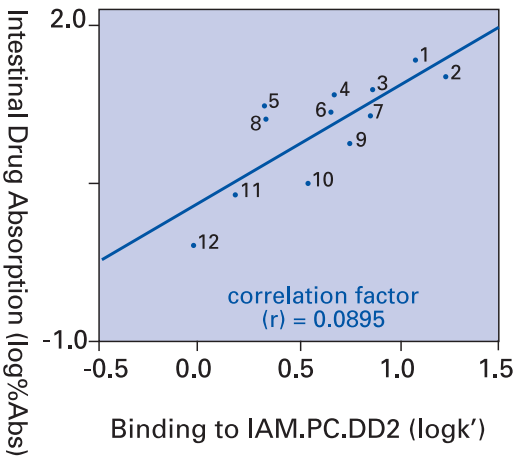
Intestinal Tissue Correlation

Measuring drug permeability in the intestinal tissue, where absorption is occurring, is physiologically more relevant than measuring drug permeability in Caco-2 cells. Figure 5 and table 1 illustrate that drug absorption in this inverted rat intestinal tissue model correlates with drug retention factors k'_{IAM} measured on the IAM.PC.DD2 column.

Product	Particle Size	Column Dimensions	Catalog #
IAM.PC.DD2	10 µm, 300Å	3 cm x 4.6 mm i.d.	774010
IAM.PC.DD2	10 µm, 300Å	10 cm x 4.6 mm i.d.	774011
IAM.PC.DD2	10 µm, 300Å	15 cm x 4.6 mm i.d.	774014
IAM.PC.DD2 Guard Kit*	10 µm, 300Å	1 cm x 3.0 mm i.d	774012
IAM.PC.DD2 Guard Cartridges**	10 µm, 300Å	1 cm x 3.0 mm i.d	774013

* Includes 1 holder and 2 guard cartridges
** Includes 3 guard cartridges

IAM Correlates with Intestinal Drug Absorption	
Column:	IAM.PC.DD2
	10 cm x 4.6 mm i.d.
Mobile Phase:	0.01 M DPBS Buffer, pH 5.4
Flow Rate:	1.0 mL/min
Load:	10 µL
Detection:	UV 220 nm



1. *m*-Nitroaniline
2. *p*-Nitroaniline
3. Salicylic acid
4. *p*-Toluidine
5. Aniline
6. *m*-Nitrobenzoic acid
7. Phenol
8. Benzoic acid
9. Acetanilide
10. Antipyrine
11. Theophylline
12. Acetylsalicylic acid

Figure 5. IAM.PC.DD2 columns measure drug absorption in inverted rat intestinal tissue.

IAM Fast Screen Mini Column

Method	Number of Compounds Evaluated	Correlation (r) with IAM Fast-Screen Mini Column
Partitioning into liposomes	23	0.831
Intestinal drug permeability	12	0.839
Caco-2 cell permeability	8	0.909

Table 2. Comparing k'IAM data with other methods for estimating permeability.

Compound	% Absorption of Inverted Rat Intestine	IAM Fast-Screen Mini Column Retention Time	
m-nitroaniline	77	133.1	15.29
p-nitroaniline	68	177.9	21.84
salicylic acid	60	93.8	9.54
p-toluidine	59	79.7	7.48
aniline	54	52.1	3.45
m-nitrobenzoic acid	53	68.1	5.79
phenol	51	94.6	9.66
benzoic acid	51	43.7	2.22
acetanilide	42	76.2	6.97
antipyrine	32	51.8	3.40
theophylline	29	39.3	1.58
acetylsalcylic acid	20	36.1	1.11
r (correlation factor)*			**0.8385**

Table 3. Correlating drug partitioning into IAM with rat intestinal drug absorption.

* r is calculated by plotting log k' vs. log % absorption of inverted rat intestine.

Packed with the Ester PC Ligand phase, IAM Fast-Screen Mini columns are a rapid and economically viable screening method for the high throughput estimation of drug permeability. Their benefits include excellent reproducibility, short analysis time and low cost. This can be of great use in characterizing large libraries of compounds. The structure of the esterIAM.PC.C10/C3 packing, selected for the Fast-Screen Mini Column, is shown in figure 6. This PC analog demonstrates superiority in retention times and stability–essential features for short columns and mass drug screening. The IAM.PC Fast-Screen Mini Column, 1 cm in length by 3.0 mm in internal diameter, was specifically designed by Regis for rapid estimation of drug permeability in high throughput screening programs. When connected to an HPLC system with an autosampler, a single column can be used in the analysis of hundreds of samples per day with highly reproducible results. The 1 cm Fast-Screen Mini Column is offered not as a separation tool, but rather as a tool for characterizing the chromatographic retention factor (k') of individual analytes. The measured k' of analytes on this column can be used to estimate a value for drug permeability.

Column Advantages

Regis Technologies' 1 cm Fast-Screen Mini Column for Drug Discovery provides the following advantages:

- Excellent correlation to traditional methods
- Rapid indication of drug absorption
- High sample throughput
- Highly reproducible results
- Durability
- Cost effectiveness
- Ability to establish absorption zones for high throughput screening

IAM Fast-Screen Mini Column Structure

Excellent Correlation To Traditional Methods

The traditional means of predicting permeability include use of Caco-2 cell line cultures, intestinal tissue, or liposome assays. These are laborious and costly to perform. Data obtained from the IAM Fast-Screen Mini Column correlate well to data obtained from traditional assays. This is summarized in table 2.

Chromatographic Conditions:	
Column:	IAM Fast-Screen Mini Column 1 cm x 3.0 mm i.d.
Mobile Phase:	Dulbecco's Phosphate Buffered Saline, pH 5.4
Flow Rate:	0.3 mL/min
Load:	10 µL
Detection:	UV 254 nm, 0.1 AUFS

IAM Fast Screen Mini Column

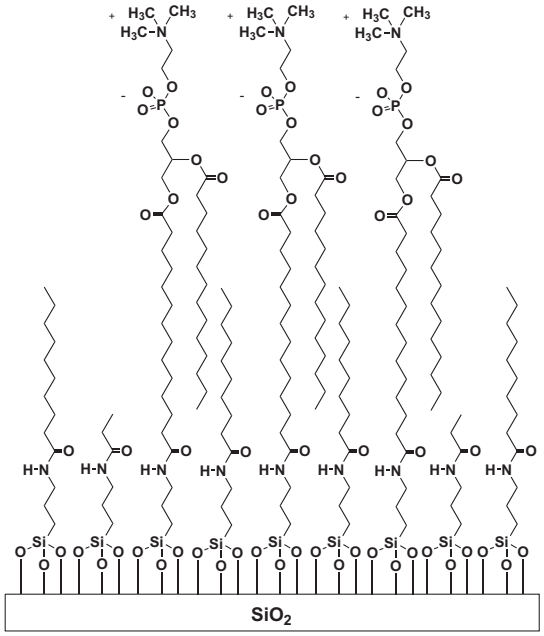
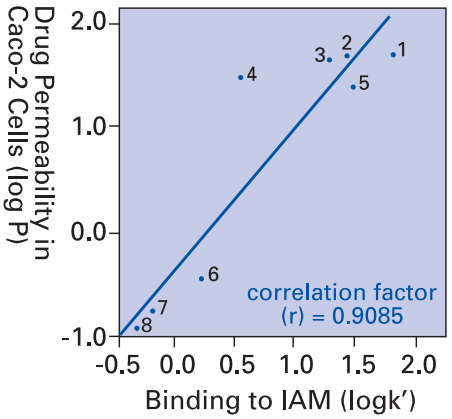


Figure 6. IAM.PC Fast-Screen Mini Column provides rapid estimation of drug permeability in high thoughput screening programs.

