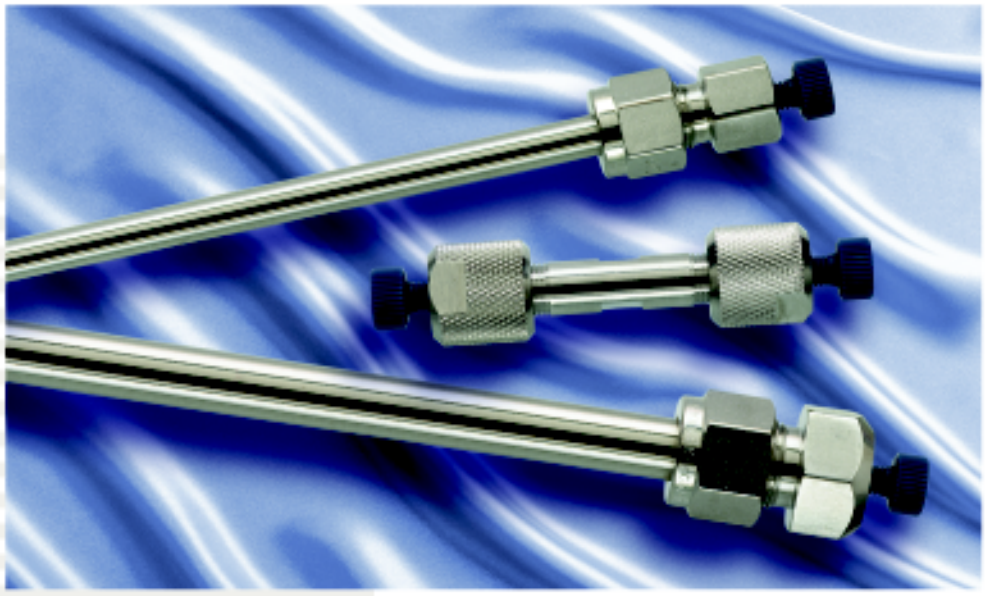
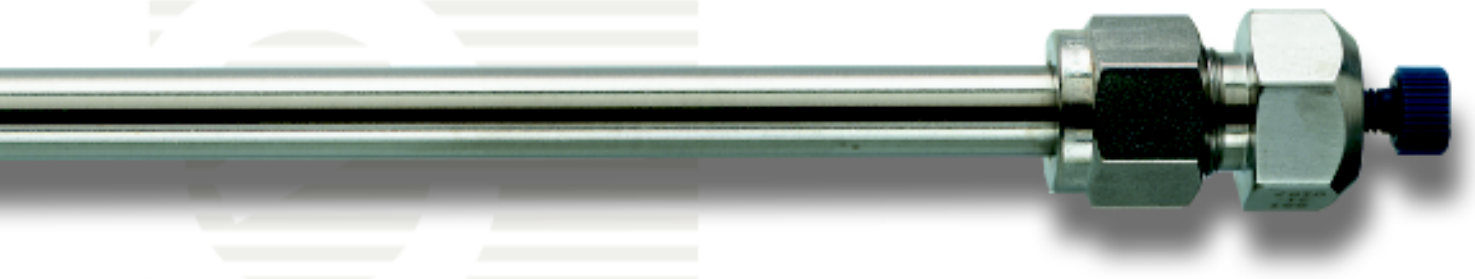
Volume 3

eichrom TECHNOLOGIES INC



MICRA® hplc columns



EICHROM TECHNOLOGIES, INC.
MICRA® HPLC Columns

"Creating Knowledge From Science"

ORDER INFORMATION:

In the United States contact:
Eichrom Technologies, Inc.
8205 S. Cass Ave. Suite 111
Darien, IL. U.S. 60561
Phone: 630-963-0320
FAX: 630-963-1928
Toll free: 800-422-6693
info@eichrom.com

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Eichrom Europe SARL.
50 rue de Paradis
75010 Paris - FRANCE
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EichromEurope@eichrom.com

For other international inquires contact:
Eichrom's U.S. office or the web site. (All Eichrom international distributors are listed.)

WWW.EICHROM.COM

Visit **www.eichrom.com** for information on all Eichrom products. The site includes product descriptions, part numbers, applications, and e-commerce. Technical questions or literature requests can be sent via email to: **info@eichrom.com**.

TECHNICAL ASSISTANCE

Call us for all of your application needs at 800-556-4272. We offer assistance in the area of HPLC methods development or any type of HPLC analysis as well as general information on specific applications. Eichrom's technical assistance group also offers customer sample submissions to help determine the best column to use for your specific assay needs.

WARRANTY

The entire MICRA-HPLC column line, manufactured by Eichrom, is warranted to be free from defects in material and workmanship for 30 days from date of receipt. All columns are individually tested and packaged with a quality assurance chromatogram.

RETURNS

Product returns will not be accepted without prior authorization. There is a 15% restocking charge for returned items.

LIABILITY

Eichrom's products are intended for laboratory use only, not as drugs, food, or for household usage. These products must be handled with due care and only by professional laboratory staff.

LITERATURE

Application notes, chromatograms, technical notes, poster reprints, literature reprints and a reference bibliography for both porous and non-porous phases are available upon request.

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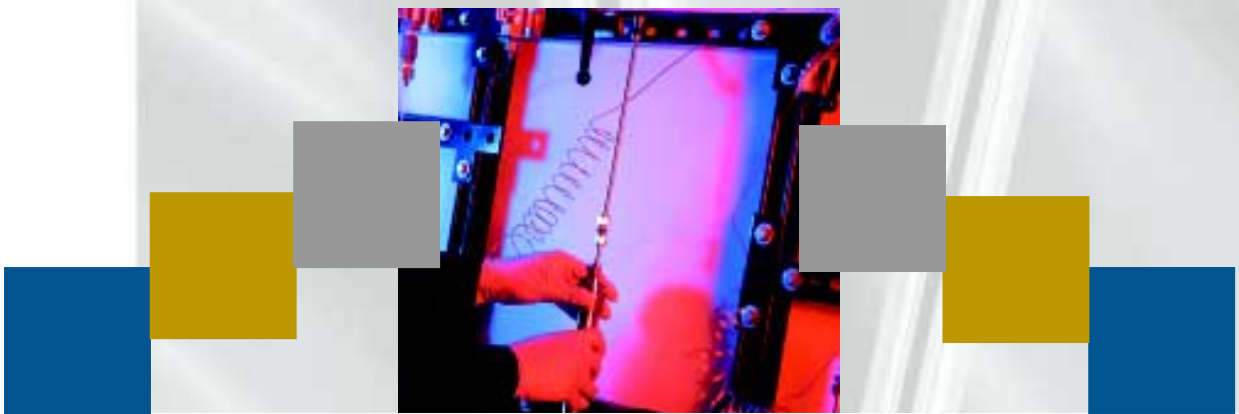


Who is Eichrom?

Eichrom Technologies, Inc. is a world leader in separation chemistry and brings diversity to the HPLC marketplace with its recent introduction of MICRA®-Platinum, MICRA®-Gold, and MICRA®-Silver HPLC columns. This introduction is made possible by the 1998 acquisition of the world-renowned SynChropak® bonding chemistry and the revolutionary ***NPS***® non-porous silica particle technology. Eichrom's leadership in the separations industry began with breakthrough technologies developed at Argonne National Laboratory for radioactive isotopes. Eichrom's founding, on Feb. 7, 1990, brought the commercialization of products which deliver efficient separations and pre-concentration of radionuclides from environmental and bioassay samples. Further innovative research in particle manufacturing and immunoassay has resulted in significant company developments and products for the food safety industry as well as in areas of clinical diagnostics, and cell separation.

HPLC Markets Served

HPLC products are manufactured to meet the application needs in the areas of life science/proteomics, pharmaceuticals, cosmetics, and environmental separations. Offices in both the U.S. and Europe as well as a worldwide network of international distributors supports an international customer base.

Chromatographic Techniques/Product offering

HPLC (High Performance Liquid Chromatography) and LC-MS (Liquid Chromatography-Mass Spectrometry) products are offered for Reversed Phase, Anion and Cation Exchange (Both weak and strong), Size Exclusion (GPC), and Hydrophobic Interaction.

Eichrom's MICRA®-Platinum, MICRA®-Gold, and MICRA®-Silver are manufactured to meet the demand for exceptional performance in analytical applications. All porous phases are based on SynChropak bonding chemistry, known for over two decades of excellent resolution, stability, and reproducibility.The ***NPS***® particles are derived from a patented, "metal free" manufacturing process. The spherical non-porous silica is 1.5µ, very pure, highly uniform, and brings advantages to the HPLC marketplace which complement standard porous supports.

Column Performance Options

MICRA®-Platinum Columns

- Reversed Phase and Normal Phase
- 1.5µ non-porous silica/3µ porous silica
- High throughput/LC-MS applications
- Three C-18 bonded phases for ***NPS***
- ***NPS***® TAS offers extra resolving power
- C18 and SCD (Short Chain, Base Deactivated) for porous silica

MICRA®-Gold Columns

- Reversed Phase - Ion Exchange - Size Exclusion
- 5µ, 7µ, 10µ Spherical Silica
- 100, 300, 500, 1000, 4000 Å Pore Sizes
- Unique to the industry:
  - "SCD100" for drug applications
  - "CATSEC" for cationic polymers

MICRA®-Silver

- Reversed Phase - Ion Exchange - Hydrophobic Interaction
- 6µ, 300 Å, Spheroidal Silica
- Economical Benefits for Methods Development
- Well Suited for Preparative Formats

Guide to Eichrom Technologies MICRA-HPLC Supports

MICRA-Line	Particle size (µ)	Pore size (Å)	Phase Description	Phase Characteristics	MW Range	Specific Application	USP XXII
SILVER RP							
RP4 300	6	300	C4	polymeric	>1000	peptides/proteins	L26
RP8 300	6	300	C8	monomeric	>1000	peptides/proteins	L7
RPP300	6	300	C18	monomeric	>1000	peptides	L1
SILVER Ion Ex.							
AX 300	6	300	WAX	polyethyleneimine	<200,000	peptides/proteins	
Q 300	6	300	SAX	quaternary amine	<200,000	proteins	
CM 300	6	300	WCX	carboxymethyl	<200,000	proteins/hemoglobin	
S 300	6	300	SCX	sulfonic acid	<200,000	basic peptides/proteins	
SILVER HIC							
HIC Propyl	6	300	C3	monomeric	>1000	proteins	
GOLD RP							
RP4 300	5	300	C4	polymeric	>1000	proteins	L26
RP4 1000	7	1000	C4	polymeric	>1000	large proteins	L26
RP4 4000	10	4000	C4	polymeric	>1000	large proteins/biopolymers	L26
RP8 HC	5	300	C8	monomeric	>1000	peptide mapping/protein digests	L7
RP8 1000	7	1000	C8	monomeric	>1000	proteins	L7
RPP 100	5	100	C18	monomeric e/c	<1000	small molecules/drugs	L1
RPP 1000	7	1000	C18	monomeric	>1000	peptides/proteins	L1
RPP 4000	10	4000	C18	monomeric	>1000	peptides/proteins	L1
SCD 100	5	100	Proprietary	base deactivated e/c	<1000	small molecules/ basic drugs	
GOLD Ion Ex.							
AX 100	5	100	WAX	polyethyleneimine	<10,000	organic acids/nucleotides	
AX 1000	7	1000	WAX	polyethyleneimine	>200,000	large proteins	
Q 100	5	100	SAX	quaternary amine	<10,000	peptides	
CM 100	5	100	WCX	carboxymethyl	<10,000	amines	
S 1000	7	1000	SCX	sulfonic acid	>200,000	proteins	
GOLD SEC					For MW range:		
GPC Peptide	5	50	SEC	Glyceryl	See page 27	peptides	L20,33
GPC100	5	100	SEC	Glyceryl	See page 27	proteins/polymers	L20,33
GPC300	5	300	SEC	Glyceryl	See page 27	proteins/polymers	L20,33
GPC500	7	500	SEC	Glyceryl	See page 27	polymers	L20,33
GPC1000	7	1000	SEC	Glyceryl	See page 27	polymers	L20,33
GPC4000	10	4000	SEC	Glyceryl	See page 27	polymers	L20,33
GPC Linear	7	100/1000	SEC	Glyceryl	See page 27	proteins/polymers	L20,33
CATSEC100	5	100	SEC	Polyamine	See page 29	cationic polymers	
CATSEC300	5	300	SEC	Polyamine	See page 29	cationic polymers	
CATSEC1000	7	1000	SEC	Polyamine	See page 29	cationic polymers	
CATSEC4000	10	4000	SEC	Polyamine	See page 29	cationic polymers	
PLATINUM RP							
RP-MS	3	100	C18	monomeric e/c	<1000	rapid / LC-MS small molecule analysis	
SCD-MS	3	100	Proprietary	base-deactivated, e/c	<1000	rapid / LC-MS pharmaceutical analysis	
ODS-I	1.5	non-porous	C18	polymeric	<200,000	rapid / LC-MS hydrophobic sm molecule & proteins analysis	
ODS-II	1.5	non-porous	C18	monomeric	<200,000	rapid / LC-MS hydrophilic, polar, sm molecule & proteins analysis	
ODS-IIIE	1.5	non-porous	C18	monomeric e/c	<200,000	rapid / LC-MS pharmaceutical analysis	
PAH	1.5	non-porous	C18	polymeric	<1000	PAH certified, rapid analysis	
TAS	1.5	non-porous	C30	monomeric	<1000	rapid carotene analysis	
PLATINUM NP							
NPS-SIL	1.5	non-porous			<1000	rapid normal phase separations	
PLATINUM Chiral							
Mobile phase selector	1.5	non-porous	C18	polymeric	<1000	rapid enatiomeric separation	

MICRA-Platinum columns represent the state-of-the-art in column chromatography. This performance category includes column supports containing the ***NPS***® non-porous silica particle technology available in a 1.5µ size, as well as 3µ porous column supports derived from the SynChropak® bonding chemistry. These columns are designed for the analysis and purification of peptides/proteins, basic/acidic molecules, and pharmaceutical compounds by high throughput analysis or LC-MS techniques. This line of columns provides exceptional performance in speed of analysis, sensitivity, stability/reproducibility and low sample/solvent waste***. NPS***® is a very pure silica, utilizing the MICRA patented, metal free manufacturing procedure.

Three *NPS*® C18 bonding chemistries are offered to meet selectivity needs:

- ODS-I, a polymeric C18, offers the larger operating pH range and high column stability.
- ODS-II, a monomeric C18, provides a less hydrophobic surface (due to exposed surface silanols) for improved selectivity of polar and neutral analytes.
- ODS-IIIE, an endcapped, monomeric C18, is designed for analysis of basic compounds and pharmaceuticals.

Three *NPS*® specialty columns include:

- ***NPS***® TAS, a C30 extended chain length is suited for caratenoid analysis and fat soluble vitamins.
- ***NPS***® PAH, offers excellent resolution of the 16 priority pollutants in less than 8 minutes.
- ***NPS***® SIL, is well suited for normal phase analysis and LC-MS applications.

Porous supports for high throughput and LC-MS applications:

- 3µ RP-MS is a highly hydrophobic C18, 100Å endcapped phase designed for small molecules, amino acids, and nucleotides.
- 3µ SCD-MS is a base deactivated phase designed for pharmaceutical analysis.

MICRA-Gold columns are Eichrom's standard line of porous HPLC columns. They are based on SynChropak bonding chemistry and designed for the analysis and purification of proteins, peptides, polymers, basic/acidic molecules, and pharmaceuticals.

Reversed Phase

RP4 is optimal for the loading and high resolution of peptides and yields excellent recovery for proteins. RP-P, known for greater resolution and superior selectivity, is commonly used for the separation of smaller peptides, low hydrophobicity proteins, nucleotides, and amino acids. The very hydrophobic RP-P100 and the less hydrophobic SCD100 (base-deactivated) offer a combination which effectively resolves drug mixtures.

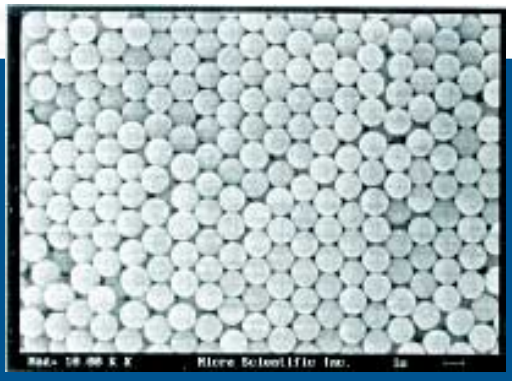
Ion exchange

These supports offer excellent selectivity based on ionic properties of a solute. They are appropriate for separating closely related organic acids, nucleotides ranging from mono to triphosphates and proteins which differ by only one or two amino acids.

Size Exclusion

The GPC series contains products with 7 pore diameters (50Å-4000 Å) allowing analysis of solutes with molecular weights ranging from 0.5kDa to 10,000kDa. The glycerol bonded phase is designed for the rapid analysis of proteins, carbohydrates, nucleic acids, and other water soluble anionic or neutral polymers. The MICRA-Gold CATSEC series, exclusive to Eichrom Technologies, is a polymerized polyamine support available in four diameters (100Å -4000Å) allowing the analysis of cationic polymers such as polyvinylpyridines to elute according to size and without adsorption.

