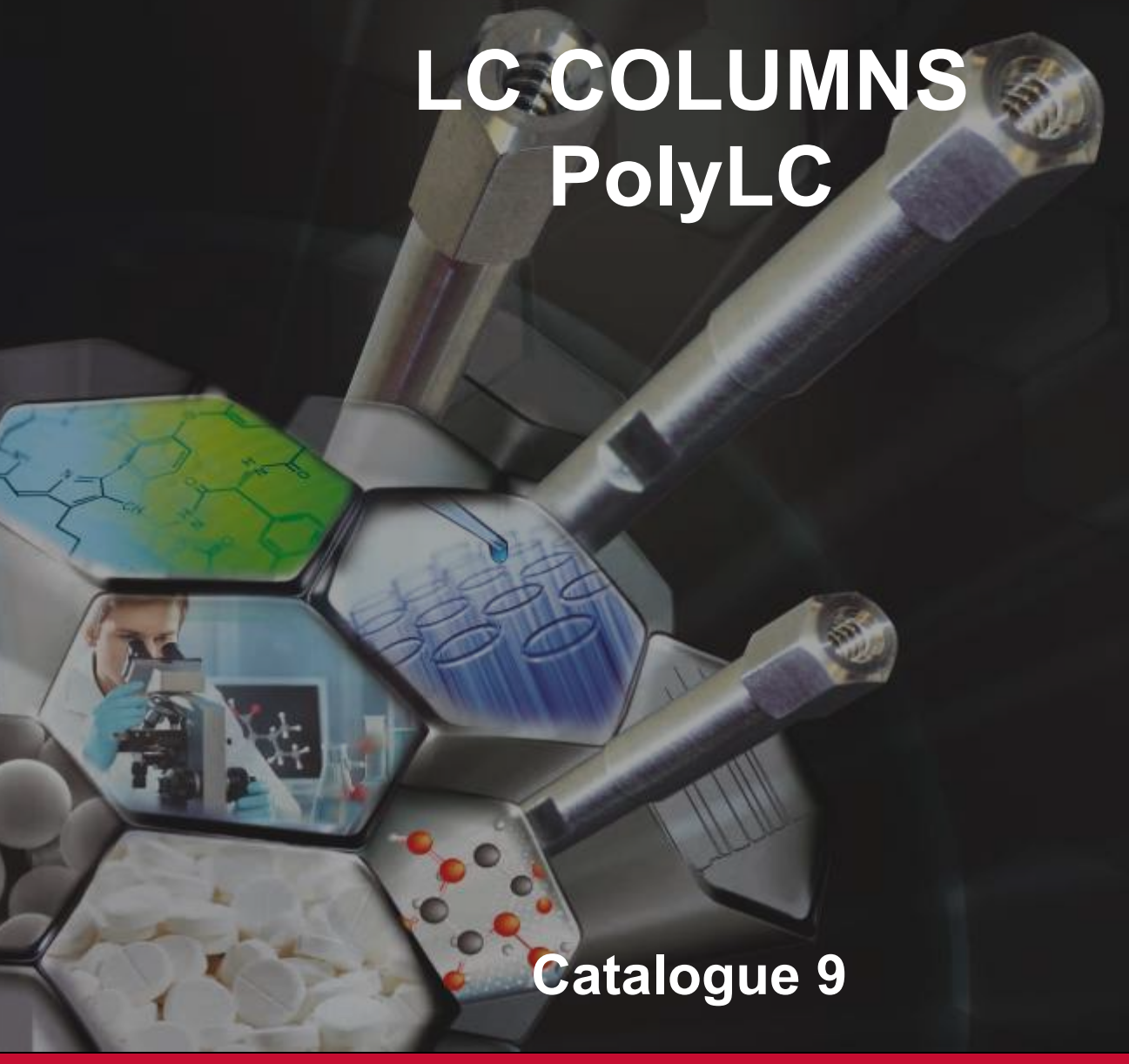
HICHROM

Chromatography Columns and Supplies

LC COLUMNS
PolyLC



Catalogue 9

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POLYLC

- Spherical silica, 60 - 1500Å pore size
- Polypeptide covalently bound coating
- High recovery for sensitive, labile proteins

PolyLC of Maryland, USA manufactures a range of unique columns for the more challenging HPLC biochemical applications. Their phases are characterised by the attachment of a polypeptide coating to wide pore silica.