IAM Fast-Screen Correlates with Drug Permeability in Caco-2 Cells	
Column:	IAM Fast-Screen Mini Column 1 cm x 3.0 mm i.d.
Mobile Phase:	0.01 M DPBS Buffer, pH 7.4
Flow Rate:	0.5 mL/min
Load:	10 µL
Detection:	UV 220 nm



1. Propanolol
2. Alprenolol
3. Warfarin
4. Metoprolol
5. Hydrocortisone
6. Terbutaline
7. Atenolol
8. (AVP) Arginine-Vasopressin

Figure 7. Correlating drug partitioning into IAM with intestinal drug permeability (log P) through Caco-2 cells.

Intestinal Tissue Correlation

Table 3 shows that drug permeability predicted by Inverted Rat Intestines correlates well to drug retention factors, k'_{IAM} measured on the IAM Fast-Screen Mini Columns. Note the short retention times.

Caco-2 Cell Correlation

Figure 7 illustrates that drug permeability predicted by Caco-2 cells correlates well to k'_{IAM} measured on the IAM Fast-Screen Mini Columns.

Rapid Indication of Drug Absorption

IAM Chromatography is a more rapid alternative to other methods. In a recent study completed by Regis, k'_{IAMs} of 12 compounds were compared with absorption data obtained in situ using rat intestines. Retention times for the compounds tested were between 20 and 180 seconds, while retention factors correlated well to the intestinal absorption data.

High Sample Throughput

IAM chromatography is of increasing importance in combinatorial chemistry, where it is used to provide an initial estimate of a drug candidates' membrane permeability. Hundreds of samples can be injected into a single Fast-Screen Mini Column using an automated HPLC system. Recently a group of 12 test analytes was evaluated in 10 runs over the course of eight hours. Total run time for the 12 test analytes was only 42 minutes.

IAM Fast Screen Mini Column

Highly Reproducible Results

The measured values for k'_{IAM} show excellent reproducibility, both from run to run and from day to day (figure 8).

Durability

IAM Fast-Screen Mini Columns are extremely durable. Correlation factors, r, for the original k′, and k′ after 5000 column volumes were identical.

Cost Effectiveness

Because the IAM Fast-Screen Mini Column is inexpensive, has a very short analysis time, and provides drug permeability estimates for hundreds of drug candidates in a fraction of the time of conventional methods, the IAM Fast-Screen Mini Column becomes the economical alternative for high throughput screening.

Ability to Establish Permeability Zones for High Throughput Screening

Permeability zones can be determined for different analytes when performing large-scale drug absorption screening. Thus, rapid IAM analyses can characterize a drug as having low, medium, or high membrane permeability (figure 9).

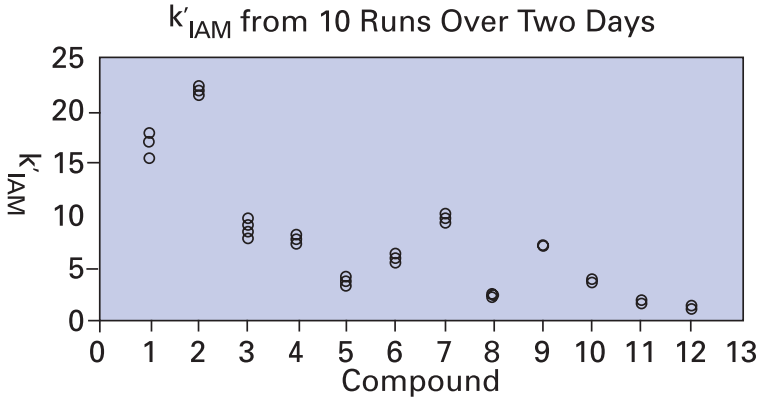


Figure 8. Highly reproducible k'_{IAM} from 10 runs over a two-day period.

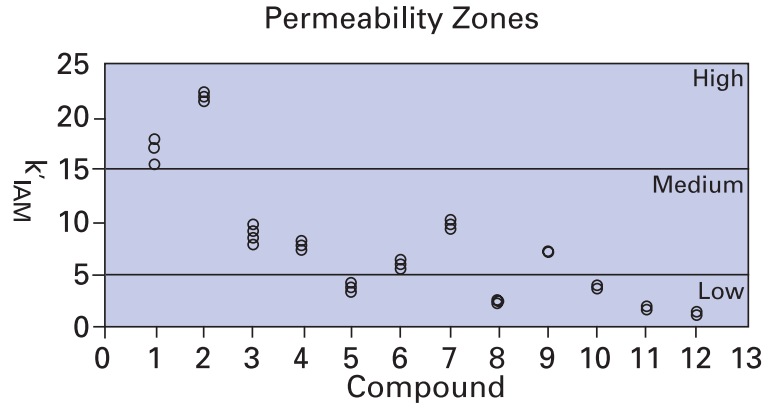


Figure 9. Permeability zones established large-scale drug absorption screening.

IAM Fast Screen Mini Column

Regis Technologies manufactures the IAM Fast-Screen Mini Column on-site in its manufacturing facility. This column, as well as all of our other products, must adhere to rigorous manufacturing and quality control specifications before release.

Regis' technical support staff, with years of chromatography experience, is available to answer any questions regarding the new IAM Fast-Screen Mini Column.

Product	Particle Size	Column Dimensions	Catalog #
IAM Fast-Screen Mini Column Kit • 2 columns, 1 cm x 3 mm • 1 cartridge holder • Care and use booklet	10 µm, 300Å	1 cm x 3.0 mm i.d	775014
IAM Fast-Screen Mini Columns, Pkg. of 6 • 6 columns, 1cm x 3 mm • Care and use booklet	10 µm, 300Å	1 cm x 3.0 mm i.d	775015
IAM Fast-Screen Mini Columns, Pkg. of 12 • 12 columns, 1 cm x 3 mm • Care and use booklet	10 µm, 300Å	1 cm x 3.0 mm i.d	775016

IAM References

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11. Schanker, L. S.; J. Pharmacol. Exp. Ther. 1958, 123, 81–88.

Ion Pairing Reagents and Buffers

Ultrapure Ion Pairing Reagents and Buffers

Ion Pair Chromatography is a method for improving the separation of charged analytes. In the resolution of organic ions with conventional HPLC methods, use of ion pair reagents can enhance peak shape and retention time when common remedies such as modifying eluent ratios or changing stationary phase fail.

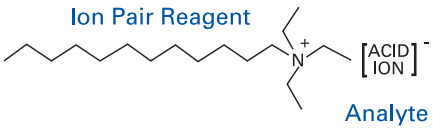


Figure 1. Quaternary Amine (Q-Series) Ion Pair Reagent.

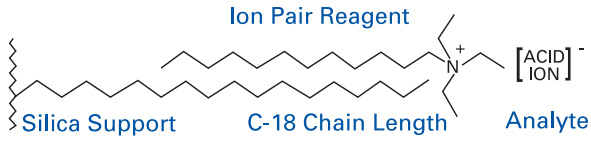


Figure 2. Quaternary Amine (Q-Series) Ion Pair Reagent interacting with C-18 Support.

The Advantages of Ion Pair Chromatography

In the past, chromatographic separation of charged analytes has been achieved by ion suppression (the careful adjustment of the mobile phase pH to result in a nonionized analyte). Determining the optimum mobile phase pH in ion suppression, however, often requires extensive method development. Samples containing more than one ionizable component were often unusable. The imitations of ion suppression led to the development of a new, more generally applicable approach to separation of ionized components: *ion pair chromatography*.

Developed by Dr. Gordon Schill in 1973, ion pair chromatography relies upon the addition of ionic compounds to the mobile phase to promote the formation of ion pairs with charged analytes. These reagents are comprised of an alkyl chain with an ionizable terminus (figure 1). When used with common hydrophobic HPLC phases in the reversed-phase mode, ion pair reagents can be used to selectively increase the retention of charged analytes (figure 2).

