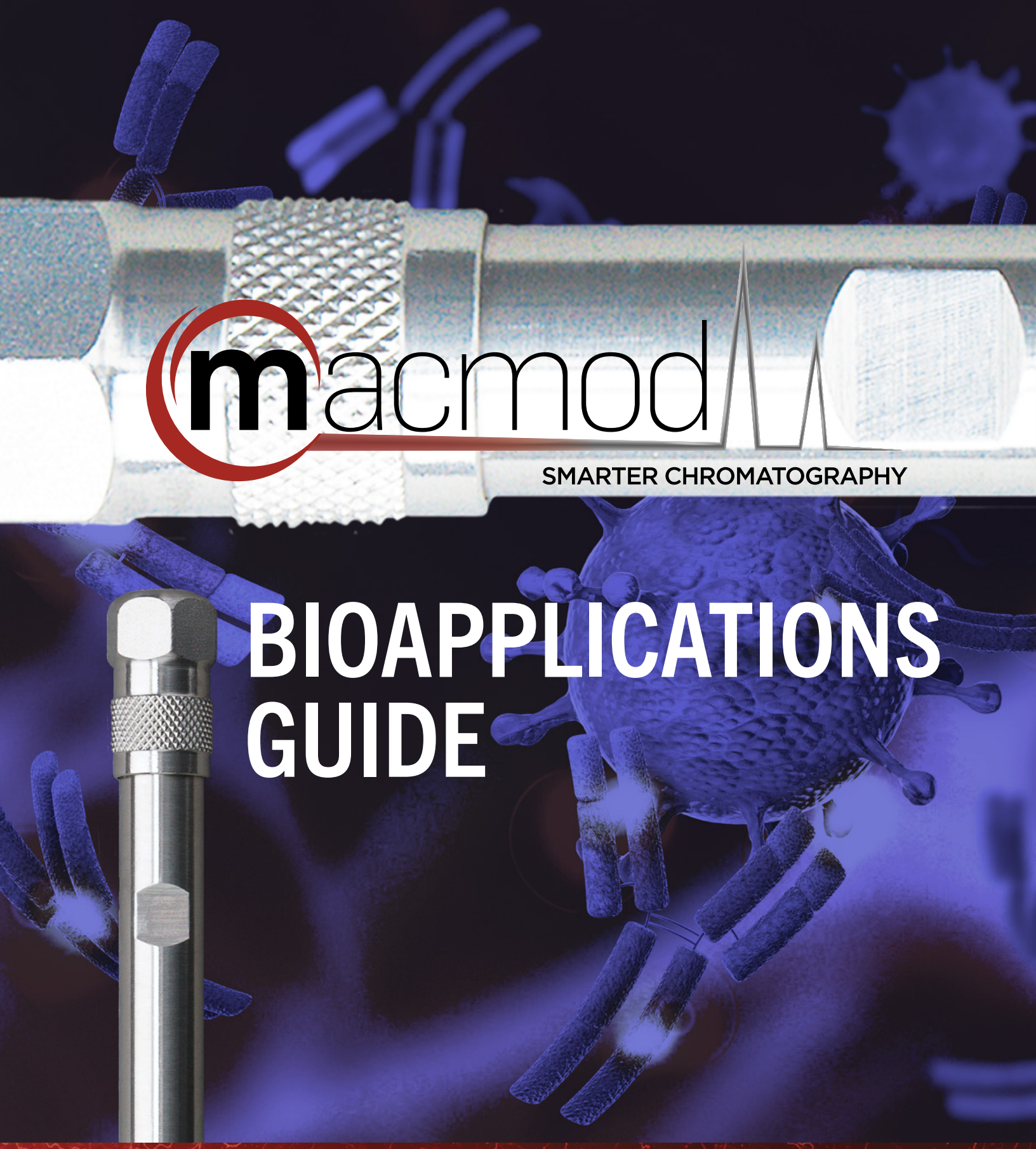


BIOAPPLICATIONS GUIDE

INFORMATION & RESEARCH COLLECTED IN COLLABORATION WITH:

avantor™ | advancedmaterialstechnology

CONTENTS

PEPTIDES

PEPTIDES & PROTEINS

PROTEINS

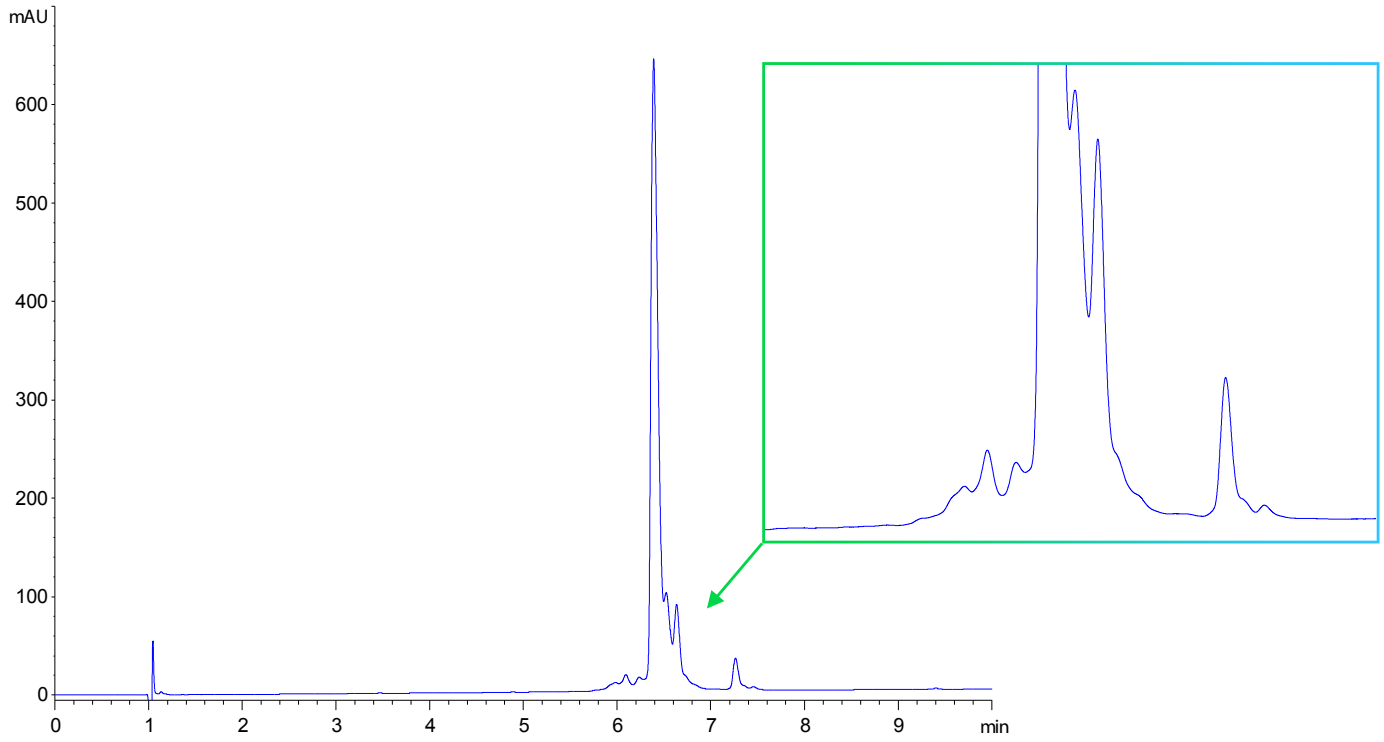
ALPHA-CHYMOTRYPSINOGEN

TEST CONDITIONS

Column: Avantor® ACE® UltraCore BIO C4, 500 Å, 2.5 µm, 3.0 x 100 mm
Part Number: BIO-251-1030
Mobile Phase: A: 0.1% TFA in H_2O
B: 0.1% TFA in Acetonitrile/H_2O 90:10 v/v
Gradient:

Time (min)	%B
0.0	40
10.0	50
12.0	100
13.0	100
13.5	40

Flow Rate: 0.43 mL/min
Temperature: 80 °C
Injection Volume: 5 µL
Detection: UV, 220 nm



DEXTRAN LADDER STANDARD IN HILIC WITH FLUORESCENCE DETECTION (II)

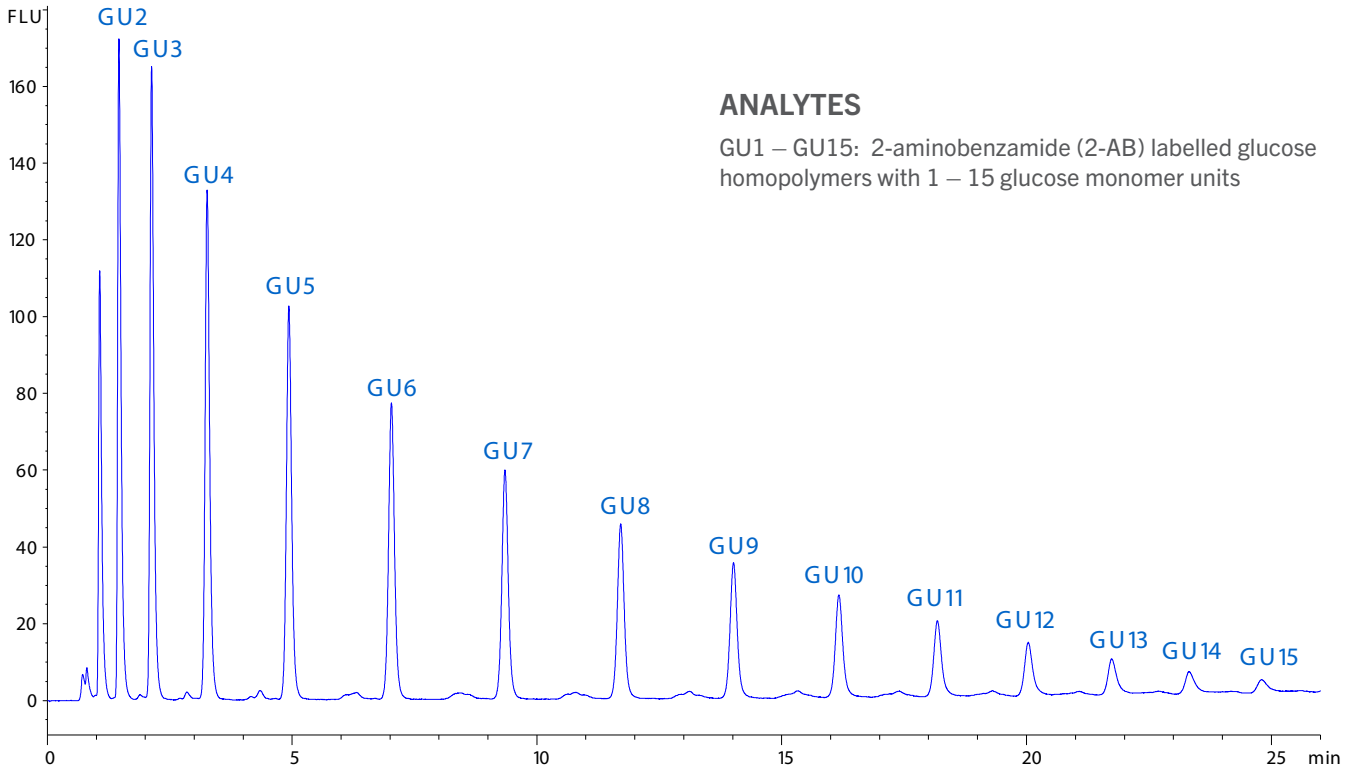
TEST CONDITIONS

Column: Avantor® ACE® Excel Glycan, 100 Å, 3 µm, 2.1 x 150 mm
Part Number: EXL-1116-1502
Mobile Phase: A: 100 mM Ammonium formate in H_2O (pH 4.5)
B: Acetonitrile

Gradient:

Time (min)	%B
0	75
24.0	60
24.3	40
24.6	40
24.9	75
45.0	75

Flow Rate: 0.5 mL/min
Temperature: 55 °C
Injection Volume: 1.5 µL
Detection: FLD $\lambda_{ex} = \lambda_{em} = 430$ nm
LC Instrument: VWR Chromaster with FLD
Dwell Volume: 1950 µL



ANALYTES

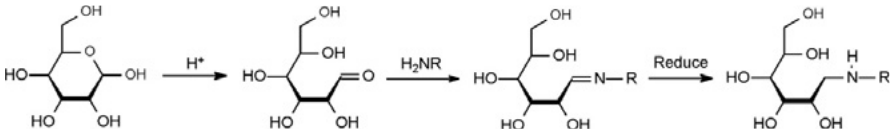
GU1 – GU15: 2-aminobenzamide (2-AB) labelled glucose homopolymers with 1 – 15 glucose monomer units

PNGASE-RELEASED AND LABELED N-GLYCANS BY HILIC

TEST CONDITIONS

Column:	HALO BioClass Glycan, 90 Å, 2.7 µm, 2.1 x 150 mm
Part Number:	92922-705
Mobile Phase:	A: 50 mM Ammonium formate, pH 4.45 B: Acetonitrile
Gradient:	80–55% B in 25 min
Flow Rate:	0.6 mL/min
Temperature:	60 °C
Pressure:	190 bar
Detection:	UV 300 nm
Injection Volume:	3 µL
Sample Solvent:	70/30 Acetonitrile/H_2O
Response Time:	0.5 sec
Data Rate:	3.3 Hz
Flow Cell:	2.5 µL semi-micro
LC System:	Shimadzu Nexera

Digestion of N-linked proteoglycans using PNGase F releases oligosaccharides, which can be reacted with an amine via Schiff base formation. The Schiff's base derivatives (imines) can be easily reduced to form stable amine derivatives for analysis.

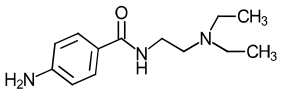


Many amines have been applied for labeling glycans (Harvey, 2011, J. Chromatogr. B, 879, 1196-1225). In this application brief, procainamide was chosen because of reported improvements in ESI-MS detection. (Klapoetke, et. al., 2010, J. Pharm. Biomed. Anal., 53, 315-324)

TYPICAL LABELING CONDITIONS

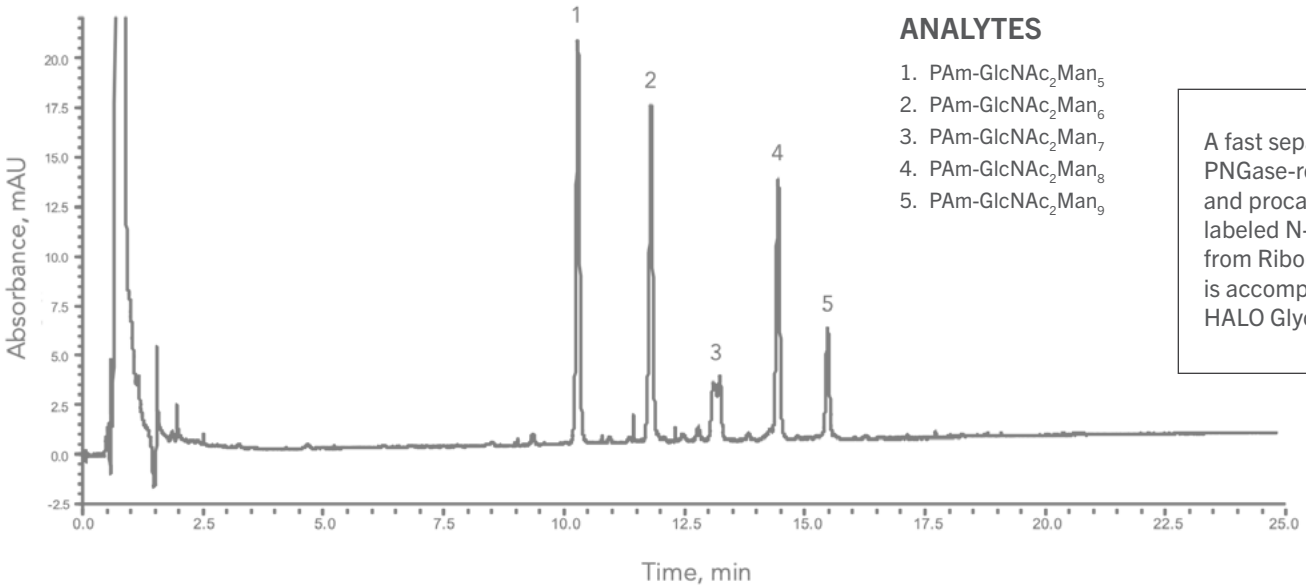
1. Glycan in H_2O (up to 10% volume)
2. 90+% volume of:
   - 0.4 M procainamide
   - 1M sodium cyanoborohydride in 30% glacial acetic acid/70% DMSO

12-16 hr reaction at 37 °C
SEC cleanup on Sephadex G-10 minicolumn
Absorbance Detection @300 nm or Fluorescence with Ex 330/Em 380 nm



Procainamide (PAm)

Ribonuclease B N-Glycans



ANALYTES

1. PAm-GlcNAc$_2$Man$_5$
2. PAm-GlcNAc$_2$Man$_6$
3. PAm-GlcNAc$_2$Man$_7$
4. PAm-GlcNAc$_2$Man$_8$
5. PAm-GlcNAc$_2$Man$_9$

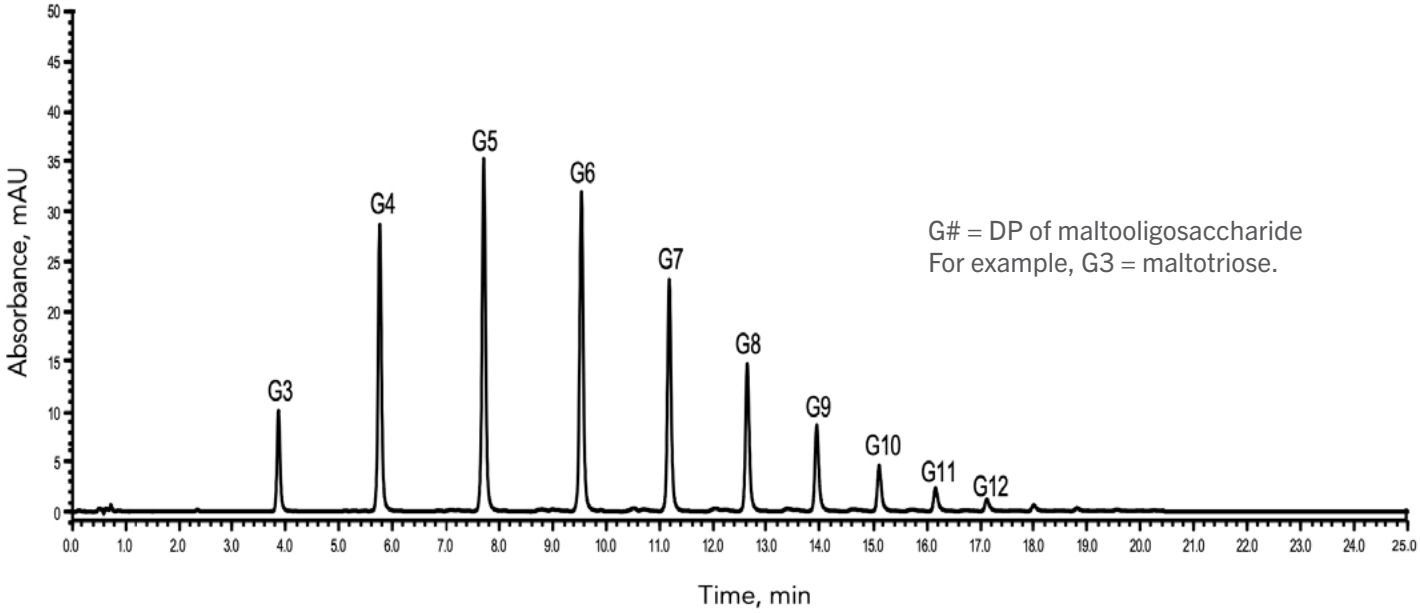
A fast separation of PNGase-released and procainamide-labeled N-Glycans from Ribonuclease B is accomplished with a HALO Glycan column.

