



AMBERCHROM™ Profile™ Columns

For Analysis and Preparative Purification of Biomolecules

PRODUCT DATA SHEET

Amberchrom Profile HPLC columns, packed with 10, 20, or 30 µm particle size macroporous, polystyrene divinylbenzene polymers, are intended for high resolution reversed phase liquid chromatography. These resins are chemically and physically stable across the complete pH range. Amberchrom Profile columns are designed for analysis and purification of peptides, proteins, and oligonucleotides, and are an excellent alternative to RPC silica. Amberchrom Profile columns are available in three different formats, and the properties of these columns are listed below in Table 1.

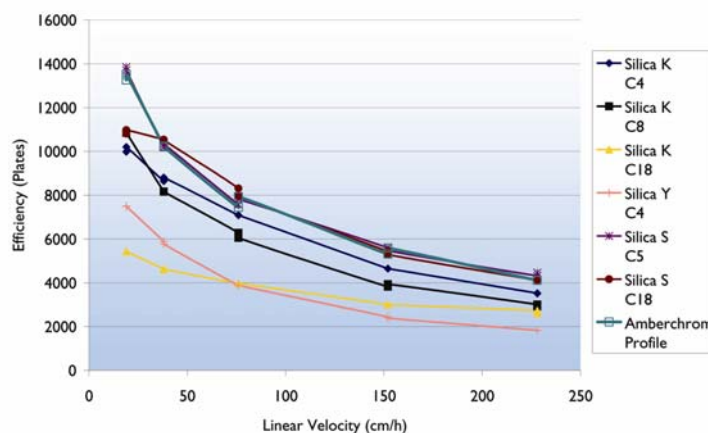
Table 1: Amberchrom™ Profile™ HPLC Column Properties

Bead Form _____	Spherical, macroporous
Pore Size _____	300 angstroms
Particle Size _____	10, 20 or 30 µm
Column Dimensions _____	4.6 mm I.D x 25 cm L 10 mm I.D x 25 cm L 20 mm I.D x 25 cm L
Maximum Pressure _____	60 bar (900 psi)
pH Range _____	1 – 14
Temperature Range _____	4 – 60°C

HIGH EFFICIENCY

Older polymeric materials could not match the high efficiency of silica packings. However, Amberchrom Profile columns provide efficiencies that are identical or better than commercially available silica products. Efficiencies of several competitive 10 µm silica columns were compared to that of a 10 µm Amberchrom Profile column using human insulin. Figure 1 demonstrates the results of this comparison, and the high efficiency of Amberchrom Profile columns.

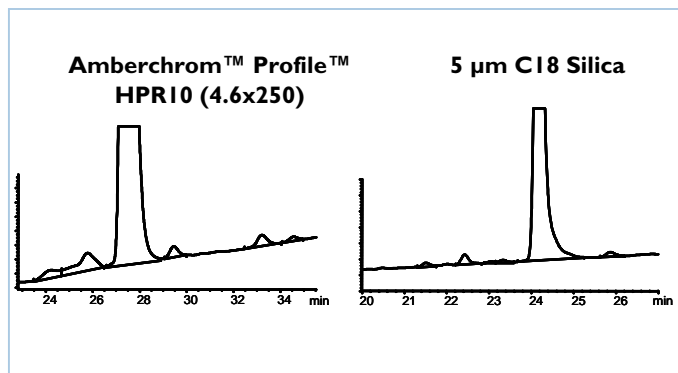
Figure 1: Column Efficiency Comparison using Human Insulin



ANALYSIS OF PEPTIDES

Amberchrom Profile columns are ideal for the analysis of peptides. Figure 2 shows a blow up of a chromatogram of an influenza peptide (Influenza peptide M1, [H]-Gly-Ile-Leu-Gly-Phe-Val-Phe-Thr-Leu-[OH]) using a standard 5 µm C18 silica column and a 10 µm Amberchrom Profile column.

Figure 2: Analysis of Influenza Peptide

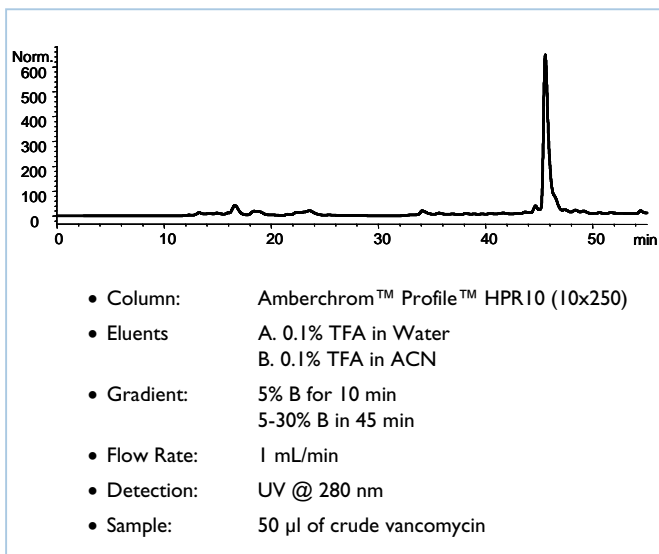


Amberchrom Profile can separate contaminants from the peak of interest and demonstrates slightly different selectivity, as the elution order is somewhat different from the C18 column. In addition, there is less tailing of the main peak in the Amberchrom Profile chromatogram compared to the C18 silica.