Although ion exchange chromatography has become a popular mode of separation, it is not useful in all situations. The advantages of ion pair chromatography over ion exchange chromatography are:

- Simple preparation of buffers
- Wide choice of carbon chain lengths for improved retention and separation
- Significantly reduced separation time
- Simultaneous separation of both ionized and nonionized solutes
- Highly reproducible results
- Improved peak shape

Regis Provides a Choice of Reagents

Regis manufactures both ultrapure anionic Sulfonate (S-Series) and cationic Quaternary Amine (Q-Series) ion pair concentrates in the following alkyl chain lengths: pentyl, hexyl, heptyl, octyl, and dodecyl. Alkyl chains are represented by cardinal numbers in the naming of our products, i.e., 5, 6, 7, 8, and 12. (See product descriptions on the following pages.)

Ultrapure Ion Pairing Reagents and Buffers

Optical Absorbance (AUFS) S-Series	200 nm	210 nm
S5	0.006	0.002
S6	0.048	0.018
S7	0.008	0.001
S8	0.001	0.003
S12	0.002	0.003
CH_3CN	0.076	0.013
CH_3OH	0.940	0.510

Optical Absorbance (AUFS) Q-Series	200 nm	210 nm
Q5	0.060	0.001
Q6	0.059	0.006
Q7	0.022	0.009
Q8	0.082	0.003
Q12	0.102	0.013

Table 1. Typical optical absorbances (AUFS) at 0.005 M.

	Retention Times (min)		Retention Ratio
Q-Series	Benzoic Acid	Benzyl Alcohol	Acid/ Alcohol
Q5	4.53	9.17	0.49
Q6	6.50	8.60	0.76
Q7	8.24	9.13	0.90
Q8	12.36	8.94	1.38
Q12	79.53	8.52	9.33

Table 2. Retention vs. chain length.

[benzoic acid/benzyl alcohol in (60/40) water/ methanol]					
Q6		Q7		Q8	
pH	R	pH	R	pH	R
7.50	0.59	7.50	0.88	7.51	1.06
6.50	0.70	6.51	1.00	6.54	1.29
5.50	0.96	5.52	1.23	5.50	1.59

Table 3. Retention ratio R as a function of pH.

Purity is a Key Ingredient

Purity is of key importance in the manufacture of our Ion Pair Reagents. Regis S- and Q-Series products are synthesized in accordance with the industry's highest quality standards, resulting in exceptional purity and integrity. This is demonstrated in table 1: UV transparency as low as 200 nm can be achieved for both the S- and Q-Series reagents. In most cases, these absorbances are lower than those for HPLC grade acetonitrile and methanol. Although the S- and Q-Series ion pair reagents can be used at wavelengths less than 210 nm, the crucial factors in determining what wavelength to use are the integrity of the detector optics and the purity of the organic modifiers.

Regis also supplies bulk Sulfonate and several additional bulk Ion Pair Reagents to complement the separation capabilities of the Sulfonate S-Series and Quaternary Amine Q-Series.

How to Select a Regis Ion Pair Reagent For Method Development

To choose the proper reagent, alkyl chain lengths must be taken into consideration. The chain lengths enable selective separation of the analyte. The longer the chain, the more hydrophobic the counterion, and therefore, greater the retention. Retention may increase by a factor of almost 20 when going from pentyl (Q5) to dodecyl (Q12), as illustrated in table 2 and figure 3. Both table 2 and figure 3 demonstrate that the Q-reagent chain length governs benzoic acid retention times, but does not affect the benzyl alcohol retention times. Similar behavior can also be achieved with the S-Series.

The following are guidelines to developing a successful method using Regis' ion pair reagents:

- Select a column — endcapped ODS (octadecylsilyl) is most common.
- Use only HPLC-grade water and chromatography grade reagents in mobile phase preparation.
- Choose the mobile phase components and concentrations that give the best separation.
- If nonionic components are present in the sample, optimize the resolution prior to attempting ionic separations.
- Select the appropriate ion pair series to provide the necessary counterion. Use the Q-series for acidic compounds and the S-series for basic compounds.
- Through a process of elimination, choose the alkyl chain length which results in the best separation (figure 4).
- Once the reagent has been selected, adjust the pH of the mobile phase to maximize resolution. Because slight modification of pH can profoundly effect retention and selectivity, make all adjustments in small increments and monitor carefully (table 3).
- Ideally, the ion pair reagent concentration in the mobile phase should be 0.005 M. However, small adjustments in reagent concentration may increase retention slightly and optimize the separation (figure 5).

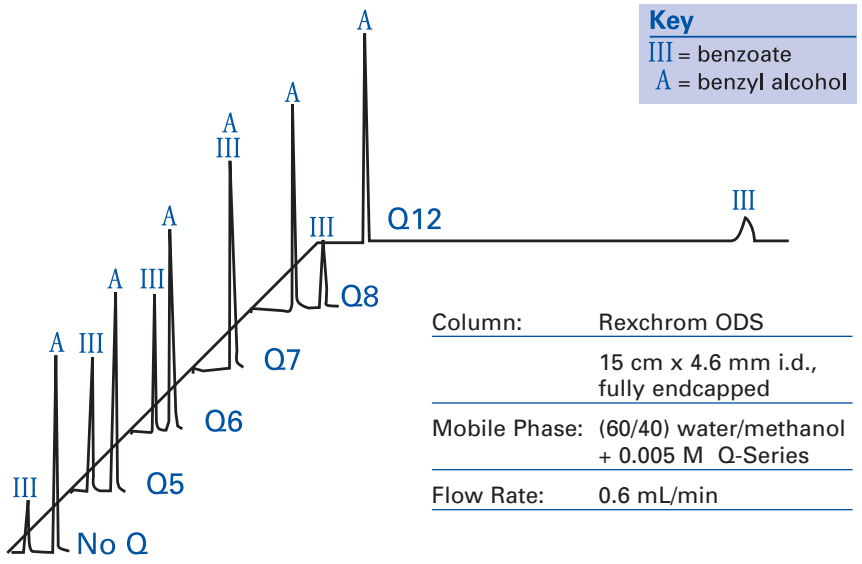


Figure 3. Retention increases with Q-Reagent chain length.

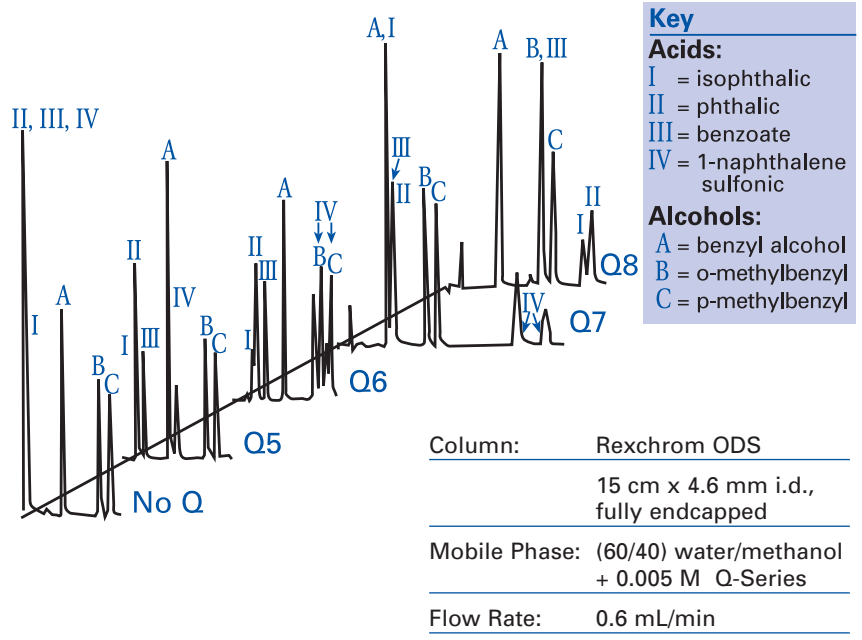


Figure 4. In a mixture of ionic and nonionic compounds, first separate the nonionic compounds from each other (See above). Then choose the ion pair reagent that retains the ionic compounds as desired. Here, Q6 seems to be the reagent of choice since all peaks are visibly separated.

