

ANALYTICAL (4.6 and 4.0mm i.d.) Columns

Traditionally columns of either 4.6 or 4.0 mm internal diameter (i.d.) and 150 or 250 mm length have been used as the industry's standard for analytical applications. Such columns are still the most popular although there is an on-going shift to shorter and narrower i.d. columns.

Silica

Silica is the most popular base material for HPLC phases. Despite its necessary porosity it has a high physical strength and a surface to which a broad range of ligands can be attached using well established silanization technology. The majority of separations are performed using these bonded materials under reversed-phase conditions.

Today most silicas are spherical in shape. They are easier to pack into columns than irregular silicas. High performance, stable and low back pressure columns can be reproducibly achieved.

Organic polymer, alumina and titanium phases are also available from Life Science.

Guard Cartridge Columns

The HI-161 universal guard cartridge system is recommended for use with analytical columns (p.22,23).

Optimum Flow

A flow rate of 0.76 ml/min through a 4.0 mm i.d. column gives the same relative flow rate as 1 ml/min through a 4.6 mm i.d. column.

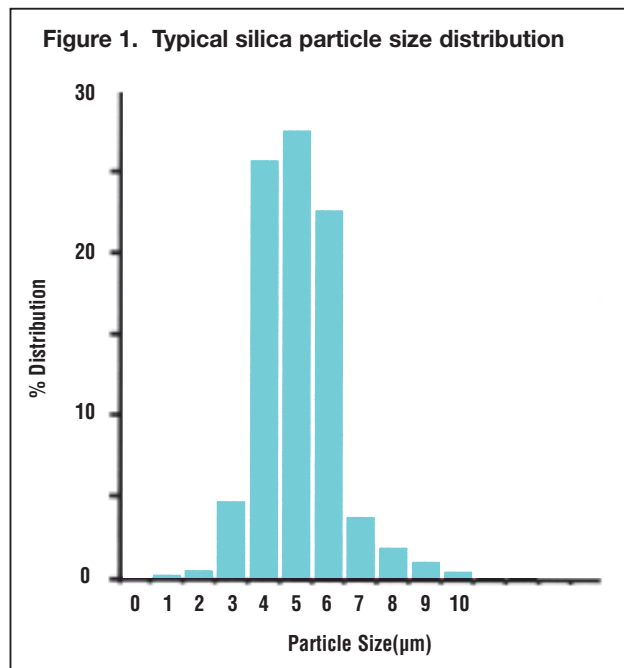


Please contact
Canadian Life Science
for application and column
selection advice

Performance

The efficiency of a column depends on the choice of particle size and column length. The mode and quality of manufacture will also affect its performance.

Particle size refers to the average diameter of the spherical silica particle. Commonly used silicas have a distribution of diameters. Hence a material of nominal particle size 5 μ m can typically contain silica particles between 4.0 and 6.5 μ m diameter (Figure 1).



Smaller particles give higher efficiencies for constant column length. However at the same time column back pressure increases significantly resulting in an effective maximum column length.

The pore size of a silica particle determines the retentivity and capacity characteristics.

Silica Phases

Silica Particle Size (μ m)	Maximum Recommended Column Length (mm)	Plate Efficiency (plates/metre)	Use of Columns
3	150	120 - 200,000	Fast analysis without loss of efficiency or sacrificing column life.
5	250	80 - 120,000	Routine analysis requiring a high separation performance under moderate operating pressure.
10	>250	40 - 60,000	Moderate efficiency at low operating pressures. Routine Q.C. labs or as scout column for scaling-up methods.
>10	>250	<40,000	Primarily for preparative applications.

MEDIUM BORE (3.2mm i.d.) Columns

- Solvent consumption reduced by 50%
- Sensitivity increased up to x2
- Uses standard analytical HPLC equipment

Initial interest in the use of analytical columns of narrower bore than the industry standard 4.6 mm arose for scientific reasons. The ability to couple HPLC with techniques capable of providing characterization data of the solute molecules necessitated the use of lower solvent volumes. Increased assay sensitivity and the need for less sample were added features.

The limited general availability of low dispersion volume equipment and the greater difficulties of manufacturing narrow bore HPLC columns have restricted developments. However the cost and environmental issues associated with the purchase and disposal of solvents is becoming increasingly important.

Consequently the utility of the intermediate 3.2 mm i.d. column is now being strongly recommended by a number of organizations. The choice represents a compromise between using standard 4.6 mm i.d. columns and microbore columns of 2.1 mm i.d. or less.

The 3.2 mm i.d. columns offer a 50% saving in solvent consumption for the same linear dynamic flow without necessarily requiring a change to lower dispersion volume injectors and flow cells. For satisfactory use of 2.1 mm or lower i.d. columns such a change is essential. When a 3.2 mm i.d. column is used with a Rheodyne valve injector model 7125 or 7725 and an 8 μ l flow cell some loss in performance is observed (Table 1). The loss decreases with increase in retention time.

In practice chromatograms obtained from 3.2 and 4.6 mm i.d. columns are very similar in appearance (Figure 1).

Guard cartridge columns

The HI-161 universal guard cartridge system is recommended for use with medium bore columns (p.23).

- Significant cost-saving
- Performance comparable to standard 4.6 mm i.d. columns

Equipment

A Rheodyne 7125 or 7725 valve injector and an 8 μ l flow cell can be used with 3.2 mm i.d. columns containing 5 μ m particles. For critical separations a Rheodyne 8125 valve injector and a micro flow detector cell (<5 μ l) are recommended.

The loss in efficiency of biphenyl and phenanthrene peaks at various retention times in going from 4.6 to 3.2 mm i.d. columns using the above standard equipment is shown below (Table 1).

