

# RAM CHROMATOGRAPHY



## RAM DIRECT INJECTION (RESTRICTED ACCESS MEDIA)

### 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). With RAM Direct Injection HPLC columns, a variety of complex sample matrices can be injected directly, without prior sample clean-up, and drugs, drug metabolites, peptides and other analytes can be separated and detected.

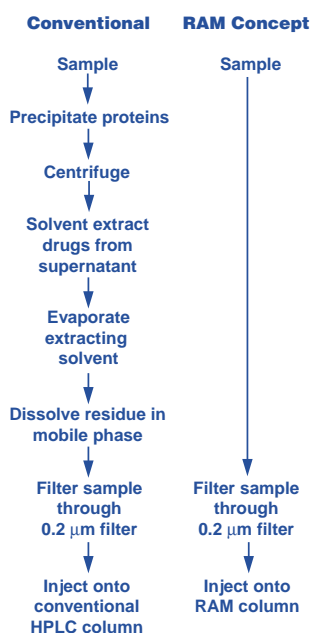
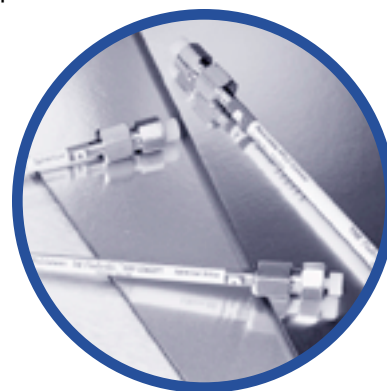


Figure 1. RAM Direct Injection eliminates the lengthy pretreatment steps needed in conventional methods.

### RAM Direct Injection Advantages

RAM Direct Injection technology offers the following benefits:

- **Elimination of 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.
- **Compatibility with automated sample processing**  
Simplified sample preparation and use of HPLC columns allows the use of automated systems.
- **Reduction of 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.

- **Reduction of 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 Phases**

Porous silica supports are characterized as consisting 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 majority of the silica's surface area is in the pores.



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.

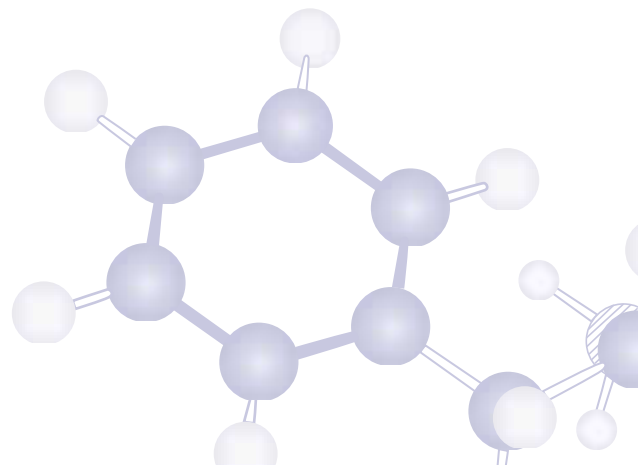
There are two RAM Direct Injection Technologies:

### **ISRP (Internal Surface Reversed Phase)**

- GFF
- GFF II

### **SPS (Semi-Permeable Surface)**

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







## 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

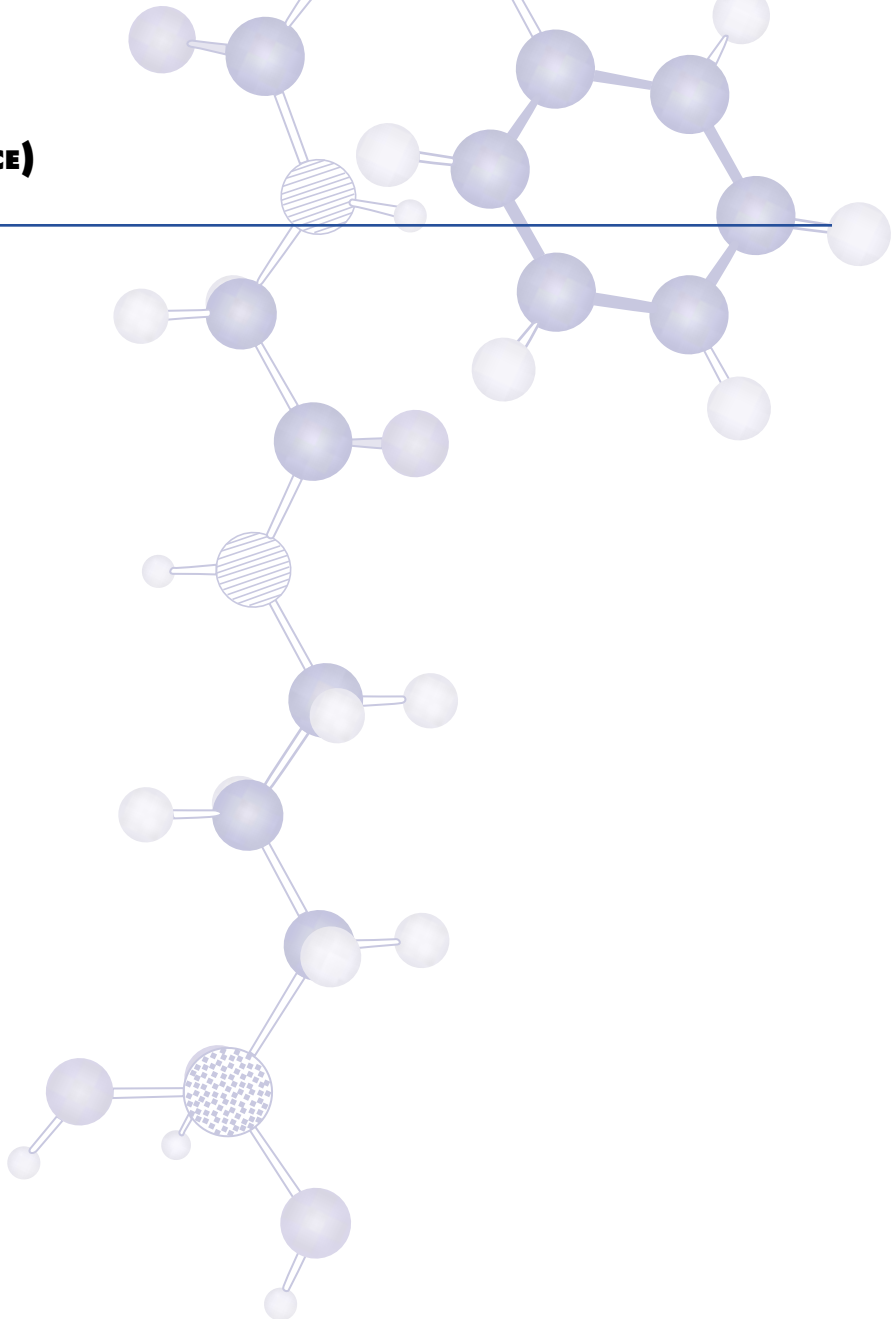
### SPS Selectivity

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

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

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

The SPS phases allow use of buffered, normal-phase, and reversed-phase eluents. The actual composition is limited only by the pH and organic modifier parameters dictated by the proteins 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/](http://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

Product information and applications are available online at:  
[www.registech.com/ram/](http://www.registech.com/ram/).

## RAM DIRECT INJECTION APPLICATION/ISRP

### Separation of Barbiturates in Human Serum

**Column:** ISRP GFF II, 5  $\mu\text{m}$ , 80Å  
15 cm x 4.6 mm i.d.

**Mobile Phase:** (95/5) 0.1 M potassium phosphate buffer, pH 7.5/  
methanol

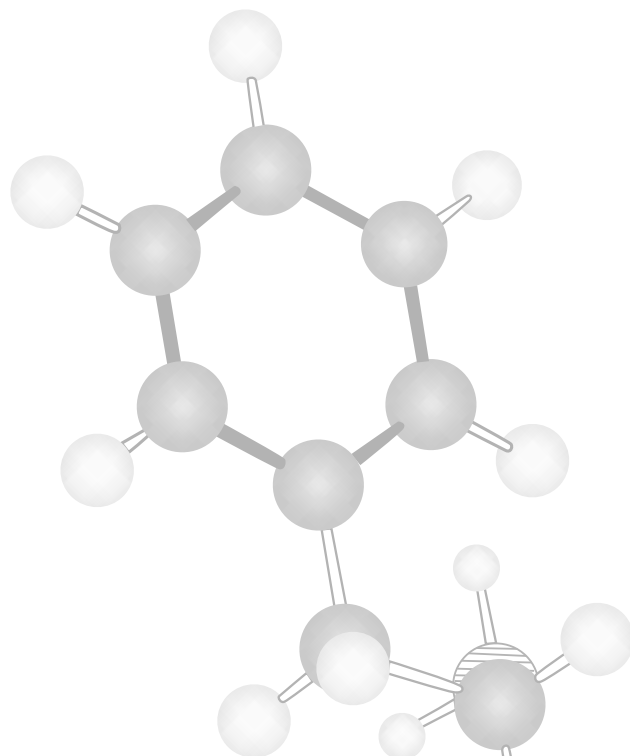
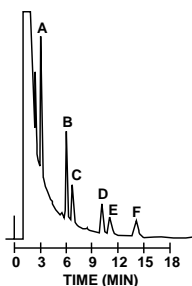
**Flow Rate:** 1.0 mL/min