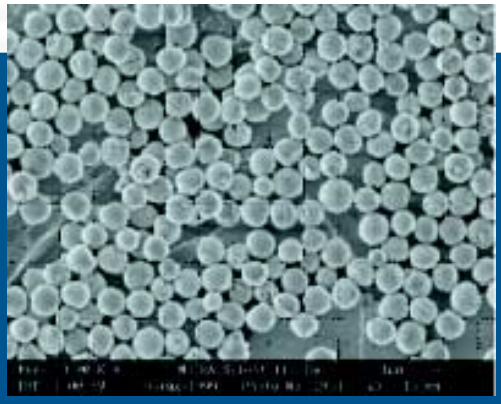
The MICRA-Silver series offers economical benefits to method development and preparative work. It is well suited for dedicating columns to specific analyses and scaling up from analytical to preparative applications. RP-4 is a polymeric bonding with a C4 ligand chain and is a popular choice for protein/peptide separations, particularly large hydrophobic solutes. RP-8 and RP-P are monomeric bondings and are appropriate for separating smaller proteins/peptides with a less hydrophobic nature. Both weak and strong Anion and Cation exchange supports are available for separating ionic compounds. They are compatible with aqueous buffers and many organic solvents in the pH 2-8 range. Hydrophobic Interaction Chromatography (HIC), uses descending salt gradients for elution, preserving the tertiary structure of the proteins and their enzymatic activity.



- 1.5µ and 3µ highly spherical and uniform,
- porous and ***NPS*** non-porous silica.
- Short run times, low solvent consumption
- High sensitivity
- Available phases include

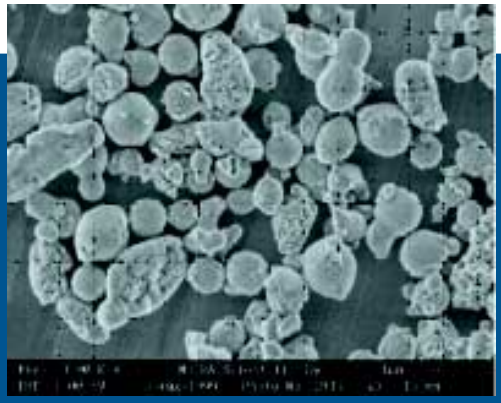
NPS (non-porous):
Reversed phase: ODS-I, ODS-II, ODS-IIIE, TAS, PAH
Normal phase: SIL

Porous:
Reversed phase: RP-MS, SCD-MS



- Highly spherical silica of very uniform size
- Particle sizes 5, 7 & 10µ
- Pore sizes 50Å ,100Å, 300Å, 500Å, 1000Å, 4000Å
- Phases available include:

Reversed phase: RP4 (300, 1000, 4000)
RP8 (300, 1000) RP-P 100,
RPP (100, 1000, 4000), SCD100
Anion Exchange: AX (100, 1000), Q100
Cation Exchange: CM100, S1000
Size Exclusion: GPC PEP, GPC Linear
GPC (100, 300, 500, 1000, 4000)
CATSEC (100, 300, 1000, 4000)



- 6µ, 300Å, Spheroidal silica
- Column sizes: 2-10mm ID & 50-300mm length
- Phases available include:

Reversed phase: RP4, RP8, RPP (RP18)
Anion exchange: AX300, Q300
Cation exchange: CM300, S300
Hydrophobic Interaction: HIC-Propyl

MICRA-Platinum Reversed Phase HPLC columns

MICRA® ***NPS***® 1.5µ non-porous silica

FAST CHROMATOGRAPHY AND RESOLUTION

The goal of fast chromatography is to reduce analysis time while still maintaining good resolution. Several ways to improve speed are possible, the most widely used means have centered on using shorter columns packed with smaller particles and increasing flow rate. These methods do not alter the selectivity nor the retention factor (**k**) of the components, the analyte resolution will change only with changes in column efficiency (**N**), directly linked to the column length (**L**) and particle size ($\mathbf{d_p}$) by the equations (**H = L/N**) and ($\mathbf{N \propto 1/d_p}$). The ideal case for fast chromatography is to use short columns packed with small particles to maximize speed while maintaining column efficiency. Column efficiency, however, can be further enhanced by minimizing mass transfer effects associated with the support material. The use of nonporous particles eliminates the pore diffusion (**B & C**) terms of the van Deemter equation (**H = A + B/u + Cu**). As shown in the van Deemter plots below, columns packed with 1.5µ ***NPS*** particles exhibit very high efficiencies that are insensitive to changes in flow rate or linear velocity (**u**). MICRA ***NPS*** represents the ideal HPLC column support for fast separations at the highest resolution possible.

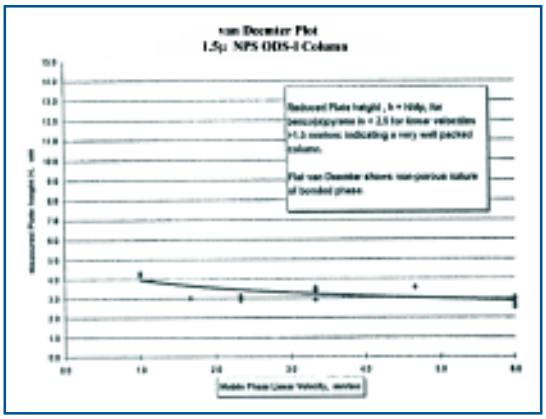
RETENTION FACTOR AND SPEED

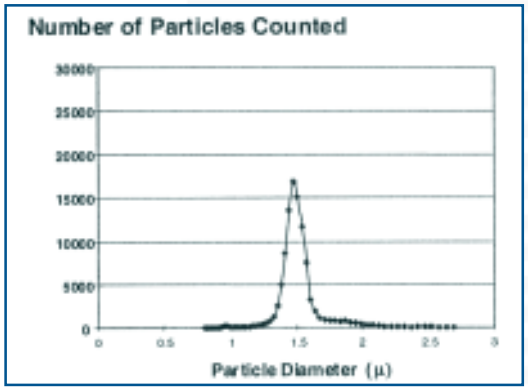
Eliminating pores also reduces the available surface area for C-18 bonding. For a given diameter of highly uniform nonporous silica particles, the surface area available for bonding is calculated from the density and particle diameter of the silica. For ***NPS*** we typically measure a density of 2.1 ± 0.1 g/mL and for a 1.5µ ***NPS*** material the surface area then is 1.9 m^2/g (a typical porous support surface area of around 200 m^2/g and a silica density of 0.4 g/mL).

In reversed phase separations, the equation below can be used to compare the expected values of k for the two supports : $\mathbf{k = KV_s/V_m}$

The distribution coefficient (**K**), the support volume ($\mathbf{V_s}$) and the mobile phase concentration ($\mathbf{V_m}$) contribute to the distribution of solute between the stationary surface and moving liquid. The pores themselves do not change the solute-solvent equilibrium to any significant degree. The surface area of the support is, therefore, very representative of the support contribution to the equilibrium of the column. Eliminating the porosity eliminates 50% of the available liquid volume ($\mathbf{V_m}$) in a packed column ($\mathbf{V_{m,NPS} \approx 1/2\ V_{m,porous}}$).

NPS has 5 times the density and 1/100 the surface area of porous supports. For the same separation and mobile phase composition, ***NPS*** will exhibit capacity factors, k, roughly 10 times smaller compared to a typical porous silica.



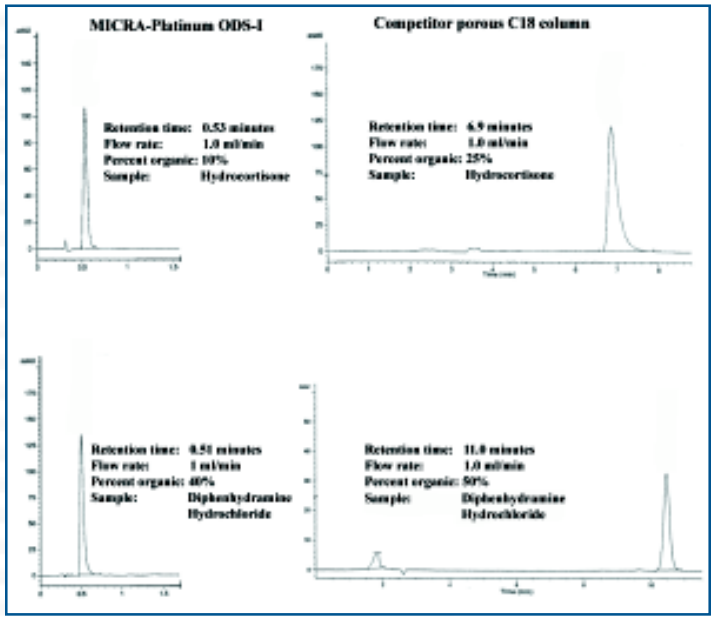


MICRA-Platinum Reversed Phase HPLC columns

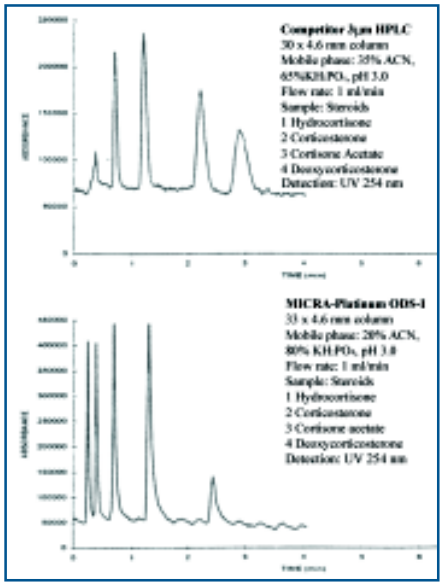
MICRA® *NPS*® 1.5µ non-porous silica

Illustrations show advantages of using columns packed with non-porous silica over columns packed with the more traditional porous silica.

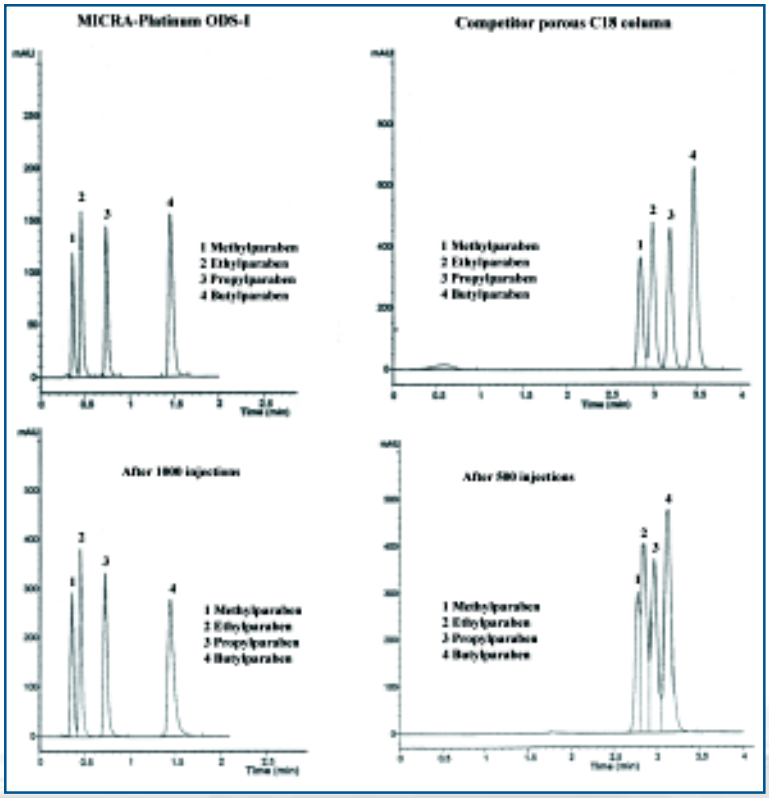
Faster



High Sensitivity



Stability Improved



MICRA-Platinum Reversed Phase HPLC columns

NPS ODS-I

The 1.5µ ***NPS*** ODS-I is a premium support of choice for most separations. It offers an excellent combination of stability, versatility, efficiency and shape selectivity for a broad range of separations. This is a polymeric bonding and yields low surface silanol activity, without endcapping. ODS-I offers fast and efficient separation opportunities in the areas of pharmaceutical, life science, and environmental.

Pharmaceutical

With the addition of less organic modifier than on conventional porous columns, it is possible to separate basic compounds as is shown in the analysis of Barbiturates. Most USP methods can be modified to use up to 90% less organic and obtain up to 20 times faster separations. This translates to most drug types including analgesics, β -blockers, antidepressants, vitamins, cough and cold, hormones, antibiotics, derivatives, and sedatives.

Life Science

Rapid analysis in the area of life science is illustrated with the separation of molecules such as amino acids, peptides, proteins and tryptic digest. See pages 16-17 for LCMS applications as well as a fast alternative to the time consuming and labor intensive method to measure and quantify protein expression in cells, 2-D polyacrylamide gel electrophoresis.

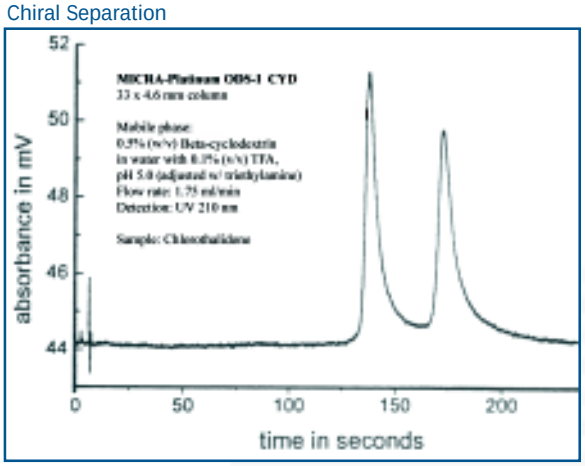
Environmental

ODS-I PAH specialty column demonstrates a reproducible and rugged method for the separation of 16 PAHs in less than 8 minutes. The U.S. EPA classifies the compounds as priority pollutants requiring routine monitoring for regulatory administration in drinking water, waste water and soil using methods 610, SW-846, and 8310.

Chiral

Enantiomeric separations using a chiral eluent selector yields fast results with good resolution.

Chiral Separation



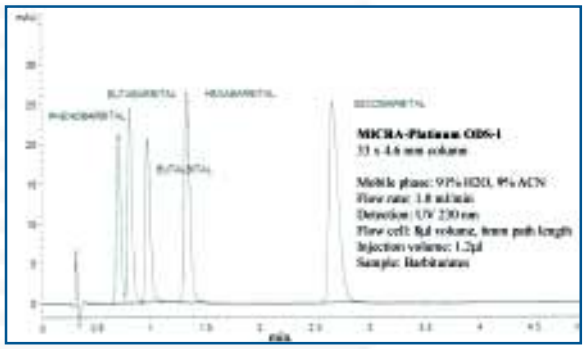
ORDERING INFORMATION				
MICRA-Platinum	**1.5µ *NPS* silica**	**ODS-I**	**ODS-I PAH**	**ODS-I CYD**
			(PAH Certified)	**(Chiral Mobile Phase Selector)**
	Format (mm)	Item Number	Item Number	Item Number
Mini-bore	**33x3**	0430ODS101.5		
	53x3	0630ODS101.5		
Fast Analysis	**14x4.6**	0146ODS101.5		
Standard	**33x4.6**	0446ODS101.5	0446PAH101.5	0446CYD101.5
High Capacity	**53x4.6**	0646ODS101.5		

MICRA-Platinum Reversed Phase HPLC columns

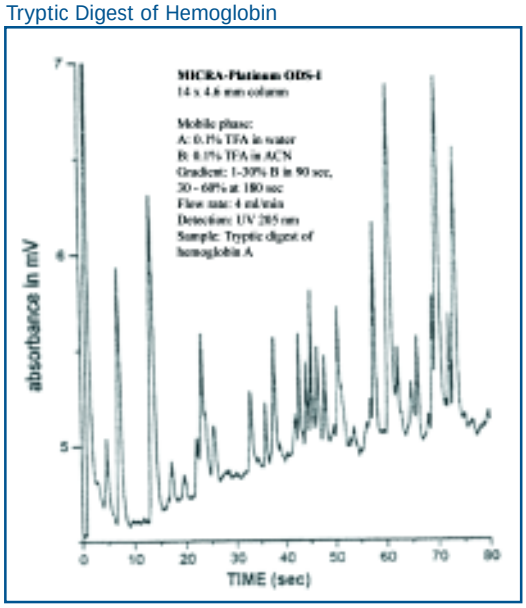
NPS ODS-I

Suggested and available Pharmaceutical, Life science, and Environmental applications for MICRA Platinum ***NPS*** ODS-I supports include amino acids, analgesics, antibiotics, barbiturates, benzodiazepines, benzotriazole, β - blocking drugs, capillary formats, catecholamines, epirubicin, explosives, food additives, mini-bore formats, on-line process monitoring, polynuclear aromatic hydrocarbons, preservatives, steroids, sun screens, tricyclic antidepressants, and water soluble vitamins.