Table 1

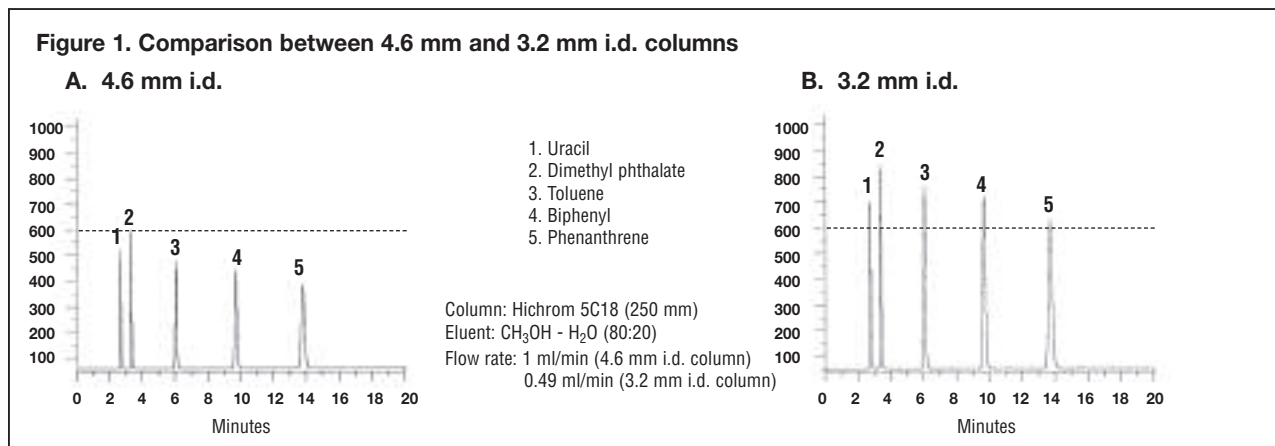
Biphenyl	k'	0.93	2.47	7.09
	Efficiency Loss (%)	39	18	8
Phenanthrene	k'	1.23	3.32	10.12
	Efficiency Loss (%)	32	14	7

Optimum flow

A flow rate of 0.48 ml/min through a 3.2 mm i.d. column gives the same relative flow rate as 1 ml/min through a 4.6 mm i.d. column. The optimum flow rate for the medium bore column will be < 0.4 ml/min.

Sample size

Existing methods can be readily adapted for use with 3.2 mm columns at the appropriate flow rate. However as an effective increase in detector sensitivity will be observed, care should be taken not to exceed detector linearity. It may be necessary to dilute samples to lower concentration levels.



MICROBORE (2.1 and 1.0mm i.d.) COLUMNS

- Solvent consumption reduced by 80-95%
- Sensitivity increased x5 to x21
- Significant cost-saving
- LC-MS applications

Microbore columns (1.0 and 2.1 mm i.d. traditionally packed) and capillary columns (<1 mm i.d.) offer significant theoretical advantages over conventional 4.6 mm i.d. columns. However, current instrumental constraints and manufacturing difficulties have limited their application, especially the narrower 1mm internal diameter and capillary columns.

With this in mind, Life Science currently offer a comprehensive range of 1.0 and 2.1 mm i.d. microbore columns. Life Science's microbore columns offer minimal loss of performance compared to the corresponding analytical columns and can be used with optimized conventional equipment.

Column Design

Columns are manufactured from high quality 316 stainless steel tubing. Both column ends are terminated with standard $\frac{1}{4}$ - $\frac{1}{16}$ " female reducing unions. Stainless steel frits inset into a PEEK ring (traditional) or PEEK cap (modular cartridge) are used to retain the packing material.

Column design varies from manufacturer to manufacturer.

Comparison of Microbore and Standard Column Parameters

Column ¹		Relative Flow Rate (mL/min)	Solvent Reduction (%)	Theoretical Sensitivity Increase	Recommended	
I.D. (mm)	Internal Volume (µL)				Flow Cell (µl)	Injection Volume (µl)
4.6	1500	1.0	-	-	8.0	20
4.0	1133	0.76	24	1.3	8.0	15
3.2	725	0.48	52	2	2.0 - 8.0	10
2.1	300	0.20	80	5	1.0 - 2.0	5
1.0	71	0.047	95	21	≤1.0	1

¹ 150 mm length

Column Evaluation

Microbore columns often perform poorly because of excessive system dead volume.

Most HPLC systems in use today are designed for standard bore columns and cannot effectively use columns with internal volumes less than 0.5 ml. The extra column volume measured from the injector through to the detector reduces the achievable efficiency of the column. If the calculated theoretical plate value is less than 90% of that measured by the manufacturer, there is probably an excessive amount of extra column volume in the system for the column being used. Early eluting peaks are less diluted and have smaller volumes than later eluting peaks and will consequently be more susceptible to the detrimental effects of excessive extra column volume.

Table 1 Connection tubing internal volumes

Tubing i.d. (inches)	Volume per 5m length (µl)
0.006 (0.152 mm)	0.9
0.010 (0.254 mm)	2.5
0.020 (0.508 mm)	10.1
0.030 (0.762 mm)	22.8

MICROBORE COLUMNS

If excessive extra column volume is a problem, several measures can be undertaken to improve the performance of the system.

- Use connecting tubing that has an internal diameter of 0.010 inches (0.254 mm) or less. Tubing with an i.d. of 0.007 inches (0.177 mm) or lower is preferable for use with microbore columns (see Table 1, p.14).
- Keep the connecting tubing as short as possible between the injector and the column and the column and the detector. For microbore columns, it is desirable to keep these distances less than 5 cm.
- Use only 'low dead volume' fittings and unions. A fingertight column coupler (throughbore 0.007") HI-081 is recommended to connect guard and microbore columns.
- Make sure that all fittings are undamaged and correctly made.
- Use a low volume detector flow cell.
- Use Rheodyne valve injector model 8125.

Microbore columns normally require detector flow cells with volumes of $2\mu\text{l}$ or less. The design of the flow cell, however, can be just as important as the cell volume. Some low volume flow cells perform worse than larger volume flow cells because of their inadequate design.

Figure 1 provides an example of what can happen when a microbore column is used with a typical HPLC system. The microbore column is unable to achieve the baseline separation (B) provided by the standard bore column (A). However, when the microbore column is used in an 'optimized' HPLC system that has less than $10\mu\text{l}$ of extra column volume, excellent resolution and peak shape is obtained (C).

Most of our manufacturers microbore columns are evaluated using a Rheodyne model 8125 injector fitted with a $5\mu\text{l}$ loop manufactured from special 0.020" i.d. tubing.

A detector of low dispersion volume fitted with a $1\mu\text{l}$ flow cell monitors column performance.

