UTILIZING SPECIFICITY AND SELECTIVITY IN BIO-ANALYTICAL SEPARATIONS

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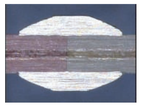
Introduction

Biological macromolecules such as proteins contain a number of chemical properties which can be targeted by changing the column packing material. Most commonly the different hydrophobicities of peptides are utilized to separate samples on C18 reversed phase columns or the number of basic amino acids per molecule to bind to strong cation exchangers. More specifically, IMAC columns loaded with Fe(III) can be used to target protein phosphorylation.
On the other end of the specificity scale are the affinity columns, which use biospecific recognition as means of separation. An example given is the enrichment of glycosylated peptides by a lectin (Con-A) modified trap column and specific elution with a competitive substrate. Isolated glycans can then be analysed with a porous graphitized carbon column which shows a unique ability to separate structural isoforms of branched oligo-saccharides.

Features of the ProteCol Range of Columns and Accessories

- Integrated connection tubing
- Smooth fused silica internal surfaces
- Zero - volume connections
- Robust 1/32" capillaries of PEEKsil (PEEK coated fused silica)



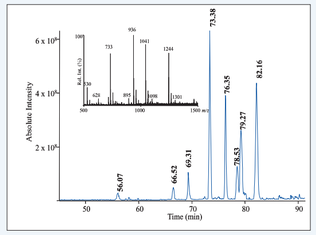
Porous Graphitized Carbon Columns for the Separation of Glycan Isomers

Protein glycosylation is a important and interesting yet very complex field in biochemistry. The demands on LC columns are unique in terms of selectivity because of the chemistry of the analyte as well as its complex isomeric nature.

Conditions:

Column:	ProteCol-Hypercarb 150µm ID x 100mm
Sample:	Oligosaccharides from rat small intestine mucin (MUC2) isolated by 1D SDS PAGE
Injection Volume:	10µL
Flow rate:	0.6 µL/min
Mobile phase A:	10mM ammonium carbonate
Mobile phase B:	methanol
Gradient:	0-30% B in 60 minutes

Column format:
Length 50, 100 and 150mm
ID: 75, 150, 300 and 530µm
Packing:
porous graphitized carbon
5µm; 250Å



Fuc a1—2Gal b1—3GlcNAc b1 → 6 GalNAcol / Fuc a1—2Gal b1—3GlcNAc b1
[M-H]⁻: 1244
RT: 73.38
S/N: 32

Fuc a1—2Gal b1—3GlcNAc b1 → 6 GalNAcol / Fuc a1—2Gal b1—4GlcNAc b1
[M-H]⁻: 1244
RT: 76.35
S/N: 98

Fuc a1—2Gal b1—4GlcNAc b1 → 6 GalNAcol / Fuc a1—2Gal b1—4GlcNAc b1
[M-H]⁻: 1244
RT: 81.87
S/N: 26

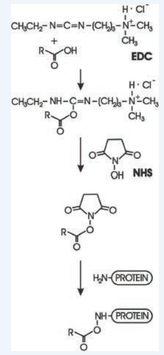
Concanavalin-A modified CMD Columns for the Enrichment of Glycosylated Peptides

CMD columns are columns packed with macro-porous (2000Å) silica coated with carboxymethylated dextran (CMD). CMD allows the end-user to attach any biospecific ligand or enzyme onto the surface to form a tailor-made affinity column or immobilized enzymatic reactor. The surface modification using EDC and NHS is very fast and can be performed with any HPLC autosampler for reagent delivery.

Conditions for the affinity purification of glycosylated peptides from bovine muscle tissue digest.

Mobile phase: 50mM phosphate buffer pH = 5.6
with 20mM each of Ca2+, Mg2+ and Mn2+

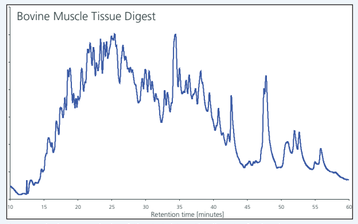
Equilibrate Con-A modified column
Inject tryptic digest sampole
Wash column
Elute trapped glycopeptides with 10 mg/mL
methyl-a-D-mannopyranoside
Collect eluate
Analyze on C18 column



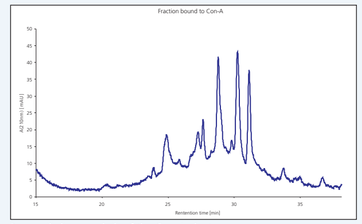
Mobile phase: 50mM phosphate buffer pH 6.5
Equilibrate column
Make stock solutions:
4 mg/mL EDC in mobile phase
6mg/mL NHS in mobile phase
10mg/mL ligand in mobile phase
10mg/mL ethanolamine in mobile phase

Just prior to the immobilization
mix EDC and NHS stock 1:1

Inject 8µL of EDC/NHS at low flow rate (1µL/min for a 300µm ID column)
Wash column
Inject ligand at low flow rate
Wash column
Inject ethanolamine at low flow rate
Wash column

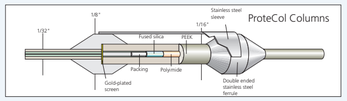


Reversed phase analysis of the total tryptic digest of a bovine muscle tissue



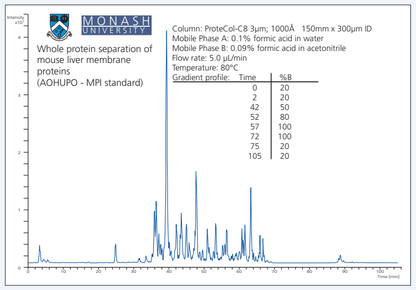
Reversed phase analysis of the Con-A eluate.