Amberchrom Profile columns can be used under conditions typically used with silica columns. Figure 3 demonstrates the use of these conditions for the analysis of a crude broth containing a cyclic peptide, vancomycin.

However, unlike silica columns, Amberchrom Profile columns can be easily regenerated using sodium hydroxide. This is especially important when performing routine analyses of crude broths like the one shown in Figure 3.

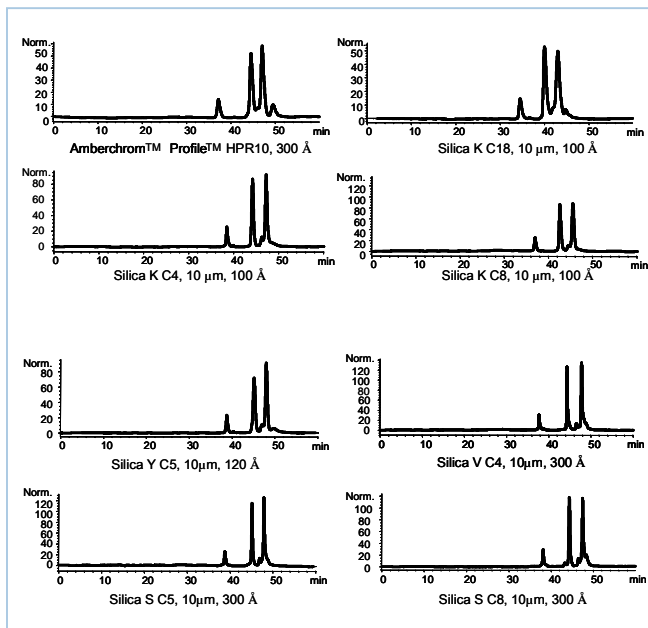
Figure 3: Analysis of Crude Broth of Vancomycin



ANALYSIS OF PROTEINS

Figure 4 is a protein separation comparison between Amberchrom Profile and conventional silica columns. Here under identical conditions with an acidic mobile phase, four standard proteins (cytochrome c, ribonuclease a, lysozyme, and BSA) are separated. There is similar retention and selectivity between the packings, however, Amberchrom Profile is able to separate BSA from lysozyme whereas most silica columns can not.

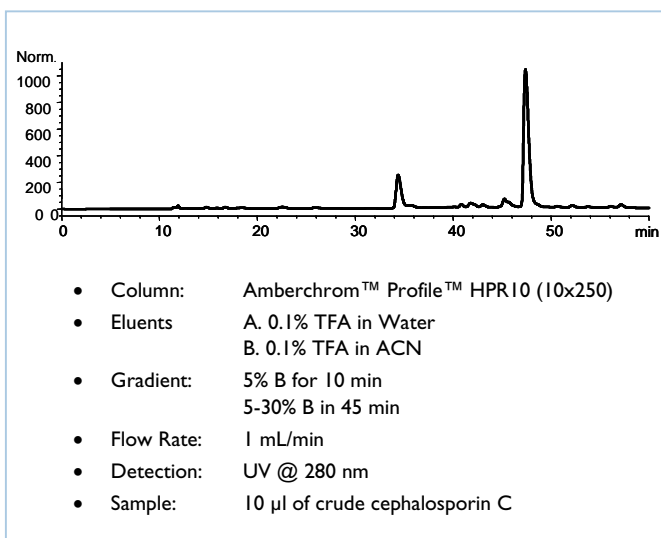
Figure 4: Protein Separation Comparison



ANALYSIS OF SMALL MW COMPOUNDS

Amberchrom Profile columns can also be used for the analysis of small molecules such as Cephalosporin C. Figure 5 demonstrates the analysis of a crude Cephalosporin C broth using standard reversed phase conditions.

Figure 5: Analysis of Crude Broth of Cephalosporin C



PURIFICATION OF PEPTIDES

Purification of the influenza peptide (Influenza peptide M1, [H]-Gly-Ile-Leu-Gly-Phe-Val-Phe-Thr-Leu-[OH]) was complicated by the poor solubility of the peptide. The peptide was solubilized in 30% acetonitrile/0.1% TFA. The peptide was retained under these conditions, and could be eluted isocratically. Overall recovery under these conditions was poor, so the loading and elution mobile phases were altered.

A mobile phase with 30% acetonitrile/70% Milli-Q® water, pH 10 was used to purify the peptide. Solubility of the peptide improved and total recovery of the peptide during elution improved to 100%. The final adjustment was in the loading concentration of the peptide. All previous purifications had employed a loading concentration of 0.75 mg/mL and a total column loading of 0.45 mg/mL. For the final small scale purification, the loading concentration was increased to 4.3 mg/mL and the total column loading was 5.2 mg/mL. Yield and purity targets were met at the initial scale, and the purification was then successfully scaled 5-fold.