Microbore Guard Cartridge Columns

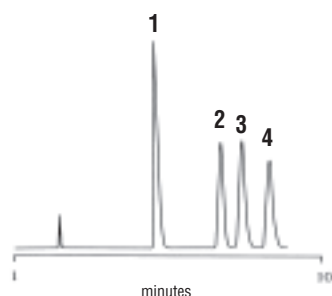
The HI-161 universal guard cartridge system is recommended for use with microbore columns. (p.23)



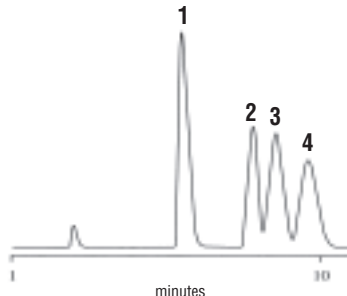
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Figure 1. HPLC systems must be optimized to obtain the best performance from microbore columns

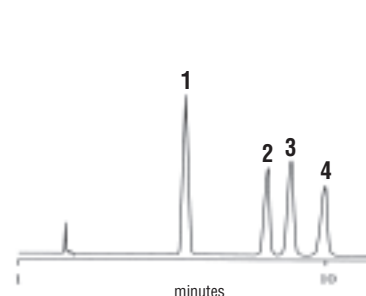
A. Typical HPLC System
Standard Bore Column, 150 x 4.6 mm



B. Typical HPLC System
Microbore Column, 150 x 2.1 mm



C. Optimized HPLC System
Microbore Column, 150 x 2.1 mm



Eluent: 0.025M KH_2PO_4 + 0.2% TFA + 0.2% TEA,
pH 2.5 - CH_3CN (64:36)

Flow rate: 1 ml/min for 150 x 4.6 mm column
0.2 ml/min for 150 x 2.1 mm column

Sample: Tricyclic antidepressants

1. Doxepin 2. Nortriptyline 3. Amitriptyline 4. Trimipramine

CAPILLARY & NANO (<1.0mm i.d.) COLUMNS

- High sensitivity
- Low sample mass and volume applications
- LC-MS and LC-MS/MS applications
- Very low solvent consumption

Capillary and nano LC is gaining acceptance in applications where limited sample amounts lead to problems in detection sensitivity. High sensitivity and high resolution separations can now be achieved for small sample volumes. This is particularly relevant in the areas of pharmacokinetics, trace analysis and particularly the rapidly expanding field of proteomics. Figure 1 shows the analysis of 7.5pmol/ μ l from a tryptic digest of horse skeletal muscle myoglobin.

Capillary columns are ideal for use with detectors requiring the lowest flow rates, such as the electrospray LC-MS detector. Indeed, the on-line coupling with a mass spectrometer has been a major driving force behind the development of capillary chromatography.

Sensitivity

The introduction of capillary columns has made possible high sensitivity and high resolution separations for small sample volumes. Table 1 shows the theoretical sensitivity increase of various i.d. capillary columns compared with a 1mm i.d. microbore column. The use of a 0.075mm (75 μ m) i.d. column, for instance, can decrease detection limits by a factor of >3500 relative to a 4.6mm i.d. column when the same sample size is used, due to lower chromatographic dilution of the sample.

Instrument Modifications

In order to fully exploit the benefits of using capillary dimensions, the HPLC system must be capable of handling sample volumes in the sub-microlitre range. The use of columns of <1mm i.d. requires either a specially designed micro-LC instrument or extensive modifications of a standard HPLC instrument. The major principles in the conversion are flow rate reduction and elimination of dead volume.

Flow rate reduction can be achieved using a high performance, low μ l/minute pump or by incorporating a flow splitting tee between a standard HPLC pump and the injector. The majority of the flow can be split to waste or recycled.

To ensure that band spreading is kept to a minimum, low dispersion column hardware must be used throughout the system. Connecting capillaries must be dead volume free and as short as possible. A micro-scale injector allowing injection of sub-microlitre sample volumes should be used. Micro flow cells with appropriate internal volume (<1 μ l) and path length should be used for UV detection.

Figure 1. Peptide map from tryptic digest of horse skeletal muscle myoglobin

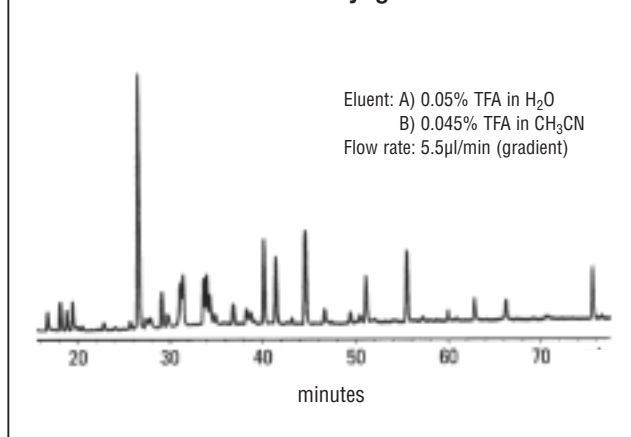


Table 1

Column Availability

Capillary columns, ranging in i.d. from 0.05mm to 0.8mm, are available from a variety of manufacturers.

Column i.d. (mm)	Typical Flow Rate (μ l/min)	Theoretical Sensitivity Increase
1.0	40 - 240	-
0.5	10 - 60	4
0.3	3 - 18	11
0.15	0.05 - 5	44
0.075	0.02 - 1.5	178
0.050	0.01 - 0.9	462

Please contact Canadian Life Science to discuss the availability of custom-packed capillary columns and new developments in capillary LC.

LC-MS COLUMNS

- Column diameter \leq 2.1 mm
- Rapid analysis
- Characterization technique

Introduction

Traditional problems associated with the inherent incompatibility of HPLC (high liquid pressure) and mass spectrometry (low vapour pressure) have been largely overcome. LC-MS has become a leading technique offering characterization of solute molecules.

Column Dimensions

LC-MS interface techniques such as Atmospheric Pressure Chemical Ionisation, Electrospray, Particle Beam or Thermospray can typically handle maximum flow rates of 200 μ l/min. As microbore columns (2 mm i.d.) utilize such flows they are commonly used in LC-MS applications.

A 50 x 2.1 mm i.d. column is used for fast speed analysis applications while a 250 x 2.1 mm i.d. column will be the one of choice for more complex separations.

Where sensitivity is an issue, as in the analysis of peptides and proteins, 1mm i.d. columns are available.

Availability

Canadian Life Science offers a variety of manufacturer's LC-MS columns in lengths from 10 to 250mm and 1.0 and 2.1mm i.d. Alternative materials and dimensions can readily be supplied. Please enquire for details.