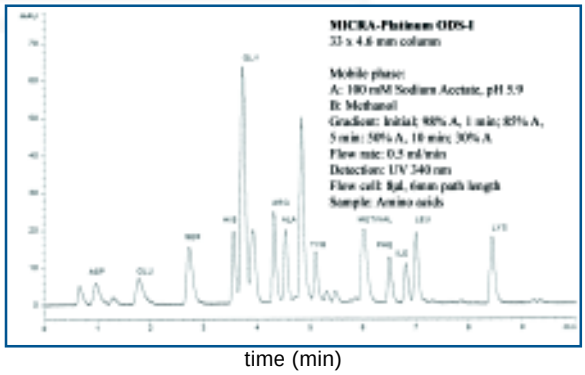
Barbiturates



Tryptic Digest of Hemoglobin

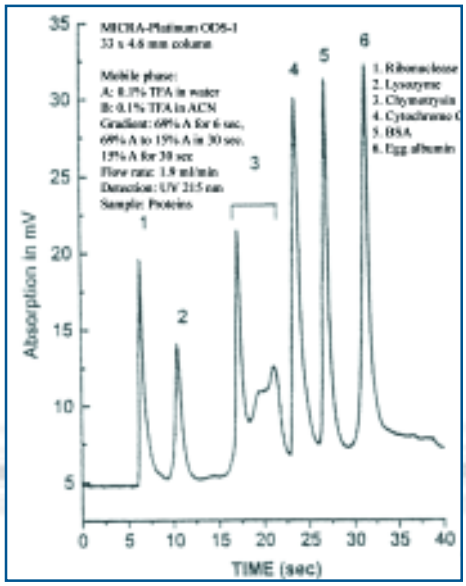


Amino Acids

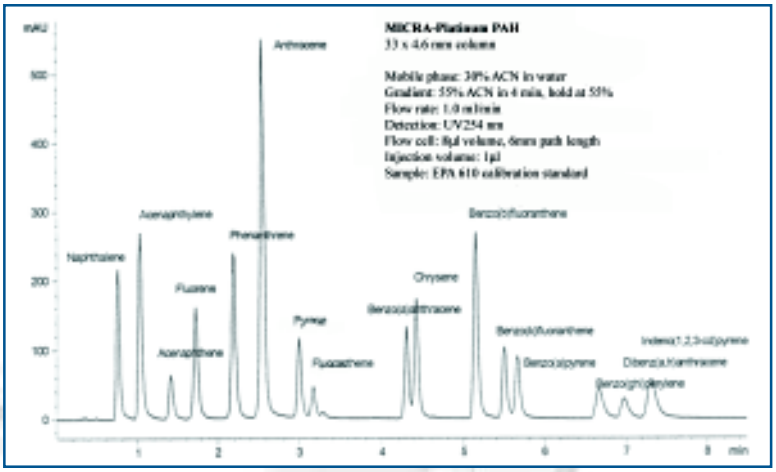


time (min)

Protein Mixture



PAH EPA 610 standard



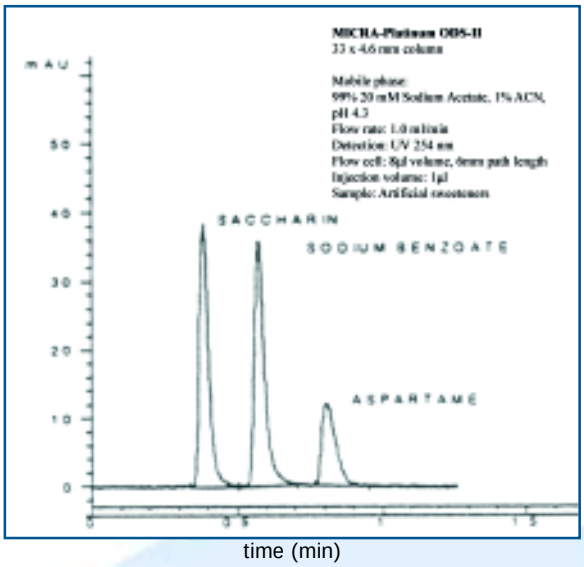
time (min)

MICRA-Platinum Reversed Phase HPLC columns

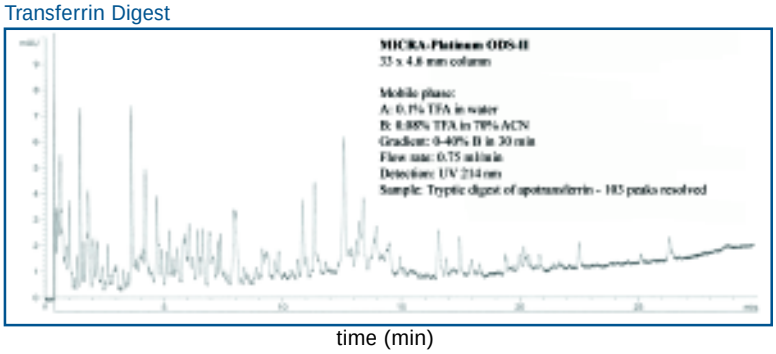
NPS ODS-II

ODS-II is monomerically bonded and offers a more hydrophilic surface than the ODS-I phase but less stability due to decreased shielding of the surface silanols. The increase in silanophilic interactions with analytes and mobile phases provides an opportunity to alter selectivity and resolve components that otherwise do not separate on the more shielded ODS-I, polymerically bonded, phase. As the ODS-II applications demonstrate, fast separations with high efficiency are obtained with this column technology. The detection of 103 peaks in the tryptic digest of apotransferrin, illustrated below, highlights the excellent resolution and high peak capacity possible with this column.

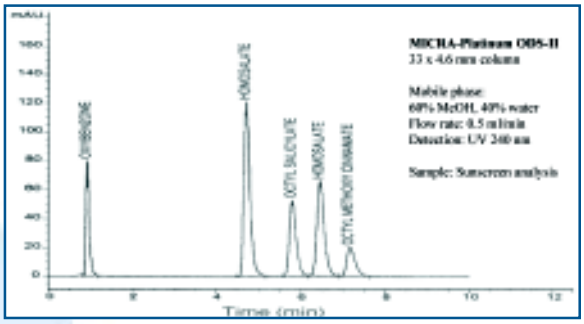
Artificial Sweetners



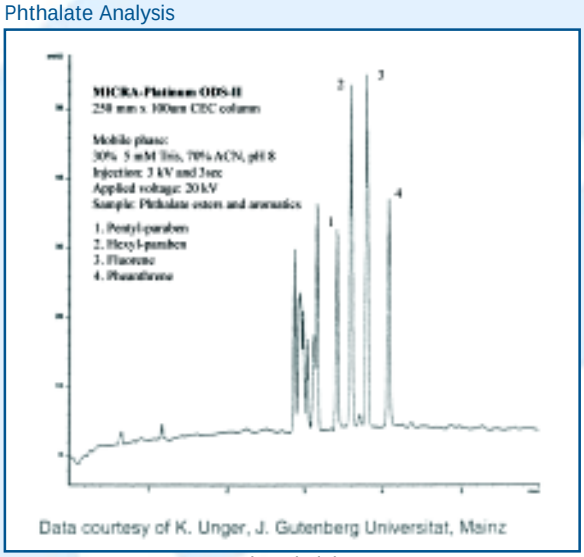
Transferrin Digest



Sunscreens



Phthalate Analysis



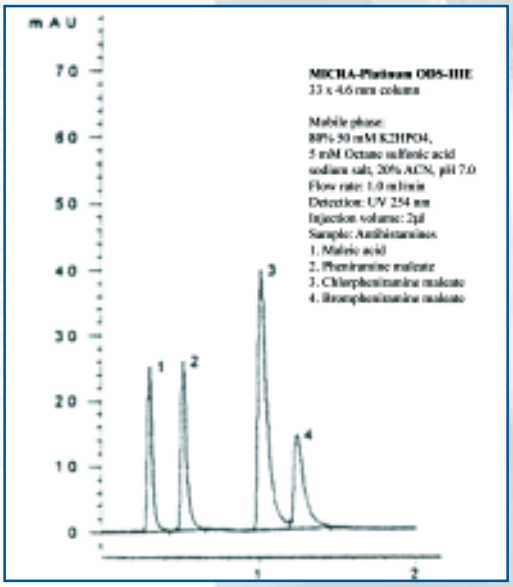
ORDERING INFORMATION		
MICRA-Platinum 1.5µ *NPS* silica		ODS-II
	Format (mm)	Item Number
Fast Analysis	14x4.6	0146ODS201.5
Standard	33x4.6	0446ODS201.5
High Capacity	53x4.6	0646ODS201.5

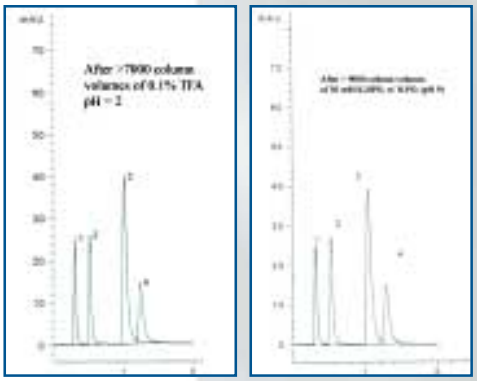
MICRA-Platinum Reversed Phase HPLC columns

NPS ODS-IIIE

ODS-IIIE is an endcapped, monomeric bonding. It is specifically designed for fast analysis and excellent resolution of basic compounds. The most critical factors in this type of analysis are peak shape and column stability. These key factors are illustrated below as well as an example of the opportunities ODS-IIIE offers for rapid protein profiling.

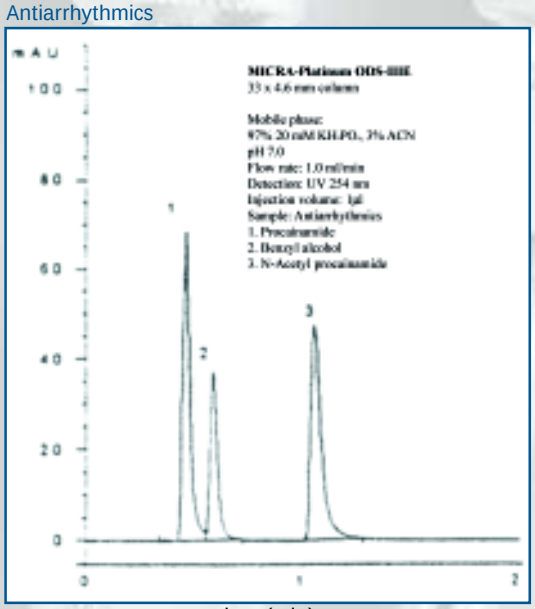
Antihistamines





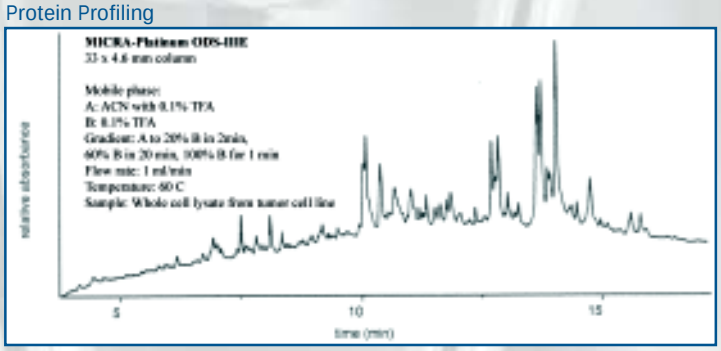
time (min)

Antiarrhythmics



time (min)

Protein Profiling



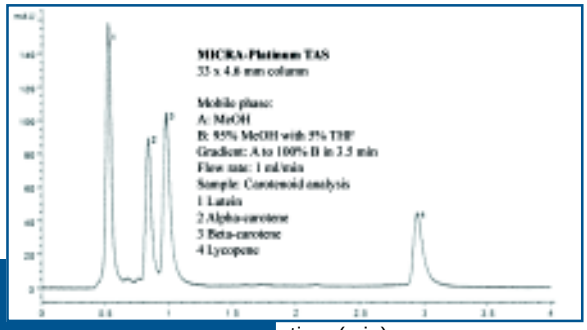
ORDERING INFORMATION		
MICRA-Platinum 1.5µ *NPS* silica		ODS-III E
	Format (mm)	Item Number
Fast Analysis	14x4.6	0146ODS3E1.5
Standard	33x4.6	0446ODS3E1.5
High Capacity	53x4.6	0646ODS3E1.5

MICRA-Platinum HPLC columns

NPS ODS-TAS:C30

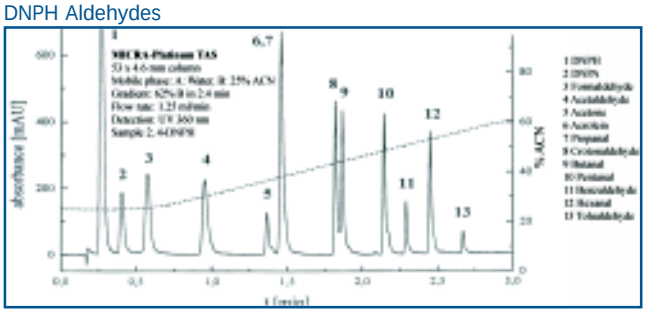
NPS-TAS is an extended C30 chain length with high carbon load for higher resolving power. It is designed for rapid analysis of carotenoids and fast separation of oil soluble vitamins as well as 2,4-DNPH and other DNPH-aldehydes and ketones in air samples. For information is available on this applications by requesting application notes AP20 an AP22.

Carotenoids



time (min)

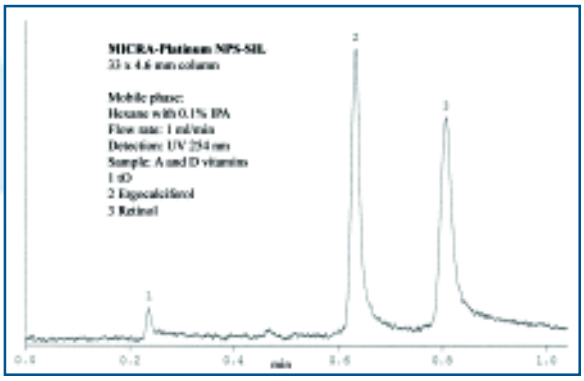
DNPH Aldehydes



NPS-Sil : normal phase

MICRA-Platinum Sil is a non-bonded phase on 1.5 non-porous spherical silica. ***NPS*** columns offer increased stability, short run times, high sensitivity, and greatly improved mass transfer. This column is excellent for high throughput and QC lab applications, as well as LC/MS separations. They are highly efficient for all normal phase applications and work particularly well with very polar compounds.

Vitamins A and D



ORDERING INFORMATION			
MICRA-Platinum		**ODS-TAS**	**ODS-Sil**
1.5µ *NPS* silica			
	Format (mm)	Item Number	Item Number
Standard	**33x4.6**	0446TAS101.5	0446NPS01.5
High Capacity	**53x4.6**	0646TAS101.5	

MICRA-Platinum *NPS* 1.5µ HPLC Columns

Information & Operating Instructions For Optimum Results

Silica Surface Bonded Phase Descriptions

ODS-I, II and IIIE have similar surface C-18 ligand densities. ODS-I is more stable in low and high pH environments due to increased surface shielding provided by the polymeric surface chemistry. The ODS-II is a monomeric C18 silane resulting in a more accessible silica surface making it more hydrophilic in nature with improved selectivity between closely related polar components. ODS-IIIE is an endcapped version of ODS-II . It provides excellent peak shape and is well suited for basic drug and pharmaceutical analysis. 100% aqueous mobile phases can be used with all of these phases. The 33x4.6 mm columns yield an approximate plate efficiency of 5000 plates/column on standard LC systems. Column loading capacities is 5000ng/column. For optimum efficiencies, use injection volumes of 2µl.

Particle Size Considerations

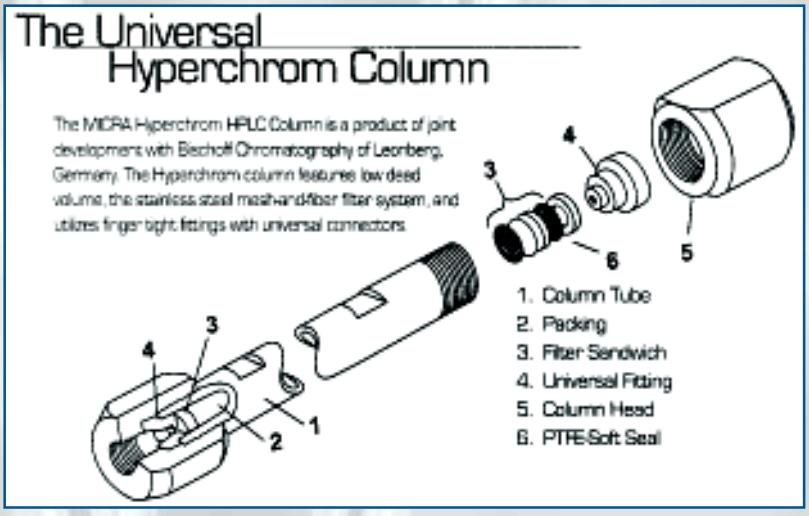
Eichrom's 1.5µ ***NPS*** columns offer increased speed of analysis, increased resolution of small samples, decreased solvent consumption, and equilibration time savings for routine analytical work. These columns are compatible with conventional equipment, however, a t_0 of <0.35ml is recommended for obtaining excellent efficiency and resolution. Due to the smaller surface area, total carbon load on the ***NPS*** C18 columns is approximately 6%. Additionally, the smaller particle size does result in typical operating pressures of 250 bar at 1.0ml/min flow rates (50% water/50% ACN) for the 33x4.6mm columns. The column void volume of a standard 5µ porous column is 70% of the empty column volume. The column void volume of Eichrom's ***NPS*** columns is 30% of the empty column volume. The highly uniform sphere size provides uniform interstitial voids, thus, no channeling, improved reproducibility, low column dead volume, flat van Deemter plot, and higher stability. Typical bare silica properties are as follows ; Fe <5ppm, Ca <10ppm, K <10ppm, Na < 10ppm, Al <10ppm, surface area <3 m^2/g, density 2.0g/ml, % residual carbon <1000 mass ppm, % residual nitrogen <1000 mass ppm.