PolyLC Phases

PolyLC Phase[1]	Particle Size (µm)	Pore Size (Å)	Functional Group	Chromatography Mode	Applications
PolyHYDROXYETHYL A™	3, 5, 12	60, 100, 200, 300, 500, 1000, 1500	Hydroxyethylaspartamide	1.Hydrophilic Interaction (HILIC) 2. Size Exclusion	Peptides, proteins, carbohydrates, polar small molecules
PolyCAT A™	3, 5, 12	300, 1000, 1500	Aspartic acid	Weak cation-exchange	Proteins with isoelectric point >6.0
PolyWAX LP™	3, 5, 12	100, 300, 1000, 1500	Linear polyethyleneimine	Weak anion-exchange	Proteins with isoelectric points <6.0, nucleic acids and oligonucleotide analogues
PolyGLYCOPLEX™	5, 12	-	-	Hydrophilic Interaction (HILIC)	Complex carbohydrates
PolySULFOETHYL A™	3, 5, 12	200, 300, 1000	Sulphoethylaspartamide	Strong cation-exchange	Peptides
PolyPROPYL A™	3, 5, 12	300, 1000, 1500	Propylaspartamide	Hydrophobic Interaction (HIC)	Proteins and peptides

[1] PolyMETHYL A™ and PolyETHYL A™ materials are also available

PolyHYDROXYETHYL A™

PolyHYDROXYETHYL A™ is a neutral polar material designed specifically for HILIC. Peptides and proteins are typically eluted with a decreasing gradient of acetonitrile or propanol for peptide mapping or multidimensional purification of synthetic and natural peptides. PolyHYDROXYETHYL A is also used for eliminating detergents, lipids and salts from samples and for the HPLC of solutes that are insoluble in aqueous media, such as membrane proteins. Figure 1 shows a typical chromatogram for the isolation of pure pathogenic prion protein from the brain of a sheep with scrapie.

Table 1.
PolyHYDROXYETHYL A - SEC fractionation ranges (Daltons)

Pore Diameter (Å)	Denaturing Eluent (e.g. 50mM formic acid)	Conventional Eluent (phosphate/sulphate buffer)
60	40-600	40-10,000
200	40-1600	200-25,000
300	40-40,000	300-100,000
500	40-150,000	400-300,000
1000	40-1,000,000	1000-2,000,000
1500	40-1,000,000	5000-2,000,000

In the absence of organic solvent, PolyHYDROXYETHYL A functions in the SEC mode. Using conventional salt buffers, the fractionation range is determined by the pore size of the packing (see Table 1). However, if the eluent contains a denaturing agent (eg. 50mM formic acid), smaller solutes can be separated by size. The 60Å pore size material permits the separation of peptides and other small solutes by SEC (Figure 2).

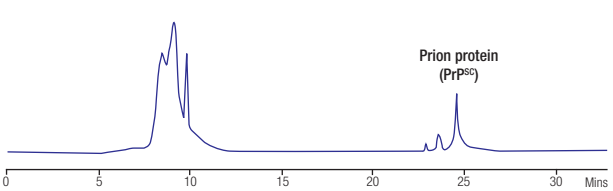


Figure 1. Extract of brain from sheep with scrapie (Proteinase K-treated)

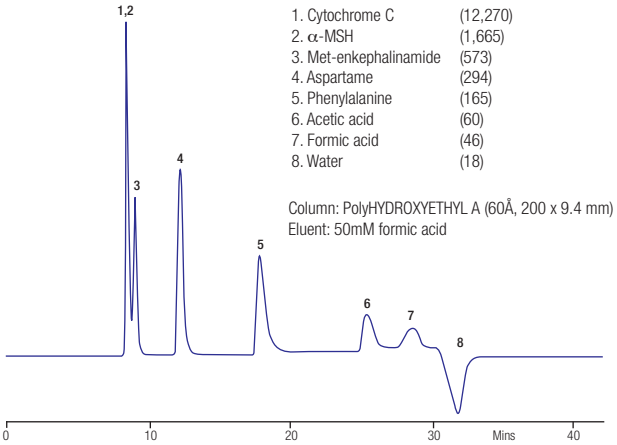
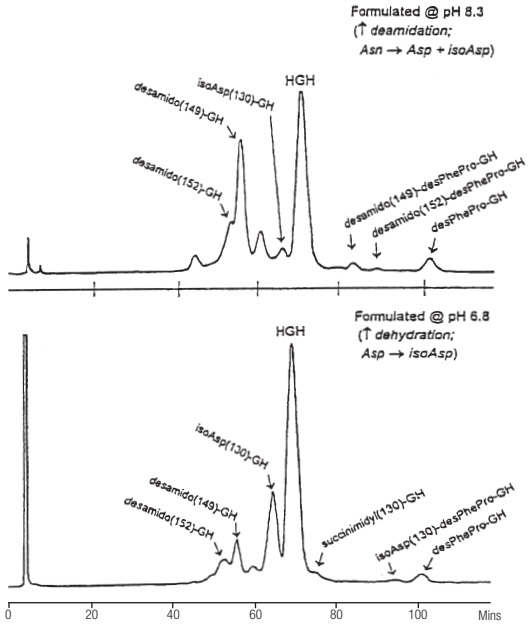