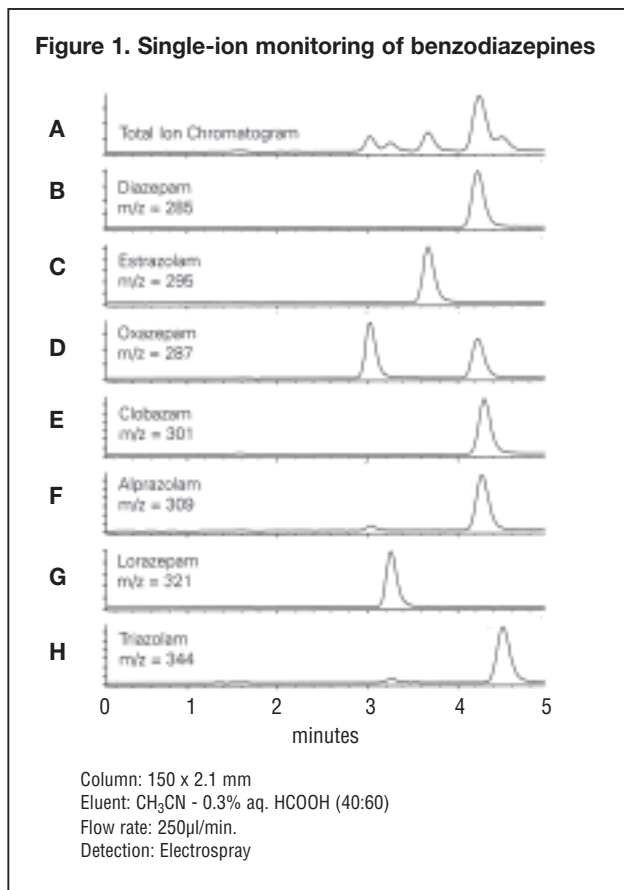
Applications

LC-MS has numerous applications. These include:

- Drug metabolism, structure elucidation and quantitative studies
- Protein and peptide identification and sequencing
- Combinatorial chemistry
- Agrochemical identification and quantitative studies
- High sensitivity
- Quantitative analysis
- Peak deconvolution ability

Figure 1 shows the use of single-ion monitoring to analyse a mixture of benzodiazepines. Although the total ion current chromatogram (A) obtained on a 150 x 2.1 mm i.d. column only resolves Oxazepam (D), Lorazepam (G) and Estrazolam (C), the four remaining unresolved benzodiazepines can be analyzed using a single-ion monitoring technique (Diazepam m/z 285 (B), Clobazam m/z 301 (E), Alprazolam m/z 309 (F) and Triazolam m/z 344 (H)).

Although the assay is complete in under 5 minutes, use of a 50 x 2.1 mm rapid analysis column will reduce the analysis time to under 2.5 minutes.

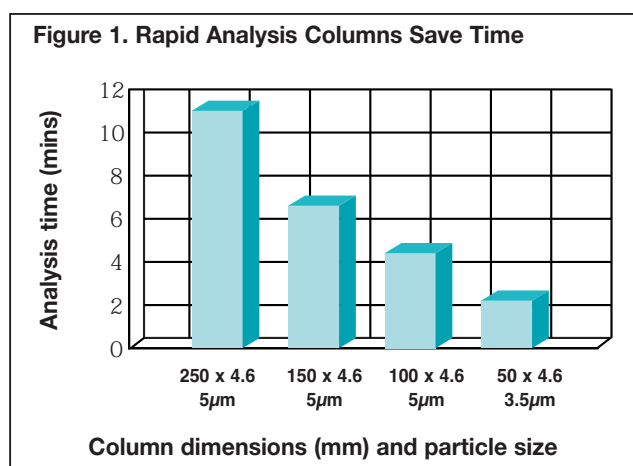


RAPID ANALYSIS (1-10CM LENGTH) COLUMNS

- Analysis time reduced by over 80%
- Increase in productivity x5
- Solvent saving up to 95%
- 3 - 5 μ m particle size silica
- Wide range of chemistries
- Baseline resolution maintained

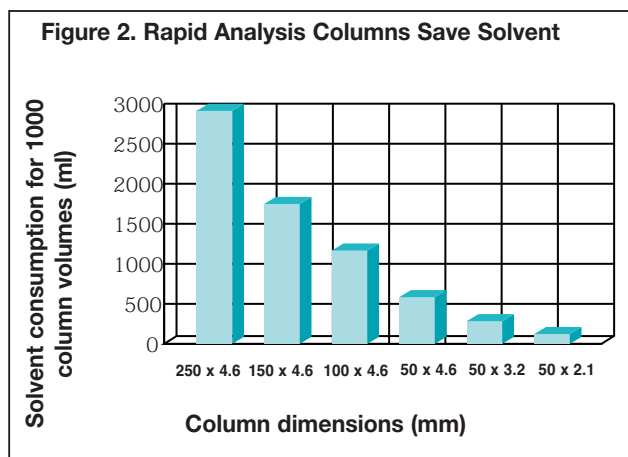
Rapid Analysis columns are designed to analyze large numbers of samples in as short a time as possible (Figure 1) without major sacrifice of column resolution.

Quality Control environments particularly benefit from the use of Rapid Analysis columns giving corresponding increases in productivity.