Mobile Phase Instructions

- Mobile phase must be filtered through a 0.2µ filter prior to use.
- Samples should be filtered through a 0.2µ filter prior to injection.
- HPLC water with less than 20% organic must be changed daily.
- Ensure constant temperatures throughout the analysis.
- Start with 1/3 the amount of organic modifier compared to a standard porous column.
- Typical flow rates are 0.5 to 1.5ml/min.
- Minimum equilibration volume is 2.75ml.
- Keep mobile phase buffers between a pH of 2-9, ODS-I columns can a tolerate pH of 10.

Hardware and Detector Instructions

For routine analysis, excellent results can be obtained with flow cells of 15µl or less. Use 0.010 I.D or smaller tubing connections between injector and detector to minimize dispersion effects. If using RI detectors, minimum heat exchanger volumes are needed for use with column. Use a precolumn filter between the injector and column inlet. DO NOT use a guard column.



Storage and Usage

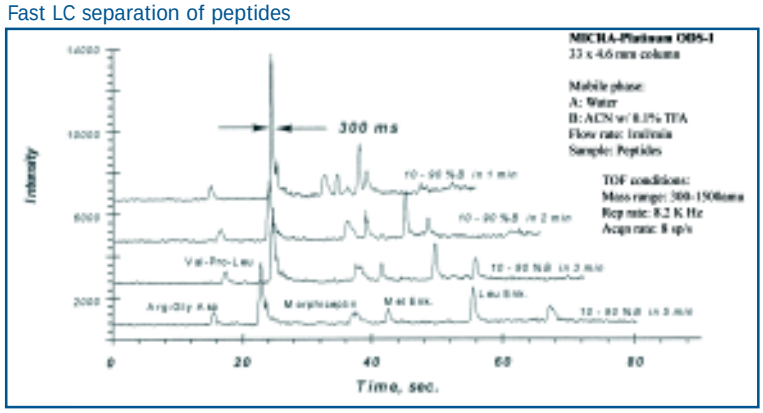
Reversed phase columns are shipped in 20% ACN. Before storing, rinse columns with water followed by methanol or acetonitrile. Storage solvents should contain at least 20% organic. Avoid storage at a pH greater than 7 and less than 5.

MICRA-Platinum Reversed Phase HPLC columns

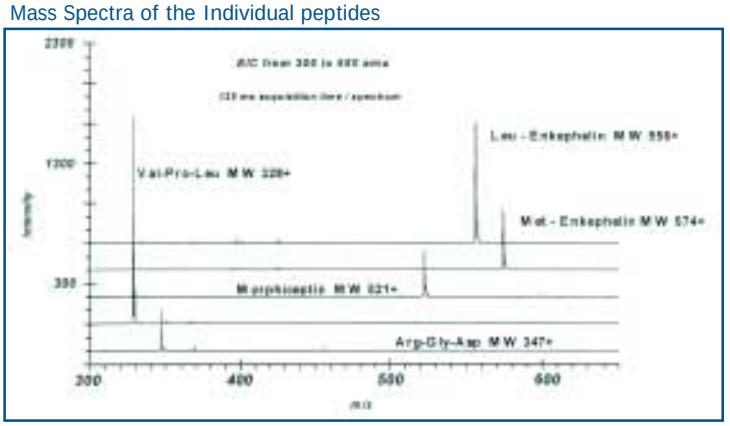
Applications in LC/MS

MICRA ***NPS*** technology provides the opportunity for a new TOF-based LC-MS system which rapidly resolves and identifies all of the individual components present while maintaining high peak capacity. As shown below, Analytica of Brandford's MS-Enterprise can analyze complex mixtures in well under 1 minute with elution time as narrow as 300 ms as shown below. Fast LC-ESI/MS analysis of herbicides using ***NPS*** ODS-I has been developed by American Cyanamid CO., Princeton, N J . For additional information on these procedures, request application notes and AP14, AP21, and/or reference the article: **Rapid Peptide Mapping by RP-LC on Non Porous Silica with On-Line Electrospray Time-of-Flight Mass Spectrometry**, Banks J . F. and Gulciek E., Anal. Chem., 69, (19), 3973-8, (1997).

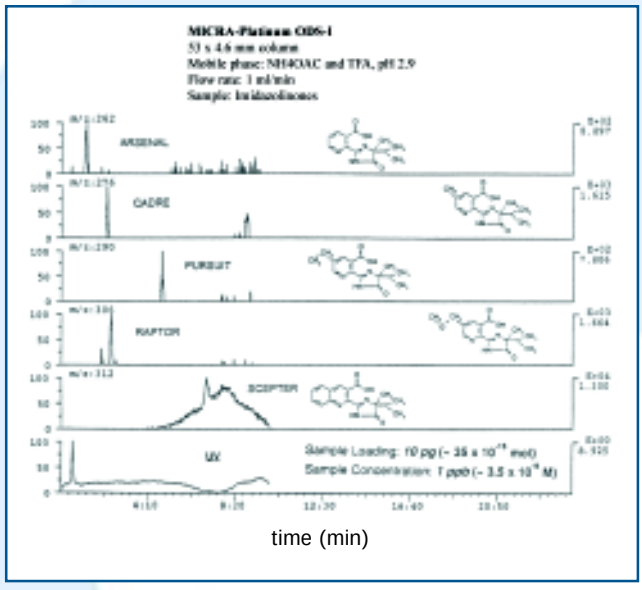
Fast LC separation of peptides



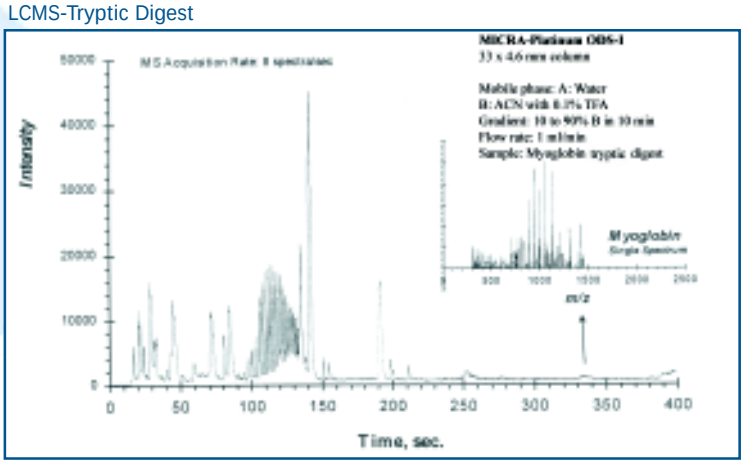
Mass Spectra of the Individual peptides



LCMS-Herbicides



LCMS-Tryptic Digest

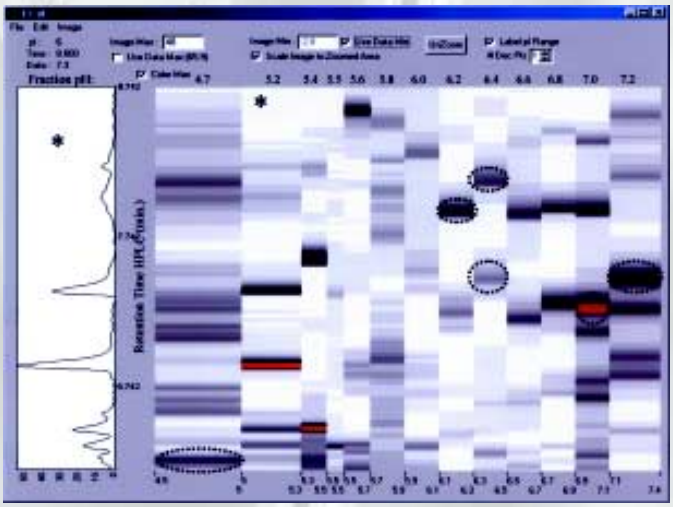


Applications in Proteomics : A novel 2D liquid phase separation method for protein mapping

The time consuming and labor intensive method to measure and quantify protein expression in cells, 2-D polyacrylamide gel electrophoresis (PAGE), is now replaceable by a novel method using ***NPS*** technology. (For additional information, request application note: AP34).

The alternative to 2-D PAGE involves a first step of separating proteins from the human erythroleukemia (HEL) cell line by pI using Rotofor (Biorad) Isoelectric Focusing (IEF) (horizontal axis) followed by a gradient HPLC separation based on hydrophobicity (vertical axis) using MICRA-Platinum ***NPS*** ODS-I. This digital image, shown below, is obtained from the UV (214 nm) chromatographic output converted to an ASCII format using a 256 step gray scale. This method of presenting the data is designed to offer the same advantages of pattern recognition and protein profiling obtained using 2-D PAGE. The entire procedure is outlined in **Isoelectric Focusing Nonporous RP HPLC : A Two-Dimensional Liquid-Phase Separation Method for Mapping of Cellular Proteins with Identification Using MALDI-TOF Mass Spectrometry**, Wall, D.B., *et.al, Anal. Chem.*, 72, (6), 1099-1111, (2000). A copy of this article and several others on protein mapping is available from Eichrom upon request.

Protein mapping has also been performed by classical 2D-HPLC using cation or anion exchange columns in the first dimension and ***NPS*** columns in the second dimension. This system offers high-resolution protein separation with a total analysis time of less than 20 min, equivalent to the run time of the first dimension. Ref.: **Protein mapping by two-dimensional HPLC**, Wagner K., Racaityte K., Unger K. K., Miliotis T., Edholm L. E., Bischoff R. and Marko-Varga, *J. Chromatogr. A*, **893**, 293-305, (2000)



NPS advantages over PAGE

Analysis times
Hours vs. Days
Eliminates labor intensive aspects of PAGE
Works well with whole cell lysates

MS Interface
Readily automated with sample collectors
Proteins isolated in liquid phase
Low chemical background noise

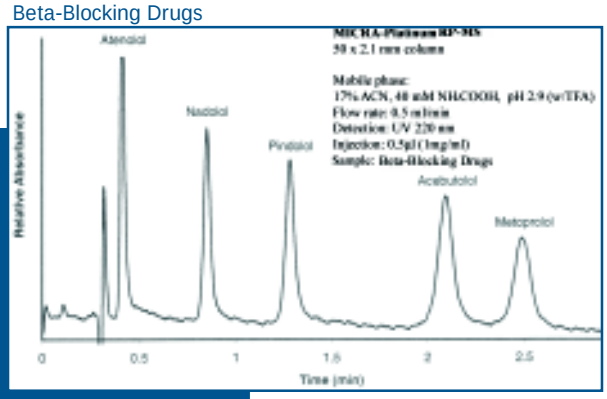
High protein recovery
Minimal non-specific adsorption using ***NPS***
High loadability for protein isolation, purification or sequencing
Superior sensitivity for low MW proteins

MICRA-Platinum Reversed Phase HPLC columns

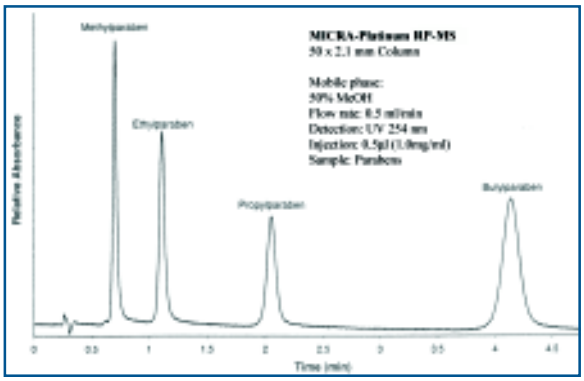
3µ, 100Å, porous silica: C18, RP-MS phase

RP-MS is a C18 endcapped, 3 micron support designed for high throughput analysis and LC/MS applications. Typical mixtures of small molecules are resolved such as amino acids, peptides, nucleotides and water-soluble vitamins. Examples below include a fast isocratic separation of β-blocking drugs performed on a 50x2.1mm column and an analysis of parabens is done in less than 4.5 min with the same size column. Application notes on these samples are available by requesting AP29 and AP30 respectively. Additional application notes using MICRA-Platinum RP-MS include AP30 for analgesics and anti-inflammatory compounds as well as AP32 for tricyclic antidepressants (TCA), and diazepams.

Beta-Blocking Drugs



Parabens



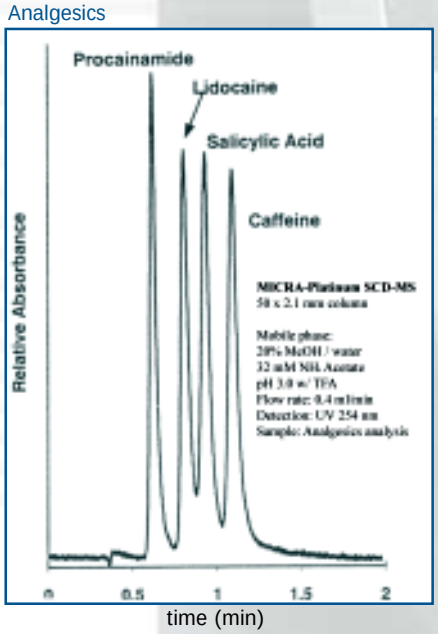
ORDERING INFORMATION		
MICRA-Platinum RP-MS 100 Å , 3µ		
	Format	Item Number
	(mm)	
LC-MS	**50x2.1**	MB2CR101-3-5
Standard	**100x4.6**	CR101-3-10
	150x4.6	CR101-3-15
Standard Guard	**50x4.6**	FCR101-3-5
Guard cartridges 3/pkg	**10x3.2**	KR101-1
Stainless Steel Guard holder		KART-G

MICRA-Platinum Reversed Phase HPLC columns

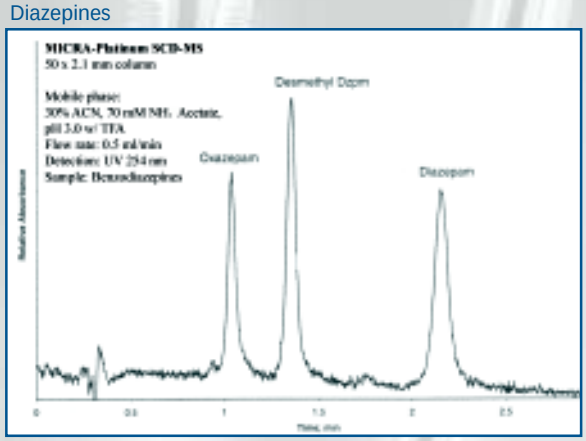
3µ, 100Å, porous silica: short chain base-deactivated, SCD-MS phase

SCD-MS is a short chain base-deactivated support designed for drug mixtures. These columns combine the unique selectivity of the SynChropak SCD-100 bonded phase with 3 particles, in a small column format ideal for fast MS applications. The SCD unique base deactivation produces excellent peak shape for drugs with high "pK_a"s. The lower level of hydrophobicity decreases the amount of organic modifier needed.

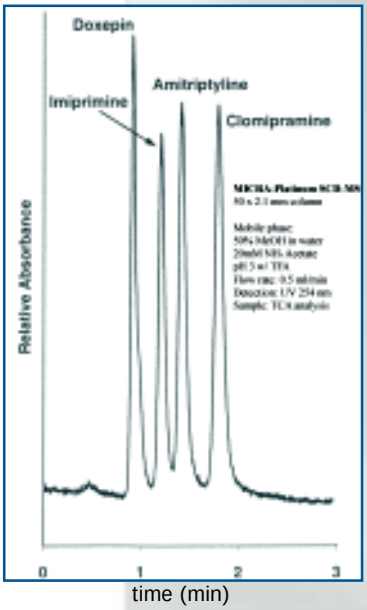
Analgesics



Diazepines



TCA analysis



ORDERING INFORMATION		
MICRA-Platinum SCD-MS	100 Å , 3µ	
	Format (mm)	Item Number
LC-MS	50x2.1	MB2SCD100-3-5
Standard	50x4.6	SCD100-3-5
	100x4.6	CSCD100-3-10
	150x4.6	CSCD100-3-15
Standard Guard	50x4.6	FSCD100-3-5
Guard cartridges 3/pkg	10x3.2	KSCD100-1
Stainless Steel Guard holder		KART-G

MICRA-Gold Reversed Phase HPLC Columns

C4 Bonding

The polymerically bonded, non-endcapped, C4 supports are a popular choice for protein and peptide separations, especially for large hydrophobic solutes. C4 ligands are often used with protein separationss due to their excellent recovery.

MICRA-Gold RP4

5µ, 300Å, Spherical silica

This 300Å pore size is optimal for the loading and high resolution of peptides.

MICRA-Gold RP4 1000

7µ , 1000Å, Spherical silica

Decreased surface area of RP4 supports results in faster analysis and increased recovery of small samples.

MICRA-Gold RP4 4000

10µ , 4000Å, Spherical silica

This 4000Å support is especially popular for very large hydrophobic solutes. It delivers high speed analysis and excellent sample recovery.

C8 Bonding

MICRA-Gold RP8 1000

7µ , 1000Å, Spherical silica

The monomerically bonded C8 support is suited for separating smaller proteins and peptides, especially those of a less hydrophobic nature.The decreased surface area of this non-endcapped, 1000Å, support results in faster analysis and increased recovery of small samples.

C18 Bonding

The monomerically bonded C18 supports offer high retention and superior selectivity.