A summary of the purification strategy is shown in Table 2.

Table 2: Amberchrom™ Profile™ XT20 Process Optimization

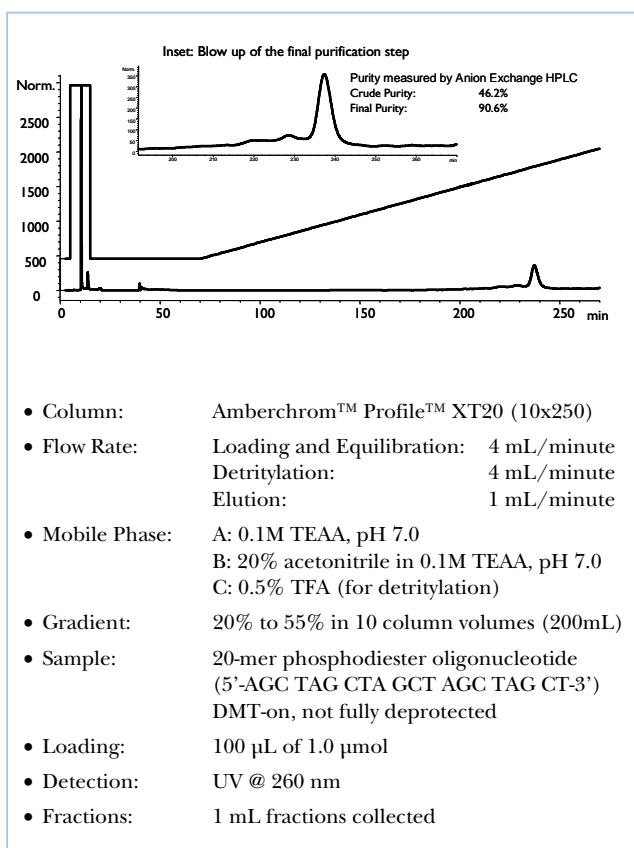
Packing Material	Column Loading mg/ml	Linear Velocity cm/h	Yield @ 95 % Purity	Total Recovery %
Acidic pH mobile phase 0.75 mg/mL conc. 4.6mm I.D. x 25cm L	0.45	180	40	48
Acidic pH mobile phase 0.75 mg/mL conc. 4.6mm I.D. x 25cm L	0.45	90	53	56
Basic pH mobile phase 0.75 mg/mL conc. 4.6mm I.D. x 25cm L	0.45	90	30	100
Basic pH mobile phase 4.3 mg/mL conc. 4.6mm I.D. x 25cm L	5.2	90	85	100
Basic pH mobile phase 4.1 mg/mL conc. 10mm I.D. x 25cm L	4.6	90	80	100

PURIFICATION OF OLIGONUCLEOTIDES

Amberchrom Profile columns are an excellent choice for the separation of synthetic oligonucleotides. A single-step purification of a 20-mer synthetic, phosphodiester oligonucleotide was developed on a 10 (I.D) x 250 mm Amberchrom Profile XT20 column. The crude DMT-on oligonucleotide, which was not fully deprotected, was loaded onto the column, detritylated, and eluted. One of the advantages of the polymeric Amberchrom Profile is the ability to detritylate on-column with the use of 0.5% trifluoroacetic acid (TFA).

Figure 6 presents the results of the separation which demonstrates the ability of Amberchrom Profile columns to purify crude synthetic oligonucleotides in a single step from a starting purity of ~46% to a final purity of ~91%.

Figure 6: 20-mer Oligonucleotide Purification



ORDERING INFORMATION

Part Number	Description
10237932	AMBERCHROM PROFILE HPR10 4.6x250 mm
10237938	AMBERCHROM PROFILE HPR10 10x250 mm
10237940	AMBERCHROM PROFILE HPR10 20x250 mm
10237952	AMBERCHROM PROFILE HPR10 50x250 mm
10225950	AMBERCHROM PROFILE XT20 4.6x250 mm
10237749	AMBERCHROM PROFILE XT20 10x250 mm
10237750	AMBERCHROM PROFILE XT20 20x250 mm
10237781	AMBERCHROM PROFILE XT20 50x250 mm
10248207	AMBERCHROM PROFILE XT30 4.6x250 mm
10237909	AMBERCHROM PROFILE XT30 10x250 mm
10237941	AMBERCHROM PROFILE XT30 20x250 mm
10237946	AMBERCHROM PROFILE XT30 50x250 mm

You can order Amberchrom™ Profile™ columns directly at our web store

<http://www.amberchrom.com>

Rohm and Haas/Ion Exchange Resins - Philadelphia, PA - Tel. (800) RH AMBER - Fax: (1)-215-409-4534
Rohm and Haas/Ion Exchange Resins - 75579 Paris Cedex 12 - Tel. (33) 1 40 02 50 00 - Fax: 1 43 45 28 19

WEB SITE: <http://www.amberchrom.com>



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