PROCAINAMIDE-LABELED DEXTRANS ON HALO GLYCAN COLUMN

TEST CONDITIONS

Column:	HALO BioClass Glycan, 90 Å, 2.7 µm, 2.1 x 150 mm
Part Number:	92922-705
Mobile Phase:	A: 50 mM Ammonium formate, pH 4.45 B: Acetonitrile
Gradient:	80–55% B in 25 min
Flow Rate:	0.6 mL/min
Temperature:	60 °C
Pressure:	190 bar
Detection:	UV 300 nm
Injection Volume:	3 µL
Sample Solvent:	70/30 Acetonitrile/H_2O
Response Time:	0.5 sec
Data Rate:	3.3 Hz
Flow Cell:	2.5 µL semi-micro
LC System:	Shimadzu Nexera

A HALO Glycan column shows an efficient separation of procainamide-labeled dextran standards (Sigma-Aldrich 1:1 (w/w) of Part numbers 00268 and 00269) at 0.5 µg/µL in 70% Acetonitrile/30% H_2O. Each lot of HALO Glycan packing is tested using this sample to assure lot-to-lot reproducibility and performance.



1000 Å C4 PROTEIN COLUMN FOR A HIGH RESOLUTION SEPARATION OF A MONOCLONAL ANTIBODY

TEST CONDITIONS

Column: HALO BioClass Protein C4, 1000 Å, 2.7 µm, 2.1 x 100 mm

Part Number: 92712-614

Mobile Phase:
A: H_2O, 0.1% TFA
B: 80/20 Acetonitrile/H_2O, 0.085% TFA

Gradient:

Time (min)	%B
0.00	40
12.0	47.5

Flow Rate: 0.4 mL/min
Pressure: 210 bar
Temperature: 80 °C
Injection Volume: 2 µL
Sample Solvent: 70/30 H_2O/Acetonitrile
Detection: UV 280 nm, PDA
Data Rate: 12.5 Hz
Response Time: 0.05 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

MONOCLONAL ANTIBODY STRUCTURE:

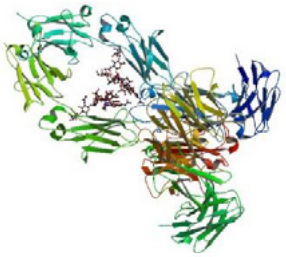
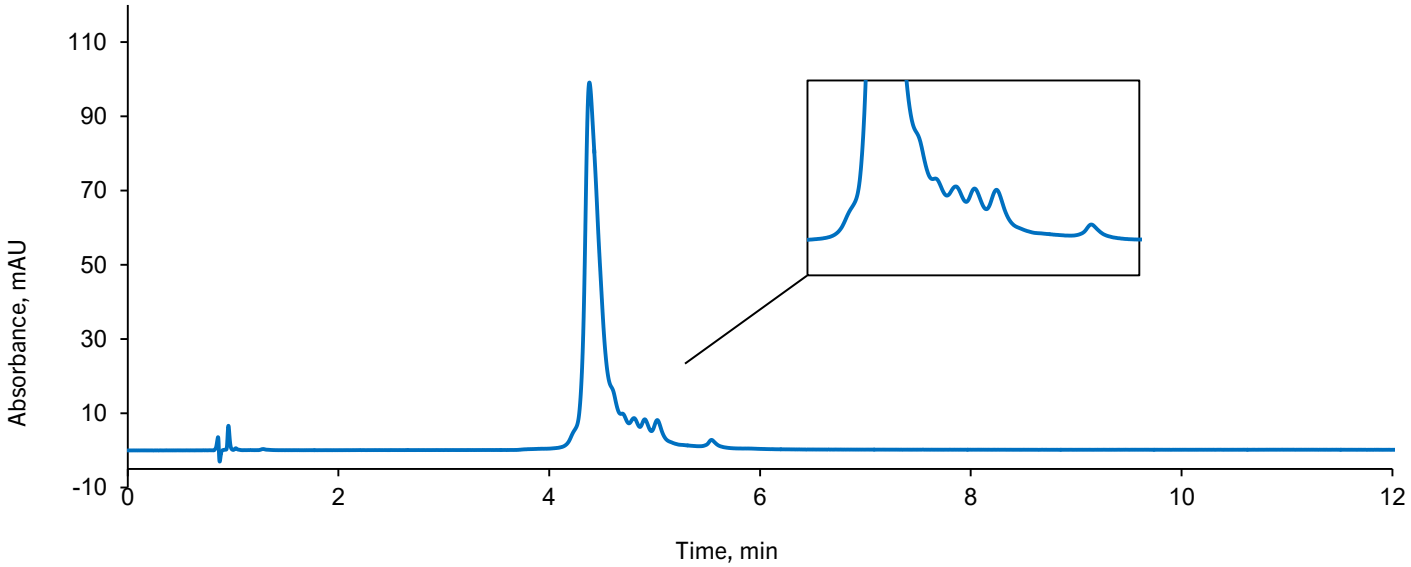


Image from the RCSB PDB (www.rcsb.org) of PDB ID 1HZH (E.O. Saphire, P.W. Parren, R. Pantophlet, M.B. Zwick, G.M. Morris, P.M. Rudd, R.A. Dwek, R.L. Stanfield, D.R. Burton, I.A. Wilson) (2001) Crystal structure of a neutralizing human IGG against HIV-1: a template for vaccine design Science 293: 1155-1159)

Trastuzumab (MW ~148 kDa) is a monoclonal antibody used to treat breast cancer. Enhanced resolution of trastuzumab and its variants is demonstrated in the chromatogram below. The pores of the HALO 1000 Å C4 Protein particles accommodate larger biomolecules enabling superior separations at high temperatures.



EFFECT OF ACID MODIFIERS ON INTACT mAb PEAK SHAPE

TEST CONDITIONS

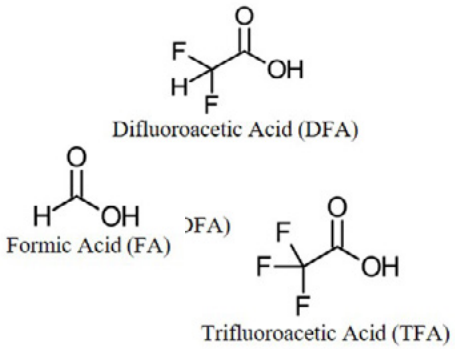
Column: HALO BioClass Protein C4, 1000 Å, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-714
Mobile Phase: A: H_2O, with 0.1% FA, DFA, or TFA as noted
B: 80/20 Acetonitrile/H_2O, with 0.1% FA, DFA, or TFA as noted

Gradient:

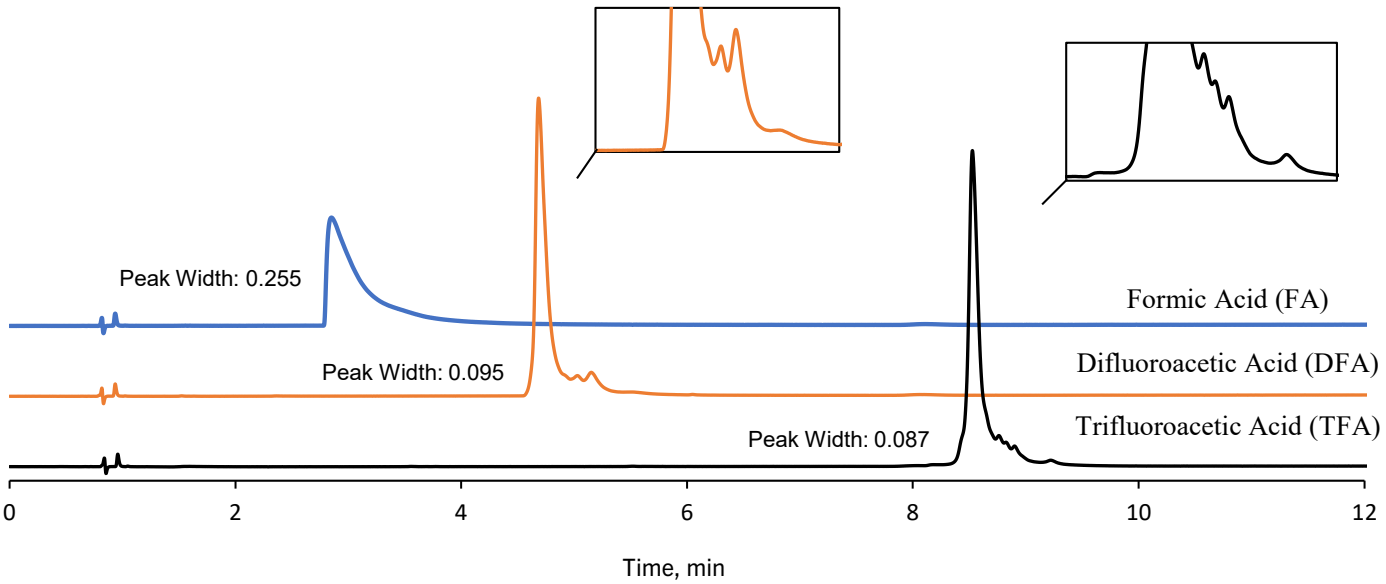
Time (min)	%B
0.00	40
12.0	47.5

Flow Rate: 0.4 mL/min
Initial Pressure: 218 bar
Temperature: 80 °C
Injection Volume: 2 µL
Sample Solvent: 30/70 Acetonitrile/H_2O Detection: UV 280 nm, PDA
Data Rate: 12.5 Hz
Response Time: 0.05 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

STRUCTURES



Trastuzumab (~148 kDa) is a monoclonal antibody (mAb) used to treat breast cancer. TFA and DFA can be used as mobile phase additives instead of formic acid to provide much narrower and more symmetrical peaks, and to allow adjustments to retention and resolution among minor variants.



IdeS DIGESTED mAb

TEST CONDITIONS

Columns: HALO BioClass Protein Diphenyl, 1000 Å, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-726

HALO BioClass Protein ES-C18, 1000 Å, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-702

HALO BioClass Protein C4, 1000 Å, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-714

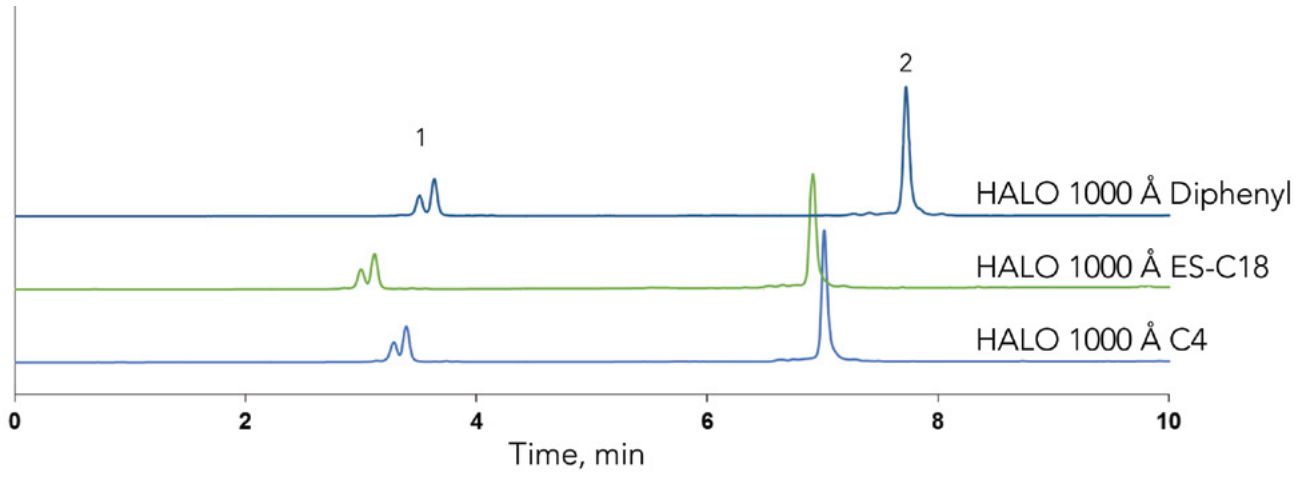
Mobile Phase: A: H_2O/0.1% TFA
B: Acetonitrile/0.1% TFA

Gradient: 30-45% B in 10 min
Flow Rate: 0.4 mL/min
Temperature: 80 °C
Detection: Fluorescence (280 nm ex, 350 nm em)
Injection Volume: 0.5 µL
LC System: UPLC, I-Class

The characterization of mAbs is critically important for protein biotherapeutic drug development. Although the analysis of the heavy and light chain can provide important information, often times site specific information is more critical and allows for a more thorough characterization of the mAb. IdeS, a cysteine protease, is often used to do a partial digestion of the mAb, and by site specific cleavage, provide heterogeneity information about the structure. Two Fc fragments (Fc/2) and one $(Fab')_2$ fragment are produced, which allows for a thorough characterization of the Fc fragment. The separation of IdeS digested cetuximab was run on the three stationary phases that are available on the 1000 Å HALO® particle. Slightly different selectivity and retention were observed for the Diphenyl, ES-C18, and C4 with all of them providing excellent resolution and peak shape for the fragments of cetuximab.

ANALYTES

1. Fc/2
2. $F(ab')_2$



IgG2 SELECTIVITY COMPARISON ON 1000 Å C4, ES-C18, AND DIPHENYL PHASES

TEST CONDITIONS

Columns:	HALO BioClass Protein C4, 1000 Å, 2.7 µm, 2.1 x 150 mm Part Number: 92712-714
	HALO BioClass Protein ES-C18, 1000 Å, 2.7 µm, 2.1 x 150 mm Part Number: 92712-702
	HALO BioClass Protein Diphenyl, 1000 Å, 2.7 µm, 2.1 x 150 mm Part Number: 92712-726
Mobile Phase:	A: 2:10:88 n-Propanol/Acetonitrile/H_2O + 0.1% Difluoroacetic acid (DFA)
	B: 70:20:10 n-Propanol/Acetonitrile/H_2O + 0.1% DFA
Gradient:	16–26% B in 20 min
Flow Rate	0.2 mL/min
Temperature:	80 °C
Instrument:	Shimadzu Nexera
Detection:	PDA 280 nm; 350 nm reference
Injection Volume:	2 µL of 2 mg/mL denosumab
Sample Solvent:	H_2O (0.1% TFA)

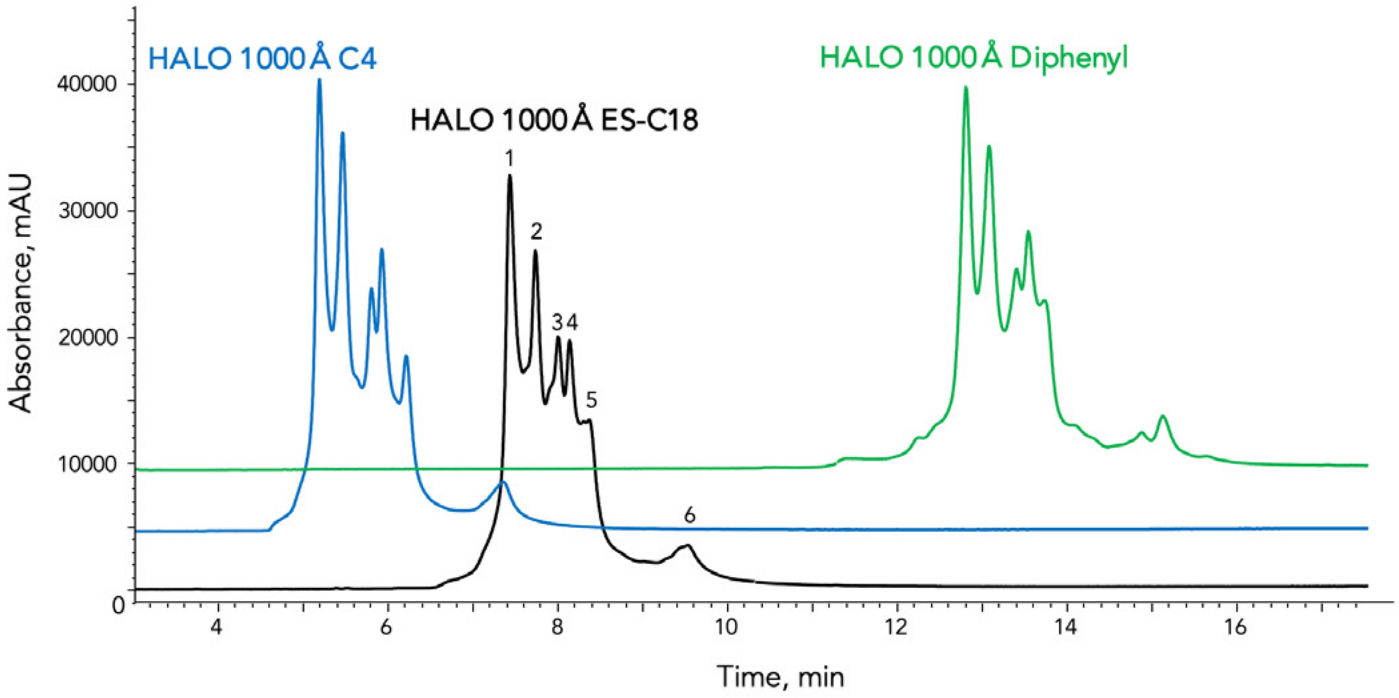
There are currently three bonded phases available on HALO 1000 Å Fused-Core® particles – C4, ES-C18, and Diphenyl. Each shows unique selectivity for the separation of monoclonal antibodies. In this example, denosumab isoforms are resolved using a shallow gradient with the addition of n-propanol. Diphenyl phase is the most retentive phase, followed by ES-C18, and then C4. All three phases are recommended to be screened to determine which one yields the optimum separation for mAbs under investigation.