MICRA-Gold RPP 100

5µ, 100Å, Spherical silica

This 100Å support is endcapped and offers excellent results for the separation of amino acids, small peptides, nucleotides, and other small molecules such as water-soluble vitamins. (See page 22 for part numbers)

MICRA-Gold RPP 1000

7µ , 1000Å, Spherical silica

This 1000Å support is ideal for the separation of small peptides, nucleotides, and amino acids. Decreased surface area results in faster analysis and increased recovery of small samples.

MICRA-Gold RPP 4000

10∝ , 4000Å, Spherical silica

RPP 4000, similar to RPP 1000 in separation results, offers higher speed and compatability with larger hydrophobic solutes.

ORDERING INFORMATION

MICRA-Gold Reversed Phase HPLC Columns

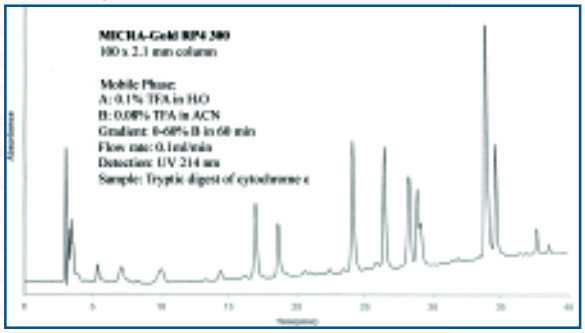
		RP4 300	RP4 1000	RP4 4000	RP8 1000	RPP 1000	RPP 4000
		300 Å , 5µ	1000 Å, 7µ	4000 Å , 10µ	1000 Å , 7µ	1000 Å , 7µ	4000 Å , 10µ
	Format (mm)	Item Number	Item Number	Item Number	Item Number	Item Number	Item Number
Narrow-bore	100x2.1	MB2C4RG103-10	MB2C4R110-10	MB2C4R140-10	MB2C8R110-10	MB2CR110-10	MB2CR140-10
	250x2.1	MB2C4RG103-25			MB2C8R110-25	MB2CR110-25	MB2CR140-25
Narrow-bore Guard	50x2.1	MB2FC4RG103-5	MB2FC4R110-5	MB2FC4R140-5	MB2FC8R110-5	MB2FCR110-5	MB2FCR140-5
Standard	50x4.6		C4R110-5	C4R140-5			
	100x4.6	C4RG103-10	C4R110-10	C4R140-10	C8R110-10	CR110-10	CR140-10
	250x4.6	C4RG103-25	C4R110-25	C4R140-25	C8R110-25	CR110-25	CR140-25
Standard Guard	50x4.6	FC4RG103-5	FC4R110-5	FC4R140-5	FC8R110-5	FCR110-5	FCR140-5
Guard cartridges 3/pkg	10x3.2	K4RG103-1	K4R110-1	K4R140-1	K8R110-1	KR110-1	KR140-1
Stainless Steel Guard holder		KART-G	KART-G	KART-G	KART-G	KART-G	KART-G

For information on Reversed Phase Peptide Standards, see page 32.

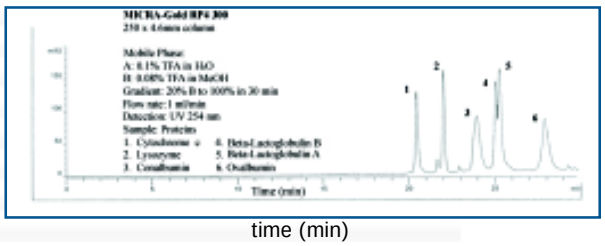
MICRA-Gold Reversed Phase Columns exhibit high stability and excellent resolution. Suggested applications include amino acids, nucleotides, peptides, and proteins.

Pore size	Optimum MW	Surface area
100Å	<2,000	250m²/g
300Å	*<70,000*	*100m²/g*
1000Å	*<300,000*	*20m²/g*
4000Å	*<1000,000*	*6m²/g*

Protein Digests

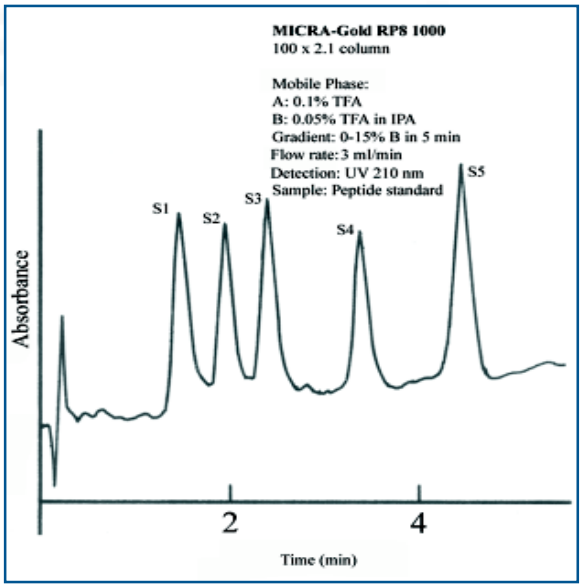


Protein Mixture

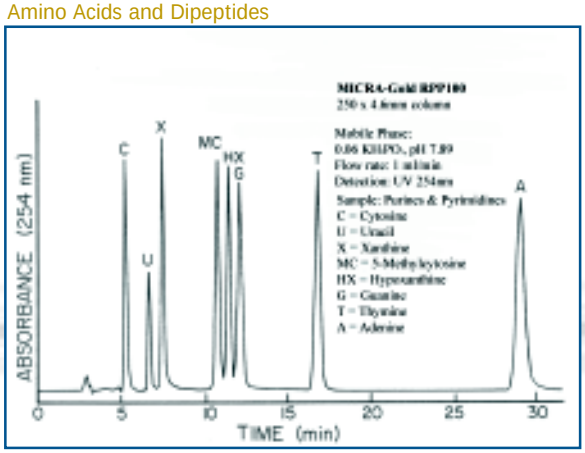


time (min)

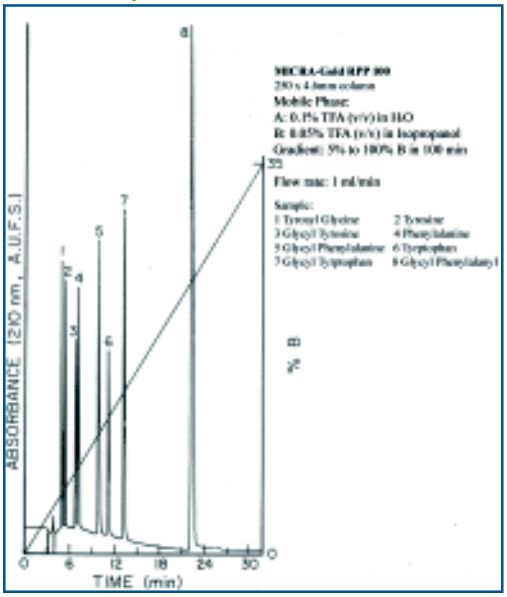
Peptide Standard



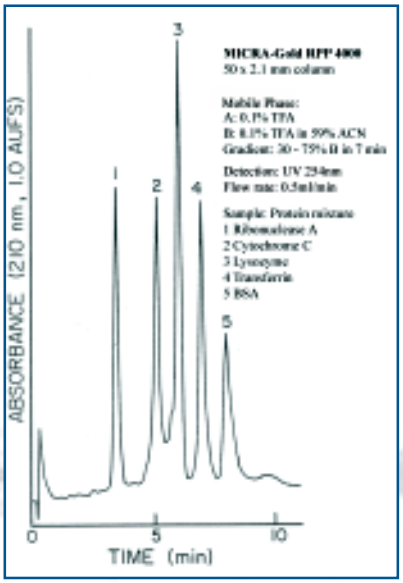
Amino Acids and Dipeptides



Purines and Pyrimidines



Protein Mixture



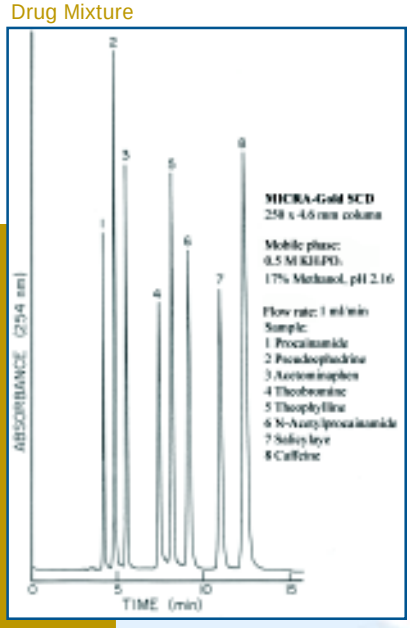
MICRA-Gold Specialty Reversed Phase HPLC Column

MICRA-Gold SCD

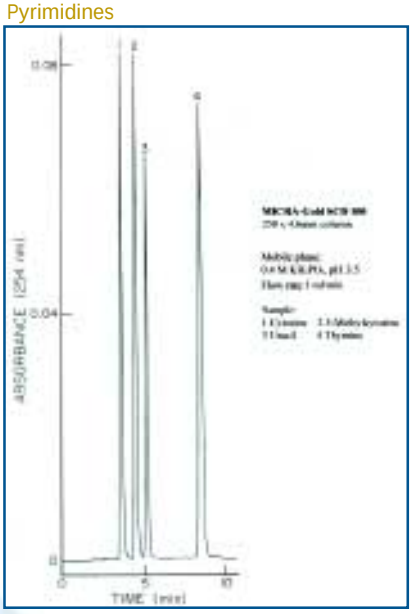
5µ , 100Å, Spherical silica

This uniquely deactivated reversed phase support is bonded with a short alkyl chain ligand and is endcapped. Excellent peak shapes are obtained for many drug mixtures without the addition of silanol suppressing agents. MICRA-Gold SCD is ideal for the analysis of positively charged molecules, as well as neutral and acidic molecules.

Drug Mixture



Pyrimidines



ORDERING INFORMATION			
MICRA-Gold Reversed Phase HPLC Columns			
		RPP 100	**SCD 100**
		100 Å , 5µ	**100 Å, 5µ**
	Format (mm)	Item Number	Item Number
Narrow-bore	**100x2.1**	MB2CR101-10	MB2SCD100-10
	250x2.1	MB2CR101-25	MB2SCD100-25
Narrow-bore Guard	**50x2.1**	MB2FCR101-5	MB2FSCD100-5
Standard	**100x4.6**	CR101-10	SCD100-10
	150x4.6	CR101-15	SCD100-15
	250x4.6	CR101-25	SCD100-25
Standard Guard	**50x4.6**	FCR101-5	FSCD100-5
Semi-Prep	**250x10**	PCR101-25	
Semi-Prep Guard	**50x10**	PFCR101-5	
Guard cartridges 3/pkg	**10x3.2**	KR101-1	KSCD100-1
Stainless Steel Guard holder		KART-G	KART-G

MICRA-Gold Ion Exchange HPLC Columns

Cation Exchange Phases

MICRA-Gold Cation exchange columns exhibit excellent resolution, high loading capacity, compatibility with nonionic detergents and organic solvents, and offer high recovery of biological activity. Suggested applications include proteins, enzymes, nucleotides, peptides, hemoglobins, and catecholamines.

WCX

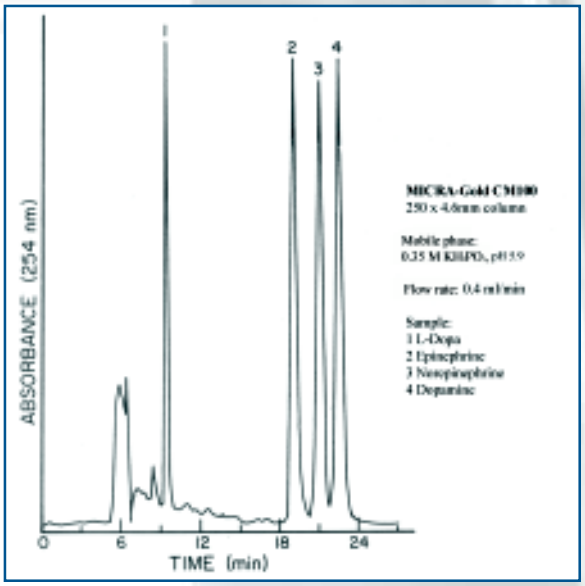
MICRA-Gold CM100

5µ, 100Å, Spherical Silica

This weak cation exchanger has a polyamide coating containing carboxymethyl groups and is compatible with aqueous and many organic solvents in the pH range of 2-8. Selectivity can be altered by mobile phase salt composition and pH affects ionization of both the support and the solute. This support is a superb choice for the separation of small cationic compounds such as catecholamines.

CM (WCX) Specifications for Solute Interaction				
Pore Size	**Optimum MW**	**Surface Area**	**Absolute Loading Cap.**	**CM (WCX) I E Capacity**
100Å	<10,000	250 m^2/g	34 mg/ml	65mg hemoglobin
				per gram support

Catecholamines



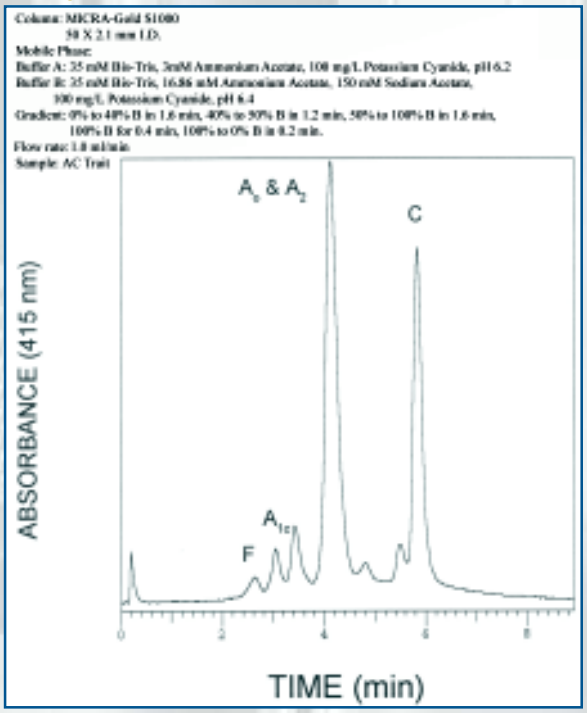
SCX

MICRA-Gold S1000

7µ, 1000Å, Spherical silica

This strong cation exchanger has a polyamide coating containing sulfonicpropyl functional groups. It is compatible with aqueous and many organic solvents in the pH range of 2-8. Ionization of just the solute is affected by pH above pH3. This is an excellent column for the fast analysis of glycosylated hemoglobins.

Hemoglobin



ORDERING INFORMATION			
MICRA-Gold Cation Exchange HPLC Columns			
		CM 100	**S 1000**
		100 Å, 5µ	**1000 Å, 7µ**
	Format (mm)	Item Number	Item Number
Narrow-bore	**50x2.1**		MB2CS110-5
	100x2.1	MB2CCM101-10	MB2CS110-10
	250x2.1	MB2CCM101-25	MB2CS110-25
Narrow-bore Guard	**50x2.1**	MB2FCCM101-5	MB2FCS110-5
Standard	**100x4.6**	CCM101-10	CS110-10
	250x4.6	CCM101-25	CS110-25
Standard Guard	**50x4.6**	FCCM101-5	FCS110-5
Semi-Prep	**250x7.8**	SPCCM101-25	SPCS110-25
	250x10	PCCM101-25	PCS110-25
Semi-Prep Guard	**50x7.8**	SPFCCM101-5	SPFCS110-5
	50x10	PFCCM101-5	PFCS110-5
Guard cartridges 3/pkg	**10x3.2**	KCM101-1	KS110-1
Stainless Steel Guard holder		KART-G	KART-G

MICRA-Gold Ion Exchange HPLC Columns

Anion Exchange Phases

WAX

MICRA-Gold AX100

5µ , 100Å, Spherical silica

This weak anion exchanger is a crosslinked polyethyleneimine support compatible with aqueous and many organic solvents in the pH range of 2-8. Selectivity can be altered by mobile phase composition and pH affects ionization of both the support and the solute. Similar to AX300, this 100Å support provides a high surface area for the separation of small molecules. It provides excellent separations of nucleotides, acidic molecules and oligonucleotides of up to 30 residues.

MICRA-Gold AX1000

7µ , 1000Å, Spherical silica

This weak anion exchanger is a crosslinked polyethyleneimine phase compatible with aqueous and many organic solvents in the pH range of 2-8. Selectivity can be altered by mobile phase composition and pH affects ionization of both the support and the solute. It is particularly well suited for the separation of proteins larger than 200,000 daltons such as estrogen receptor isoforms.

SAX

MICRA-Gold Q100

5µ, 100Å, Spherical silica

Q100 is a strong anion exchange, quaternized crosslinked polyethyleneimine support. It is compatible with aqueous and many organic solvents in the pH range of 2-8. Ionization of this support has no pH dependence. This 100Å packing material is an excellent choice for the rapid separation of peptides, small proteins and small anionic particles such as nucleotides.