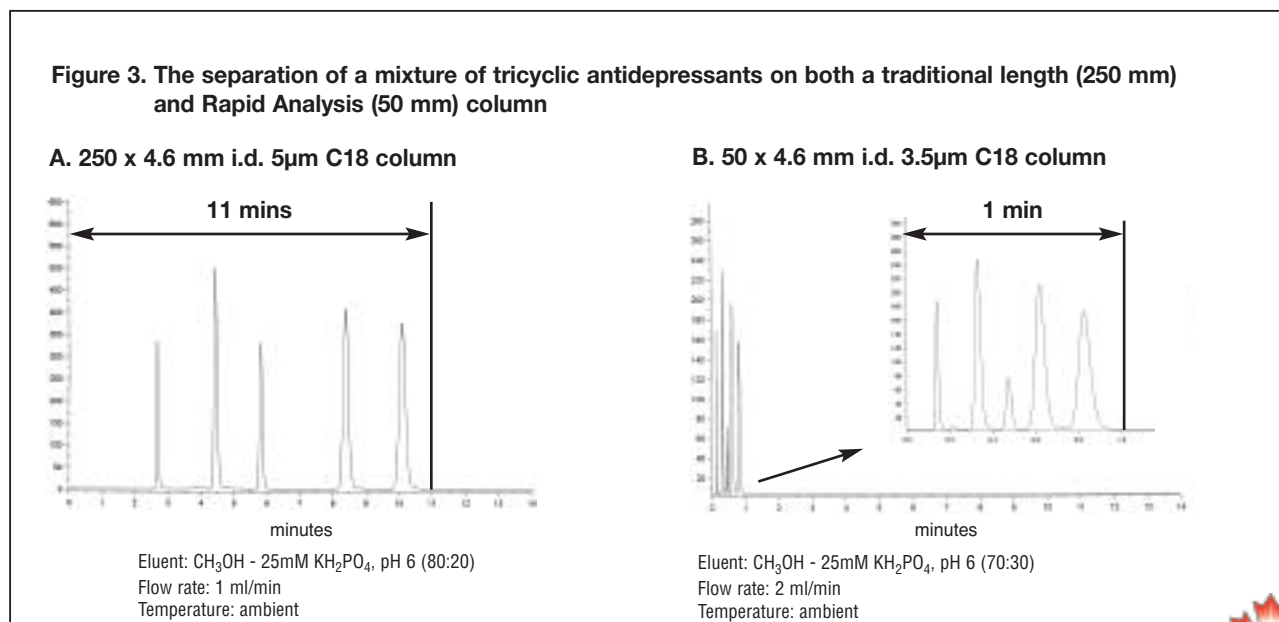
Typical reduction of column length from 250 to 50 mm will offer an 80% saving in analysis time. Often the efficiencies associated with a 250 mm length column containing 5 μ m silica particles (>20,000 plates) are not required. In such circumstances a 50 mm length column packed with smaller 3.5 μ m particles can still generate an adequate 5000 plates.

Simultaneously less solvent is required (Figure 2) especially with the added reduction of column diameter.



The advantage of combining a shorter column length with smaller particle size silica without compromising resolution is clearly shown in Figure 3. Baseline resolution is maintained and the analysis time reduced by over 90%.

We supply the following rapid analysis columns: Ace, Hichrom, Inertsil, Kromasil, Nucleosil.



COMBINATORIAL CHEMISTRY COLUMNS

- Matched analytical and preparative columns
- Easy scale-up for lead compound identification
- High sample throughput
- Rapid analysis without efficiency loss
- Fast re-equilibration

Introduction

Traditionally, potential new drugs have been individually synthesized, purified and their structure confirmed prior to the measurement of structure-activity relationships.

A combinatorial chemistry approach, in which mixtures of compounds are synthesized and initially tested, has now often replaced the older methods.

Rapid chromatographic analysis of each mixture is required to aid characterization of active and non-active components.

Columns

Combinatorial chemistry analytical columns are designed to analyze large numbers of complex mixtures in as short a time as possible without major loss of column resolution.

They will often need to re-equilibrate as soon as possible following rapid gradient changes in the eluent composition needed to accommodate sample mixtures containing a wide polarity range of molecules. Good column stability is an essential requirement.

Columns are typically 50 to 100 mm in length, of 3.2 to 4.6 mm internal diameter and packed with 5 μ m particle size material.

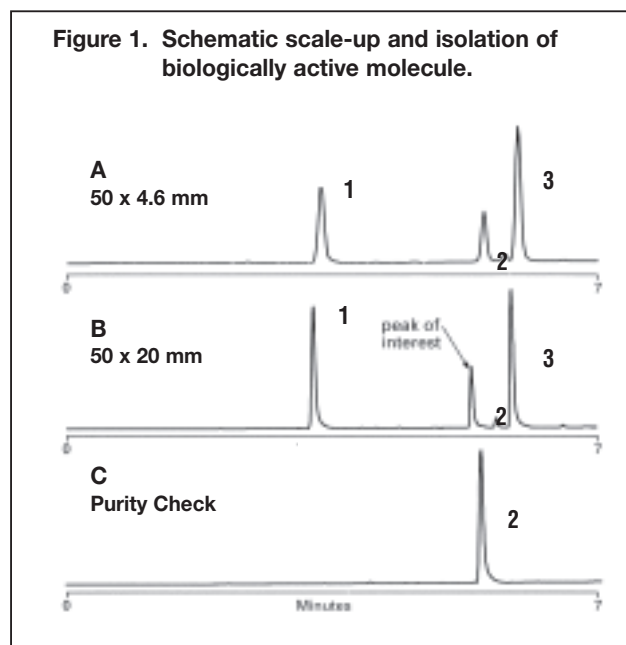
Availability

Canadian Life Science offers a wide range of Combinatorial Chemistry columns.

The standard manufactured column sizes are shown in Figure 1. Please contact Canadian Life Science for information on matched kits and alternative dimensions and materials.

Scale-up

Once a lead compound has been identified it becomes necessary to isolate it using larger diameter HPLC columns. Preparative and analytical columns need to be matched in terms of length and material content so that a one-step scale-up is easily attained. Figure 1 shows the utility of combinatorial chemistry columns for scale-up purposes. The progressive steps of analytical screening (Figure 1A), preparative isolation of Peak 2 using x20 increase in sample size (Figure 1B) and finally checking the purity of the isolated Peak 2 (Figure 1C), are shown.



Please contact Canadian Life Science for further product information or combinatorial chemistry brochures

