



▶ **RAM**
Chromatography

RAM Direct Injection

(Restricted Access Media)

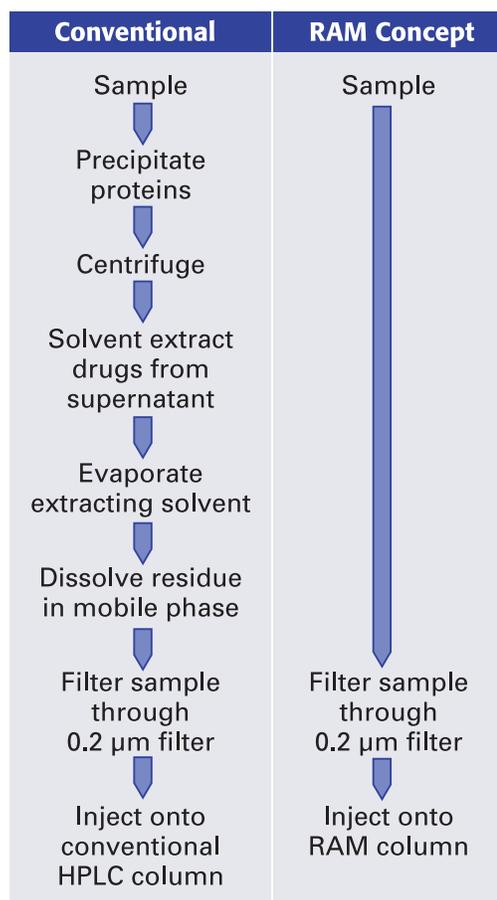


Figure 1. RAM Direct Injection eliminates the lengthy pretreatment steps needed in conventional methods.

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.

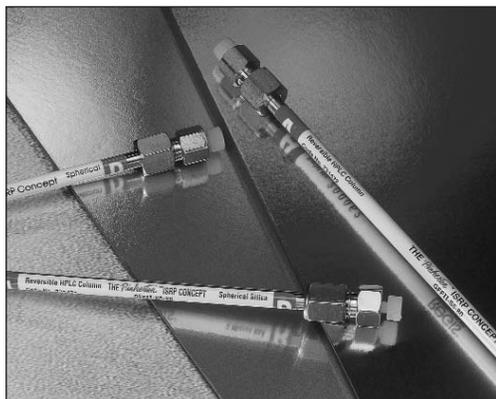
RAM Direct Injection Advantages

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

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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



(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.

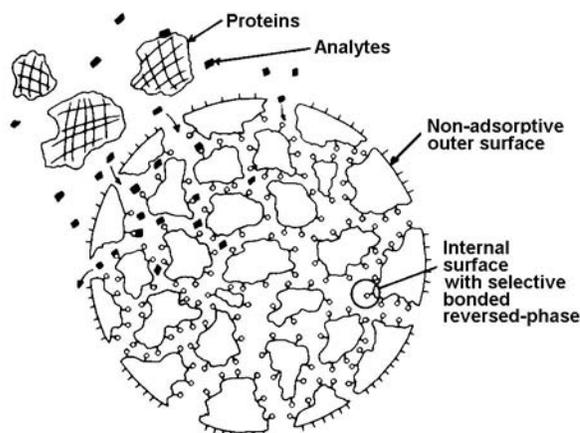
Temperature:

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 \AA	5 cm x 2.1 mm i.d.	731469
GFF II	5 μm , 80 \AA	5 cm x 4.6 mm i.d.	731470
GFF II	5 μm , 80 \AA	15 cm x 4.6 mm i.d.	731471
GFF II	5 μm , 80 \AA	25 cm x 4.6 mm i.d.	731472
GFF II Guard Kit*	5 μm , 80 \AA	1 cm x 3.0 mm i.d.	731475
GFF II Guard Cartridges**	5 μm , 80 \AA	1 cm x 3.0 mm i.d.	731474

* Includes 1 holder and 2 guard cartridges

** Includes 3 guard cartridges



Rigid porous hydrophilic particle

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

(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.