Regis Sulfonates (S-Series) For Basic Compounds

S-Series Ion Pair Concentrates (For Cations)

The sulfonates are sodium salts that act as an anionic counterion for the separation and resolution of positively charged analytes. The sulfonates are available as: Ion pair concentrates—premixed 0.5 M solutions of alkyl sulfonates. When diluted to 1 L with HPLC-grade water, a 10 mL bottle forms a 0.005 M solution.

Larger quantities are available upon request. Please call Regis for pricing.

Product	Size	Catalog #
S5 (1-pentylsodiumsulfonate)	(5) 10 mL bottles 100 mL bottle	405025 405035
S6 (1-hexylsodiumsulfonate)	(5) 10 mL bottles 100 mL bottle	405026 405036
S7 (1-heptylsodiumsulfonate)	(5) 10 mL bottles 100 mL bottle	405027 405037
S8 (1-octylsodiumsulfonate)	(5) 10 mL bottles 100 mL bottle	405028 405038
S12 (1-dodecylsodiumsulfonate)	(5) 10 mL bottles 100 mL bottle	405021 405031
0.5 M solutions of Alkyl Sulfonates *(Each 10 mL bottle, diluted to 1 L, produces a 0.005 M solution)*		
S-Series Method Development Kit *Each kit contains a 10 mL bottle of each of the following:*		
S5, S6, S7, S8, S12		405020

Bulk Ion Pair Reagents (For Cations)

Bulk powder—fine, purified crystals, for use as a buffer in large-scale mobile phase preparation.

Larger quantities are available upon request. Please call Regis for pricing.

Product	Size	Catalog #
1-Pentanesulfonate, Sodium Salt	25 gm 100 gm	403025 403125
1-Hexanesulfonate, Sodium Salt	25 gm 100 gm	403026 403126
1-Heptanesulfonate, Sodium Salt	25 gm 100 gm	403027 403127
1-Octanesulfonate, Sodium Salt	25 gm 100 gm	403028 403128

Regis Quaternary Amines (Q-Series) For Acidic Compounds

Q-Series Ion Pair Concentrates (For Anions)

The Q-series is comprised of quaternary alkyltriethylamines that can be used for the resolution of negatively charged species. This unique set of cationic reagents was developed to complement the Sulfonate Series (S-Series) and is exclusively manufactured by Regis. The Quaternary Alkyltriethylamines are available as:

Ion pair concentrates —premixed 0.5 M solutions of alkylamines. When diluted to 1 L with HPLC-grade water, a 10 mL bottle forms a 0.005 M buffered solution.

Product	Size	Catalog #
Q5 (1-pentyltriethyl-ammonium phosphate)	(5) 10 mL bottles 100 mL bottle	404025 404035
Q6 (1-hexyltriethyl-ammonium phosphate)	(5) 10 mL bottles 100 mL bottle	404026 404036
Q7 (1-heptyltriethyl-ammonium phosphate)	(5) 10 mL bottles 100 mL bottle	404027 404037
Q8 (1-octyltriethyl-ammonium phosphate)	(5) 10 mL bottles 100 mL bottle	404028 404038
Q12 (1-dodecyltriethyl-ammonium phosphate)	(5) 10 mL bottles 100 mL bottle	404021 404031
0.5 M solutions of Quaternary Alkyltriethylamines *(Each 10 mL bottle, diluted to 1 L, produces a 0.005 M solution)*		
Q-Series Method Development Kit *Each kit contains a 10 mL bottle of each of the following:*		
Q5, Q6, Q7, Q8, Q12		404020

Other Regis Bulk Ion Pair Reagents (For Anions)

Other bulk Ion Pair reagents such as Tetrabutylammonium phosphate, Trihexylamine and Triheptylamine are complementary reagents used for the resolution of negatively charged analytes.

Product	Size	Catalog #
Tetrabutylammonium phosphate 0.5 M, pH 7.5	10 mL	680502
Tetrabutylammonium phosphate 0.5 M, pH 7.5	500 mL	680503

Ion Pair References

1. Perry, J. A.; Glunz, L. G.; Szczerba, T. J.; Hocson, V. S.; Reagents For Ion Pair Reversed-Phase HPLC; American Laboratory 1984, 16(10), 114—119.
2. Eksborg, S.; Lagerstrom, P.; Modin, R.; Schill, G.; Ion Pair Chromatography of Organic Compounds J. Chrom. 1973, 83, 99.
3. Eksborg, S.; Schill, G.; Ion Pair Partition Chromatography of Organic Ammonium Compounds Anal. Chem. 1973, 45, 2092.

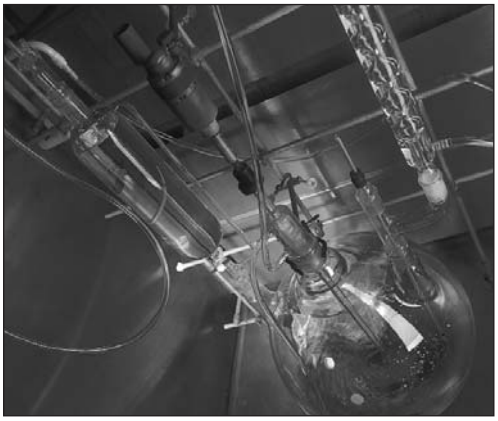
GC Derivatization Reagents

Derivatization

Derivatization is primarily performed to modify an analyte's function ality to enable chromatographic separations. For more than 40 years, Regis has been a leader in the manufacture of highly pure derivatization reagents for gas chromatography. The formation of chemical derivatives to facilitate meaningful analysis has long been a common practice in gas chromatography. For the analytical chemist, judicious use of derivatization can be the key to unlocking and simplifying a great many complex and perplexing separation problems. Derivatization, accomplished through alteration of functional groups, provides:

- Increased sample volatility
- Improved selectivity and chromatographic efficiency
- Enhanced detectability

Additional procedures and references are available on our Web site at: www.registech.com/gc.



Gas Chromatography (GC) Derivatization

Sample volatility or thermal stability is crucial in GC applications. If a sample does not possess these important characteristics, GC analysis is highly unproductive. Derivatization techniques have been developed to address these issues to insure successful separations. In GC Derivatization, replacement of active hydrogen in functional groups, such as -COOH, -OH, -NH, and -SH, is the primary area of concern and is accomplished through either silylation, acylation or alkylation.

Silylation

Silylation is the most widely used derivatization procedure for sample analysis by GC. Silylation reagents are popular because they are easy to use and readily form derivatives. In silylation, an active hydrogen is replaced by an alkylsilyl group, such as trimethylsilyl (TMS) or *t*-butyldimethylsilyl (*t*-BDMS). Compared to their parent compounds, silyl derivatives are more volatile, less polar, and more thermally stable. As a result, GC separation is improved and detection is enhanced.

Silylation reagents are generally moisture sensitive, requiring them to be sealed under nitrogen to prevent deactivation. The derivatives of TMS reagents are also moisture sensitive. In response to this difficulty, *t*-BDMS reagents were introduced, which enabled the formation of derivatives 10,000 times more stable to hydrolysis than the TMS ethers.

Both TMS and *t*-BDMS reagents are suitable for a wide variety of compounds, offer excellent thermal stability and can be used in a variety of GC conditions and applications.

Analysis by the popular combination of gas chromatography and mass spectrometry (GS/MS) often requires special sample derivatization. Particularly effective in these applications is MTBSTFA.