METHOD DEVELOPMENT KITS

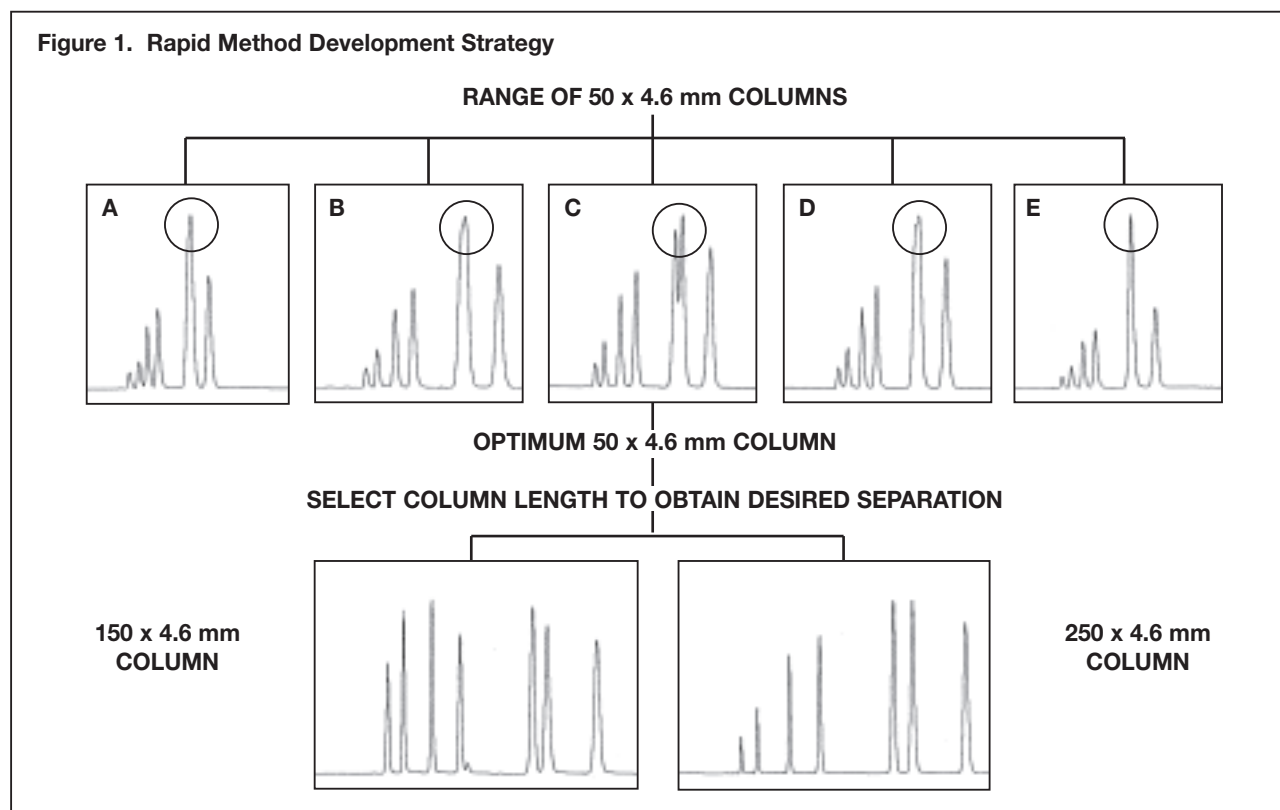
Rapid Method Development Kits

- Five 50 x 4.6 mm columns
- Wide range of phases
- Rapid economic column screening

The use of Rapid Method Development Kits allows the chromatographer to rapidly evaluate a series of bonded phases for routine analysis. Once the best partial or total resolution of the sample is obtained, when necessary a longer column packed with the same phase can be used to obtain optimum separation (Figure 1).



Selection of Optimum Bonded Phase and Method Optimization



Canadian Life Science is able to offer >70% of the world's leading brands of HPLC phases. Hence Rapid Method Development Kits can contain an extensive range of columns packed with the chromatographer's selection of phases (silica, chemistry and particle size).

Please contact Canadian Life Science for guidance on column selection or information on our applications support or column screening services.

Many leading manufacturers offer competitively priced Method Development Kits including Ace, Epic, Inertsil, Kromasil Chiral, Nucleosil Chiral, Primesep and ProntoSIL.

Please contact Canadian Life Science for the latest Method Development Kit promotional offers

METHOD DEVELOPMENT KITS (CONT'D)

Selectivity Kits

- 3 column kits
- Hydrophobicity Kit (C18, C8 and C4 columns)
- Polarity Kit (Variety of chemistries)
- Detect minor impurities

Chromatographers may wish to optimize the bonded phase on longer columns. Additionally, the use of all three columns within our Selectivity Kits enables the chromatographer to further check for impurities that may remain undetected when only one column is used.

Selectivity Kits are available in a wide range of phases customized to the user's needs from many manufacturers. Please contact us.



Validation Kits

- Three columns
- One phase, three batches
- Rapid method validation
- Confirms assay reproducibility, robustness

Method Validation Kits contain three columns of the same phase and dimensions, but which are packed with different batches of material. The kits are available in all column dimensions and phases. They can be used to assess the batch to batch variability of a chosen bonded phase.

By validating a number of batches of material, the chromatographer can obtain greater confidence in the reproducibility and long-term robustness of the method.



**Please contact Canadian Life Science
to discuss the availability of any type of Method Development Kit
custom packed to the individual scientist's requirements**

GUARD CARTRIDGES

- Protection for columns from 1.0 - 21.2 mm i.d.
- No loss in column performance or selectivity
- Significantly extends column lifetime
- Packed with the same high performance silica used in main column

Introduction

Guard cartridges are designed to protect valuable analytical and preparative HPLC columns from contamination with impurity particles and irreversibly adsorbed solutes. By placing a guard cartridge between the column and the injector valve, contaminants which would otherwise damage the column are trapped on the disposable cartridge. This procedure significantly extends the lifetime of the protected column without affecting performance or selectivity. (see Figure 1).

Silica

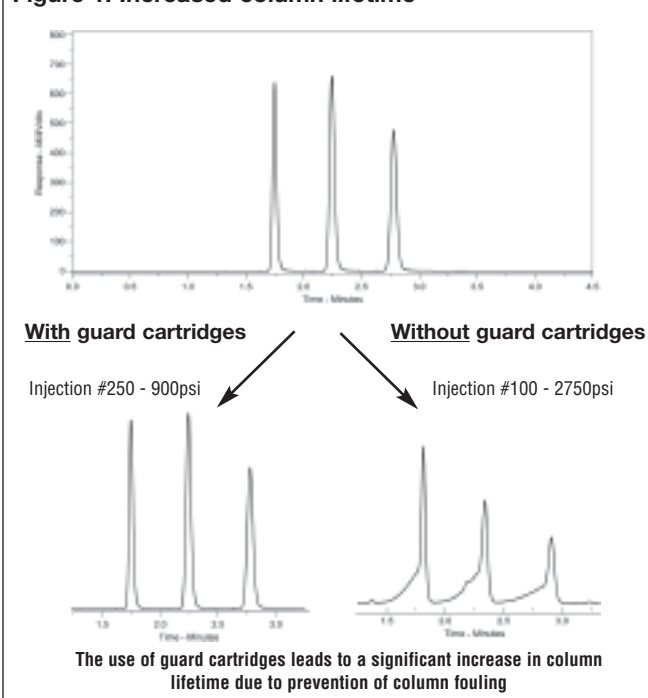
It is recommended that guard cartridges are packed with the same silica as used in the HPLC column to be protected (this eliminates the possibility of any loss of performance or selectivity). All our cartridges conform to this requirement. As we are able to supply cartridges packed with any commercially available silica, virtually all analytical and preparative columns can be suitably protected.