**Load:** 10  $\mu\text{L}$

**Detection:** UV 240 nm

**Sample Composition in Human Serum:**

- A. Barbitital
- B. Phenobarbital
- C. Butobarbital
- D. Amobarbital
- E. Pentobarbital
- F. Secobarbital



## RAM DIRECT INJECTION APPLICATION/SPS

### Determination of Antipyrine and Acetaminophen

**Column:** SPS C8, 5  $\mu\text{m}$ , 100Å  
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

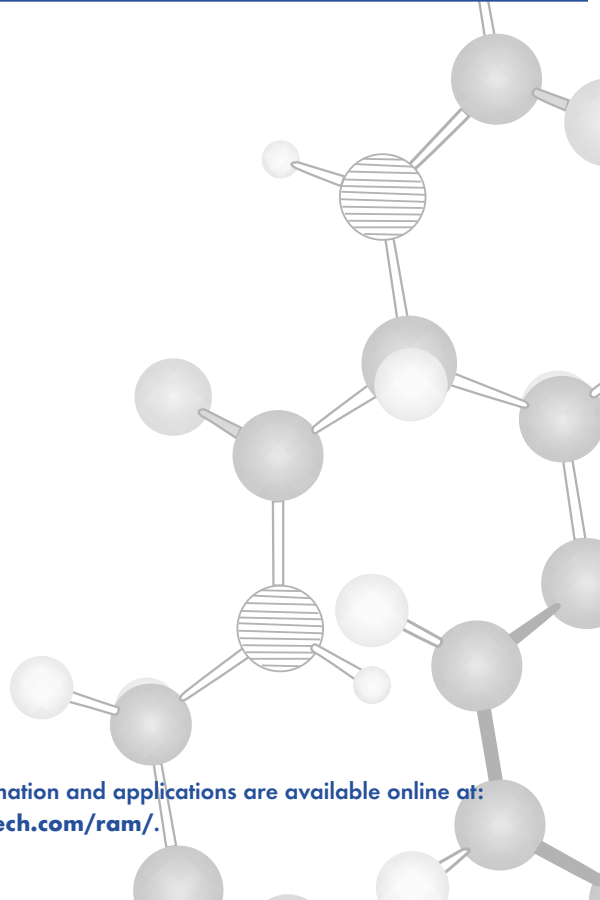
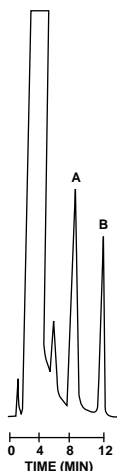
**Load:** 25  $\mu\text{L}$

**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.



Product information and applications are available online at:  
[www.registech.com/ram/](http://www.registech.com/ram/).

# RAM DIRECT INJECTION APPLICATIONS/COLUMN SWITCHING

## Column Switching with RAM Columns

### Column Switching 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.

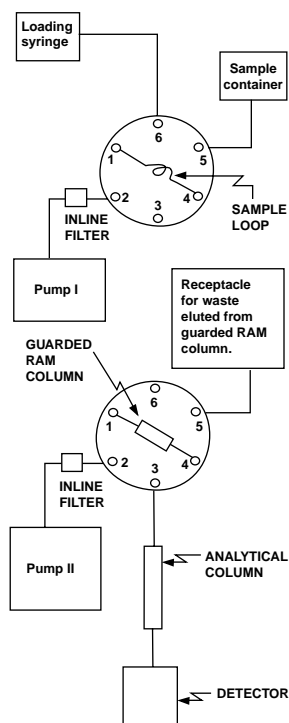


Figure 4. Column switching system.

For additional information on RAM Direct Injection, check our Web site at [www.registech.com/ram/](http://www.registech.com/ram/). You may also request a copy of the RAM Direct Injection Application Guide containing additional RAM applications, by contacting Regis at:

(800) 323-8144 ext. 649

(847) 967-6000 ext. 649

e-mail us at: [sales@registech.com](mailto:sales@registech.com).