Figure 2. Size exclusion separation of small molecules

PolyCAT A™

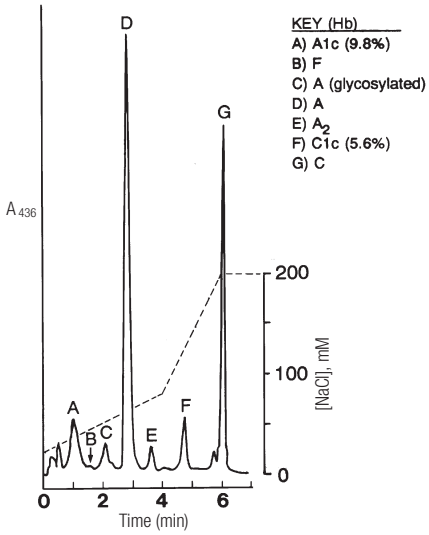
PolyCAT A™ consists of poly(aspartic acid) covalently bonded to silica. Proteins elute from the material by weak cation-exchange chromatography, resulting in high efficiency peaks and high binding capacity and recovery. PolyCAT A columns enable the separation and quantitation of many protein variants that differ by a single residue. Side products from the synthesis or degradation on storage of pharmaceutical proteins can effectively be analysed on a PolyCAT A column. Figure 3 shows the analysis of recombinant human growth hormone (HGH) incubated at two different pHs.

PolyCAT A columns are widely used in haemoglobin analyses, where all major and most minor variants are resolved (see Figure 4).



Column: PolyCAT A, 200 x 4.6mm (1000Å)
Eluent: 130-145mM NH_4acetate, pH 4.0 with 40% CH_3CN

Figure 3. Recombinant protein variant analysis -Human growth hormone after 6 days at 37°C



Column: PolyCAT A, 35 x 4.6mm (5µm, 1000Å)
Eluent: Gradient of increasing [NaCl]

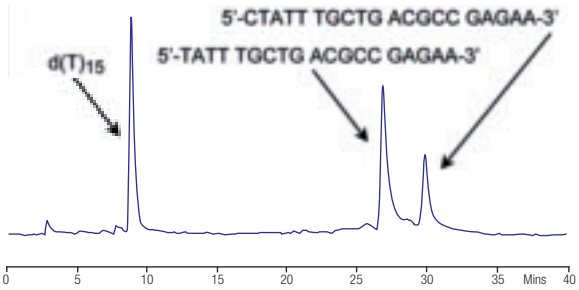
Figure 4. Analysis of diabetic haemoglobin (C trait)

PolyWAX LP™

PolyWAX LP™ is a hydrophilic weak anion-exchange (WAX) material prepared with linear polyethyleneimine (PEI), which confers greater selectivity and recovery than conventional branched polymer material. It was developed for the analysis of enzymes and other proteins. PolyWAX LP also offers excellent results for the analysis of larger oligonucleotides, their analogues and dsDNA fragments. The 3µm 1500Å version affords good resolution of oligonucleotides differing by just one base (see Figure 5).

PolyGLYCOPLEX™

PolyGLYCOPLEX™ is a neutral material with a high capacity for retaining complex carbohydrates in the HILIC mode, frequently using just acetonitrile and water. Selectivity is good for both native glycans and derivatives such as those with the 2-aminopyridine fluorophore. Sialylated and asialoglycans can be resolved using the same operating conditions.



Column: PolyWAX LP, 100 x 4.6mm (3µm, 1500Å)
Eluent: Gradient of increasing [NaCl] in 25mM Tris-Cl, pH 8 – CH_3CN (70:30)
Flow rate: 0.5ml/min
Temperature: 60°C
Detection: UV, 250nm

Figure 5. Anion-exchange of oligonucleotides

PolySULFOETHYL A™

This strong cation-exchange (SCX) material was developed specifically for the HPLC of peptides, fractionating peptides by charge rather than polarity. Selectivity complements that of reversed-phase columns. Compared to other SCX materials based on sulphopropyl groups, PolySULFOETHYL A™ is unusually hydrophilic. This leads to higher recovery and higher capacity.