ANALYTES

1. IgG2-B
2. IgG2-B
3. IgG2-A/B
4. IgG2-A/B
5. IgG2-A
6. IgG2-A*

} disulfide bridge isoforms of IgG2

Note: Labels on ES-C18 chromatogram also apply to C4 and Diphenyl chromatograms.



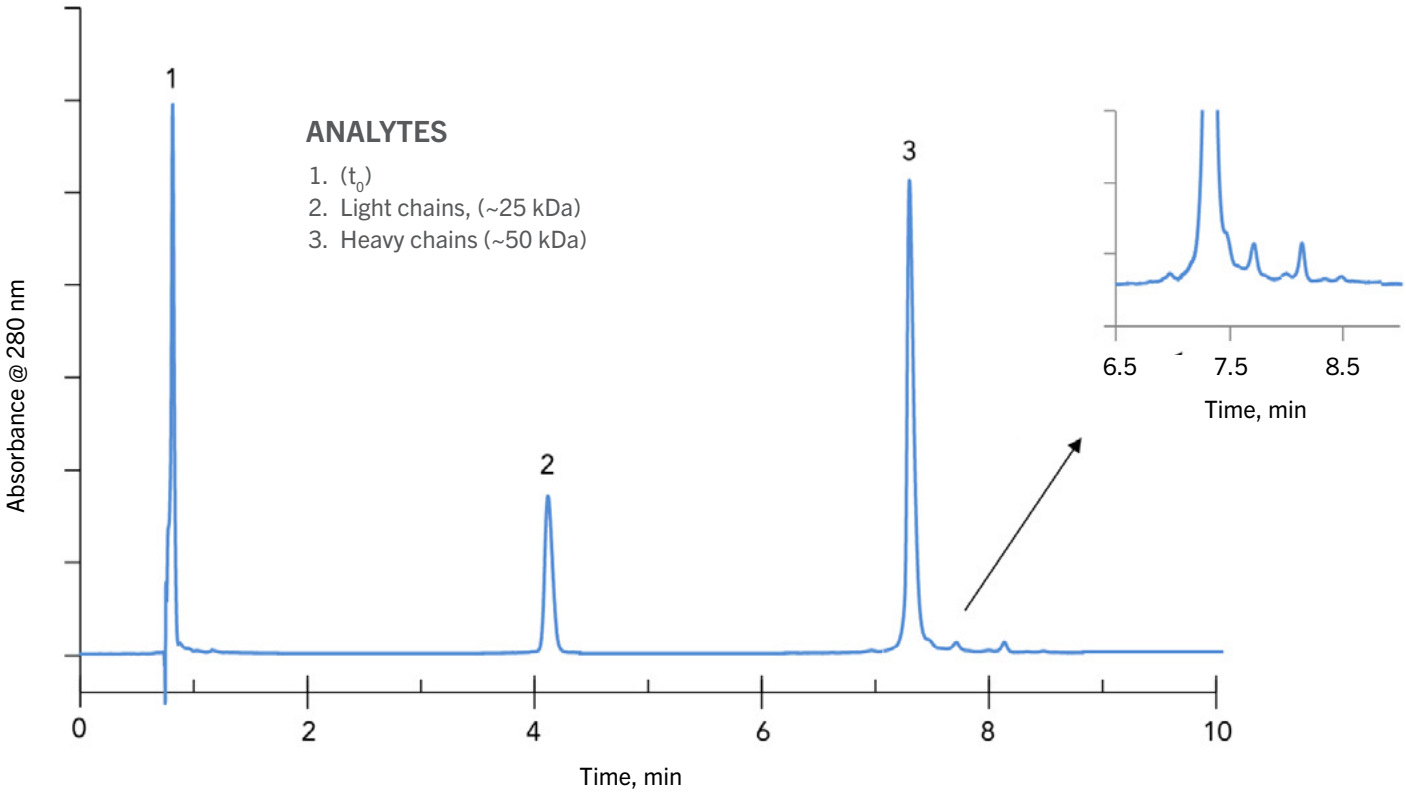
IgG2-B MONOCLONAL ANTIBODY

TEST CONDITIONS

Column:	HALO BioClass Protein C4, 400 Å, 3.4 µm, 2.1 x 100 mm
Part Number:	93412-614
Mobile Phase:	67/33: A/B to start A: H_2O ctg. 0.1% Trifluoroacetic acid (TFA) B: 80/20: Acetonitrile/H_2O/ (0.1% TFA)
Gradient:	33% B to 40%B in 10 minutes
Flow Rate:	0.25 mL/min
Initial pressure:	42 Bar
Temperature:	80 °C
Detection:	UV 280 nm, PDA
Injection Volume:	1.0 µL
Sample:	0.5 mg/mL IgG2-B treated with 100 mM DTT in 8 M guanidine-HCl @ 50 °C for 35 minutes
Response Time:	0.08 sec
Flow Cell:	1 µL micro cell
LC System:	Shimadzu Nexera
Gradient delay volume:	~ 115 µL

The HALO Fused-Core Protein C4, 400 Å, 3.4 µm stationary phase is useful for the separation of proteins up to 500 kDa in size. Shown here is the separation of light and heavy chains from a reduced IgG2-B antibody. Note the resolution of small peaks at the end of the chromatogram.

Special endcapping procedures ensure that the columns will be stable at elevated temperatures, even with aggressive mobile phases.



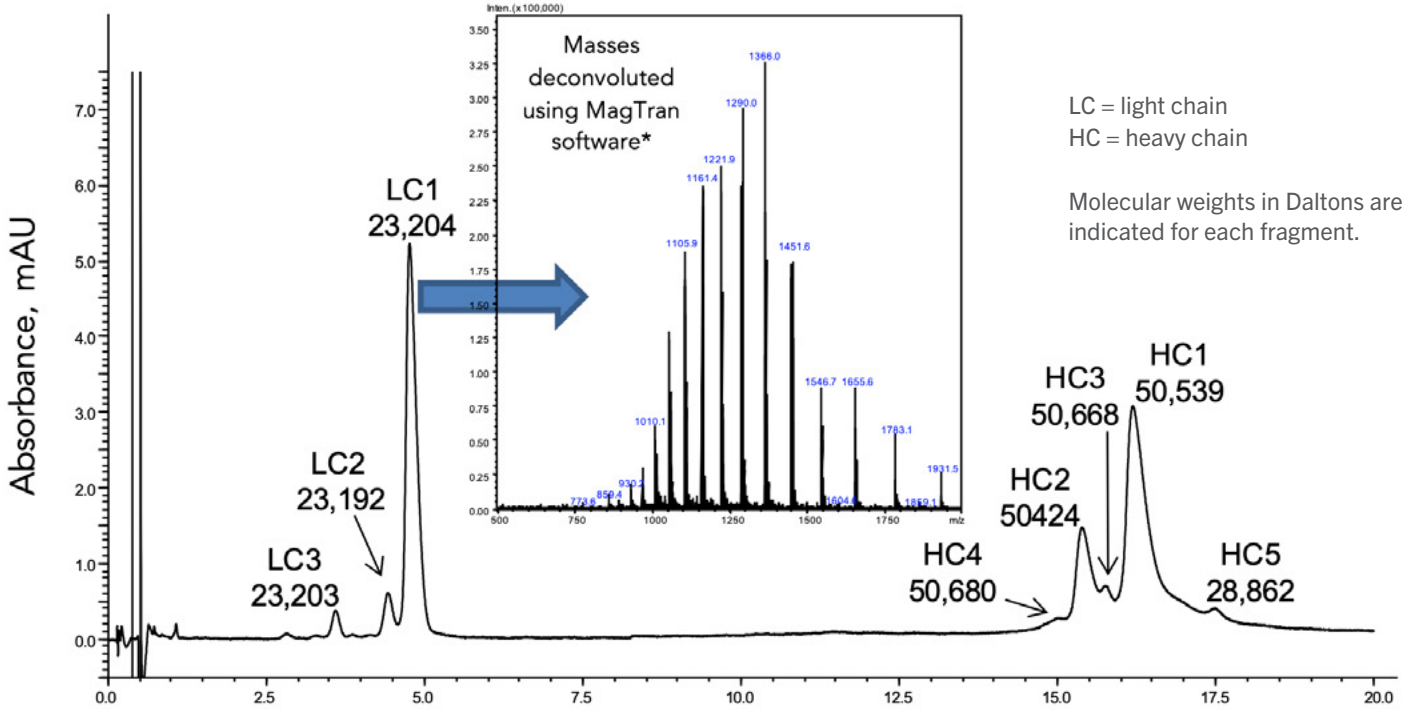
LC-MS ANALYSIS OF REDUCED IgG1 MONOCLONAL ANTIBODY FRAGMENTS

TEST CONDITIONS

Column:	HALO BioClass Protein C4, 400 Å, 3.4 µm, 2.1 x 100 mm
Part Number:	93412-614
Mobile Phase:	A: .5% (v/v) Formic acid with 20 mM Ammonium formate B: 45% Acetonitrile/45% Isopropanol/0.5% (v/v) Formic acid/9.5% H_2O with 20 mM Ammonium formate
Gradient:	29–32% B in 20 min
Flow Rate:	0.4 mL/min
Pressure:	20 bar
Temperature:	80 °C
Detection:	280 nm and MS using 2 pps scan rate from 500 to 2000 m/z
Injection Volume:	2 µL of 2 µg/µL reduced and alkylated IgG1
Sample Solvent:	0.25% (v/v) Formic acid in H_2O
MS parameters:	Positive ion mode, ESI at +4.5 kV, 400 °C heat block, 225 °C capillary
LC-MS System:	Shimadzu Nexera and LCMS-2020 (single quadrupole MS)

HALO 400 Å C4 has the low pH and high temperature stability that is required to analyze reduced and alkylated IgG1 using MS-compatible mobile phase. The use of 80 °C enables improved peak shape while the high resolution MS allows complete analysis of the IgG1 fragments that are present.

Adapted from *J. Chromatogr. A* 1315 (2013) 118-126.



LC = light chain
HC = heavy chain

Molecular weights in Daltons are indicated for each fragment.

*Z. Zhang, A.G. Marshall, J. Am. Soc. Mass Spectrom. 9 (1998) 225.

LC-MS ANALYSIS OF TRASTUZUMAB USING A 1000 Å C4 COLUMN

LC TEST CONDITIONS

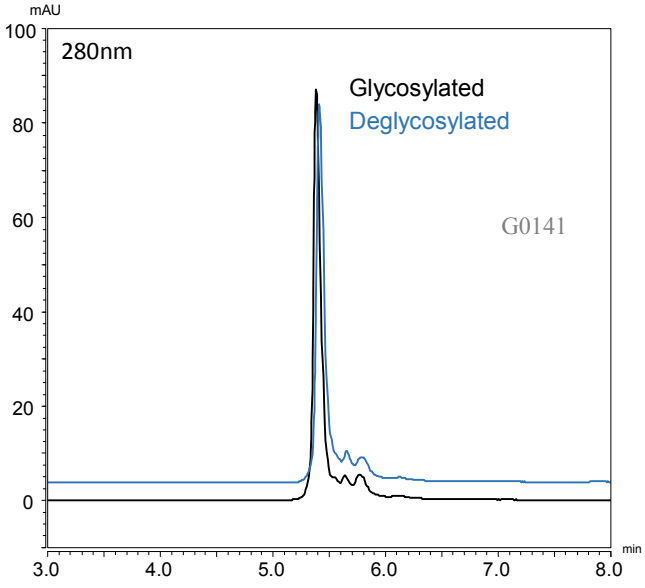
Column:	HALO BioClass Protein C4, 1000 Å, 2.7 µm, 2.1 x 150 mm
Part Number:	92712-714
Mobile Phase:	A: 10 mM Difluoroacetic acid (DFA) in H_2O B: 10 mM Difluoroacetic acid in 10/90 H_2O/acetonitrile
Gradient:	32–42% B in 10 min
Flow Rate:	0.35 mL/min
Pressure:	184 bar
Temperature:	80 °C
Detection :	280 nm
Injection Volume:	1 µL of 2 mg/mL trastuzumab (glycosylated/deglycosylated)
Sample Solvent:	0.1% DFA in 70/30 H_2O/acetonitrile
LC System:	Shimadzu Nexera

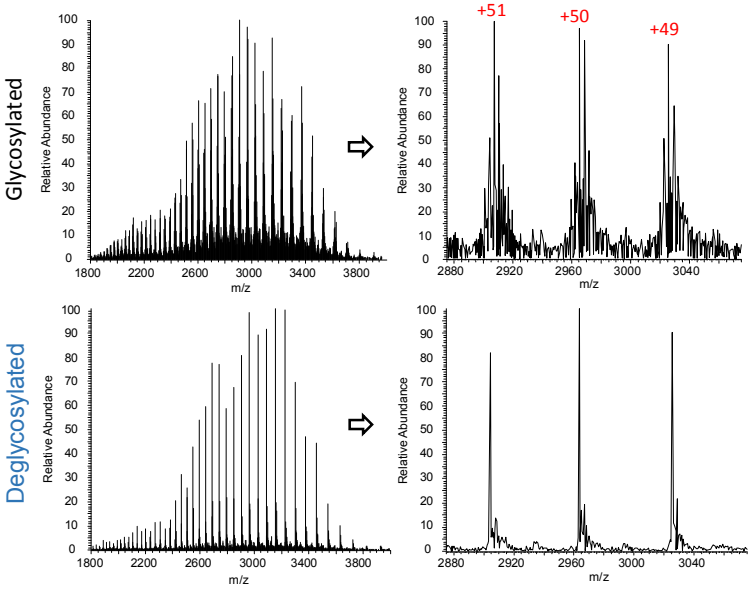
MS TEST CONDITIONS

MS System:	Thermo Fisher Orbitrap VelosPro ETD
Scan Time:	6 µscans/250 ms max inject time
Scan range:	1800 to 4000 m/z
MS parameters:	Positive ion mode, ESI at +4.0 kV, 225 °C capillary

LC-MS analysis using a HALO 1000 Å C4 Protein column has been used to analyze two samples of the monoclonal antibody, trastuzumab: glycosylated and enzymatically deglycosylated. Minor variant structures are observed in both the glycosylated and deglycosylated monoclonal IgG (small peaks after main peak), indicating that the polypeptides are structural variants.

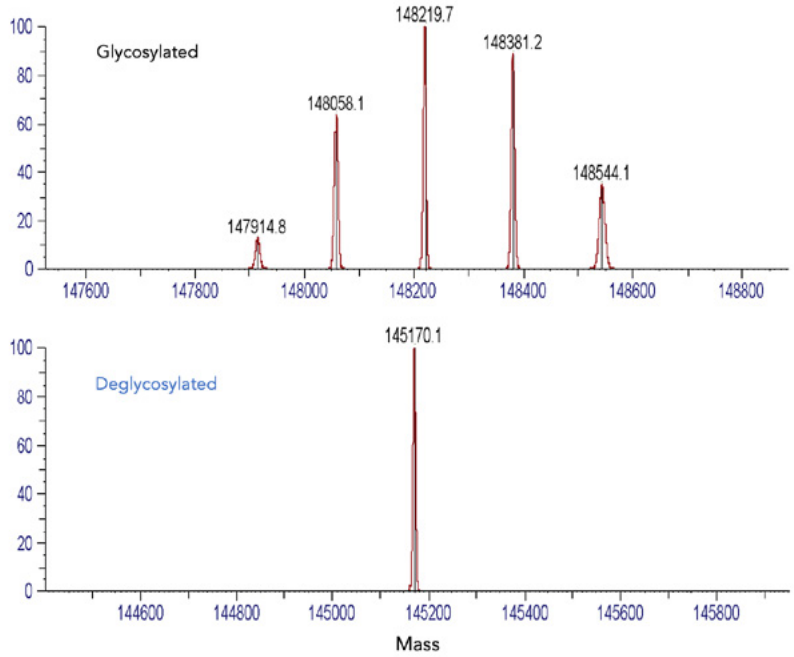
The glycosylation profile of therapeutic mAbs is an important characteristic that must be monitored throughout the manufacturing process. Determination of the mass of the deglycosylated IgG confirms the identity and integrity of the protein.