Guard Cartridge Holder System

For traditional columns (with compression end fittings) a fingertight column coupler (HI-081) is required to connect the holder (HI-161) to the column (see Figure 2).

- Stand-alone or integral design
- Tested to ensure consistent high level performance
- Cartridges individually identified
- Readily disposable and cost effective

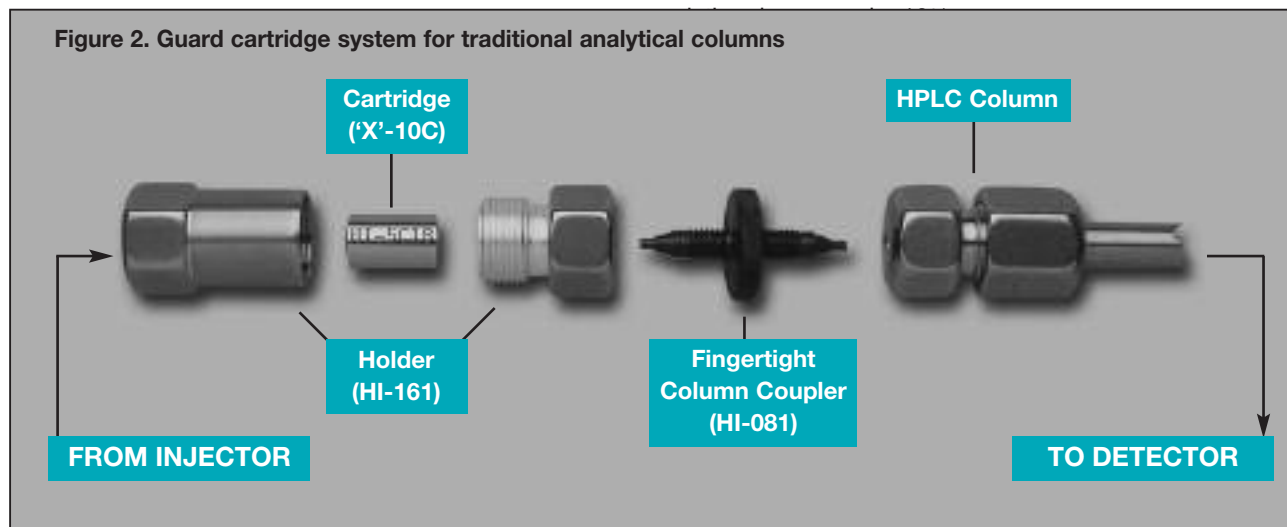
Figure 1. Increased column lifetime



Guard Cartridge Replacement

It is generally recommended that for effective column protection, guard cartridges should be replaced when the column back pressure increases by 10% or column efficiency or res-

Figure 2. Guard cartridge system for traditional analytical columns



GUARD CARTRIDGES (CONT'D)

Ordering Information - Guard Cartridges Manufactured by Hichrom

Guard Cartridges

Guard Cartridge	Catalogue No. ¹	Pack Quantity		Holder	Coupler
		3	5		
For 1.0mm i.d. columns	X-10CE5	-	\$295	HI-161 \$153	HI-081 \$60
For 2.1mm i.d. columns	X-10CM5	-	\$295	HI-161 \$153	HI-081 \$60
For 3.2 - 4.6mm i.d. columns	X-10C5	-	\$295	HI-161 \$153	HI-081 \$60
For 7.75 - 21.2mm i.d. columns	X-10CP3	\$344	-	HI-150 \$340	HI-081 \$60
For 30mm i.d. columns	X-20CP3	\$344	-	HI-183 \$910	HI-083 \$60

¹ When ordering replace 'X' with the appropriate silica code - see column listings or contact Canadian Life Science for details.

Example: For a 5 pack of Hichrom RPB guard cartridges for 3.2 - 4.6 mm i.d. columns, Catalogue No. = HIRPB-10C5 Quantity 5

Starter Kits

Starter kits (see Figure 2) contain five 10mm length guard cartridges packed with any chosen silica, a free-standing holder (HI-161) and a fingertight column coupler (HI-081).

Starter Kit	Catalogue No. ¹	Price
For 1.0mm i.d. columns	X-10CE5-SK	\$395
For 2.1mm i.d. columns	X-10CM5-SK	\$395
For 3.2-4.6mm mm i.d. columns	X-10C5-SK	\$395

¹ When ordering replace 'X' with the appropriate silica code - see column listings or contact Canadian Life Science for details.

Example: For a Hichrom RPB starter kit for 3.2 - 4.6 mm i.d. columns, Catalogue No. = HIRPB-10C-SK



Integral Guard Cartridge System

An integral guard holder is also available for 3.2mm to 4.6mm i.d. modular cartridge columns. The holder for either 10mm length cartridges (HI-175) or 30mm length cartridges (HI-176) is directly attached at the column inlet end (see Figure 3).

Please enquire for further information on the integral guard cartridge system and modular cartridge columns.



Note: Take the inlet fitting off the column (taking care not to remove the fitted frit) and replace with the integral guard holder base.

Other Guard Columns/Cartridge Systems and Starter Kits available for many of our column brands.

If this information is not available in the column section for the column you require, please contact Canadian Life Science

PREPARATIVE AND PROCESS SCALE COLUMNS

- Wide range of bulk silicas
- Particle size 5-50 μm
- Column internal diameters 10-100 mm (4")
- Analytical matched test columns
- High purity products
- Good recoveries
- High-speed technique

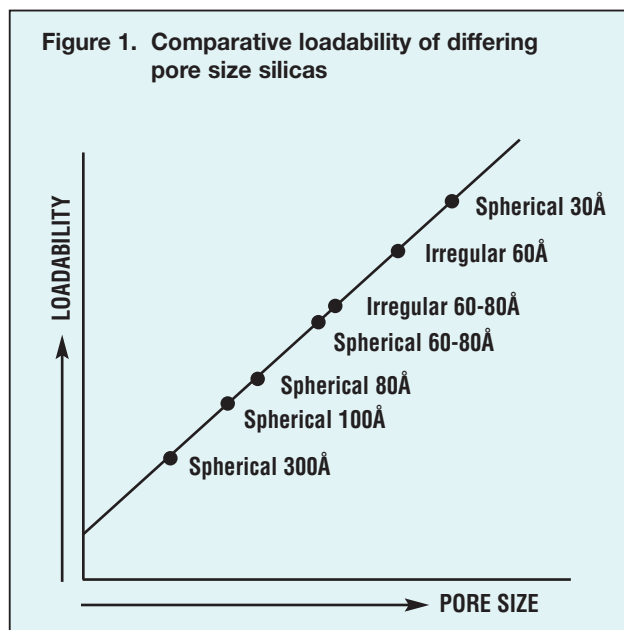
Introduction

Preparative HPLC will isolate and purify milligram to kilogram amounts of compound. The technique uses larger particle size silicas and wider internal diameter columns than in analytical HPLC. Column efficiency can be preserved on scale-up from analytical to preparative separations. However, broader lower efficiency chromatographic peaks are more often observed when the column is used in an overload state.