PolySULFOETHYL A is widely used in proteomics for the fractionation and identification of difficult proteins from complex tissue or cell extracts using 2D LC-MS/MS. By combining complementary HPLC techniques, an increased amount of information can be obtained. Different methods must be used for water-soluble and water-insoluble proteins, as shown in Figure 6. Some tryptic peptides from membrane proteins may be too hydrophobic for reversed-phase HPLC. A sequence of separations including PolySULFOETHYL A and/or PolyHYDROXYETHYL A materials provide a suitable alternative.

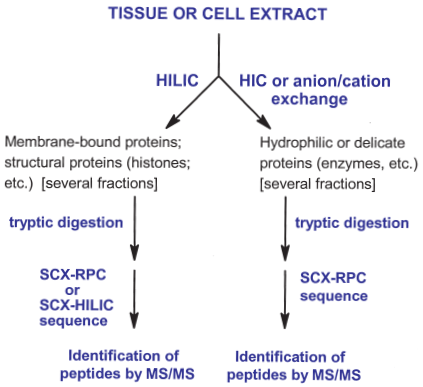


Figure 6

Electrostatic Repulsion – Hydrophilic Interaction Chromatography (ERLIC)

The term ERLIC was devised by PolyLC, for HILIC separations where an ionic column surface chemistry is used to repel a common ionic polar group on an analyte or within a set of analytes. The column has the same charge as the sample solutes. The eluent contains enough organic solvent so that hydrophilic interaction keeps the solutes on the column despite the electrostatic repulsion. The pH of the eluent is selected to ensure that the solutes do have the same charge as the column. Some separations that would nomally require a gradient can be done isocratically using ERLIC.

Applications of ERLIC include:

- Cation-exchange columns (PolySULFOETHYL A or PolyCAT A) are used for negatively charged solutes such as nucleotides and nucleic acids.
- Anion-exchange columns (PolyWAX LP) are used for amino acids, peptides and proteins. ERLIC of peptides on PolyWAX LP at pH 2 can be used to separate phosphopeptides from nonphosphorylated peptides.

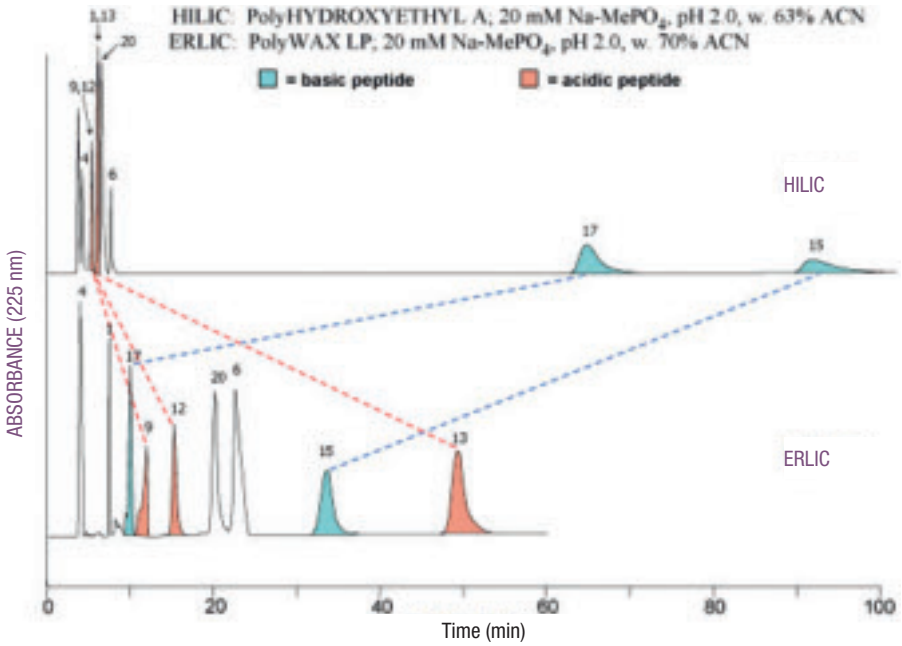


Figure 7. HILIC vs ERLIC separation of peptides

Figure 7 shows the elution of a mixture of acidic, basic and neutral peptides by HILIC on a PolyHYDROXYETHYL A column and ERLIC on a PolyWAX LP column. On the PolyHYDROXYETHYL A column, the basic peptides are much better retained than the neutral or acidic peptides, since basic solutes are the most polar of all. With the PolyWAX LP column, selective repulsion of the basic peptides puts them in the same elution time frame as the other peptides.