Acylation

Acylation reagents offer the same types of advantages available from silylation reagents: creating less polar, more volatile derivatives. However, in comparison to silylating reagents, the acylating reagents more readily target highly polar, multi-functional compounds, such as carbohydrates and amino acids. In addition, acylating reagents provide the distinct advantage of introducing electroncapturing groups, thus enhancing detectability during analysis.

Generally, these reagents are available as acid anhydrides, acyl derivatives, or acyl halides. The acyl halides and acyl derivatives are highly reactive and are suitable for use where steric hindrance may be a factor. Acid anhydrides are supplied in a number of fluorinated configurations, which improve detection. These fluorinated anhydride derivatives are used primarily for Electron Capture Detection (ECD), but can also be used for Flame Ionization Detection (FID). Fluorinated anhydrides are often used in derivatizing samples to confirm drugs of abuse. Despite the special utility of these reagents, their acidic nature requires that any excess or byproducts be removed prior to analysis to prevent deterioration of the column.

Gas Chromatography (GC) Derivatization

Alkylation

As with other derivatization reagents, alkylation reagents reduce molecular polarity by replacing active hydrogens with an alkyl group. These reagents are used to modify compounds having acidic hydrogens, such as carboxylic acids and phenols. Alkylation reagents can be used alone to form esters, ethers, and amides—or they can be used in conjunction with acylation or silylation reagents. A two-step approach is commonly used in the derivatization of amino acids, where multiple functional groups on these compounds may necessitate protection during derivatization.

Due to the availability of reagents and their ease of use, esterification (the reaction of an acid with an alcohol in the presence of a catalyst to form an ester) is the most popular method of alkylation. Alkylation reagents are available in several configurations that enable the formation of a variety of esters. Alkyl esters are stable, and can be formed quickly and quantitatively. By altering the length of the substituted alkyl group, retention of the derivative can be varied. In addition to the formation of simple esters, alkylation reagents can be used in extractive procedures where biological matrices may be present.

GC Chiral Derivatization

GC analysis of enantiomeric compounds on nonracemic or achiral stationary phases requires the use of enantiopure derivatization reagents. These reagents generally target one specific functional group to produce diastereomers of each of the enantiomeric analytes. From the resulting chromatograms, calculations are conducted to determine the enantiomeric concentration of the analyte.

Guide to GC Derivatization Methods/Functional Groups

GC Derivatization Method

Functional Group	Silylation	Acylation	Alkylation
Active Hydrogens	BSTFA, BSTFA/TMCS, Deriva-Sil, Hydrox-Sil, TBH MSTFA, MTBSTFA, TMSI	PFPOH/PFPA	DMF Dialkylacetals,
Carboxylic Acids	BSTFA, Hydrox-Sil Conc., MTBSTFA, TMSI	PFPOH/PFPA	BF_3/Methanol, BF_3/n-Butanol, DMF Dialkylacetals, TBH
Alcohols and Phenols: unhindered and moderately hindered	BSA, BSTFA/TMCS, HMDS, MTBSTFA/*t*-BDMCS	HFBI, Fluorinated anhydrides (HFBA, PFPA, TFAA), MBTFA, MCF*	DMF Dialkylacetals, PFB-Br/TBA-H-SO_4, TBH
Alcohols and Phenols: highly hindered	BSTFA/TMCS, Deriva-Sil, Deriva-Sil Conc.	Fluorinated anhydrides (HFBA, PFPA, TFAA), HFBI, PFBCl	DMF Dialkylacetals, PFB-Br/TBA-H-SO_4, TBH
Amines: primary and secondary	BSTFA, MTBSTFA/*t*-BDMCS	Fluorinated anhydrides (HFBA, PFPA, TFAA), HFBI, MBTFA, PFBCl, TPC*	DMF Dialkylacetals, TBH
Amides	BSA, BSTFA, BSTFA/TMCS, Deriva-Sil Conc.	HFBI	DMF Dialkylacetals,
Amino Acids	BSTFA, TMSI	HFBI (+ Silylation)	DMF Dialkylacetals, TBH 3N HCl in n-Butanol
Catecholamines	TMSI	Fluorinated anhydrides (HFBA, PFPA, TFAA), HFBI	
Carbohydrates and Sugars	HMDS, Hydrox-Sil AQ, TMSI	MBTFA	
Inorganic Anions	BSTFA, MTBSTFA		
Nitrosamines		HFBA	
Sulfonamides	BSTFA	Fluorinated anhydrides (HFBA, PFPA, TFAA)	DMF Dialkylacetals, PFB-Br/TBA-H-SO_4

Derivatization reagents are listed in alphabetical order, not in order of preference.

*For Chiral Analysis

Source: Knapp, D.R. Handbook of Analytical Derivatization Reactions; John Wiley and Sons: New York, 1979.

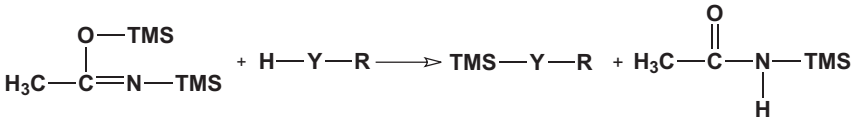
Silyation Reagents

BSA

N,O-Bis(trimethylsilyl)acetamide

- Forms highly stable TMS derivatives with most organic functional groups under mild reaction conditions.

Product	Size	Catalog #
BSA	10 x 1 gram	270501
	4 x 5 gram	270502
	25 gram	270503
	100 gram	270504



TMS= $Si(CH_3)_3$

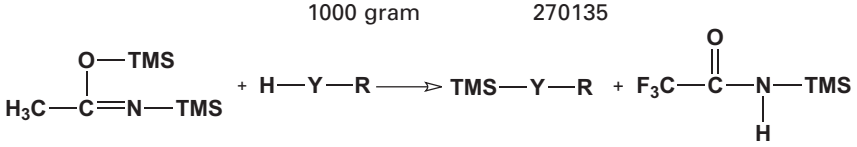
Y = O, S, NH, NR^1, COO
R, R^1 = Alk, Ar

BSTFA-Regisil®
BSTFA +TMCS (1%, 10%)

N,O-Bis(trimethylsilyl)trifluoroacetamide

- Reacts faster and more completely than BSA due to presence of trifluoroacetyl group.
- The high volatility of BSTFA and its byproducts results in separation of early eluting peaks.
- Highly volatile and stable products result in low detector noise and fouling.
- Excellent solubility.
- Addition of TMCS catalyzes reactions of hindered functional groups in secondary alcohols and amines.

Product	Size	Catalog #
Regisil® RC-1 BSTFA	10 x 1 gram	270111
	4 x 5 gram	270112
	25 gram	270113
	100 gram	270114
	1000 gram	270116
Regisil® RC-2 BSTFA +1% TMCS	10 x 1 gram	270121
	4 x 5 gram	270122
	25 gram	270123
	100 gram	270124
	1000 gram	270126
Regisil® RC-3 BSTFA +10% TMCS	10 x 1 gram	270131
	4 x 5 gram	270132
	25 gram	270133
	100 gram	270134
	1000 gram	270135



TMS= $Si(CH_3)_3$

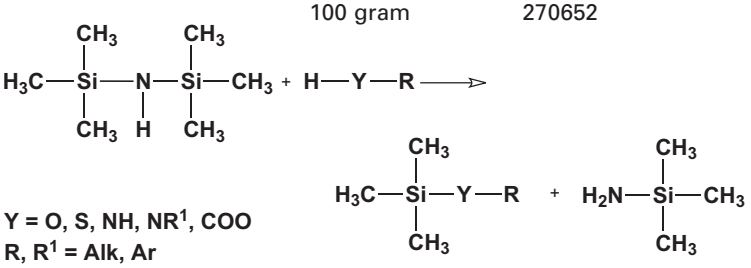
Y = O, S, NH, NR^1, COO
R, R^1 = Alk, Ar

HMDS

Hexamethyldisilazane

- Weak TMS donor, used for silylation of carbohydrates.
- Used as mixture with pyridine and trifluoroacetic acid.