LC-MS ANALYSIS OF TRASTUZUMAB USING A 1000 Å C4 COLUMN *(continued)*

DECONVOLUTED SPECTRA AND PEAK INFORMATION



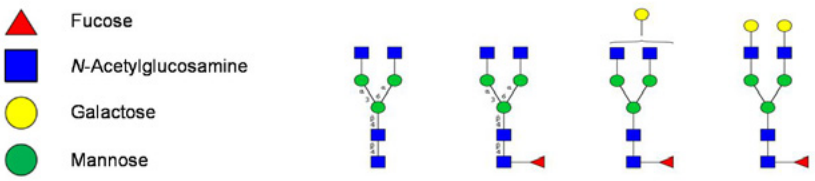
The structure of trastuzumab consists of two heavy chains and two light chains. Glycosylation occurs on the two heavy chains. One or more of the same or different carbohydrate moieties can be present on each heavy chain. Table 1 contains the combinations of sugars that correspond to the masses that were observed upon deconvolution of the mass spectrum on the previous page. The last column is the mass of the deglycosylated trastuzumab, which results from enzymatic cleavage of the glycans by PNGase F.

TABLE 1

GLYCANS	G0/G0F		G0F/G0F		G1F/G0F		G1F/G1F, G2F/G0F		G1F/G2F		Deglycosylated Trastuzumab	
	T[1]	M[1]	T	M	T	M	T	M	T	M	T	M
Trastuzumab	147911	147915	148057	148058	148219	148220	148381	148381	148543	148544	145167	145170
ΔMass (glyc) Trastuzumab	2744	2745	2890	2888	3052	3050	3214	3211	3376	3374	—	3

T = Theoretical mass
M = Measured mass
[1]All masses reported in Daltons

Glycan Descriptions:



DECONVOLUTION PARAMETERS:

Minimum Adjacent Charges 3 - 6
Noise Rejection 95% Confidence
m/z Range 1800 - 4000
Mass Tolerance 20 ppm
Charge State Range 40 - 120
Choice of Peak Model Intact Protein

NIST mAb

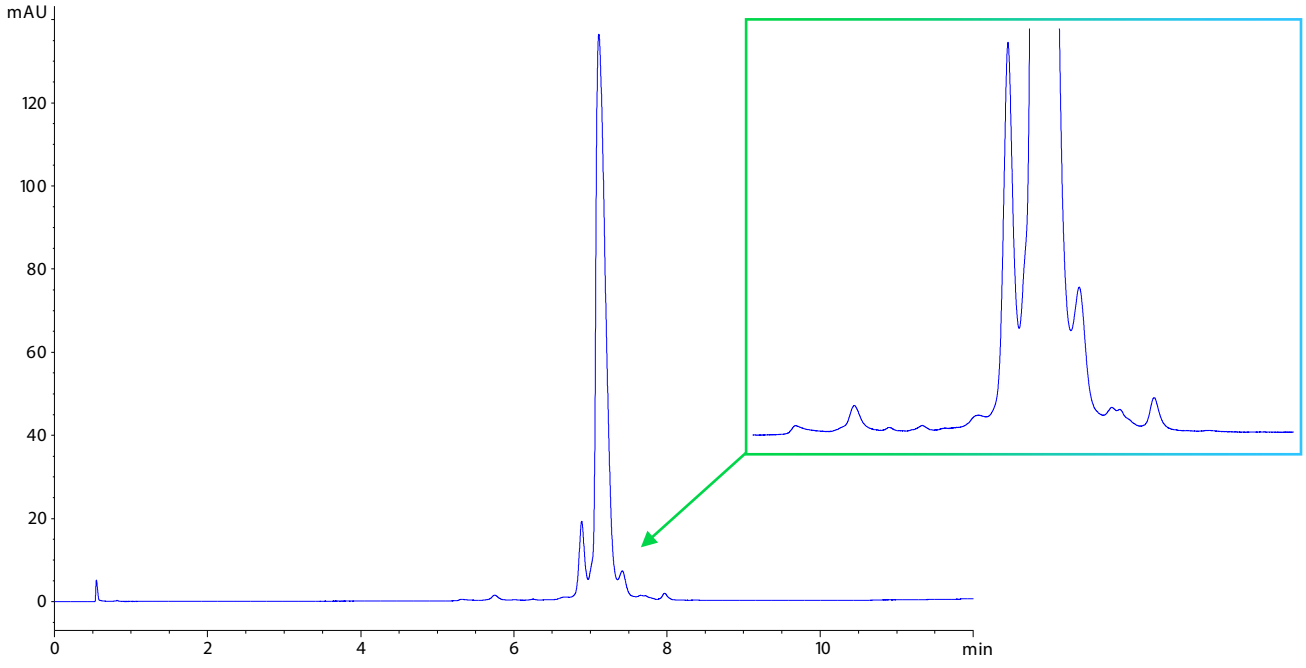
TEST CONDITIONS

Column: Avantor® ACE® UltraCore BIO C4, 500 Å, 2.5 µm, 3.0 x 100 mm
Part Number: BIO-251-1030
Mobile Phase: A: 0.1% TFA in H_2O
B: 0.1% TFA in Acetonitrile/H_2O 9:1 v/v

Gradient:

Time (min)	%B
0	36
10	45
12	80
14	80
14.5	36

Flow Rate: 0.8 mL/min
Temperature: 80 °C
Injection Volume: 1 µL
Detection: UV, 280 nm



REDUCED IgG1 (TRASTUZUMAB) RETENTION COMPARISON

TEST CONDITIONS

Column: HALO BioClass Protein Diphenyl, 1000 Å, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-726

HALO BioClass Protein C4, 1000 Å, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-714

HALO BioClass Protein ES-C18, 1000 Å, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-702

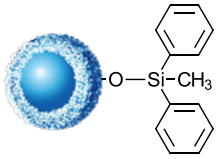
Mobile Phase: A: H_2O/ 0.1% TFA
B: Acetonitrile/ 0.1% TFA

Gradient:

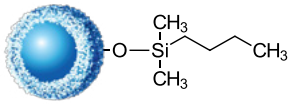
Time (min)	%B
0.0	30
14.0	40

Flow Rate: 0.4 mL/min
Temperature: 80 °C
Detection: 280 nm, PDA
Injection Volume: 2 µL
Sample Solvent: H_2O
Data Rate: 12.5 Hz
Response Time: 0.25 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

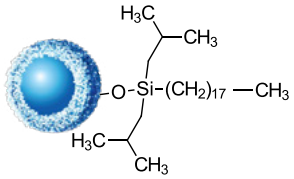
STRUCTURES



HALO Protein Diphenyl, 1000 Å

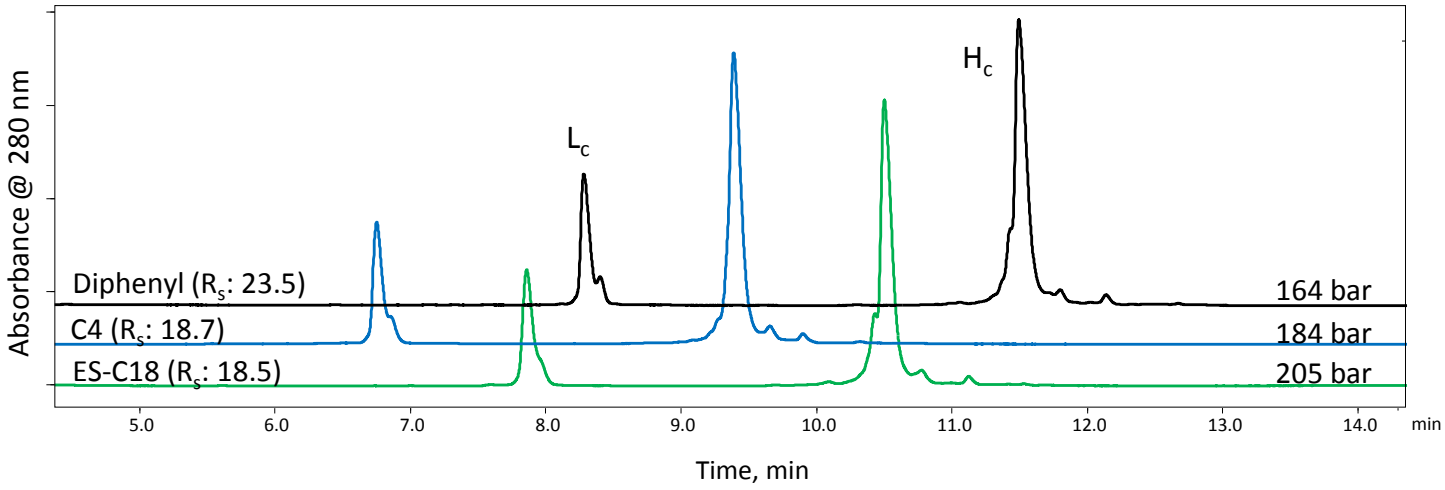


HALO Protein C4, 1000 Å



HALO Protein ES-C18, 1000 Å

Trastuzumab is a monoclonal antibody used to treat breast cancer. Enhanced resolution of trastuzumab's heavy and light chains is demonstrated in the chromatograms below using three different HALO® bonded phases. The 1000 Å pores of the HALO® Protein columns readily accommodate large biomolecules, and allow unrestricted pore access, narrower peaks and superior separations at high temperatures.



TRASTUZUMAB AND LARGE MOLECULAR MASS FRACTIONS

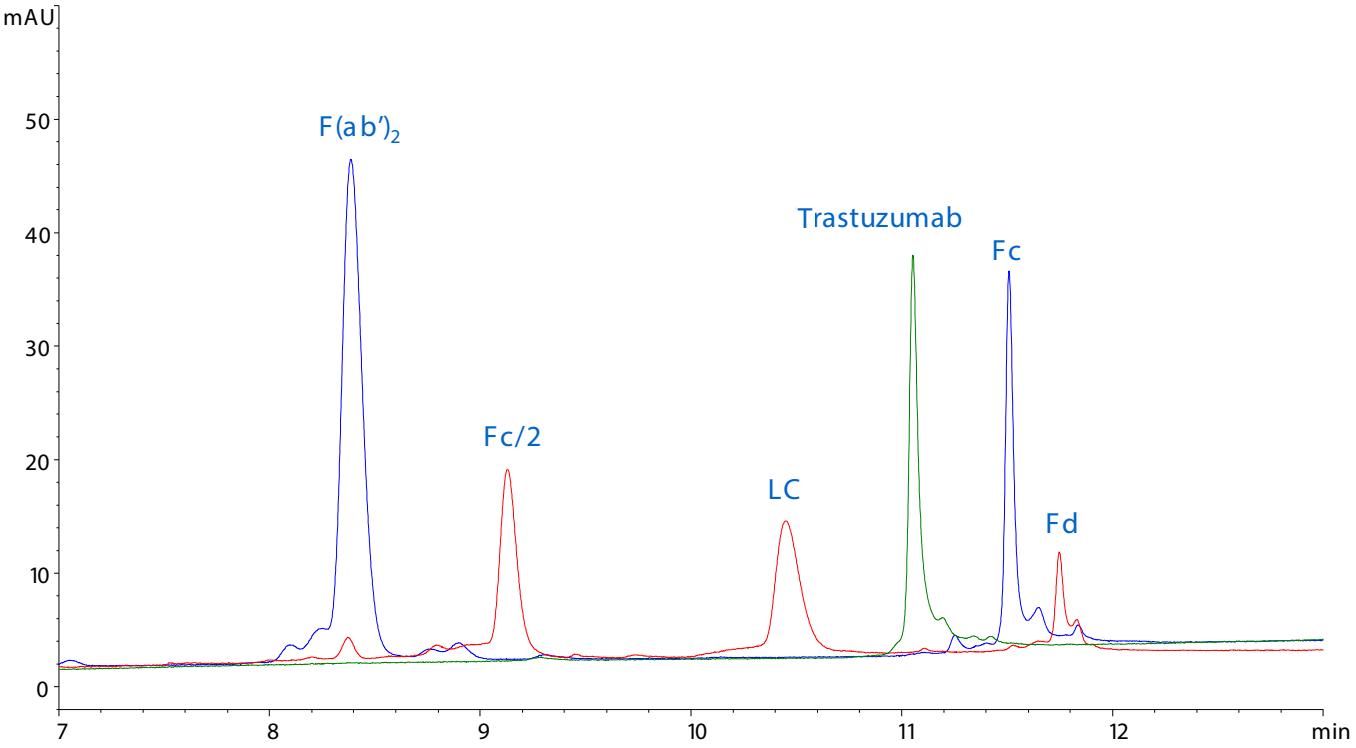
TEST CONDITIONS

Column: Avantor® ACE® UltraCore BIO C18, 300 Å, 3.5 µm, 3.0 x 100 mm
Part Number: BIO-350-1030
Mobile Phase: A: 0.1% TFA in H_2O
B: 0.1% TFA in Acetonitrile

Gradient:

Time (min)	%B
0	25
20	50
21	95
23	95
24	25
34	25

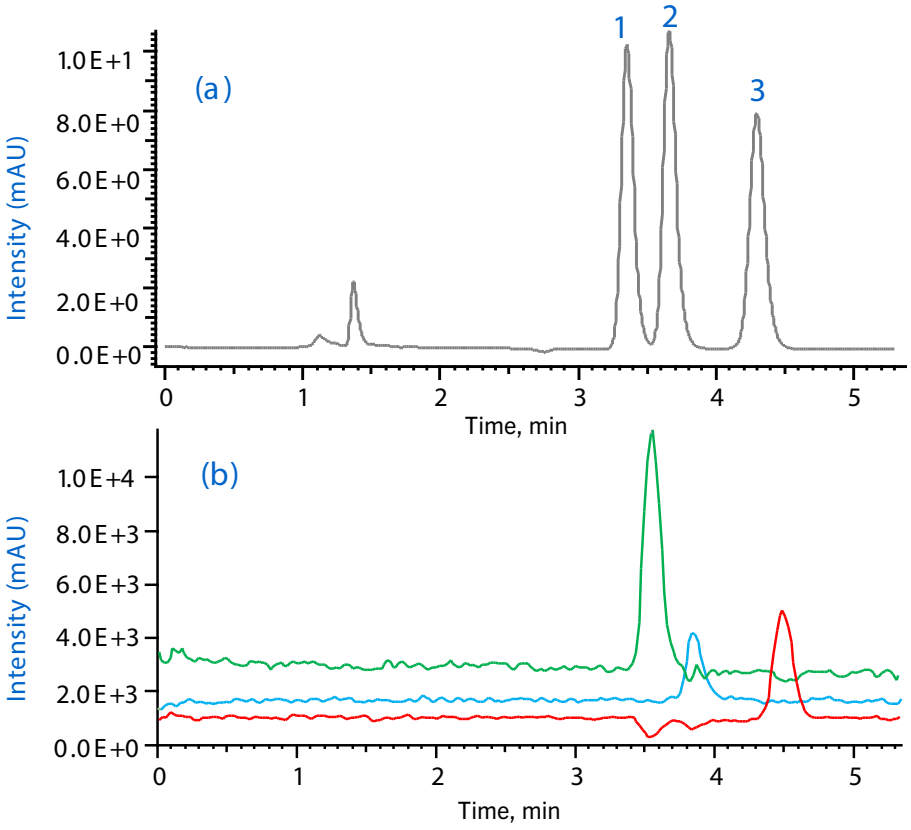
Flow Rate: 0.43 mL/min
Temperature: 60 °C
Injection Volume: 20 µL
Detection: UV, 214 nm
System Dwell Volume: 525 µL



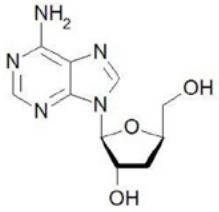
ADENINE AND NUCLEOSIDES USING HILIC-MS MODE

TEST CONDITIONS

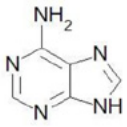
Column:	Avantor® ACE® HILIC-N, 100 Å, 5 µm, 4.6 x 150 mm
Part Number:	HILN-5-1546U
Mobile Phase:	10 mM Ammonium formate pH 4.7 in Acetonitrile/H_2O (90:10 v/v)
Flow Rate:	1.5 mL/min
Temperature:	25 °C
Injection Volume:	5 µL
Detection:	(a) UV, 254 nm (b) Chromaster MSD SIM Positive ion mode $[M + H]^+$
LC Instrument:	VWR Hitachi Chromaster and 5610 MSD single quad MS



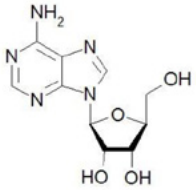
1. Deoxyadenosine (*m/z* 252.4)