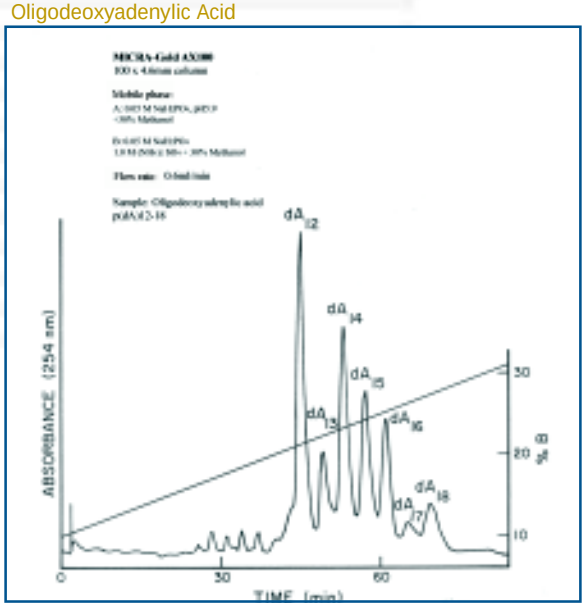
ORDERING INFORMATION				
MICRA-Gold Anion Exchange HPLC Columns				
		AX 100	AX 1000	Q 100
		100 Å , 5µ	1000 Å, 7µ	100 Å , 5µ
	Format (mm)	Item Number	Item Number	Item Number
Narrow-bore	100x2.1	MB2CA101-10	MB2CA110-10	MB2CQ101-10
	250x2.1		MB2CA110-25	MB2CQ101-25
Narrow-bore Guard	50x2.1	MB2FCA101-5	MB2FCA110-5	MB2FCQ101-5
Standard	100x4.6	CA101-10	CA110-10	CQ101-10
	250x4.6	CA101-25	CA110-25	CQ101-25
Standard Guard	50x4.6	FCA101-5	FCA110-5	FCQ101-5
Semi-Prep	250x7.8	SPCA101-25	SPCA110-25	SPCQ101-25
	250x10	PCA101-25	PCA110-25	
Semi-Prep Guard	50x7.8	SPFCA101-5	SPFCA110-5	SPFCQ101-5
	50x10	PFCA101-5	PFCA110-5	
Guard cartridges 3/pkg	10x3.2	KA101-1	KA110-1	KQ101-1
Stainless Steel Guard holder		KART-G	KART-G	KART-G

MICRA-Gold Ion Exchange HPLC Columns

MICRA-Gold Anion Exchange columns exhibit excellent resolution, high loading capacity, and compatibility with nonionic detergents and organic solvents. Suggested applications include proteins enzymes, nucleotides, chromatofocusing, and receptor purifications.

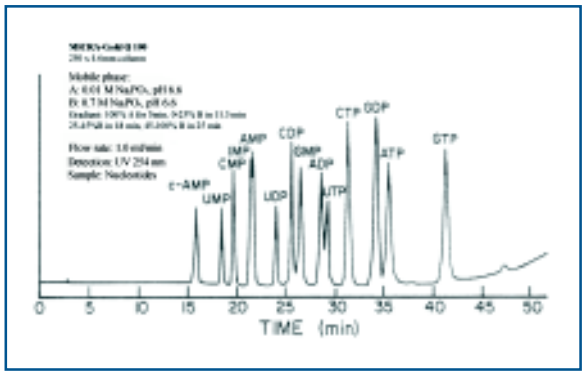
AX (WAX) Specifications for Solute Interaction				
Pore Size	**Optimum MW**	**Surface Area**	**Absolute Loading Cap.**	**AX (WAX) I E Capacity**
100Å	<10,000	250 m^2/g	34 mg/ml	30mg hemoglobin
				per gram support
1000Å	<300,000	20m^2/g	38 mg/ml	

Oligodeoxyadenylic Acid

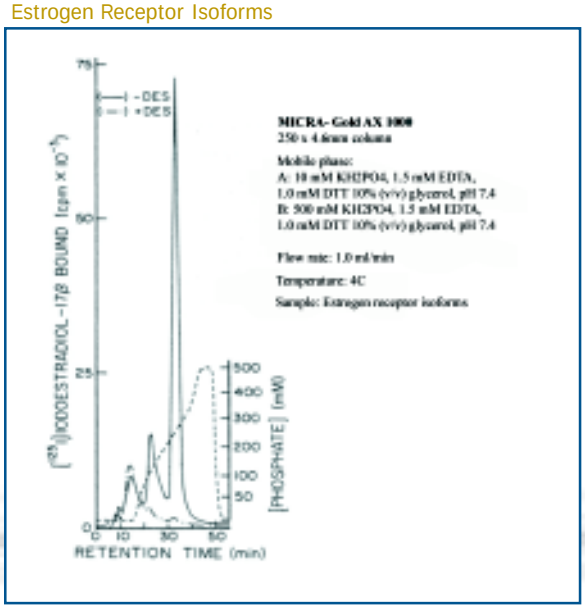


Organic Acid

Nucleotides



Estrogen Receptor Isoforms



MICRA-Gold Size Exclusion (GPC) HPLC Columns

The MICRA-Gold GPC series is derived from a glycerol bonded size exclusion support. Products are offered in seven pore diameters (50Å-4000 Å). This diversity in pore size allows for the analysis of solutes with molecular weights ranging from 0.5 kDa to 10,000 kDa. These supports have minimal interaction with anionic and neutral water-soluble polymers.

Pore sizes ranging from 100Å to 500Å are popular for the rapid analysis of proteins and smaller water-soluble polymers.

MICRA-Gold GPC100
5µ , 100Å
Appropriate for globular molecules with MW ranges from 5-160 kD and linear molecules with MW ranges from 0.5-25 kD

MICRA-Gold GPC300
5µ , 300Å
Appropriate for globular molecules with MW ranges from 10-1000 kD and linear molecules with MW ranges from 2-100 kD.

MICRA-Gold GPC500
7µ , 500Å
Appropriate for globular molecules with MW ranges from 40-2000 kD and linear molecules with MW ranges from 10-350 kD.

For effectively analyzing very large polymers by size use the 1000Å or 4000Å pore size.

MICRA-Gold GPC1000
7µ , 1000Å
GPC1000 is most frequently used with linear molecules possessing MW ranges from 40-1000 kD. It also allows for the separation of globular molecules such as protein multimers with MW ranges from 40-1,000 kD.

MICRA-Gold GPC4000
10µ , 4000Å
This 4000 Å support is appropriate for linear molecules possessing MW ranges from 70-10,000 kD

ORDERING INFORMATION

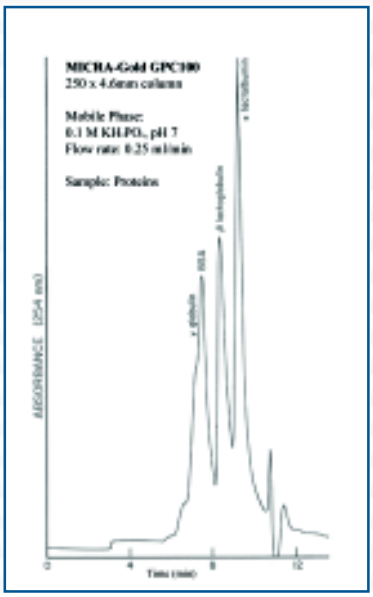
MICRA-Gold Size Exclusion HPLC Columns

		GPC 100	GPC 300	GPC 500	GPC 1000	GPC 4000
		100 Å , 5µ	300 Å, 5µ	500 Å , 7µ	1000 Å , 7µ	4000 Å , 10µ
	Format (mm)	Item Number	Item Number	Item Number	Item Number	Item Number
Standard	250x4.6	CG101-25	CG103-25	CG105-25	CG110-25	CG140-25
Standard Guard	50x4.6	FCG101-5	FCG103-5	FCG105-5	FCG110-5	FCG140-5
Semi-Prep	300x7.8	SPCG101-30	SPCG103-30	SPCG105-30	SPCG110-30	SPCG140-30
	250x10	PCG101-25	PCG103-25	PCG105-25	PCG110-25	PCG140-25
Semi-Prep Guard	50x7.8	SPFCG101-5	SPFCG103-5	SPFCG105-5	SPFCG110-5	SPFCG140-5
	50x10	PFCG101-5	PFCG103-5	PFCG105-5	PFCG110-5	PFCG140-5
Guard cartridges 3/pkg	10x3.2	KG101-1	KG103-1	KG105-1	KG110-1	KG140-1
Stainless Steel Guard holder		KART-G	KART-G	KART-G	KART-G	KART-G

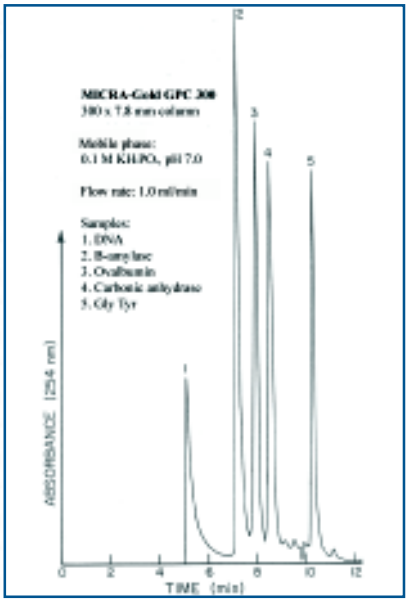
MICRA-Gold Size Exclusion (GPC) HPLC Columns

Suggested applications for Eichrom's MICRA-Gold GPC series include: peptides, proteins, carbohydrates, nucleic acids, anionic polymers and neutral polymers. MICRA-Gold GPC offers excellent resolution while maintaining biological activity. The columns are compatible with aqueous solvents (pH 2-8), surfactants, and many organic solvents including dimethylformamide, tetrahydrofuran and ethanol.

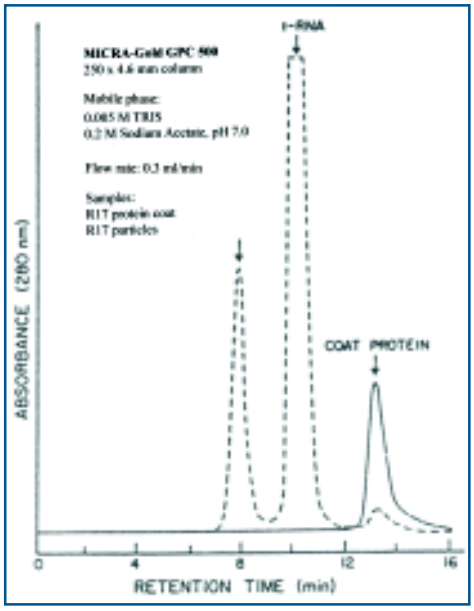
Protein Mixture



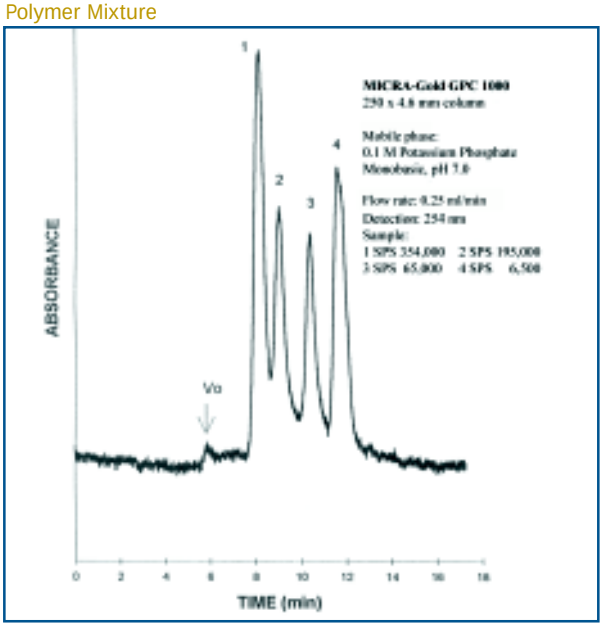
Protein Mixture



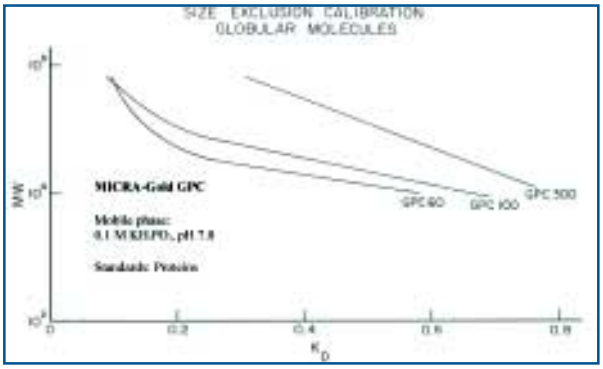
Viral Coat Protein



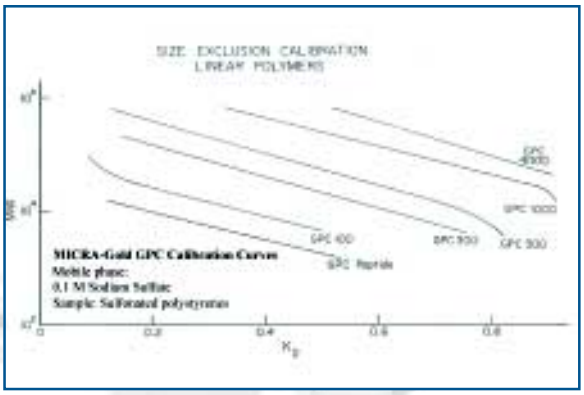
Polymer Mixture



Globular Molecules Calibration Curves



Linear Molecules Calibration Curves



Molecular Weight Range

Globular Molecules (proteins)		Linear Molecules (polymers)	
GPC Peptide	8.0×10^2 to 3.5×10^4	GPC Peptide	5.0×10^2 to 1.0×10^4
GPC100	5.0×10^3 to 1.6×10^5	GPC100	5.0×10^2 to 2.5×10^4
GPC300	1.0×10^4 to 1.0×10^6	GPC300	2.0×10^3 to 1.0×10^5
GPC500	4.0×10^4 to 1.0×10^6	GPC500	1.0×10^4 to 3.5×10^5
GPC1000	4.0×10^5 to 1.0×10^7	GPC1000	4.0×10^4 to 1.0×10^6
		GPC4000	7.0×10^4 to 1.0×10^7
		GPC Linear	1.0×10^3 to 1.0×10^6

MICRA-Gold Size Exclusion Specialty CATSEC (Cationic-polymer) HPLC Columns

Unique to Eichrom Technologies, MICRA-Gold CATSEC is a series of size exclusion supports designed specifically for analysis of cationic polymers.

The polymerized polyamine coating enables polymers such as polyvinylpyridines to elute according to size and without adsorption. Mobile phases are generally acidic and contain 0.1-0.2M salt to minimize adsorption and ion-exclusion. Supports are available in four pore diameters (100Å-4000Å) allowing analysis of solutes with molecular weights from 0.5 kDa to 10,000 kDa.

MICRA-Gold CATSEC 100
100Å, 5µ.
This support is appropriate for separating linear molecules with molecular weight ranges of 0.5-25 kD.

MICRA-Gold CATSEC 300
300Å, 5µ
The 300Å support is appropriate for linear molecules with a MW range of 2-100 kD.

MICRA-Gold CATSEC 1000
1000Å, 7µ
Designed specifically for high molecular weight ranges, this support is appropriate for MW ranges of 40-1,000 kD.

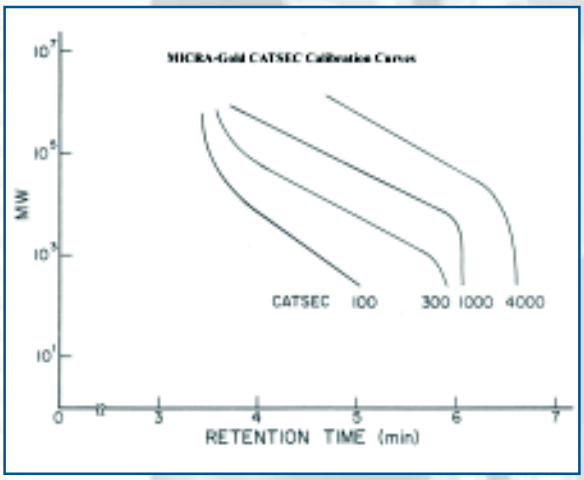
MICRA-Gold CATSEC 4000
4000Å, 10µ
CATSEC 4000 is appropriate for high molecular weight ranges of 70-10,000 kD.

ORDERING INFORMATION					
MICRA-Gold CATSEC HPLC Columns					
		CATSEC100	CATSEC300	CATSEC1000	CATSEC4000
		100 Å , 5µ	300 Å, 5µ	1000 Å , 7µ	4000 Å , 10µ
	Format (mm)	Item Number	Item Number	Item Number	Item Number
Standard	250x4.6	CCS201-25	CCS203-25	CCS210-25	CCS240-25
Standard Guard	50x4.6	FCCS201-5	FCCS203-5	FCCS210-5	FCCS240-5
Semi-Prep	300x7.8	SPCCS201-30	SPCCS203-30	SPCCS210-30	SPCCS240-30
	250x10	PCCS201-25	PCCS203-25	PCCS210-25	PCCS240-25
Semi-Prep Guard	50x7.8	SPFCCS201-5	SPFCCS203-5	SPFCCS210-5	SPFCCS240-5
	50x10	PFCCS201-5	PFCCS203-5	PFCCS210-5	PFCCS240-5
Guard cartridges 3/pkg	10x3.2	KCS201-1	KCS203-1	KCS210-1	KCS240-1
Stainless Steel Guard holder		KART-G	KART-G	KART-G	KART-G

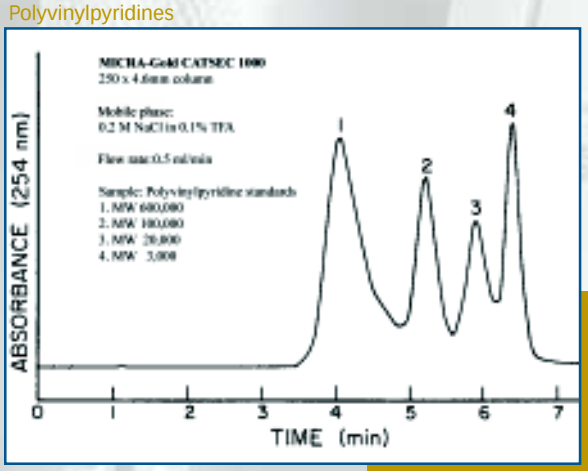
MICRA-Gold Size Exclusion Specialty CATSEC (Cationic-polymer) HPLC Columns

Suggested applications for Eichrom's unique CATSEC columns include SEC of cationic charged polymers and analysis of basic proteins.