Product	Size	Catalog #
HMDS	25 gram	270651
	100 gram	270652



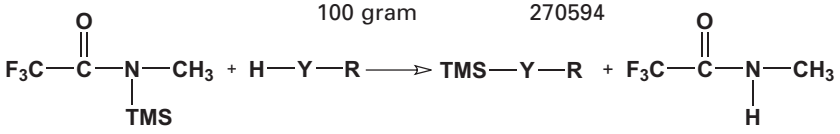
Y = O, S, NH, NR^1, COO
R, R^1 = Alk, Ar

MSTFA

N-Methyltrimethylsilyltrifluoroacetamide

- Most volatile of the TMS-acetamides.
- Useful in the analysis of volatile trace materials.

Product	Size	Catalog #
MSTFA	10 x 1 gram	270590
	10 gram	270589
	25 gram	270593
	100 gram	270594



TMS= $Si(CH_3)_3$

Y = O, S, NH, NR^1, COO
R, R^1 = Alk, Ar

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Silyation Reagents

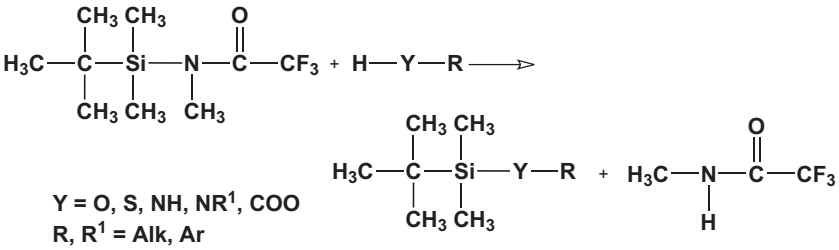
MTBSTFA

MTBSTFA + 1% t -BDMCS

N-Methyl-N-(t-butyldimethylsilyl) trifluoroacetamide

- Replaces active hydrogens to form *t*-BDMS derivatives.
- Derivatization is usually complete upon dissolution with this exceptionally strong, yet mild silylating reagent.
- MTBSTFA derivatives are 104 times more stable to hydrolysis than their corresponding TMS derivatives.
- Produces easily interpreted mass spectra for GC/MS.
- Addition of *t*-BDMCS catalyzes reactions of hindered alcohols and amines.

Product	Size	Catalog #
MTBSTFA + 1% t-BDMCS	5 x 1 gram	270141
	10 x 1 gram	270144
	2 x 5 gram	270142
	25 gram	270143
MTBSTFA no t-BDMCS	5 x 1 gram	270241
	2 x 5 gram	270242
	25 gram	270243



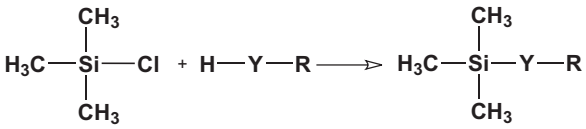
Y = O, S, NH, NR^1, COO
R, R^1 = Alk, Ar

TMCS

Trimethylchlorosilane

- Used as a catalyst to increase reactivity of other silylation reagents.

Product	Size	Catalog #
TMCS	25 gram	270601
	100 gram	270602



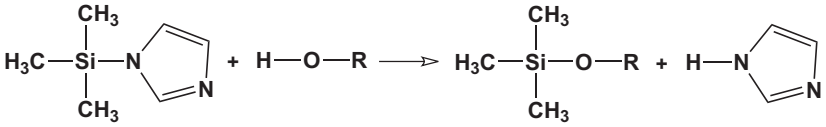
Y = O, S, NH, NR^1
R, R^1 = Alk, Ar

TMSI

Trimethylsilylimidazole

- Potent, selective TMS donor that reacts with alcohols and phenols but not amines or amides.
- Derivatizes wet sugar samples, hindered hydroxyl groups in steroids, and amino acids in fluorinated acylation reagents.
- Used in the preparation of dual perfluoroacyl and TMS derivatives.

Product	Size	Catalog #
TMSI	10 x 1 gram	270401
	5 gram	270402
	25 gram	270403



R, R^1 = Alk, Ar

Silyation Formulations

	Product	Size	Catalog #
Deriva-Sil • BSTFA:TMCS:TMSI:Pyridine (3:2:3:10) formulation. • Derivatizes sterically-hindered compounds. • Reacts with carbohydrates, hydroxy- and keto-steroids, fatty acids, and some amines and amides. • Derivatizations are complete in minutes.	Deriva-Sil BSTFA:TMCS: TMSI:Pyridine (3:2:3:10)	10 x 1 ml 25 ml	270151 270152
Deriva-Sil Concentrate • BSTFA:TMCS:TMSI (3:2:3) concentrate formulation. • Used for applications where pyridine is undesirable (i.e., 3-ketosteroids).	Deriva-Sil Concentrate BSTFA:TMCS:TMSI (3:2:3)	25 ml	270150
Hydrox-Sil • HMDS:TMCS:Pyridine (2:1:10) formulation for one-step derivatizations. • Fast formation of the TMS derivatives of organic acids, unhindered alcohols and phenols, and some amines.	Hydrox-Sil Reagent HMDS:TMCS:Pyridine (2:1:10)	25 ml	270457
Hydrox-Sil Concentrate • HMDS:TMCS (2:1) concentrate formulation. • Suited for applications where pyridine in Hydrox-Sil is undesirable.	Hydrox-Sil Concentrate HMDS:TMCS: (2:1)	25 ml	270458

Derivatization Grade Solvents

Derivatization Grade Solvents • High purity reagents packaged under nitrogen. • Sealed with Teflon®-coated septa, allowing easy access to sample without exposure to moisture and oxygen.	Acetonitrile	2 x 25 ml	270010
	Pyridine	2 x 25 ml	270013

Acylation Reagents

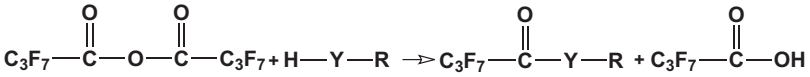
Fluorinated Anhydrides: HFBA/PFPA/TFAA

Heptafluorobutyric Anhydride/ Pentafluoropropionic Anhydride/Trifluoroacetic Anhydride

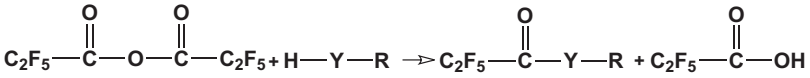
- Most commonly used for ECD.
- Reacts with alcohols, amines, and phenols.
- Bases such as triethylamine and trimethylamine can be added to promote reactivity.
- Frequently used for the confirmation of drugs of abuse.
- HFBA derivatives are the most sensitive to ECD.
- PFPA derivatives require the lowest analysis temperatures.
- TFAA is the most reactive and volatile of the anhydrides.

Product	Size	Catalog #
HFBA	10 x 1 gram	270851
	25 gram	270853
PFPA	10 x 1 gram	640110
	25 gram	640113
	100 gram	640114
TFAA	10 x 1 gram	270841
	25 gram	270843

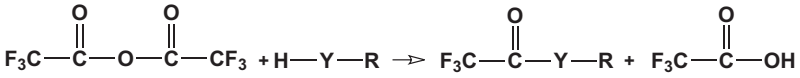
HFBA



PFPA



TFAA



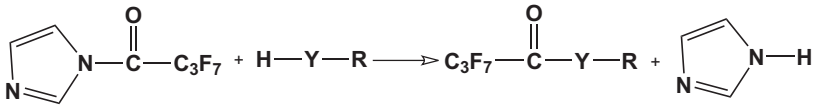
Y = O, NH, NR^1
R, R^1 = Alk, Ar

HFBI

Heptafluorobutyrylimidazole

- Readily forms derivatives with phenols, alcohols and amines suitable for ECD.
- Reactions are fast and mild.
- Imidazole is not acidic, so no decomposition or corrosion occurs on columns.