2. Adenine (*m/z* 136.1)



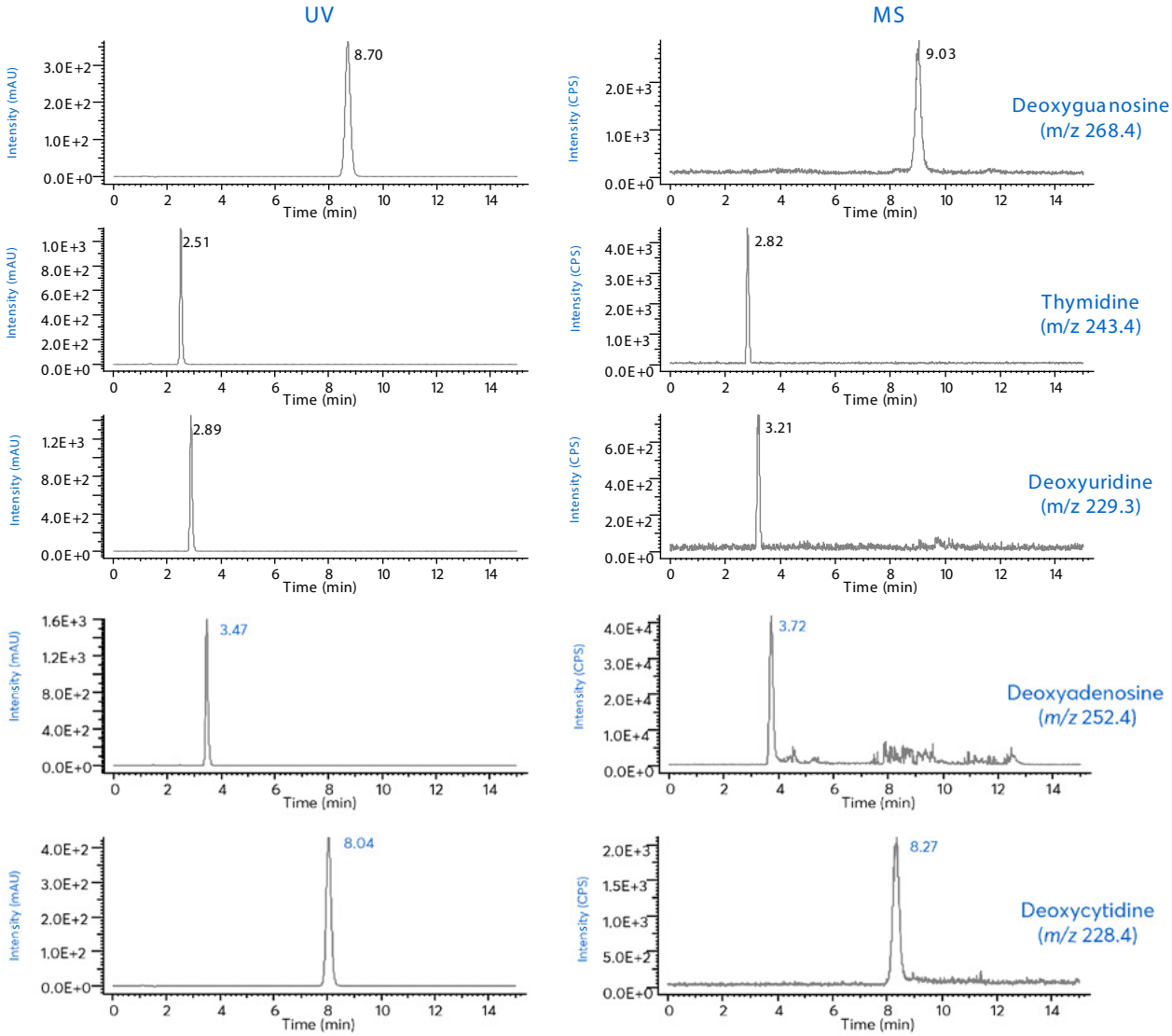
3. Adenosine (*m/z* 268.3)



NITROGENOUS BASES AND NUCLEOSIDES USING HILIC-MS MODE

TEST CONDITIONS

Column:	Avantor® ACE® HILIC-N, 100 Å, 5 µm, 4.6 x 150 mm
Part Number:	HILN-5-1546U
Mobile Phase:	10 mM ammonium formate pH 4.7 in Acetonitrile/H_2O (90:10 v/v)
Flow Rate:	1.5 mL/min
Temperature:	25 °C
Injection Volume:	5 µL
Detection:	UV, 254 nm Chromaster MSD, SIM Positive ion mode [M + H]+
LC Instrument:	VWR Hitachi Chromaster and 5610 MSD single quad MS

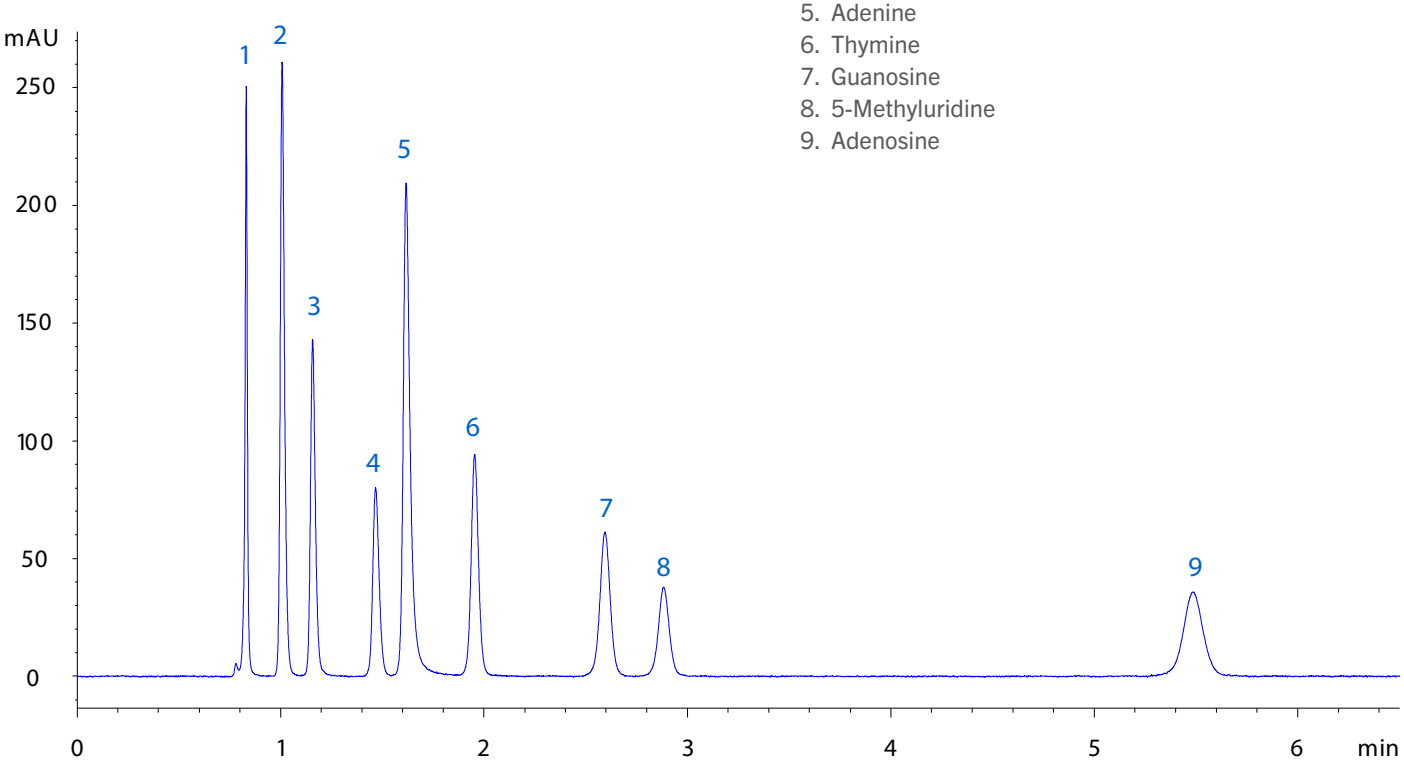


NUCLEOSIDES AND NUCLEOBASES

TEST CONDITIONS

Column:	Avantor® ACE® UltraCore Phenylhexyl, 95 Å, 3.5 µm, 3.0 x 100 mm
Part Number:	CORE-35G-1030
Mobile Phase:	10 mM ammonium formate pH 4.2 in H_2O
Flow Rate:	0.6 mL/min
Temperature:	40 °C
Injection Volume:	3 µL
Detection:	UV, 254 nm

Analytes
1. Cytosine
2. Uracil
3. Guanine
4. Uridine
5. Adenine
6. Thymine
7. Guanosine
8. 5-Methyluridine
9. Adenosine



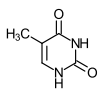
NUCLEOSIDES AND NUCLEOBASES ON PENTA-HILIC

TEST CONDITIONS

Column:	HALO Penta-HILIC, 90 Å, 2.7 µm, 4.6 x 100 mm
Part Number:	92814-605
Mobile Phase:	8/92: H_2O/Acetonitrile with 0.01 M Ammonium formate, pH=6 (adj.)
Flow Rate:	1.5 mL/min
Pressure:	99 Bar
Temperature:	35 °C
Detection:	UV 260 nm, DAD
Injection Volume:	2.0 µL
Sample Solvent:	Mobile phase
Response Time:	0.02 sec
Flow Cell:	2.5 µL semi-micro
LC System:	Shimadzu Nexera

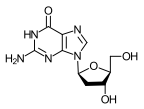
The new HALO Penta-HILIC stationary phase is an HPLC phase having a hydroxyl-rich surface for performing separations in the hydrophilic interaction chromatography mode. Here, a mixture of 13 nucleosides and nucleobases are separated isocratically in a short time with excellent resolution. These bonded superficially porous 2.7 µm HALO particles allow high resolution with modest back pressure.

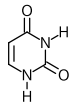
STRUCTURES

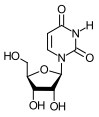

Thymine

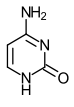

Adenine

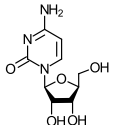

Hypoxanthine

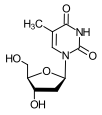

2′-Deoxyguanosine

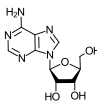

Uracil

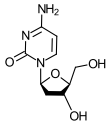

Uridine

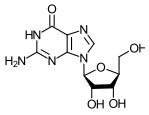

Cytosine

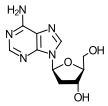

Cytidine


Thymidine


Adenosine

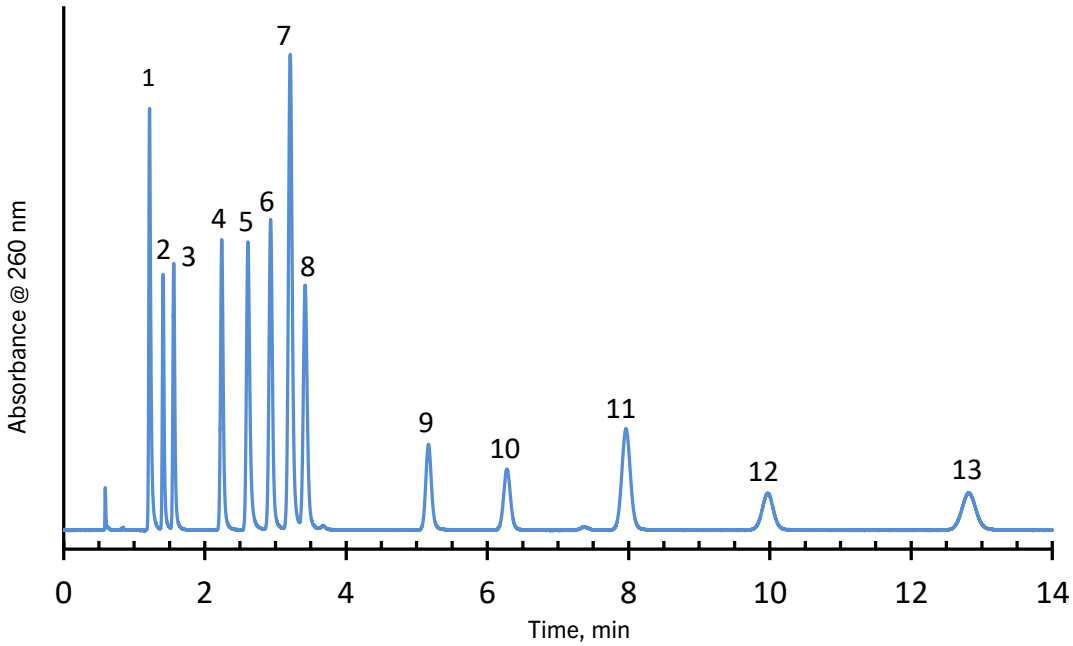

2′-Deoxycytidine


Guanosine


2′-Deoxyadenosine

ANALYTES

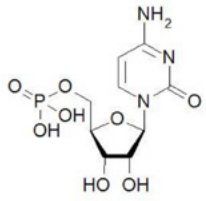
1. Thymine
2. Uracil
3. Thymidine
4. 2-Deoxyadenosine
5. Adenine
6. Uridine
7. Adenosine
8. Hypoxanthine
9. Cytosine
10. 2-Deoxycytidine
11. 2-Deoxyguanosine
12. Cytidine
13. Guanosine



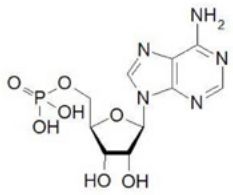
MONOPHOSPHATE NUCLEOTIDES

TEST CONDITIONS

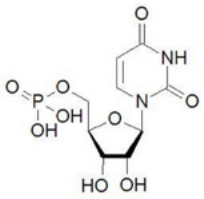
Column:	Avantor® ACE® Excel NH_2, 100 Å, 3 µm, 4.6 x 150 mm
Part Number:	EXL-1114-1546U
Mobile Phase:	10 mM Potassium dihydrogen phosphate pH 2.0 in Acetonitrile/H_2O (50:50 v/v)
Flow Rate:	1 mL/min
Temperature:	40 °C
Injection Volume:	10 µL
Detection:	UV, 260 nm



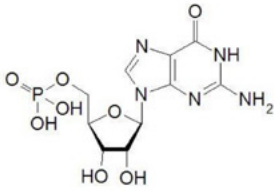
Cytidine monophosphate



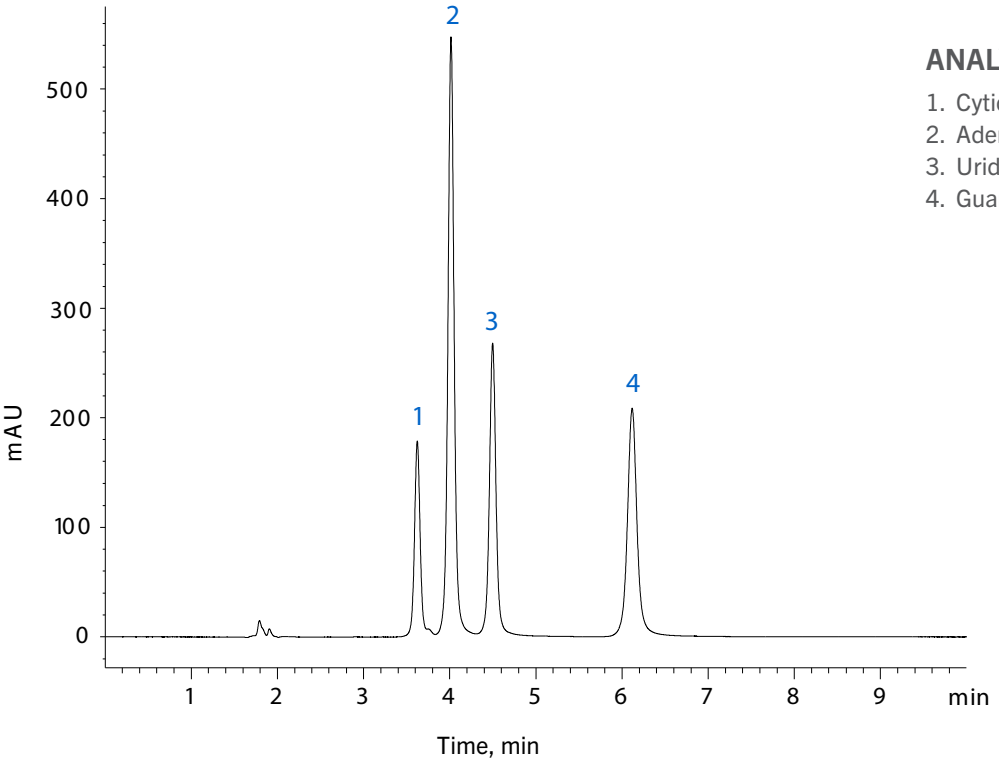
Adenosine monophosphate



Uridine monophosphate



Guanosine monophosphate



ANALYTES

1. Cytidine monophosphate
2. Adenosine monophosphate
3. Uridine monophosphate
4. Guanosine monophosphate

NUCLEIC ACIDS AND DISEASE BIOMARKER PROFILING I

TEST CONDITIONS

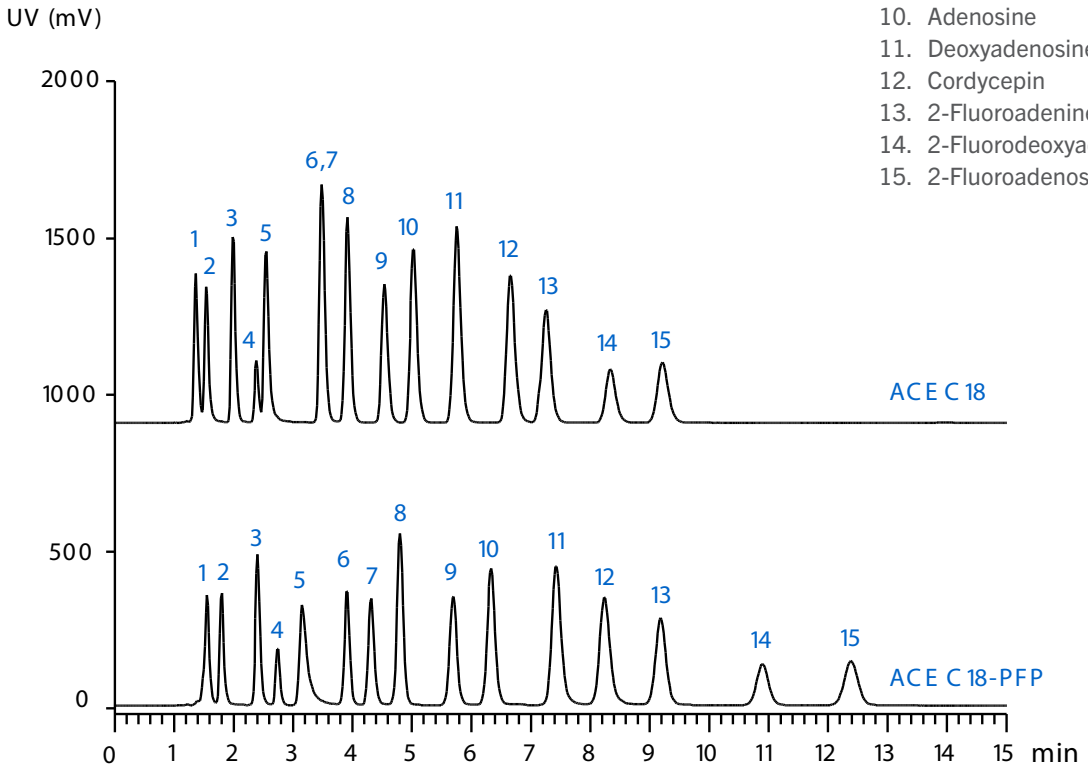
Column: Avantor® ACE® C18-PFP, 100 Å, 3 µm, 4.6 x 100 mm
Part Number: ACE-1110-1046

Avantor® ACE® C18, 100 Å, 3 µm, 4.6 x 100 mm
Part Number: ACE-111-1046

Mobile Phase: 33 mM potassium phosphate pH 6.2 with KOH/MeOH (88:12 v/v)
Flow Rate: 1 mL/min
Temperature: Ambient
Detection: UV, 260 nm

ANALYTES

1. dATP
2. dADP
3. dAMP
4. 5-Fluorodeoxyuridine
5. Adenine
6. Thymine
7. 2-Fluorodeoxyuridine
8. Adenine arabinoside
9. 2'-C-Methyladenosine
10. Adenosine
11. Deoxyadenosine
12. Cordycepin
13. 2-Fluoroadenine arabinoside
14. 2-Fluorodeoxyadenosine
15. 2-Fluoroadenosine



NUCLEOTIDES

TEST CONDITIONS

Column: HALO Penta-HILIC, 90 Å, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-605
Mobile Phase: A: 50/50: Acetonitrile/0.025 M Ammonium phosphate, pH=6
B: 75/25: Acetonitrile/0.025 M Ammonium phosphate, pH=6

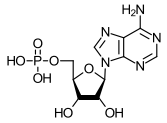
Gradient:

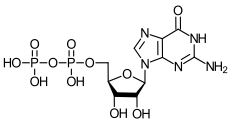
Time (min)	%B
0.0	90
8.0	40

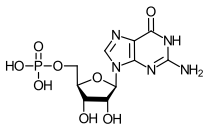
Flow Rate: 0.3 mL/min
Initial Pressure: 76 Bar
Temperature: 50 °C
Detection: UV 260 nm, DAD
Injection Volume: 1.0 µL
Sample Solvent: Mobile phase B
Response Time: 0.02 sec
Flow Cell: 1.0 µL micro
LC System: Shimadzu Nexera
Data rate: 40 Hz

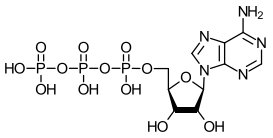
This separation demonstrates the utility of the HALO Penta-HILIC phase for analysis of nucleotides. Fused-Core technology gives high resolution separations at moderate pressures without the difficulties of using sub-2-micron-particle columns.

