HAISIL 300 *For Biomolecules*

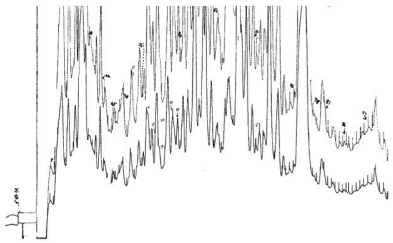
Phases	C18
Particle Sizes	5 and 10µm*
Pore Size	300Å
Pore Volume	0.7mL/gm
Surface Area	80m^2/gm
%Carbon (w/w)	C18 = 6%
Phase type	Monofunctional and fully endcapped
Silica Class	Type A
USP Class	L1

Guide to HAISIL 300 Part Numbers

Hx-xxxx-W185 HAISIL 300 C18 5µm

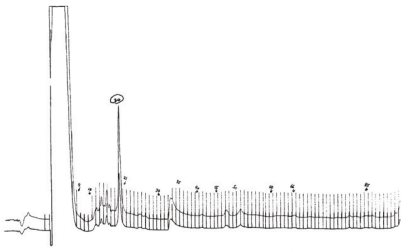
See Page 23 for complete Part Number information

* *10µm is offered in HAISpeed 20 Guard Cartridges only. See pages 68-69.*



Fractionation of a Purified Tumor Cell Extract

Peptides ranging from 100 fmol to 1pmol are collected in 40sec intervals during a 70min gradient from 0 - 60% MeCN with HFBA modifier. A 40x2.1mm HAI*SIL* 300 C18 5µm cartridge column (HK-0421-W185) is used.



Reanalysis of a Fraction from the Chromatogram above

The same 40x2.1mm HAI*SIL* 300 cartridge is used for a 70min gradient from 0-60% MeCN with TFA modifier. Tic marks indicate 40sec fraction collections.

Applications

HAISIL 300 columns and cartridges are ideal for biomolecule separations such as peptides and small proteins. Optimized phase bonding and column packing techniques assure unsurpassed performance and column-to-column reproducibility. Short cartridge columns are economical and are ideally suited for LC/MS applications. The column and cartridge offering ranges from 150µm to 20mm I.D. which means that bioseparations can be optimized by selecting a format that best fits your analytical requirements.

Micro-Preparative HPLC

Higgins Analytical Cartridge Columns are helping researchers understand the immune response of living cells. An investigation to understand the genetic control of the immune response systems in organisms involves the extraction, isolation, and identification of more than 10,000 peptides. These distinct peptides are bound to a major histocompatibility complex (MHC) molecule on tumor cells.

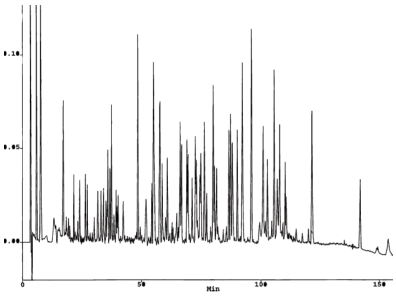
Extraction of approximately $5x10^{10}$ cells yield several nanomoles of a very complex peptide mixture. Several rounds of RP-HPLC with different organic modifiers on a 40x2.1mm PEEK cartridge column (HAISIL 300 C18 5µm, P/N: HK-0421-W185) are performed to fractionate the mixture into individual components.

The chromatograms on the left illustrate how this technique is being used to narrow the search for the one peptide that marks a particular tumor cell as being diseased enabling it to be recognized by the immune cells.

Peek and Titanium cartridge columns used in the application described above. P/N: HK-0421-W185

Very High Peak Capacity

Depending upon the type of information a protein chemist is seeking, the HPLC analysis of a complex digest can take 1 to 2 hours. The following chromatogram

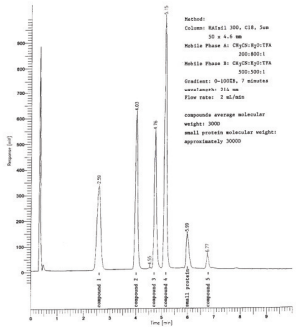


Tryptic Map of BSA
on HAISIL 300 C18 5µm 250x4.6mm
P/N HS-2546-W185

illustrates a high resolution tryptic digest map of BSA on a 250x2.1mm HAISIL 300 C18 5µm column indicates peak capacities in excess of 100. Small proteins, on the other hand can yield fewer fragments, and if only a "finger-print" of a digest is required, high peak capacity columns can be a waste of time.

Fast Analysis of a Complex Drug Delivery System

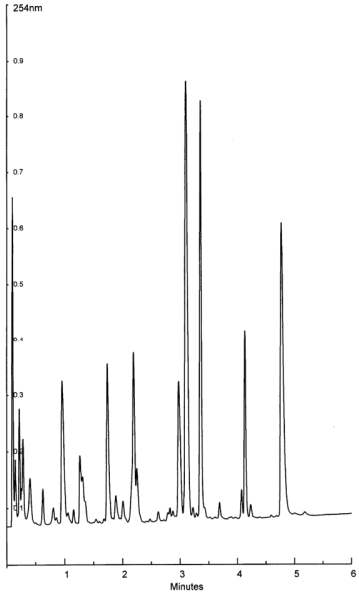
Most pharmaceutical QC laboratories are faced with rapidly increasing demands for high throughput capability. A good example of one laboratory's



approach to addressing a large number of samples for analysis on a daily basis is summarized below. This novel drug delivery system contains a small protein and other components that span an order of magnitude in molecular weight. All the compounds of interest were easily resolved in a single analysis with a fast linear gradient on a 5cm long HAISIL 300 C18 5µm column.

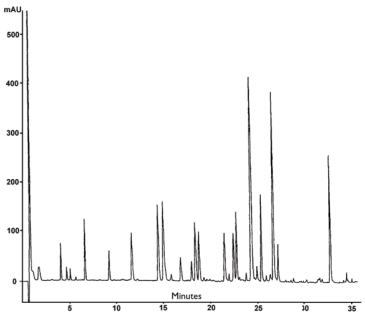
Peptide Map in 5 minutes

The figure below illustrates a very fast



peptide map of cytochrome-C. The information that can be gleaned from this five minute profile is more than adequate for many analytical requirements. The robust 50x4.6mm HAISIL 300 C18 column provides hundreds of runs without performance loss even under the aggressive 6ml/min flow conditions used in this example.

The cytochrome-C is a good example of pushing a column beyond its optimum operating conditions (more than six times optimum linear velocity) and still being able to get desired results. The same column can be run under more conventional conditions with a flatter gradient to optain even higher resolution separations. The rhGH tryptic map below was done on a 5cm long column that replaced the conventional 25cm column that was used initially.



Fast Tryptic Map of rhGH
50x4.6mm HAISIL 300 C18

The Role of Column Length in Gradient Separations

Changes in column length have a direct and linear effect on theoretical plates and analysis time in isocratic separations. Column length plays a less important role, however, in gradient techniques. The three chromatograms below illustrate how well packed short HPLC columns yield very similar peptide separations under identical gradient conditions.

For this study, the 2.1mm I.D. HAISIL 300 C18 5µm columns ranged from 250 to 100mm in length. While solvent and analysis time savings may be minimal, column cost savings can be significant when using as short a column as possible. Short columns also exhibit lower pressure at high flow rates.