PolyPROPYL A™, PolyETHYL A™ and PolyMETHYL A™

These materials separate proteins on the basis of hydrophobicity, using totally aqueous buffers and retaining tertiary structure and biological activity. Elution is typically with a decreasing salt gradient of sulphate or phosphate. The relative hydrophobic character of PolyPROPYL A™, PolyETHYL A™ and PolyMETHYL A™ is 100, 60 and 15 respectively.

Sodium Dodecyl Sulphate (SDS) Removal

SDS is sometimes used to solubilise proteins. However, its presence interferes with subsequent bioanalysis. It can be removed by either:

1. Use of PolyHYDROXYETHYL A in HILIC mode
2. Use of specific PolyLC SPE cartridges in reversed-phase mode

Ordering Information – PolyLC Phases

Formulating Catalogue Numbers

Select column dimensions and phase from the table, then complete the catalogue number by adding a suffix to specify pore diameter as follows:

Pore Diameter (Å)	60	100	200	300	500	1000	1500
Catalogue No. Suffix	-006	-01	-02	-03	-05	-10	-15

Example:
PolyCAT A column (200 x 4.6mm) with 300Å pores would be 204CT0503.

Please note that not all phases are available in all pore sizes.

For bulk material part numbers, specify pore diameter with the same suffixes as for column materials.

Example: Bulk material of PolyCAT A with 300Å pores and 5µm particle size would be BMCT0503.

Columns

PolyLC 5µm Phase[2]	Column Dimensions[1] (mm)							
	100 x 2.1	200 x 2.1	35 x 4.6	100 x 4.6	200 x 4.6	200 x 9.4	250 x 9.4	250 x 21.0
	£395	£469	£330	£395	£469	£920	£972	£3,420
PolyCAT A	102CT05- -	202CT05- -	3.54CT05- -	104CT05- -	204CT05- -	209CT05- -	259CT05- -	2521CT05- -
PolyPROPYL A	102PR05- -	202PR05- -	3.54PR05- -	104PR05- -	204PR05- -	209PR05- -	259PR05- -	2521PR05- -
PolyETHYL A	102ET05- -	202ET05- -	3.54ET05- -	104ET05- -	204ET05- -	209ET05- -	259ET05- -	2521ET05- -
PolyMETHYL A	102ME05- -	202ME05- -	3.54ME05- -	104ME05- -	204ME05- -	209ME05- -	259ME05- -	2521ME05- -
PolyWAX LP	102WX05- -	202WX05- -	3.54WX05- -	104WX05- -	204WX05- -	209WX05- -	259WX05- -	2521WX05- -
PolyHYDROXYETHYL A	102HY05- -	202HY05- -	3.54HY05- -	104HY05- -	204HY05- -	209HY05- -	259HY05- -	2521HY05- -
PolyGLYCOPLEX	102GL0500	202GL0500	3.54GL0500	104GL0500	204GL0500	209GL0500	259GL0500	2521GL0500
PolySULFOETHYL A	£430 102SE05- -	£499 202SE05- -	£339 3.54SE05- -	£430 104SE05- -	£499 204SE05- -	£989 209SE05- -	£1,041 259SE05- -	£3,569 2521SE05- -

Guard Cartridges[3], Solid Phase Extraction Cartridges and Bulk Material

PolyLC 5µm Phase[2]	Guard Cartridge Dimensions[4,5] (mm)		Solid Phase Extraction Cartridges (10/pk)	Bulk Material/g
	10 x 2.1	10 x 4.0		
	£61	£61	£59	£53
PolyCAT A	J22GCCT05- -	JGCCT05- -	SPECT1203	BMCT05- -
PolyPROPYL A	J22GCPR05- -	JGCPR05- -	SPEPR1203	BMPR05- -
PolyETHYL A	J22GCET05- -	JGCET05- -	SPEET1203	BMET05- -
PolyMETHYL A	J22GCME05- -	JGCME05- -	SPEME1203	BMME05- -
PolyWAX LP	J22GCWX05- -	JGCWX05- -	SPEWX1203	BMWX05- -
SDS Removal	J2SDS	J4SDS	SPESDS1203	BMSDS05- -
PolyHYDROXYETHYL A	J22GCHY05- -	JGCHY05- -	SPEHY1203	BMHY05- -
PolyGLYCOPLEX	J22GCGL0500	JGCGL0500	SPEGL1200	BMGL0500
PolySULFOETHYL A	£66 J22GCSE05- -	£66 JGCSE05- -	£59 SPESE1203	£61 BMSE05- -

[1] Packed capillaries and 1mm i.d. columns also available
[2] 3 and 12µm particle size material also available
[3] Disposable Javelin design. No additional holder required
[4] 20 x 4.0 and 10 x 1.0mm cartridges also available
[5] Waters compatible cartridges available