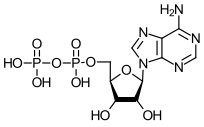
STRUCTURES

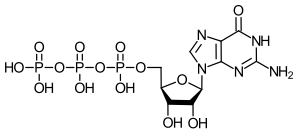

Adenosine Monophosphate

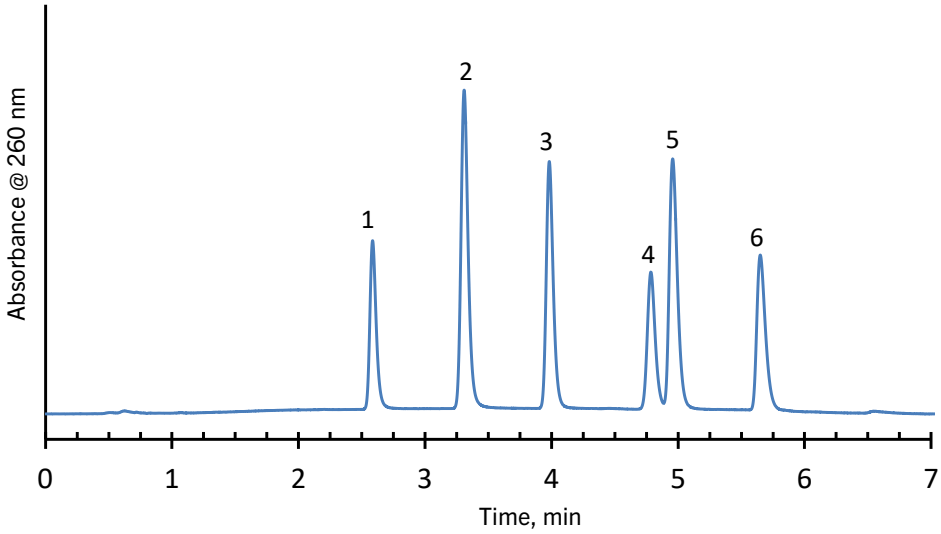

Guanosine Diphosphate


Guanosine Monophosphate


Adenosine Triphosphate


Adenosine Diphosphate


Guanosine Triphosphate



ANALYTES

1. Adenosine monophosphate (AMP)
2. Guanosine monophosphate (GMP)
3. Adenosine diphosphate (ADP)
4. Guanosine diphosphate (GDP)
5. Adenosine triphosphate (ATP)
6. Guanosine triphosphate (GTP)

ANALYSIS OF DEGRADED OLIGONUCLEOTIDE LADDER STANDARD

TEST CONDITIONS

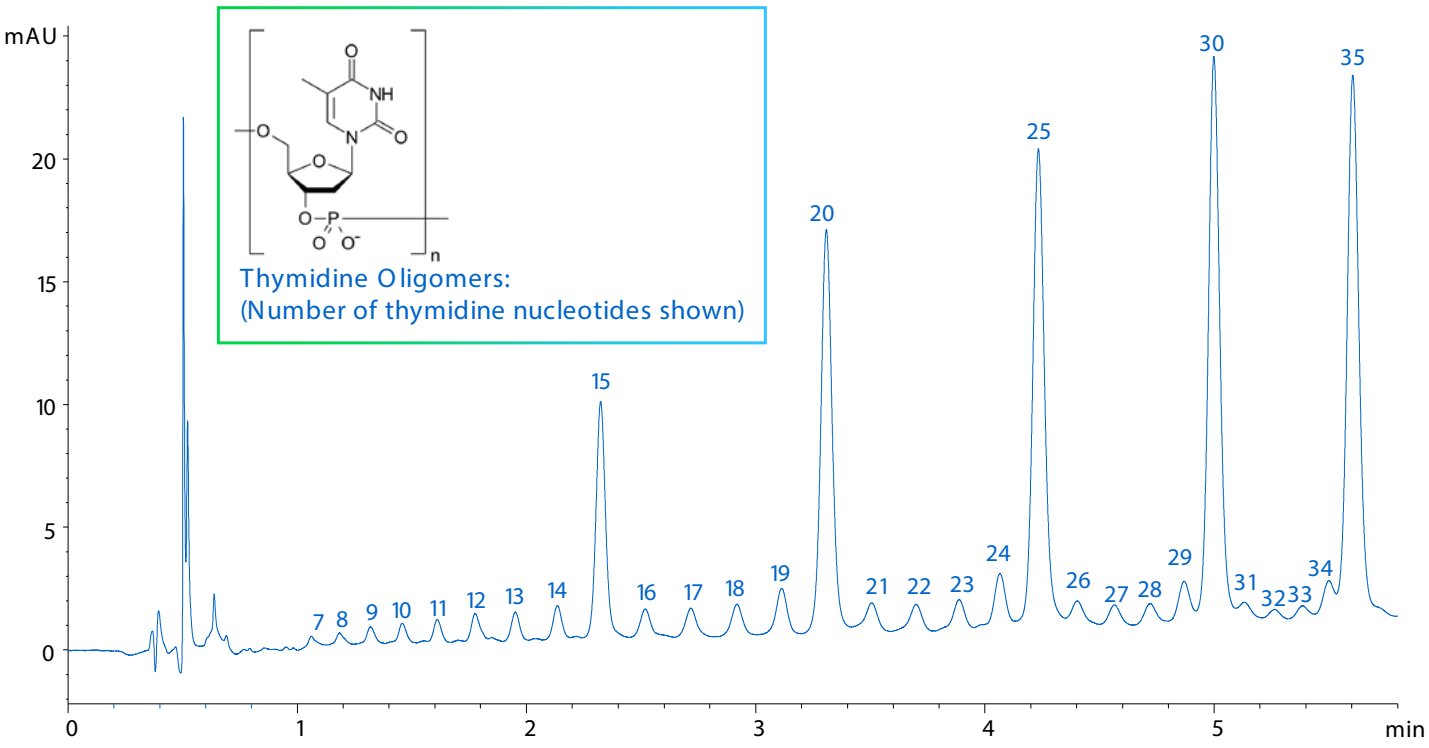
Column: Avantor® ACE® Excel Oligo, 100 Å, 1.7 µm, 2.1 x 100 mm
Part Number: EXL-1715-1002

Mobile Phase:
A: 80 mM Triethylammonium acetate in H_2O (pH 7.0)
B: 80 mM Triethylammonium acetate in Acetonitrile

Gradient:

Time (min)	%B
0.00	12
6.95	15
8.34	15
8.45	12
15.40	12

System Dwell Volume: 204 µL
Flow Rate: 0.6 ml/min
Temperature: 60 °C
Injection Volume: 2.8 µL
Detection: UV, 260 nm



MS-FRIENDLY OLIGONUCLEOTIDE SEPARATION

TEST CONDITIONS

Column: Avantor® ACE® Excel Oligo, 100 Å, 1.7 µm, 2.1 x 100 mm
Part Number: EXL-1715-1002

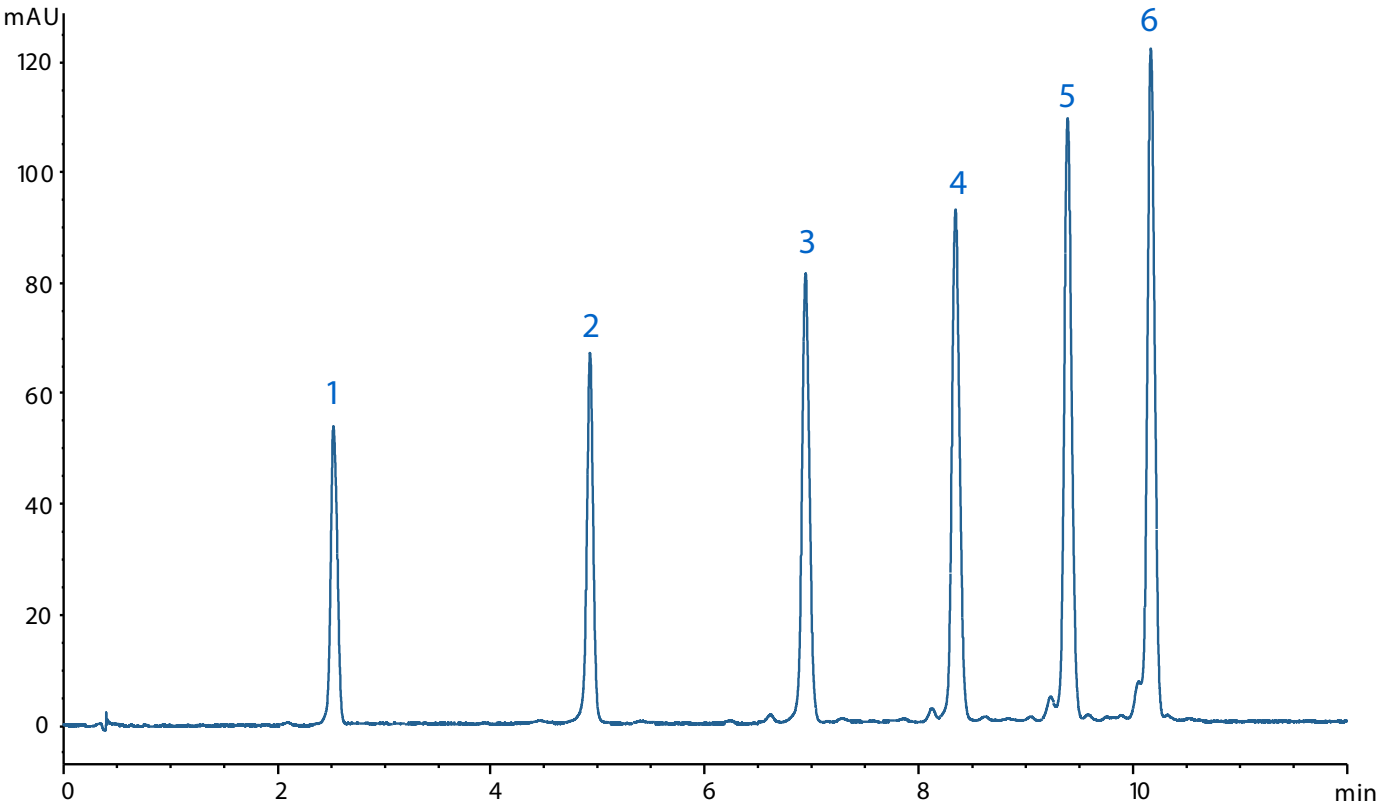
Mobile Phase:
A: 15 mM Dibutylamine + 25 mM 1,1,1,3,3,3-Hexafluoropropan-2-ol (HFIP) in H_2O
B: 15 mM Dibutylamine + 25 mM 1,1,1,3,3,3-Hexafluoropropan-2-ol (HFIP) in MeOH

Gradient:

Time (min)	%B
0.0	30
15.0	50
17.0	50
17.5	30
25.0	30

Flow Rate: 0.6 mL/min
Temperature: 60 °C
Injection Volume: 10 µL
Detection: UV, 260 nm
System Dwell Volume: 550 µL

1. 15-mer 4.5 kDa
2. 20-mer 6.0 kDa
3. 25-mer 7.5 kDa
4. 30-mer 9.1 kDa
5. 35-mer 10.6 kDa
6. 40-mer 12.1 kDa



OLIGONUCLEOTIDE LADDER STANDARD (II)

TEST CONDITIONS

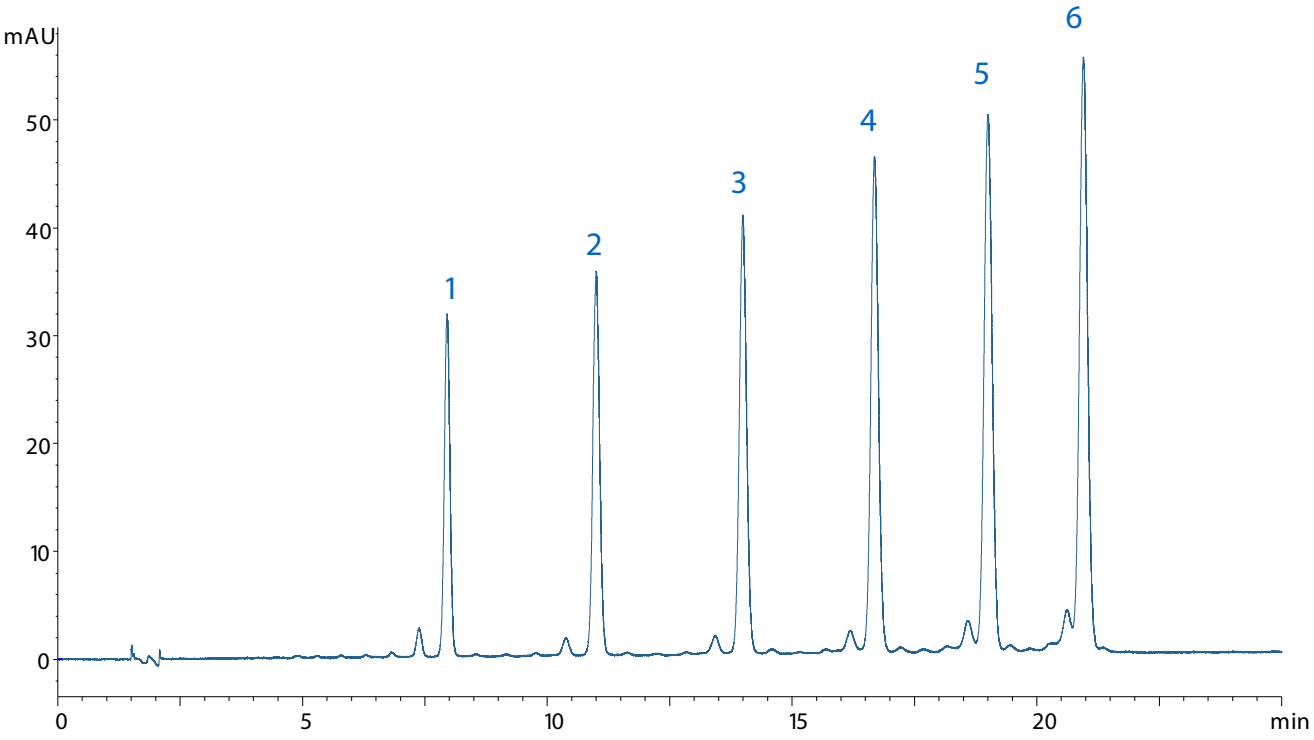
Column: Avantor® ACE® Excel Oligo, 100 Å, 3 µm, 4.6 x 150 mm
Part Number: EXL-1115-1546
Mobile Phase: A: 80 mM Triethylammonium acetate (TEAA) in H_2O (pH 7.0)
B: 80 mM Triethylammonium acetate (TEAA) in Acetonitrile

Gradient:

Time (min)	%B
0.0	12
30.0	15
36.0	15
26.5	12
66.5	12

Flow Rate: 1.0 mL/min
Temperature: 60 °C
Injection Volume: 20 µL
Detection: UV, 260 nm

1. 15-mer 4.5 kDa
2. 20-mer 6.0 kDa
3. 25-mer 7.5 kDa
4. 30-mer 9.1 kDa
5. 35-mer 10.6 kDa
6. 40-mer 12.1 kDa