Product	Size	Catalog #
HFBI	5 x 1 gram	270611
	5 gram	270612



Y = O, S, NH, NR^1
R, R^1 = Alk, Ar

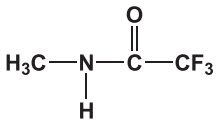
MBTFA

N-Methyl-N-bis(trifluoroacetamide)

- Reacts rapidly under mild conditions with primary and secondary amines.
- Reacts more slowly with alcohols, phenols, and thiols.
- Works well in the analysis of sugars.

Product	Size	Catalog #
MBTFA	10 x1 gram	270092
	5 gram	270091
	25 gram	270095
	100 gram	270093

Y = O, S, NH, NR^1
R, R^1 = Alk, Ar

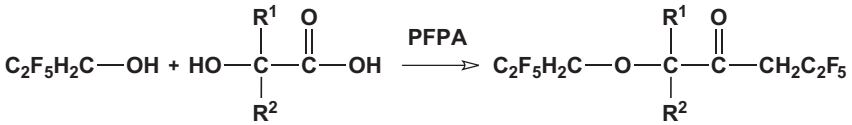


PFPOH

2,2,3,3,3-Pentafluoropropanol

- Used in combination with PFPA to make derivatives of the most common functional groups, especially polyfunctional bio-organic compounds.
- Formed derivatives are highly suitable for ECD.

Product	Size	Catalog #
PFPOH	5 gram	270815
	25 gram	270816



R^1, R^2 = H, Alk, Ar

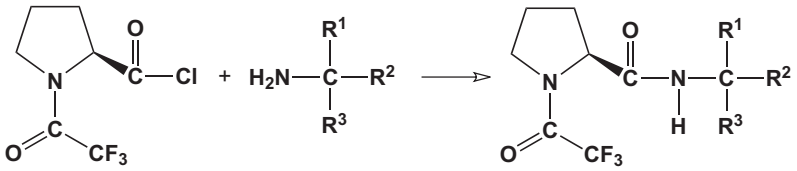
GC Chiral and Specialty Derivatization Reagents

TPC

N-Trifluoroacetyl-L-Prolyl Chloride

- Couples with amines to form diastereomers which can be separated on GC columns.
- Provides sample volatility.
- Used for confirmation of drugs of abuse testing.

Product	Size	Catalog #
TPC	25 gram	440001



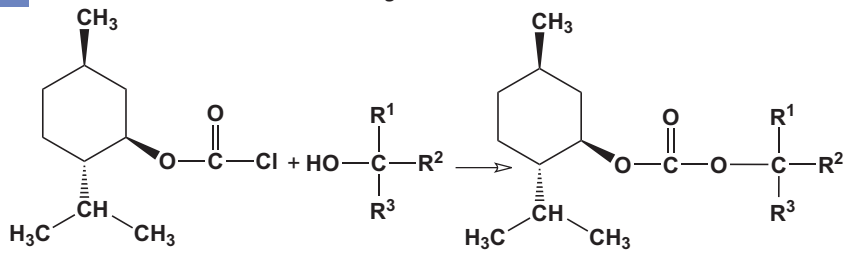
R^1, R^2, R^3 = H, Alk, Ar

MCF

(1R, 2S, 5R)-(-)-Menthylchloroformate

- Resolves enantioenriched alcohols.

Product	Size	Catalog #
MCF	25 gram	440003



R^1, R^2, R^3 = H, Alk, Ar

HFIP

1,1,1,3,3,3-Hexafluoro-2-Propanol

- Esterification reagent for the determination of aromatic acids in tissue by GC and electron capture detection.

Product	Size	Catalog #
HFIP	25 gram	270702
	100 gram	270704

(R)-(-)-MTPA-Cl

Mosher's acid chloride
(R)-(-)-α-(trifluoromethyl) phenylacetyl chloride

- (R)-(-)-MTPA-Cl is a ready to use reagent for the determination of enantiomeric purity of alcohols and amines.

Product	Size	Catalog #
(R)-(-)-MTPA-Cl Mosher's acid chloride	100 mg	270900
	500 mg	270901
	1000 mg	270902

Teflon is a registered trademark of DuPont Company.

GC Chiral and Specialty Derivatization Reagents

3.0 N HCL in n-Butanol

- Most commonly used for rapid diagnosis of neonatal blood spots by Tandem Mass Spectrometry.

Product	Size	Catalog #
3.0N HCl in n-Butanol	4 x 25 ml	201007
	100 ml	201009
	500 ml	201010

3N HCl in n-Butanol is a derivatization reagent required for newborn screening for metabolic disorders. Neonatal screening, which has become a standard procedure in many countries, measures amino acids and acylcarnitines from a single drop of blood. Blood concentration of one or several of these compounds is either abnormally high or low in a variety of metabolic disorders in newborns. Derivatization with 3N HCl in n-Butanol ensures butylation of the carboxyl acid group of the analyte and formation of butyl ester, which forces ionization or makes charging of the analytes more efficient. Although direct analysis of extracted acycarnitine without derivatization is possible, according to different reports, butylesterification is superior with regard to sensitivity and specificity. Methods that include derivatization with 3N HCl in n-Butanol is the only validated procedure at this time.

Many factors contribute to the success of a newborn screening process. Any impurities in derivatization reagent can potentially interfere with the analysis. 3N HCl in n-Butanol from Regis Technologies is manufactured under cGMP protocols to assure highest quality and lot-to lot consistency for this reagent. Each lot is tested by tandem mass spectrometry to ensure absence of contaminants which may interfere with analysis. Our Quality Assurance department reviews and approves all production documentation and test results. Regis takes necessary precautions that assure the quality of our 3N HCl in n-Butanol.

Notes

Please note: Certain GC Derivatization products are hazardous chemicals and additional shipping and handling charges could apply.

RegisSEP™ GMP SFC Separations Services

Supercritical Fluid Chromatography

Regis Technologies has added Supercritical Fluid Chromatography (SFC) to its GMP-approved chiral separations services, offering pharmaceutical and other industries an expanded separation service that uses a superior technique with cost and time efficiencies.

Superior Method

Supercritical Fluid Chromatography (SFC) is now gaining increased acceptance the method-of-choice for the analysis and purification chiral separations. Although a well-established method for over 20 years, renewed interest emerged with the recent introduction of high throughput SFC systems required for pharmaceutical applications. With the method's ability to accommodate flexible solvent conditions, SFC provides a superior chiral purification method compared to traditional HPLC.

Cost Efficient

Several factors combine to reduce the cost of SFC separations without sacrificing purity, including:

- Faster run times
- Flexible solvent conditions
- Lower solvent use as SFC typically utilizes carbon dioxide as the mobile phase
- Reduced waste for disposal
- Less ancillary equipment required

Complete SFC Service

Regis Technologies now offers RegisSEP™ SFC Separations Services. This valuable addition to our chromatographic separations group delivers benefits that no other separation method can provide. Turn to our expert staff to help solve your separations problems. Experience excellence in project management all you separations projects. Expand your capabilities overnight with our GMP compliant service that can tackle projects for the scale you need—from milligram- to kilo-scale.

RegisSEP™ GMP SFC Separation Services

Leverage the Added Value from Regis Technologies

• Reliability and Experience

Regis Technologies has been serving the pharmaceutical, biotechnology, and related industries for over 50 years. As a leader in separations services, Regis has been involved in chiral and achiral separations for over 25 years and supplies specialty chiral columns and other chromatography products. The company's decades of experience are unmatched in the field.

• GMP Compliant Organization

Regis Technology has been a fully compliant GMP organization since 1993. We manufacture APIs under GMP and are regularly inspected by the FDA. In this manner, we become an FDA approved extension of your manufacturing facility.