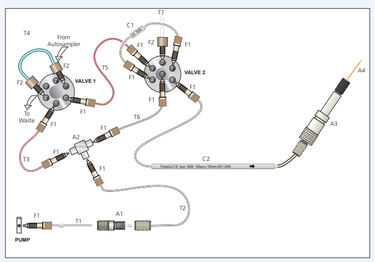
Capillary C8 and C18 Columns for the Routine Analysis of Peptides and Proteins

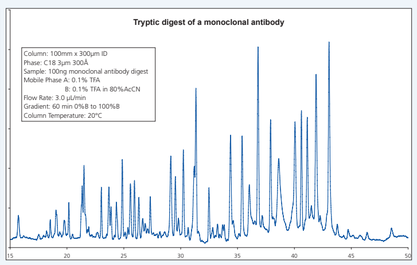


Column Formats:

Length:	5, 10, 50, 100 and 150mm
IDs:	75, 150, 300 and 530µm
Packing material:	3µm/300Å; C4, C8 and C18 3µm/1000Å; C8

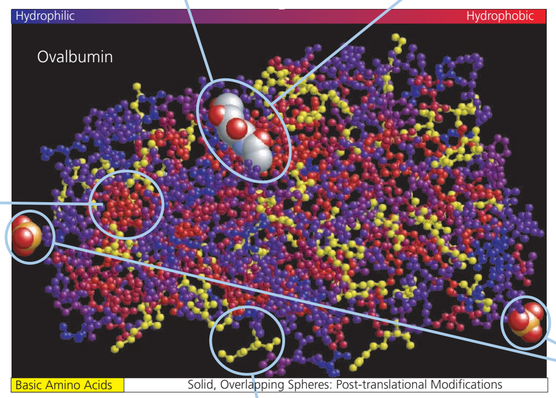






Summary:

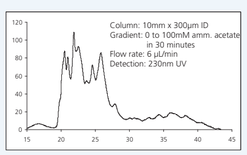
Biological samples are so complex in their composition and in their range of concentrations, that utilizing specific affinities to enrich a target group of analytes or to remove unwanted sample components becomes almost imperative. Especially when analyzing post-translational modifications there are columns available, which show a much higher specificity and better selectivity than standard reversed phase material. Furthermore, columns like the carboxymethylated dextran coated CMD column allow the user to tailor the surface specificity by immobilizing specific ligands and open up a range of affinity media which would otherwise not be commercially viable. There are yet more chemistries feasible and will be developed in the near future to increase the number of options available to the analyst.

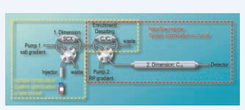


SCX Trap/Pre Columns for First Dimension MuDPIT Experiments

Column format:
Length: 5 or 10 mm
ID's: 75,150, 300 or 530µm
Packing material: strong cation exchanger; 5µm/300Å
Integrated 70mm connection tubing on each side to fit standard valve arrangements.

SCX separation of BSA digest





Possible configuration of a 2D-LC setup with desalting column

IDA-IMAC Columns for the Enrichment of Phosphorylated Peptides

Protein Phosphorylation

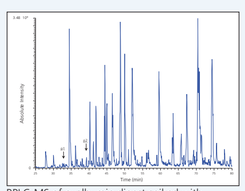
Proteins can be phosphorylated by adding a phosphate group to a serine, threonine or tyrosine residue
=> Activation or deactivation of enzymes
=> Trans-membrane transportation
=> Signal trafficking
The affinity of phosphate for certain metal ions can be utilized to enrich phosphorylated proteins or peptides

Column Specifications:

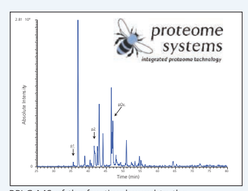
Length:	5 or 10mm
ID:	75, 150, 300 or 530µm
Connection Tubing:	2 x 70mm; 25 or 50µm ID; 1/32"OD
Tubing material:	PEEKsil (PEEK coated fused silica)
Packing:	Imino diacetic acid (IDA) modified silica; 3µm particle size; 300Å pore size
Suitable metal ions:	Fe(III), Ga(III) or Zr(IV)

IMAC phosphopeptide enrichment procedure

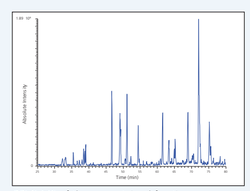
Step	Solution	Volume	Comment
1	50 mM EDTA, 1M NaCl, pH 8.5	25 µL	Stripping column
2	HPLC grade H_2O	10 µL	
3	0.1 % HAc	25 µL	
4	100 mM $FeCl_3$	50 µL	Cation charging
5	0.1 % HAc	25 µL	
6	Sample	variable	Flow-through collected
7	0.1 % HAc	25 µL	Wash fraction
8	25 % Acetonitrile, 1 % HAc, 100 mM NaCl	25 µL	Wash fraction
9	0.1 % HAc	25 µL	Wash fraction
10	50 mM K_2HPO_4	20 µL	Eluted material collected



RPLC-MS of ovalbumin digest spiked with two phosphopeptides (denoted p1 and p2) at ratio 5:1



RPLC-MS of the fraction bound to the IMAC-Fe(III) column. The 3rd marked phosphopetide comes from Ovalbumin



RPLC-MS of the non-retained fraction

Acknowledgements:
Dr. Tzong Hsien Lee, Monash University, for the whole protein separation of mouse liver membrane proteins.
Proteome Systems Ltd for their applications on the IMAC- and the Carbon columns.

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