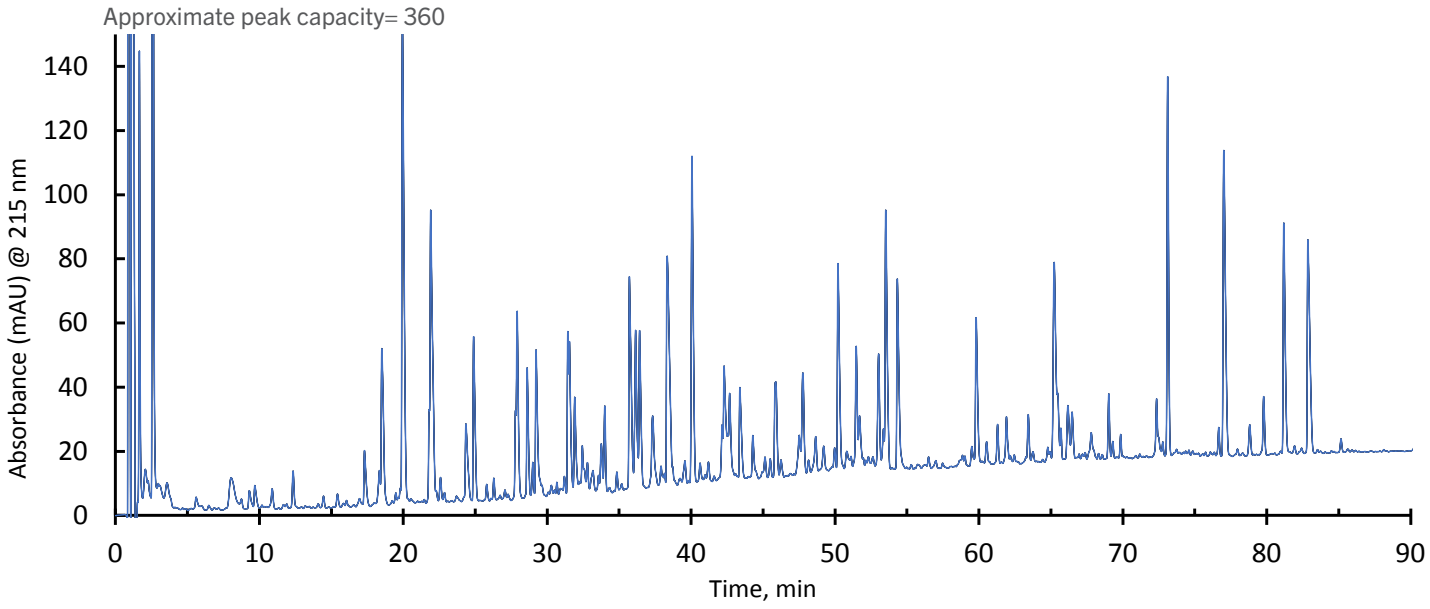
ANALYSIS OF APOTRANSFERRIN TRYPTIC DIGEST

TEST CONDITIONS

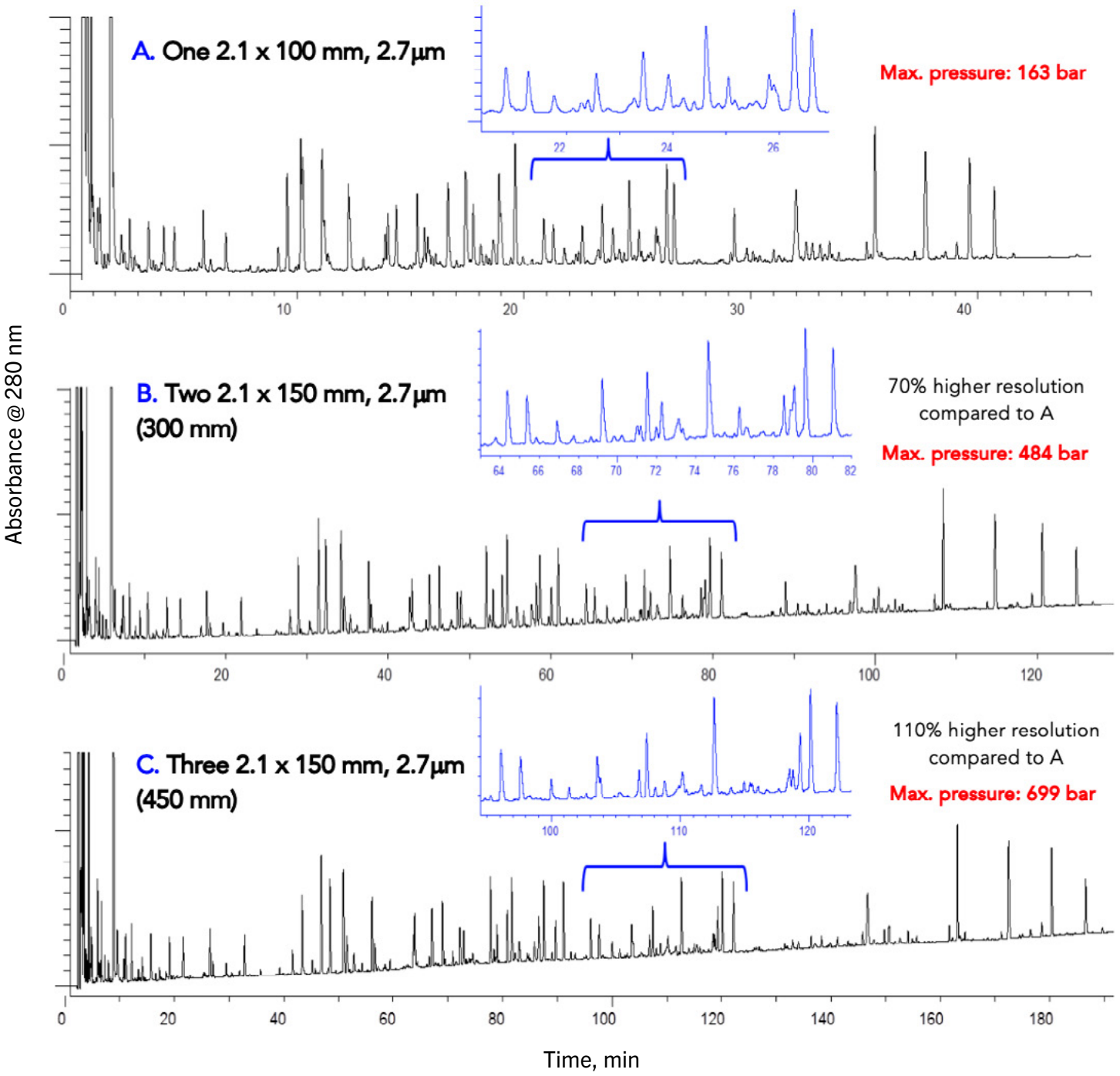
Column:	2 coupled HALO BioClass Peptide ES-C18, 160 Å, 2.7 µm, 2.1 x 100 mm
Part Number:	92122-602
Starting Mobile Phase:	95/5
Mobile Phase:	A: H_2O with 0.1% Trifluoroacetic acid (TFA) B: H_2O/Acetonitrile: (80/20) with 0.1% TFA
Gradient:	5% B to 60% B in 120 minutes
Flow Rate:	0.5 mL/min
Pressure:	380 bar maximum
Temperature:	60 °C
Detection:	UV 215 nm, PDA
Injection Volume:	35 µL
Sample Solvent:	Mobile phase A
Response Time:	0.1 sec
Flow Cell:	2 µL micro cell
LC System:	Agilent 1200 SL
Data rate:	40 Hz

This separation shows the separation of the products from a tryptic digest of apotransferrin on coupled HALO Peptide ES-C18 columns, (2.7 µm) in less than 90 minutes. Two columns were coupled to increase the peak capacity.

The use of elevated temperature improves the peak sharpness and aids in resolution. The excellent stability of this phase at elevated temperature is a result of the use of a sterically protected silane in the stationary phase synthesis.



APOTRANSFERRIN TRYPTIC DIGEST



APOTRANSFERRIN TRYPTIC DIGEST *(continued)*

TEST CONDITIONS

Column: HALO BioClass Peptide ES-C18, 160 Å, 2.7 µm

Part Number: 92122-602 (2.1 x 100 mm)
92122-702 (2.1 x 150 mm)

Mobile Phase
A: H_2O with 0.1% TFA
B: 80/20 Acetonitrile/ H_2O with 0.1% TFA

Gradient A:

Time (min)	%B
0	5
60	60

Gradient B:

Time (min)	%B
0	5
180	60

Gradient C:

Time (min)	%B
0	5
270	60

Flow Rate: 0.4 mL/min
Temperature: 60 °C
Detection: UV 215 nm, PDA Injection
Volume: 10 µL
Sample Solvent: H_2O
Data Rate: 40 Hz
Response Time: 0.05 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

The chromatograms on the preceding page show a comparison of an apotransferrin tryptic digest sample analyzed on three different lengths of HALO® Peptide ES-C18 columns: a single 2.1 x 100 mm, two 2.1 x 150 mm columns in series, and three 2.1 x 150 mm columns in series. The insets show examples of the improved performance obtained using longer column lengths along with longer gradient times for demanding samples. Resolution increases of approximately 70% and 110% are achieved by increasing column length by 3-fold and 4.5-fold respectively.

Gradient times of 60, 180 and 270 minutes were used for the top, middle and bottom chromatograms, respectively.

Lower pressures afforded by both 2.7 and 5 µm HALO® Peptide particles allow two or more columns to be used in series for additional resolution and peak capacity for challenging peptide mapping analyses. HALO® Peptide ES-C18 is also available in 2 µm particle sizes in 2.1 and 3.0 mm IDs up to 150 mm length for additional options in run time and peak capacity.

BSA TRYPTIC DIGEST ANALYSIS USING COLUMN COUPLING

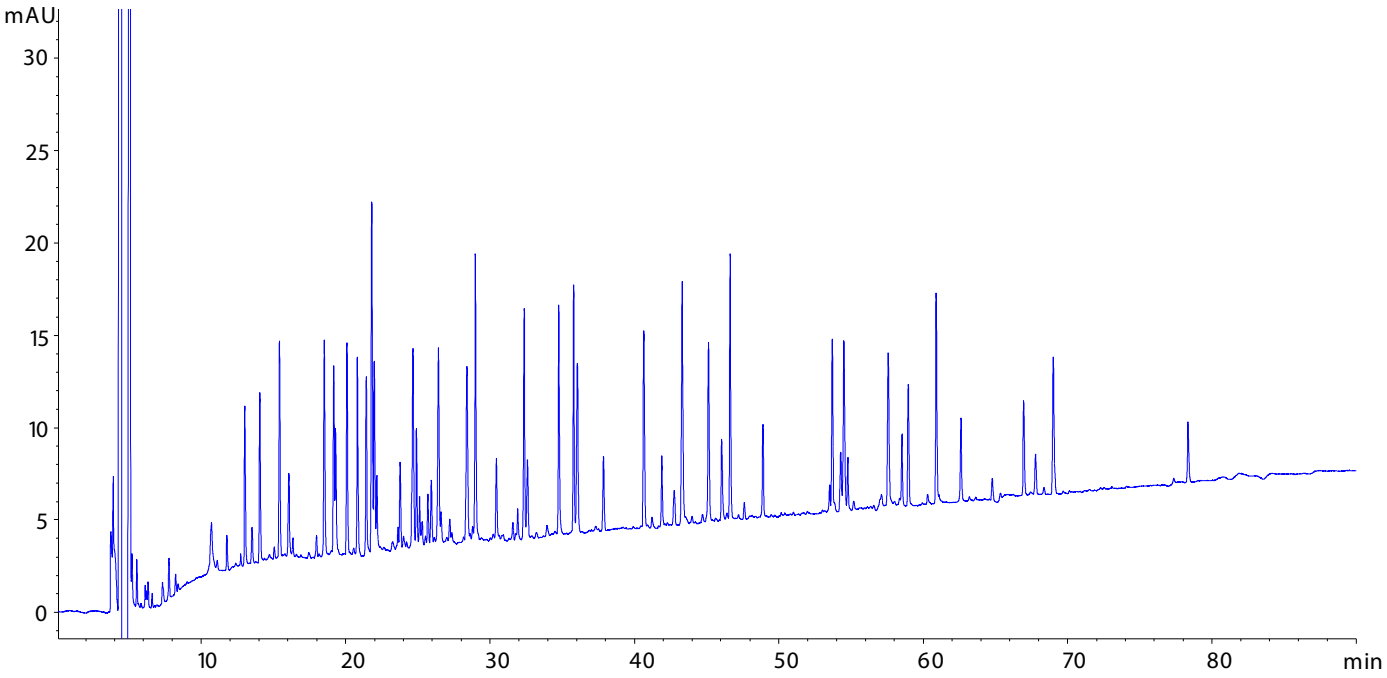
TEST CONDITIONS

Column: 3 coupled Avantor® ACE® UltraCore SuperC18, 95 Å, 2.5 µm, 2.1 x 150 mm
Part Number: CORE-25A-1502U
Mobile Phase: A: 0.05% TFA in H_2O
B: 0.05% TFA in Acetonitrile

Gradient:

Time (min)	%B
0	10
90	40
120	65
125	95
130	95
132	10
180	10

Flow Rate: 0.21 mL/min
Temperature: 60 °C
Injection Volume: 20 µL
Detection: UV, 214 nm



CAPILLARY SCALE HILIC SEPARATION OF DEAMIDATION PRODUCTS OF TRASTUZUMAB

TEST CONDITIONS

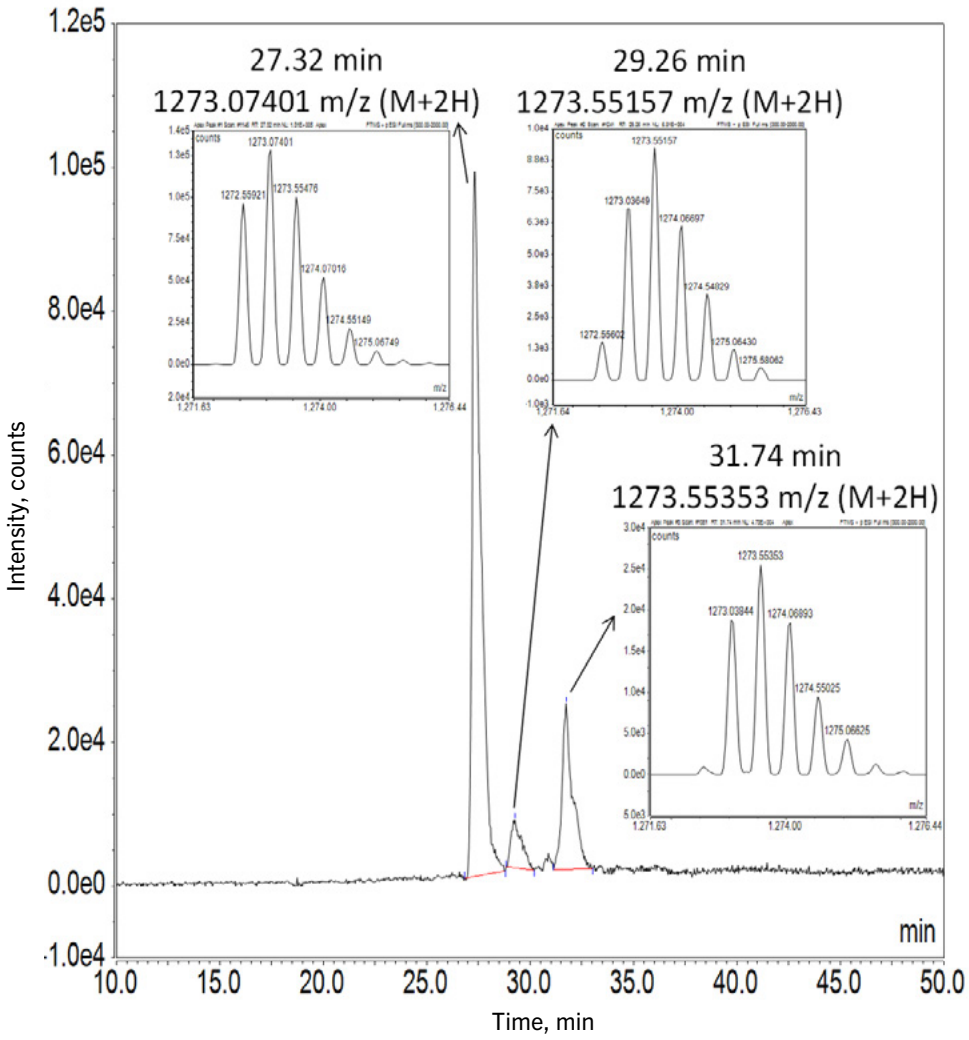
Column: HALO Penta-HILIC, 90 Å, 2.7 µm, 0.5 x 150 mm
Part Number: 98215-705
Mobile Phase: A: 50 mM Ammonium formate in H_2O
B: Acetonitrile/0.1% Formic acid

Gradient:

Time (min)	%B
0.0	80
4.0	80
64.0	48

Flow Rate: 12 µL/min
Pressure: 123 bar
Temperature: 60 °C
Detection: ESI+
Injection Volume: 1 µL
Sample Solvent: 50 mM Tris-HCl /1.5 M Guanidine-HCl, 0.5% Formic acid

LC System: Thermo Ultimate 3000
MS System: Thermo Orbitrap Velos



MS CONDITIONS

Spray Voltage (kV): 3.8
Capillary temperature: 300 °C
Sheath gas: 40
Aux gas: 10
RF lens: 50

ANALYTES

Peptide fragments of
GFYPSDIAVEWESNGQPENNYK
1.m/z= 1273.07401
2.m/z= 1273.55157
3.m/z= 1273.55353

The capillary HALO® Penta-HILIC column facilitated coupling of microflow LC conditions of 12 µL/min and a higher organic HILIC gradient separation. The column's high resolution capabilities resolved similarly charged species required for examining peptide deamidation and isomerization products of Asn, Asp, and isoAsp forms of a peptide fragment of a trastuzumab tryptic digest.