• Production Capabilities

Regis' Production department works hand-in-hand with the separations group to provide GMP productions support for the many separation projects included in your synthesis. Regis' Production department is headed by a 23-year industry veteran. Production equipment ranges from small glassware to kilogram suites to reactors ranging from 25 to 500 gallons. Regis production also utilizes large glass gravity chromatography columns with a total capacity of up to 175 kg of silica.

• Bulk Phases

Cut lead time and expense. As a manufacturer of Chiral Stationary Phases, we inventory our bulk material so it is readily available for your separation projects.

• High-Throughput SFC Systems

Regis Technologies uses Thar SFC Method Station with Regis Technologies columns. Regis chose the Thar system for its quality, and because of their ease of validation.

• Supportive Departmental Services

Your project will benefit from Regis' extensive organization including QA, Analytical Method Development, QC, Stability, and Project Management. These departments all contribute according to the needs of the project and of the customer.

RegisSEP™ GMP SFC Separation Services

Screening & Assessment Process

- Execute a CDA (1 day)
- Perform screening of the compound
- Free screening using Regis phases

Fee for screening using other phases

Report of findings is transferred to the customer

- A critical assessment of the data

is performed by Regis and presented to the customer to determine a plan of action for the compound.

• Fast Project Execution

Our experience and proven processes translate into efficient project executions, thereby reducing your time to market.

• Screening & Assessment Process

Regis' formal screening and critical assessment process is quick and easy, and ensures agreement for best results. For the initial screen, our experienced chromatographers use the wealth of their experience to select the best column performers of more than 50 available for your separations. This initial phase is most critical, enabling the project to move forward.

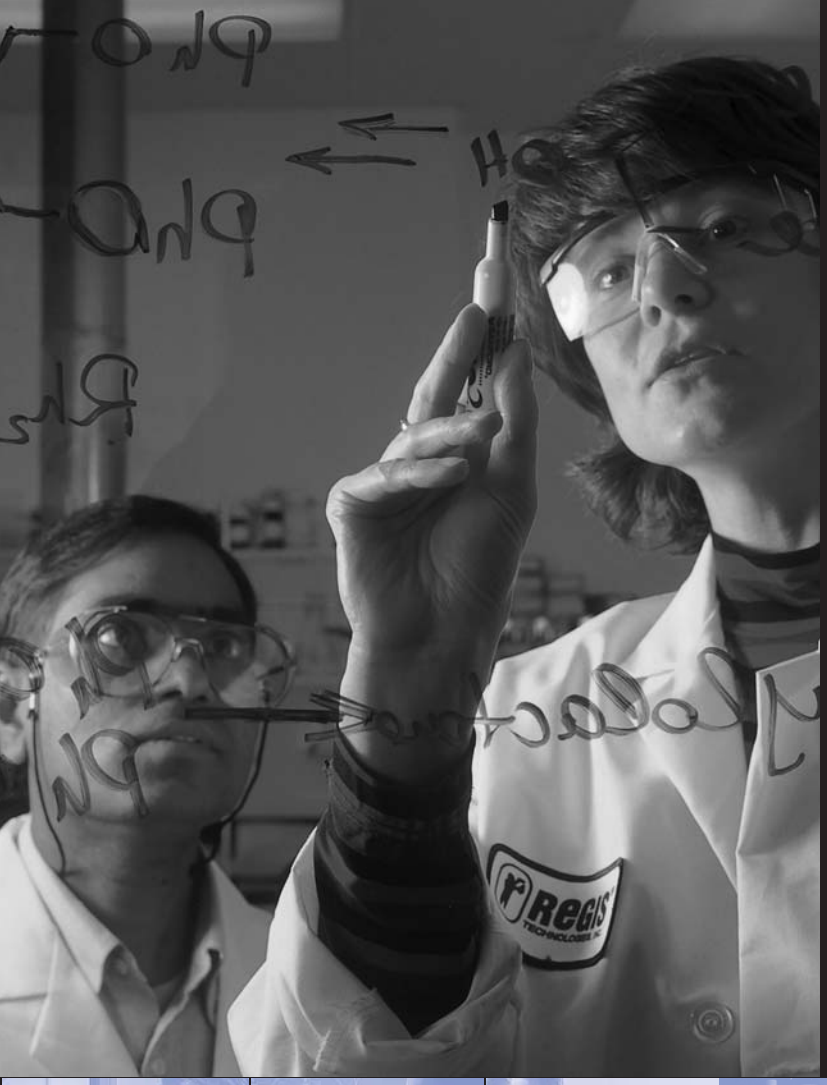
Regis continues to offer confidential free chiral screening services using its proprietary phases on both LC and SFC units—your first and only stop in determining which stationary phase meets your compound's needs.

• Scaling Up Process

Our GMP compliant service can tackle projects for the scale you need—from milligram- to kilo-scale. Regis Technologies formal process enables us to provide you with accurate quotes and yields, and shows the basic steps for how we progress from small-scale analytical to kilogram preparative separations.

This process enables us to provide you with accurate quotes and yields, and shows the basic steps for how we progress from small-scale analytical to kilogram preparative separations.

Small-Scale Separations	10-100g Separations	Kilogram Separations
Perform a solubility and loading study	Separations available on a GMP or non-GMP basis	Separations available on a GMP or non-GMP basis
Isolate desired enantiomers	Confirm purity by HPLC	Calculate machine time and raw materials
Confirm purity by HPLC	Provide a detailed separations report	Pack or procure the required columns
Provide a detailed separations report	Estimate costs for kilogram scale	Confirm purity by HPLC
Estimate costs for 10-100g scale		Provide a detailed separations report



GMP Manufacturing
Custom Synthesis

Custom Synthesis

The Chemistry of Partnership

Regis Technologies has served the pharmaceutical community for over 50 years. Partner with Regis to bring your regulatory starting materials, pre-clinical, phase I-III and commercial API to market.

Development Chemistry

Begin your synthesis in our newly constructed non-GMP development labs. For non-GMP development, we synthesize at scales from less than one gram, to 12L.

Scale up and GMP production

Regis production staff has scaled up hundreds of compounds from less than a gram to the multi kilo level and up to the metric ton scale. Regis will work with you as the project scales up from 50L flasks up to 2000L reactors in individual suites. Regis has manufactured high value intermediates and API's under GMP for over 14 years.

Analytical Method Development Group

Experienced scientists–skilled in the transference, development and validation of analytical methods–work together to meet our customers' complex analytical needs.

Quality Control

Regis maintains a modern, in-house QC department responsible for the release of raw materials, in process and final release testing. Regis offers additional QC services including fully compliant ICH stability studies.

Quality Assurance

Regis maintains a GMP compliant organization with a fully staffed, two shift Quality Assurance Division. Company wide GMP training is fundamental to a compliant facility therefore all Regis employees undergo GMP training. Regis' positive track record with the FDA and other foreign regulatory agencies assures customers that their manufacturing will be compliant with regulations. Regis welcomes your audit of our quality systems.

Project Management

Full time project managers, all with bench and analytical chemistry experience, act as the customer's internal champion steering projects through Regis in an efficient, professional manner.

Index and Order Information

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Listed Alphabetically

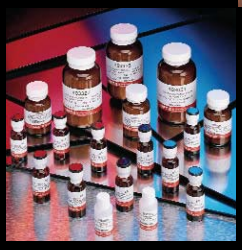
REGIS®
TECHNOLOGIES, INC.











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- www.greyhoundchrom.com

REGIS® TECHNOLOGIES, INC.

About Regis Technologies, Inc.

Regis Technologies, Inc. is a privately held company that provides synthesis and separations services to the pharmaceutical, biotechnology and other related industries. Regis provides innovative chromatography products and services, especially those with a chiral emphasis, through the utilization of our extensive organic expertise and collegiate collaborations.

Serving the Scientific Community Since 1956