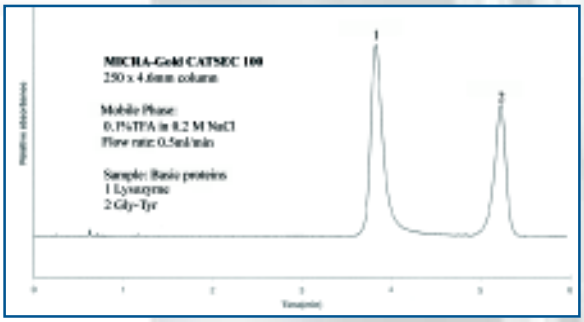
CATSEC Calibration Curves



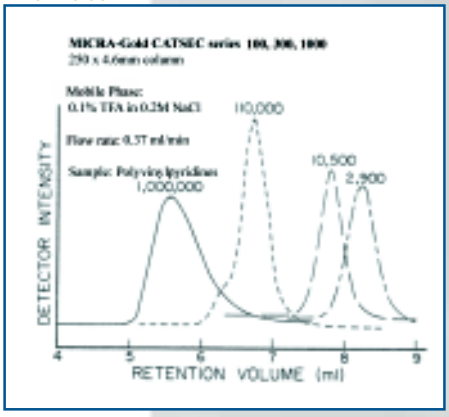
Polyvinylpyridines



Basic Proteins



Polyvinylpyridines



MICRA-Gold Size Exclusion (GPC)
Specialty GPC PEP, GPC Linear HPLC Columns

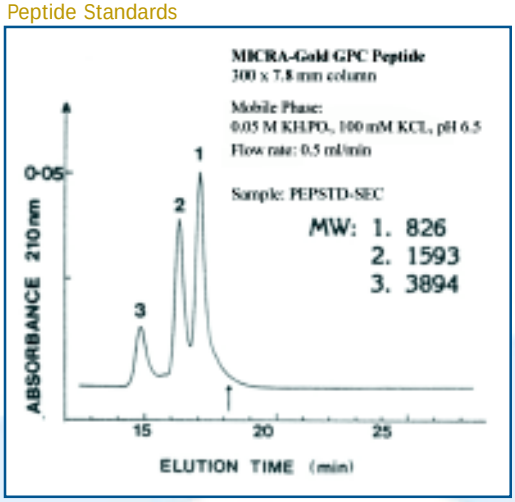
MICRA-Gold GPC Peptide
50Å , 5µ

This is a glycerol bonded size exclusion support which can resolve small peptides (MW 0.8-30 kD). It is successful in resolving peptides which have at least a two-fold MW difference. Mobile phase optimization may be necessary due to the high variability in solubility, charge, and hydrophobicity of peptides. GPC Peptide also works well as a “desalting column”. The exclusion limit for GPC Peptide makes it an effective column for rapid desalting of protein or buffer exchange samples, particularly in the 100mm length format.

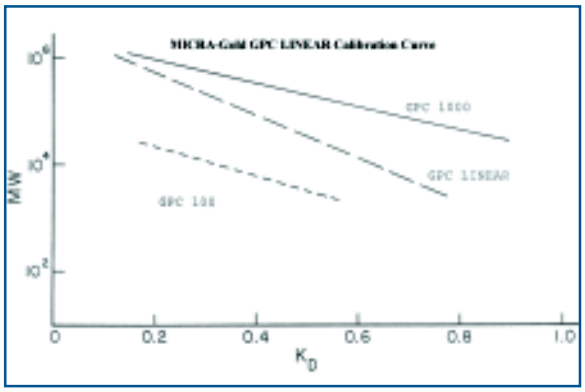
MICRA-Gold GPC Linear
100/1000Å , 7µ

GPC Linear is a glycerol bonded size exclusion support with a mixed pore distribution allowing analysis of samples with a broad range of molecular weights. This support, designed to effectively analyze samples by size, has minimal interaction with anionic and neutral water-soluble polymers. It is appropriate for organic polymers and denatured proteins possessing MW ranges from 1-1000 kD.

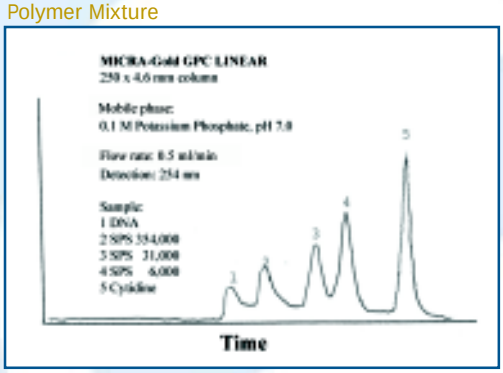
Peptide Standards



GPC Linear Calibration Curves



Polymer Mixture



ORDERING INFORMATION			
MICRA-Gold Size Exclusion GPC PEP and GPC LINEAR HPLC Columns			
		GPC PEPTIDE	**GPC LINEAR**
		50 Å , 5µ	**100 Å, 5µ/1000 Å, 7µ**
	Format (mm)	Item Number	Item Number
Standard	100x4.6	CGPEP-10	
	250X4.6	CGPEP-25	CG111-25
Standard Guard	50x4.6	FCGPEP-5	FCG111-5
Semi-Prep	300x7.8	SPCGPEP-30	SPCG111-30
	100x10	PCGPEP-10	
	250x10	PCGPEP-25	PCG111-25
Semi-Prep Guard	50x7.8	SPFCGPEP-5	SPFCG111-5
	50x10	PFCGPEP-5	PFCG111-5
Guard cartridges 3/pkg	10x3.2	KGPEP-1	KG111-1
Stainless Steel Guard holder		KART-G	KART-G
Item number for Size Exclusion peptide standard: PEPSTD-SEC			

MICRA-Silver HIC HPLC Column

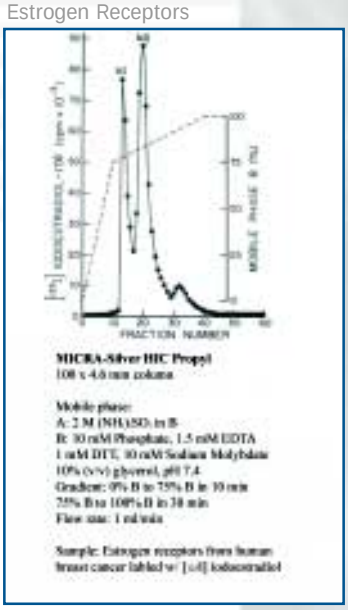
Hydrophobic Interaction Chromatography (HIC) is a mild method which binds surface amino acids of a protein to alkyl ligands on a support. HIC uses descending salt gradients for elution, preserving the tertiary structure of the proteins and their enzymatic activity.

MICRA-Silver HIC-Propyl
300Å, 6µ Spheroidal silica

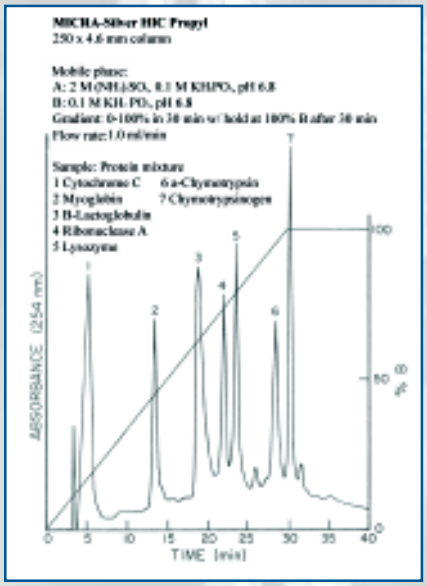
MICRA-Silver HIC-Propyl is a C3, polymerically bonded support. It is compatible with aqueous solvents with a pH range of 3-8, surfactants, and many organic solvents such as methanol and ethylene glycol. HIC- Propyl has a high loading capacity (15-35mg ovalbumin on a 250x4.6mm column) and excellent resolution of proteins as well as smaller molecules such as nucleotides.

Suggested Applications for MICRA-Silver HIC Propyl Include: labile proteins, receptors, nucleotides, and separations requiring the recovery of biological activity.

Estrogen Receptors



Protein Mixture



ORDERING INFORMATION		
MICRA-Silver Hydrophobic Interaction HPLC Columns		
		HIC Propyl
		300 Å , 6µ
	Format (mm)	Item Number
Standard	100x4.6	CH103-10
	250X4.6	CH103-25
Standard Guard	50x4.6	FCH103-5
Guard cartridges 3/pkg	10x3.2	KH103-1
Stainless Steel Guard holder		KART-G

MICRA-Silver Reversed Phase HPLC Columns

C4

MICRA-Silver RP4

6µ, 300Å, Spheroidal silica

RP4 is a polymerically bonded, non-endcapped, support with a C4 ligand. C4 is a popular choice for protein and peptide separations especially for large hydrophobic solutes. The 300Å pore size is optimal for the loading and high resolution of peptides. C4 ligands are often used for protein separations due to their excellent recovery.

C8

MICRA-Silver RP8

6µ, 300Å, Spheroidal silica

RP8 is a monomerically bonded, non-endcapped, support with a C8 ligand. As the carbon chain length has little effect on selectivity in reversed phase chromatography of peptides and proteins, MICRA-Silver RP8 is suited for separating smaller proteins and peptides, especially those of a less hydrophobic nature.

C18

MICRA-Silver RPP

6µ, 300Å, Spheroidal silica

RPP is a monomerically bonded, non-endcapped, support with a C18 ligand. Due to their greater retention and superior selectivity, C18 supports are commonly used for the separation of smaller peptides, low hydrophobicity proteins, nucleotides and amino acids.

RP Peptide Standard

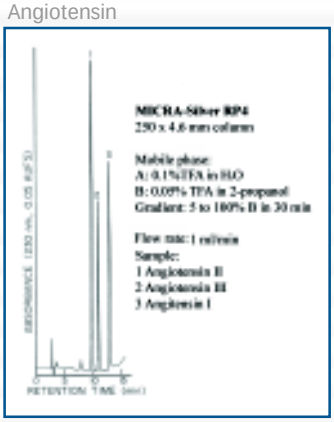
A five component standard used for monitoring column performance and predicting peptide retention. The vial contains 1mg each of 5 C-terminal amide decapeptides. One peptide contains a free N-amino group. The other peptides are N-acetylated with the sequence variation as follows: Gly_3-Gly_4, Ala_3-Gly_4, Val_3-Val_4, Val_3-Gly_4. 100 injection size is available.

ORDERING INFORMATION				
MICRA-Silver Reversed Phase HPLC Columns				
		RP4	**RP8**	**RPP**
		300 Å , 6µ	**300 Å , 6µ**	**300 Å , 6µ**
	Format (mm)	Item Number	Item Number	Item Number
Narrow-bore	**100x2.1**	MB2C4R103-10	MB2C8R103-10	MB2CR103-10
	250x2.1	MB2C4R103-25	MB2C8R103-25	MB2CR103-25
Narrow-bore Guard	**50x2.1**	MB2FC4R103-5	MB2FC8R103-5	MB2FCR103-5
Standard	**100x4.6**	C4R103-10	C8R103-10	CR103-10
	250x4.6	C4R103-25	C8R103-25	CR103-25
Standard Guard	**50x4.6**	FC4R103-5	FC8R103-5	FCR103-5
Semi-Prep	**250x10**	PC4R103-25	PC8R103-25	PCR103-25
Semi-Prep Guard	**50x10**	PFC4R103-5	PFC8R103-5	PFCR103-5
Guard cartridges 3/pkg	**10x3.2**	K4R103-1	K8R103-1	KR103-1
Stainless Steel Guard holder		KART-G	KART-G	KART-G
Item number for Reversed Phase Peptide Standard: PEPSTD-1				

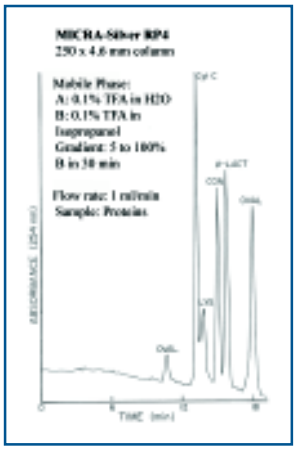
MICRA-Silver Reversed Phase HPLC Columns

Suggested Applications for MICRA-Silver Reversed Phase Columns Include: proteins, peptides, oligonucleotides, glycoproteins, antibodies, peptide hormones, insulin variants, tryptic digests, biopolymers, membrane proteins, and ribosomal proteins.

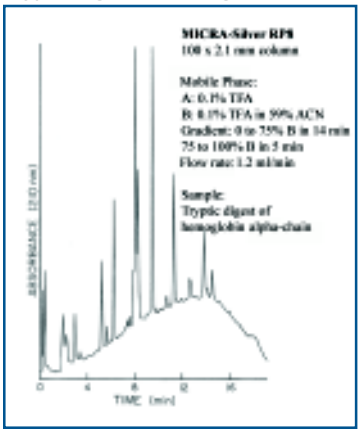
Angiotensin



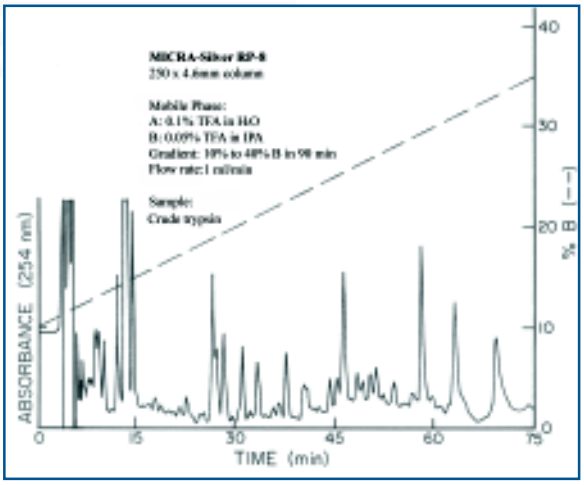
Protein Mixture



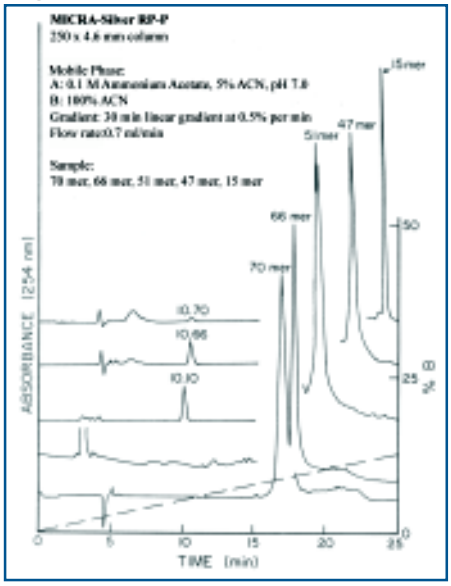
Tryptic Digest of Hemoglobin



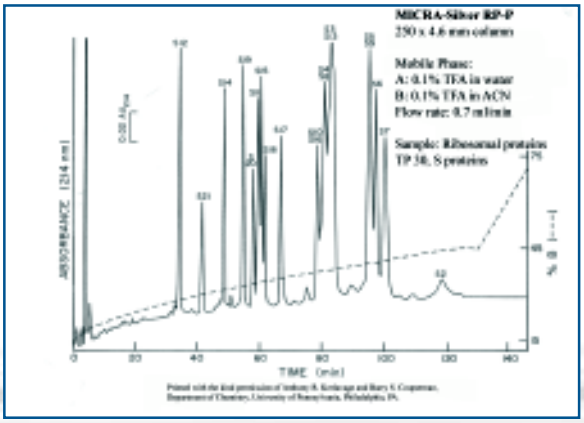
Crude Trypsin



Oligonucleotides



Ribosomal Proteins



MICRA-Silver Anion Exchange HPLC Columns

MICRA-Silver AX300

6µ, 300Å, Spheroidal silica

This weak anion exchanger is a crosslinked polyethyleneimine support. It is compatible with aqueous and many organic solvents in the pH range of 2-8. Selectivity can be altered by mobile phase composition, and pH affects ionization of both the support and the solute. AX300 permits analysis of proteins up to molecular weights of 200,000 daltons. AX300 has excellent recoveries and loading capacity. For example, 22mg ovalbumin can be loaded onto a 250x4.6 column with no overloading effects. Chromatofocusing can also be performed on AX300 due to its broad titration curves and high buffering capacity.