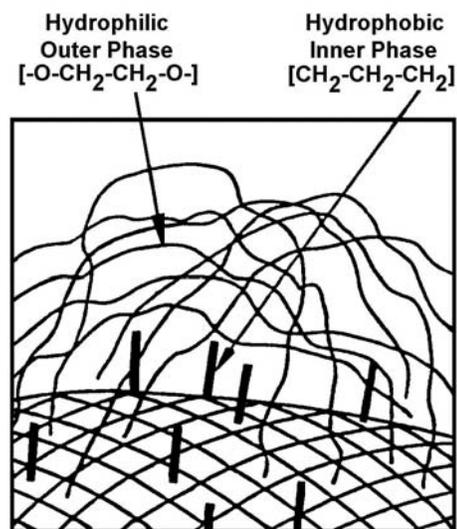


Figure 3. SPS structure highlighting the inner and outer phases.

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

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

* Includes 1 holder and 2 guard cartridges

** Includes 3 guard cartridges

(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.



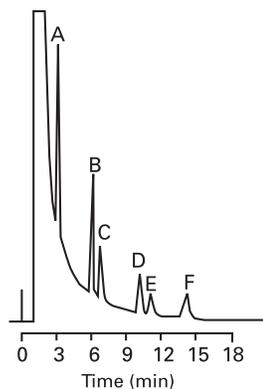
RAM Direct Injection Applications

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 μ m 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

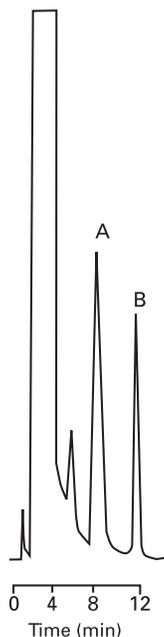
Load: 10 μ L

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 μ m 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 μ L

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 to 100 samples. The RAM guard column is an inexpensive 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.

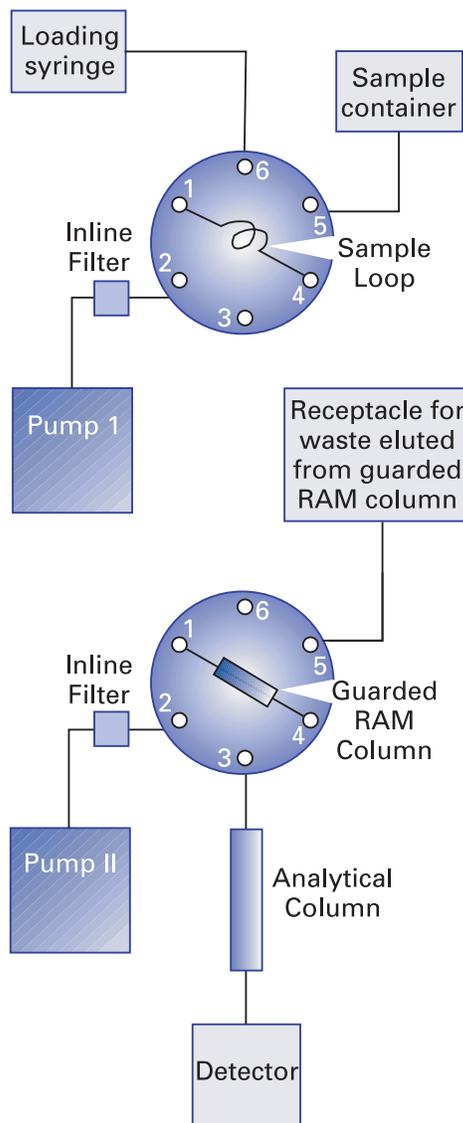


Figure 4. Column switching system.

To request a copy of the RAM Direct Injection Application Guide containing additional RAM Applications contact us by phone,

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847.967.6000 ext. 662

or e-mail,

sales@registech.com