Separation Criteria

The criteria governing preparative separations are very similar to those influencing analytical HPLC. Economic considerations become more important. They are governed by four factors.

- **Resolution**
By optimizing the separation between the peak of interest and the nearest contaminant, high sample loads can be achieved without compromising product purity.
- **Loadability**
Loadability is controlled by the silica particles' pore size and available surface area. The smaller the pore size the larger the surface area. The comparative loadability of different pore size silicas is shown in Figure 1. Application of the smaller pore size silicas is limited by the range of molecular weight materials they can chromatograph.
- **Chemical Stability**
The lifetime of a column is often dependent on the silica's chemical stability. Conditions of use will be very important.
- **Physical Stability**
The robustness of a silica will determine how many times a material can be repacked into a column.



Separation Strategy

Reversed-phase is the dominant technique used in analytical HPLC. Normal-phase HPLC is still often used in preparative separations due to the high cost of reversed-phase materials and the easier recovery of solute from the organic solvents.

Two strategies dominate the approach to preparative HPLC.

1. In the 'scale-up' approach a method developed for analytical purposes is directly applied to a larger i.d. column. Although typical 3 - 5 μm particles may be replaced with 10 μm material of identical selectivity, high preparative efficiencies are maintained. Such an approach is particularly suitable for purifying gram quantities of material with low k' -values.

2. In the alternative 'overload' approach resolution is sacrificed by operating the column in an overload situation. Such high loadings maximize column capacity. Separations are poorer but gram to kilogram amounts of material may be purified.

PREPARATIVE AND PROCESS SCALE COLUMNS_(CONT'D)

Table 1. Column Capacity

Column Size	Column Internal Diameter (mm)	Relative Flow Rate (ml/min)	Volume of 250mm Length Column (ml)	Weight of Phase (g)	Maximum Column Capacity per Injection	
					Optimum ²	Practical ³
Analytical ¹	4.6	1.0	4.2	2.5	-	-
Semi-Preparative	10	4.7	20	12	10mg	-
Preparative 1"	20	19	79	47	50mg	2g
Preparative 2"	48	110	450	268	250g	10g
Preparative 4"	96	440	1800	1072	1g	4 ⁰ g

¹ Assumes 250 x 4.6 mm column contains 2.5g material² Scale-up approach³ Overload approach

Bulk Preparative Materials

Canadian Life Science Inc. distributes commercially available preparative HPLC bulk materials. The physical properties of Daisogel, Kromasil, MCI GEL, Nucleodur and PolyLC materials are listed.

Material	Manufacturer	Particle Size	Particle Shape	Pore Size (Å)	Surface Area (m ² /g)	Chemistry
Daisogel	Daiso Co.	7 10 15 20 40-60	S	60, 120, 200, 300	450, 300, 200, 100	Sil, C1, C4, C8, C18, Ph, CN, NH ₂
Kromasil	Eka Chemicals	7	S	60	540	Sil, CN, Diol
		10		100	320	Sil, C4, C8, C18, NH ₂ , Phenyl
		13 16		300	110	Sil, C4, C8, C18
MCI GEL	Mitsubishi Chemical Corp.	5	S	600	Polyhydroxymethacrylate based ion-exchange resins	
		10 30		250, 450	Non-functionalized styrene-divinylbenzene copolymer for RP	
		Various		-	Styrene-divinylbenzene copolymer ion exchange resins	
Nucleodur	Macherey-Nagel	10 12 16 20 30 50	S	100	320	Sil, C18

¹ S=Spherical, I=Irregular² Irregular material also available³ Polymeric materials

Please contact Canadian Life Science for more bulk silica and preparative or process scale column information.

**Daiso silica is only available in bulk

PREPARATIVE AND PROCESS SCALE COLUMNS_(CONT'D)

Bulk Preparative Materials (continued)

Material	Manufacturer	Particle Size	Particle Shape	Pore Size (Å)	Surface Area (m ² /g)	Chemistry
PolyCAT A*	PolyLC	3	S	1500	10	Polyaspartic Acid - Weak cation-exchange
		5		300, 1000	100, 25	
		12		300, 1500	100, 10	
PolyETHYL A*		5		300, 1000	100, 25	Hydrophobic Interaction (HIC) of Proteins and Peptides
		12		300, 1500	100, 10	
PolyGLYCOPLEX		5, 12		-	-	Hydrophilic Interaction
PolyHYDROXYETHYL A*		3		60, 100, 200, 300, 500, 1500	430, 350, 200, 100, 35, 10	1. Hydrophilic Interaction (HILIC) 2. Size Exclusion
		5		60, 100, 200, 300, 500, 1000	430, 350, 200, 100, 35, 25	
		12		100, 300, 1500	350, 100, 10	
PolyMETHYL A*		5		300, 1000	100, 25	Hydrophobic Interaction (HIC) of Proteins and Peptides
		12		300, 1500	100, 10	
PolyPROPYL A*		3		1500	10	Hydrophobic Interaction (HIC) of Proteins and Peptides
		5		300, 1000	100, 25	
		12		300, 1500	100, 10	
PolySULFOETHYL A*		3		300, 1500	100, 10	Sulfoethylaspartamide- Strong cation-exchange
	5	200, 300, 1000	200, 100, 25			
	12	300	100			
PolyWAX LP	3	300, 1500	100, 10	Linear Polyethyleneimine- Weak anion-exchange		
	5	100, 300, 1000	350, 100, 25			
	12	300, 1500	100, 10			

A* = substituted polyaspartamide