MICRA-Silver Q300

6µ, 300Å, Spheroidal silica

This strong anion exchanger is a quaternized, crosslinked polyethyleneimine support. It is compatible with aqueous and many organic solvents in the pH range of 2-8. Ionization of Q300 has no pH dependence. This support is an excellent column for the rapid separation of proteins and enzymes.

AX (WAX) Specifications for Solute Interaction

Pore Size	Optimum MW	Surface Area	Absolute Loading Cap.	I E Capacity
300Å	<200,000	100m^2/g	80 mg/ml	30mg hemoglobin per gram support

ORDERING INFORMATION

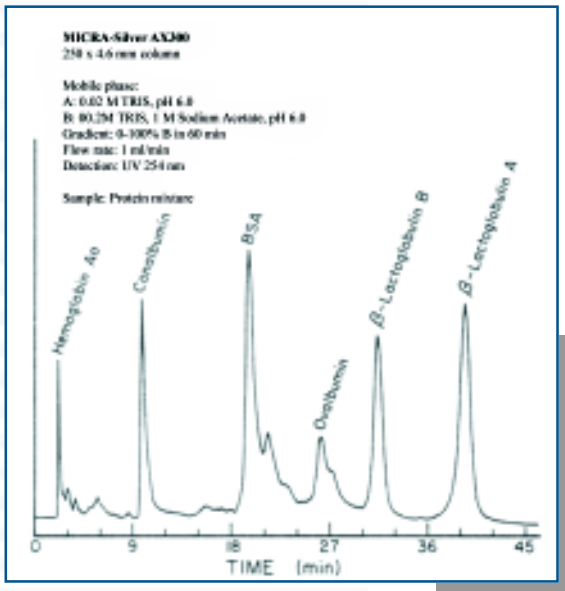
MICRA-Silver Anion Exchange HPLC Columns

		AX300	Q300
		300 Å , 6µ	300 Å, 6µ
	Format (mm)	Item Number	Item Number
Narrow-bore	100x2.1	MB2CA103-10	MB2CQ103-10
	250x2.1	MB2CA103-25	MB2CQ103-25
Narrow-bore Guard	50x2.1	MB2FCA103-5	MB2FCQ103-5
Standard	100x4.6	CA103-10	CQ103-10
	250x4.6	CA103-25	CQ103-25
Standard Guard	50x4.6	FCA103-5	FCQ103-5
Semi-Prep	250x7.8	SPCA103-25	SPCQ103-25
	250x10	PCA103-25	PCQ103-25
Semi-Prep Guard	50x7.8	SPFCA103-5	SPFCQ103-5
	50x10	PFCA103-5	PFCQ103-5
Guard cartridges 3/pkg	10x3.2	KA103-1	KQ103-1
Stainless Steel Guard holder		KART-G	KART-G

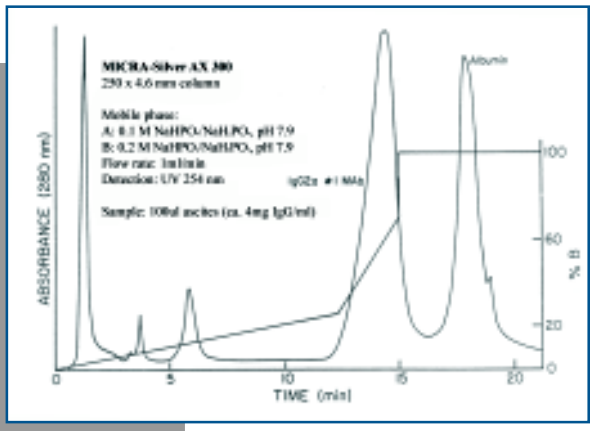
MICRA-Silver Anion Exchange HPLC Columns

MICRA-Silver Anion Exchange columns offer high resolution and loading capacity as well as excellent recovery of biomolecules. Suggested applications include the analysis of enzymes, proteins, nucleotides, oligonucleotides, chromatofocusing, carbohydrates, isoenzymes, isoforms of hormone receptor proteins, phosphorylated sugars, immunoglobulins and enzyme assays.

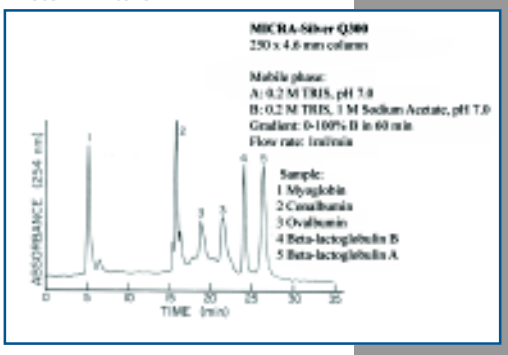
Protein Mixture



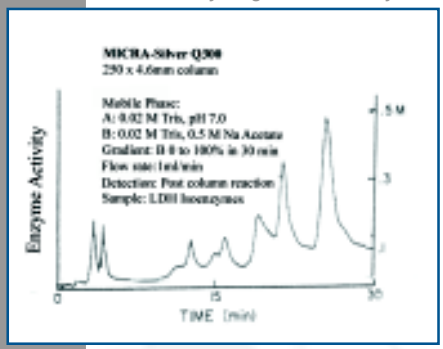
Ascite Protein



Protein Mixture



Lactate Dehydrogenase Isoenzymes



MICRA-Silver Cation Exchange HPLC Columns

MICRA-Silver CM300
6µ, 300Å, Spheroidal silica

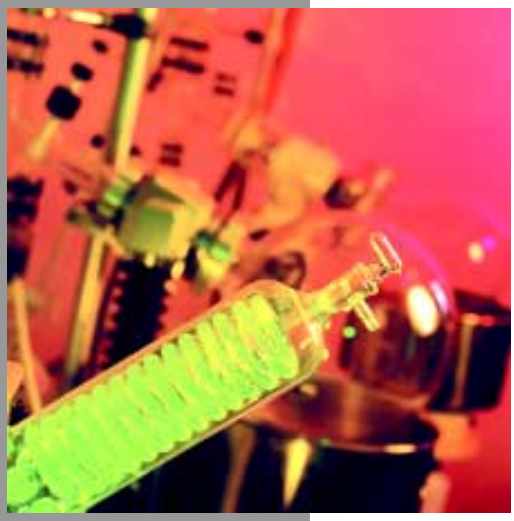
This weak cation exchanger has a polyamide coating containing carboxymethyl groups. It is compatible with aqueous and many organic solvents in the pH range of 2-8. Selectivity can be altered by mobile phase salt composition, and pH affects ionization of both the support and the solute. CM300 permits analysis of proteins up to molecular weights of 200,000 daltons and offers excellent recoveries as well as high loading capacity.

MICRA-Silver S300
6µ, 300Å, Spheroidal silica

This strong cation exchanger has a polyamide coating containing sulfopropyl functional groups. It is compatible with aqueous and many organic solvents in the pH range of 2-8. Ionization of the functional groups on this bonded phase is constant above pH 3. S300 is a good choice for separations of basic proteins.

CM (WCX) Specifications for Solute Interaction

Pore Size	Optimum MW	Surface Area	Absolute Loading Cap.	I E Capacity
300 Å	<200,000	100m^2/g	80 mg/ml	65mg hemoglobin per gram support



ORDERING INFORMATION

MICRA-Silver Cation Exchange HPLC Columns

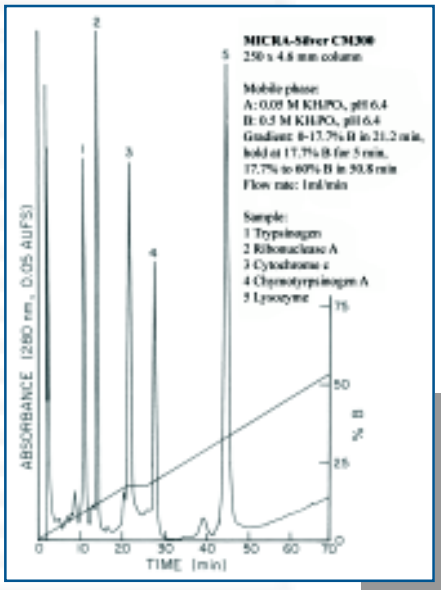
		CM300	S300
		300 Å, 6µ	300 Å, 6µ
	Format (mm)	Item Number	Item Number
Narrow-bore	100x2.1	MB2CCM103-10	MB2CS103-10
	250x2.1	MB2CCM103-25	MB2CS103-25
Narrow-bore Guard	50x2.1	MB2FCCM103-5	MB2FCS103-5
Standard	100x4.6	CCM103-10	CS103-10
	250x4.6	CCM103-25	CS103-25
Standard Guard	50x4.6	FCCM103-5	FCS103-5
Semi-Prep	250x7.8	SPCCM103-25	SPCS103-25
	250x10	PCCM103-25	PCS103-25
Semi-Prep Guard	50x7.8	SPFCCM103-5	SPFCS103-5
	50x10	PFCCM103-5	PFCS103-5
Guard cartridges 3/pkg	10x3.2	KCM103-1	KS103-1
Stainless Steel Guard holder		KART-G	KART-G

Item number for Cation Exchange Peptide Standard: PEPSTD-CX

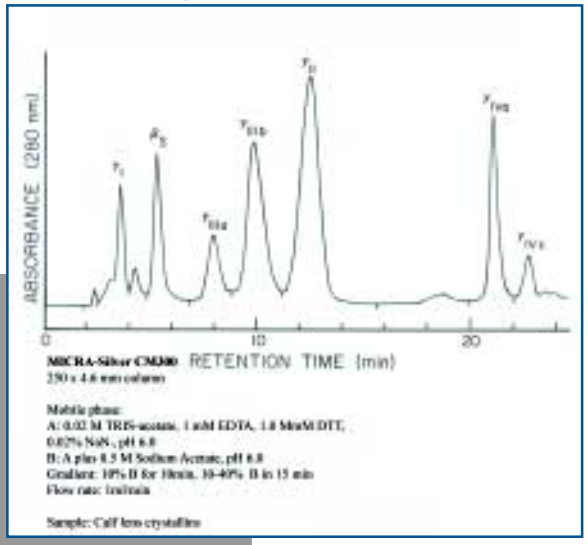
MICRA-Silver Cation Exchange HPLC Columns

MICRA-Silver Cation Exchange columns offer high resolution and loading capacity as well as excellent recovery of biomolecules. Suggested applications include the analysis of enzymes, proteins, catecholamines, peptides, hemoglobin variants, glycosylated hemoglobins, and crystallins.

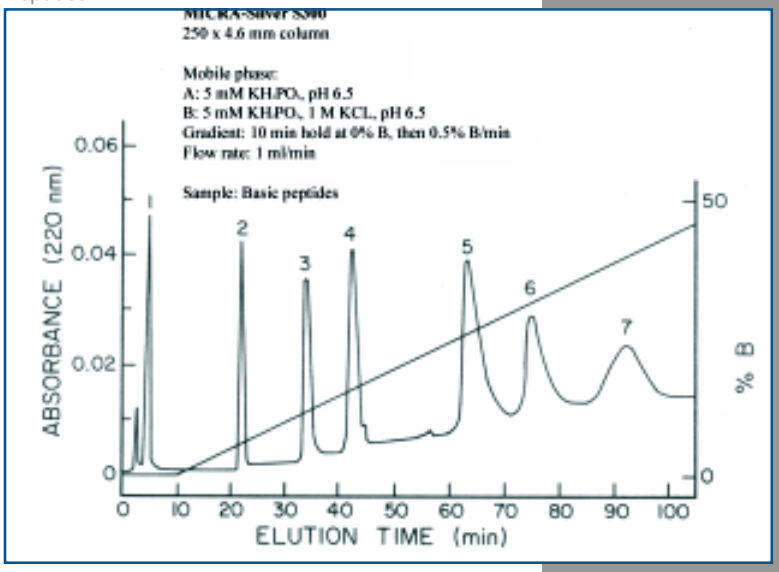
Protein Mixture



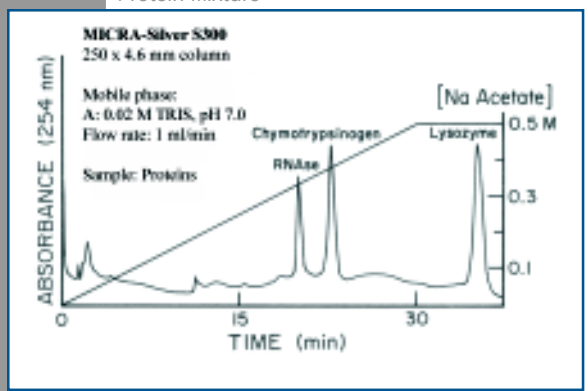
Calf Monomeric Crystallins



Peptides



Protein Mixture



Technical Information Section

Column Use Guidelines

Upon receipt of your HPLC column:

- Examine the label for part number, format, and phase verification.
- Examine the box as well as the column for any signs of physical damage that may have occurred during shipping.
- Test the column for quality verification. Each column is tested prior to packaging and is shipped with a QC performance chromatogram.

Eichrom guarantees the performance of its HPLC columns. To insure quality, all Eichrom Technologies HPLC columns are individually tested and shipped with a copy of the specific QC performance chromatogram, along with a usage protocol sheet. All columns are labeled with the column name, format, catalog number, and unique serial number identifier.

Columns in sealed boxes can be returned by obtaining a return authorization number from Eichrom's customer service, and will be assessed a 15% restocking fee. Columns in unsealed boxes cannot be returned, unless they are defective. A QC authorization is needed prior to a defective product exchange.

Column Storage Considerations

Phase	**Storage Solvent**
(Porous Silica)	
Reversed Phase	
(SCD, RP4, RP8, RPP)	>20% Alcohol or Acetonitrile
Ion Exchange	
(AX, CM, S, Q)	>10% Alcohol
Size Exclusion	
(GPC)	>10% Methanol
(CATSEC)	>10% Methanol
***NPS* (Non-Porous Silica)**	
Reversed Phase	
(ODS-I, ODS-II, ODS-IIIE)	>20% organic (pH< or =7)
Normal Phase	
(Sil)	100% organic
Specialty Phase	
(TAS)	>20% organic (pH< or =7)

Using correct column storage conditions can increase column lifetime.
Never store columns with buffers or ion pair reagents. Always flush with 5-10 column volumes of mobile phase without buffers to completely remove remaining buffers or salts.

Stationary Phase Information

(Please reference page 15 for guidelines specific to columns packed with ***NPS*** non-porous silica.)

pH issues

Silica-based HPLC columns are sensitive to pH. Mobile phase pH should be maintained between 2.0 and 8.0 for porous supports, and between 2.0 and 9.0 on non-porous supports. Low pH (<2.0) can lead to hydrolysis of the bonded groups resulting in loss of phase. High pH (>8.0) can dissolve the silica forming voids and fines. For conditions requiring lower or higher mobile phase pH's, a presaturator column should be installed between the pump and the injector to minimize effects on the stationary phase.

Back Pressure Issues

To minimize column damage, porous columns should not be run in a continuous fashion at pressures higher than 4000psi. Pressures over 5000psi will result in damage. Columns with large pore sizes should be used at even lower pressures to prevent damage to the packing material. In contrast, the non-porous silica columns routinely operate at approximately 3500psi. These columns tolerate higher pressures, however, pressures over 6000psi can damage these columns as well. For both types of silica, avoid any sudden pressure fluctuations. If back pressures increase, flush the column by reversing the flow. Add a precolumn filter and a guard between the injector and the analytical column. When using an ***NPS*** column only employ the precolumn filter, as the use of a guard will greatly increase extra column effects and degrade the separation.

Mobile Phase Guidelines

Use only HPLC-Grade solvents due to the drastic effect impurities can have on the performance of HPLC columns. When changing solvents, insure that the solvents are miscible. Using immiscible solvents can damage the stationary phase. Salts or buffer precipitation can also damage the stationary phase. The sample should be soluble in the mobile phase, and when possible, the mobile phase should be the sample solvent.

Typical flow rates and load capacities

I.D. (mm)	Flow Rate (ml/min)	Dynamic Loading (mg protein/25cm length)		
		IEC	RPC	SEC
2.0	0.2	3.0	1.5	0.05
4.6	1.0	15	8	0.2
7.8	2.0	50	20	2.0
10.0	5.0	80	30	4.0

Column Cleaning Protocols

Reversed Phase Columns

Columns can be cleaned by washing with repeated gradients of 0.1% TFA in water to 100% IPA or ACN.

Non-Porous Reversed Phase Columns

Columns can be cleaned / flushed, by inverting the column and washing with repeated gradients of 0.1% TFA in water to 100% ACN. After cleaning, reinstall the column in the correct flow direction (as indicated by the arrow on column) and repeat the procedure.

Ion Exchange Columns

Columns can be cleaned by washing with at least 10 column volumes of 1M salt at the operating pH. If the column efficiency has not improved after the above treatment, the column should be further washed at a different pH between 2-8 with an intrinsically stronger salt and/or with 0.1% TFA and then 0.1% TFA and 10% IPA mixture, each for at least 10 column volumes.

Size Exclusion Columns

If components of the sample have become non-specifically adsorbed to the column, it can be cleaned with solvents that would remove them. Detergents, organic solvents or 0.1% TFA can be used.

Hydrophobic Interaction Columns

The column can be cleaned by washing with 10 column volumes of 0.05M potassium phosphate monobasic, pH7 in water, and then another 10 volumes of this mobile phase containing 10% IPA. If this procedure is not effective, the use of 0.1% TFA with or without 10% IPA may remove insoluble compounds.

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SynChropak® bonding chemistry

NPS® particle technology