COMPARISON SEPARATION OF SEVEN PEPTIDES

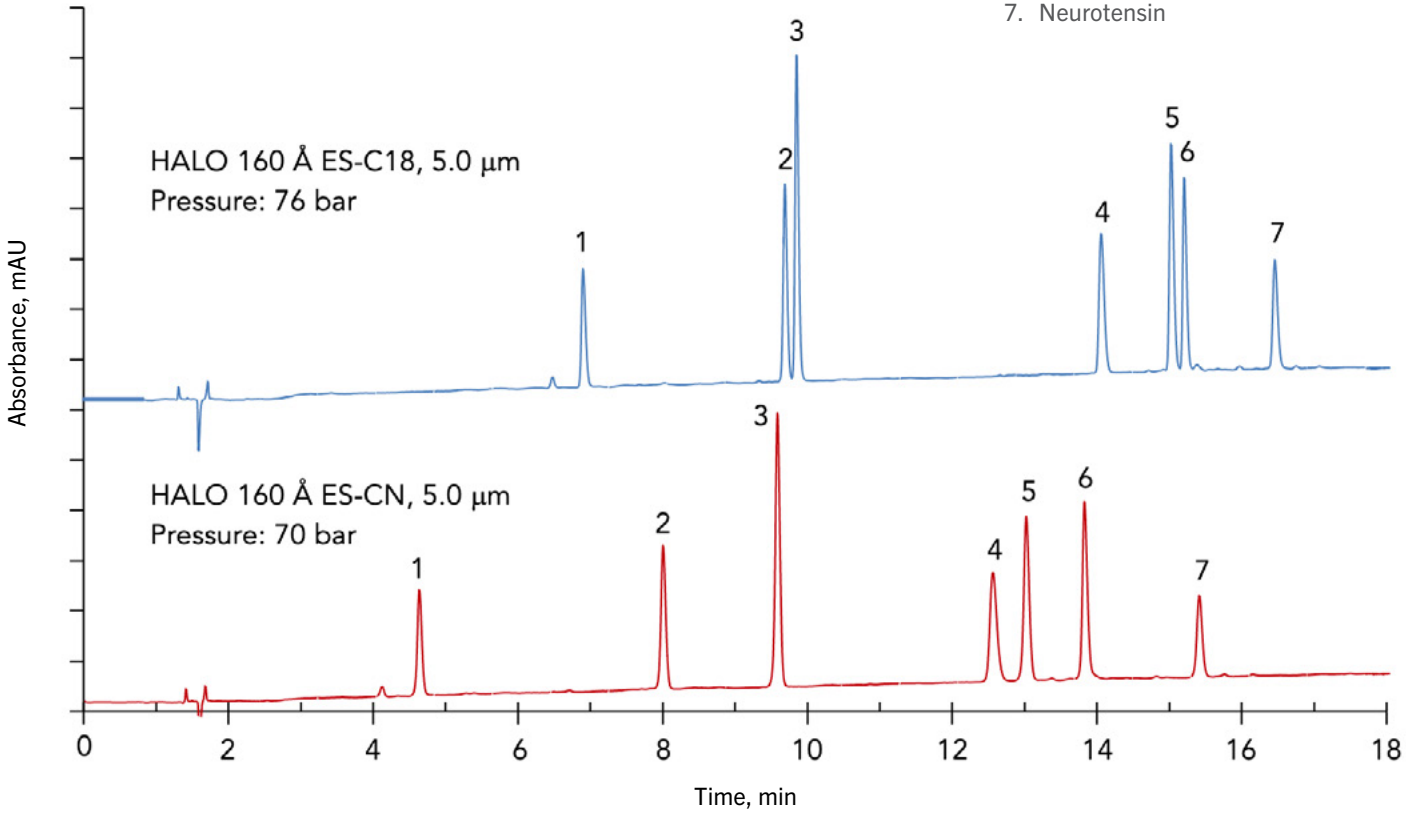
TEST CONDITIONS

Columns:	HALO BioClass Peptide ES-C18, 160 Å, 5 µm, 4.6 x 150 mm Part Number: 95124-702
	HALO BioClass Peptide ES-CN, 160 Å, 5 µm, 4.6 x 150 mm Part Number: 95124-704
Mobile Phase:	A: 0.1% Trifluoroacetic acid in H_2O B: 0.1% Trifluoroacetic acid in Acetonitrile
Flow Rate:	1.0 mL/min
Gradient:	5% B to 50% B in 30 minutes
Starting pressure:	As indicated on chart
Temperature:	40 °C
Detection:	UV 215 nm, VWD
Injection Volume:	10 µL
Sample Solvent:	Mobile phase A
Response Time:	0.12 sec
LC System:	Agilent 1100 Quaternary
Flow Cell:	5 µL semi-micro

HALO-5, 160 Å pore, HPLC column phases are suitable for the separation of molecules up to about 20 kDa in size. Shown here are two different bonded phases that allow for different selectivities that can enhance separation capabilities. These two C18 and cyano bonded phases are made using sterically- hindered silanes for increased stability at elevated temperatures and low pH.

ANALYTES

1. Asp-Phe
2. Angiotensin (1-7) amide
3. Tyr-Tyr-Tyr
4. Bradykinin
5. Leu-Enk
6. Angiotensin II
7. Neurotensin



DEAMIDATION PRODUCTS OF THE NIST mAb

TEST CONDITIONS

Column:	HALO BioClass Peptide ES-C18, 160 Å, 2.7 µm, 2.1 x 100 mm
Part Number:	92122-602
Mobile Phase:	A: H_2O/0.1% Formic acid B: Acetonitrile/0.1% Formic acid

Gradient:

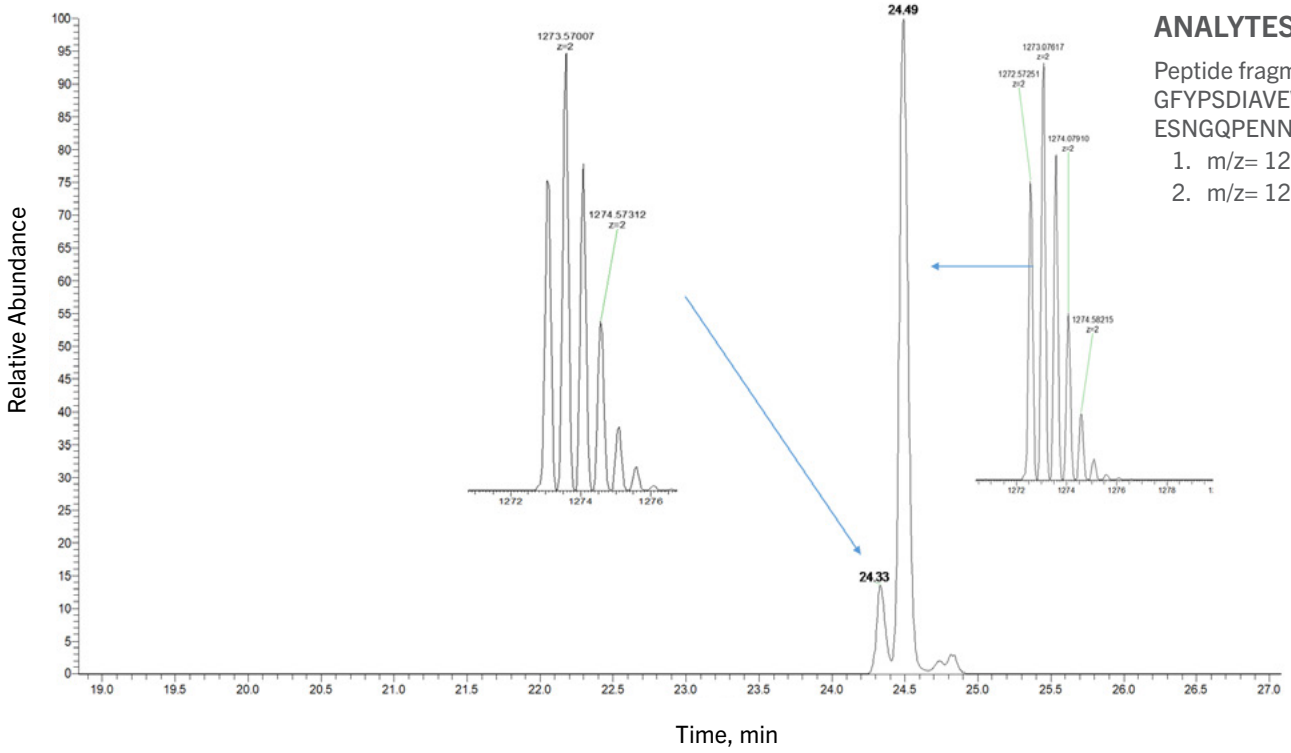
Time (min)	%B
0.0	2
45.0	40
45.5	80
48.0	80
48.5	2
55.0	2

Flow Rate:	0.3 mL/min
Pressure:	124 bar
Temperature:	60 °C
Detection:	ESI+
Injection Volume:	5 µL
Sample Solvent:	50 mM Tris-HCl /1.5 M Guanidine-HCl, 0.5% Formic acid
LC System:	Shimadzu Nexera X2
MS System:	Orbitrap Velos Pro

MS CONDITIONS

Spray Voltage (kV):	4.0
Capillary temperature:	300 °C
Sheath gas:	40
Aux gas:	10
RF lens:	50

Deamidation is a reaction in which an amide functional group in the side chain of the amino acids asparagine or glutamine is removed or converted to another functional group. Deamidation products are of increasing importance in proteomics because they can alter a protein's structure, or possibly its function and stability, resulting in degradation. This is especially of interest in monoclonal antibody (mAb) development as well. The HALO® ES-C18 has the high effeciency necessary to separate the deamidation products of the NIST mAb.



ANALYTES

Peptide fragments of GFYPSDIAVEW-ESNGQPENNYK

1. m/z= 1273.57007
2. m/z= 1273.07617

DETECTION OF OXYTOCIN AND VASOPRESSIN IN SERUM BY LC-MS-MS

TEST CONDITIONS

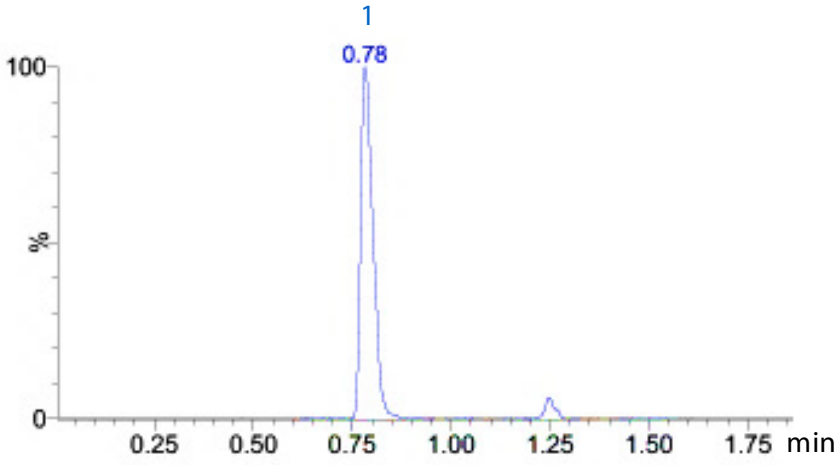
Column: Avantor® ACE® C18-300, 3 µm, 2.1 x 50 mm
Part Number: ACE-211-0502

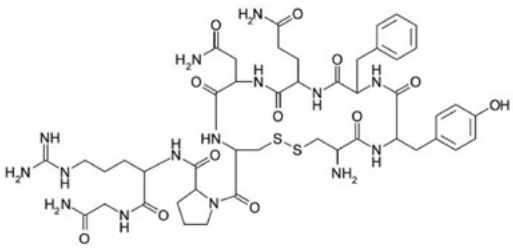
Mobile Phase: A: 0.1% Formic acid in H_2O
B: 0.1% Formic acid in Acetonitrile

Gradient:

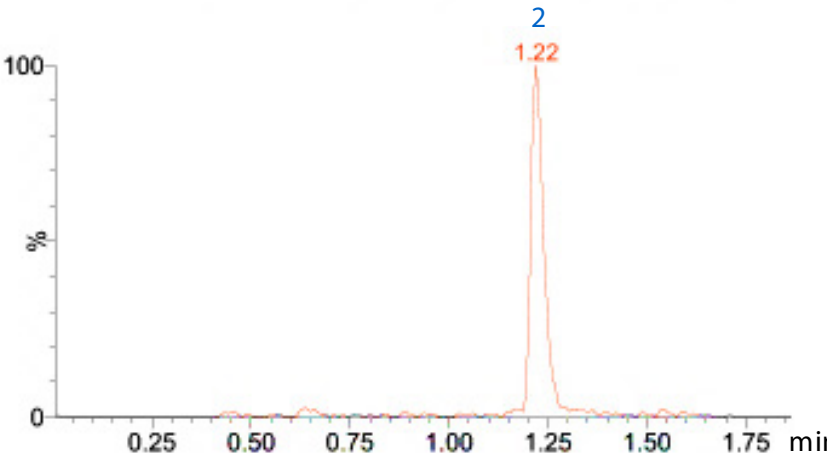
Time (min)	%B
0.0	10
1.5	33
1.6	10

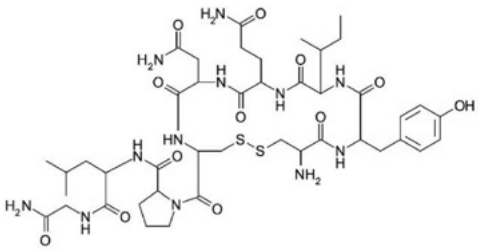
Flow Rate: 0.4 mL/min
Temperature: 40 °C
Injection Volume: 10 µL
Detection: Positive ion mode ESI, MRM
Desolvation temperature: 500 °C
Ion source temperature: 150 °C
MS Instrument: XEVO TQ-S triple quad MS





1. Vasopressin
(m/z 542.8 → 328.2)





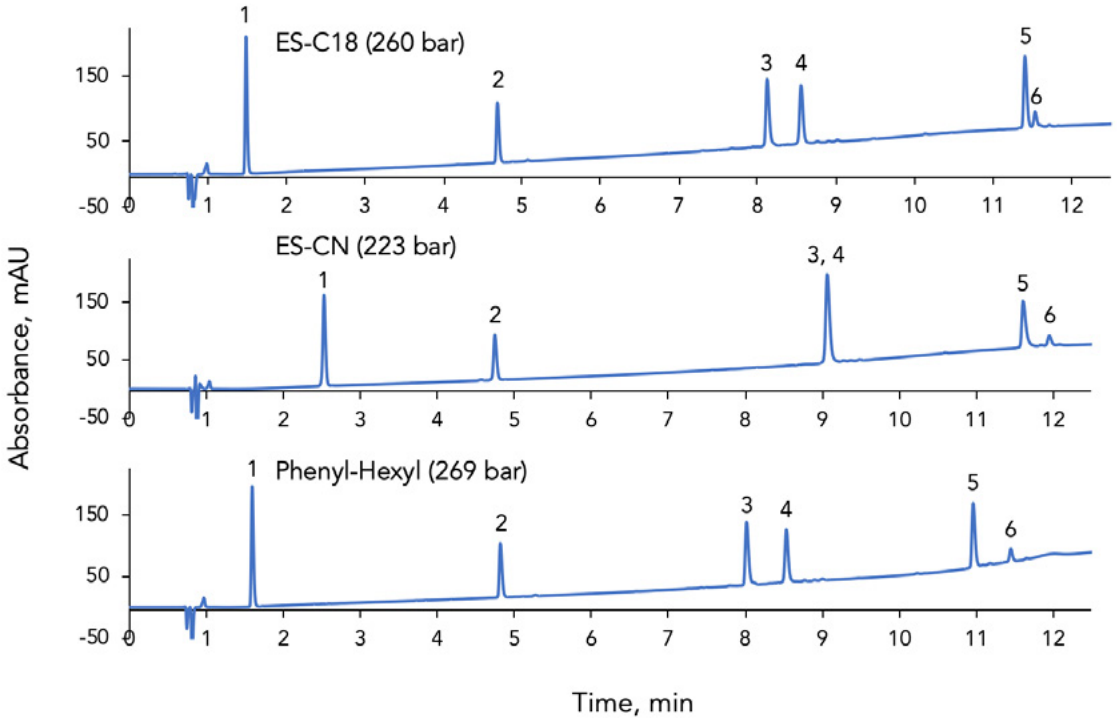
2. Oxytocin
(m/z 504.2 → 285.2)

ENHANCED SELECTIVITY FOR THE SEPARATION OF PEPTIDES COMPARING THREE DIFFERENT 160 Å BONDED PHASES

TEST CONDITIONS

Column:	HALO BioClass Peptide ES-C18, 160 Å, 2.7 µm, 2.1 x 150 mm Part Number: 92122-702
	HALO BioClass Peptide ES-CN, 160 Å, 2.7 µm, 2.1 x 150 mm Part Number: 92122-704
	HALO BioClass Peptide Phenyl-Hexyl, 160 Å, 2.7 µm, 2.1 x 150 mm Part Number: 92112-706
Mobile Phase:	A: 0.1% Formic acid in H_2O + 10 mM Ammonium formate B: 50/50 n-Propanol/H_2O + 0.1% Formic acid + 10 mM Ammonium formate (pH: 3.45)
Flow Rate:	0.4 mL/min
Gradient:	10-60 %B in 15 min
Temperature:	60 °C
Detection:	UV 220 nm, PDA
Injection Volume:	2 µL
Sample Solvent:	H_2O, 0.1% TFA
Response Time:	0.24 sec
Data Rate:	12.5 Hz
LC System:	Shimadzu Nexera Flow Cell: 1 µL

The initial separation using a HALO 160 Å ES-C18 column showed inadequate resolution of peaks 5 and 6. The same separation was attempted on a 160 Å ES-CN column which provided improved resolution of peaks 5 and 6, but resulted in coelution of peaks 3 and 4. The HALO 160 Å Phenyl-Hexyl column delivered excellent resolution between both peak pairs.



ANALYTES

1. Tyr-Tyr-Tyr
2. Angiotensin II
3. Angiotensin 1-12
4. Melittin
5. Sauvagine
6. β-Endorphin

ENHANCED SELECTIVITY FOR TRYPTIC DIGEST USING LC-MS

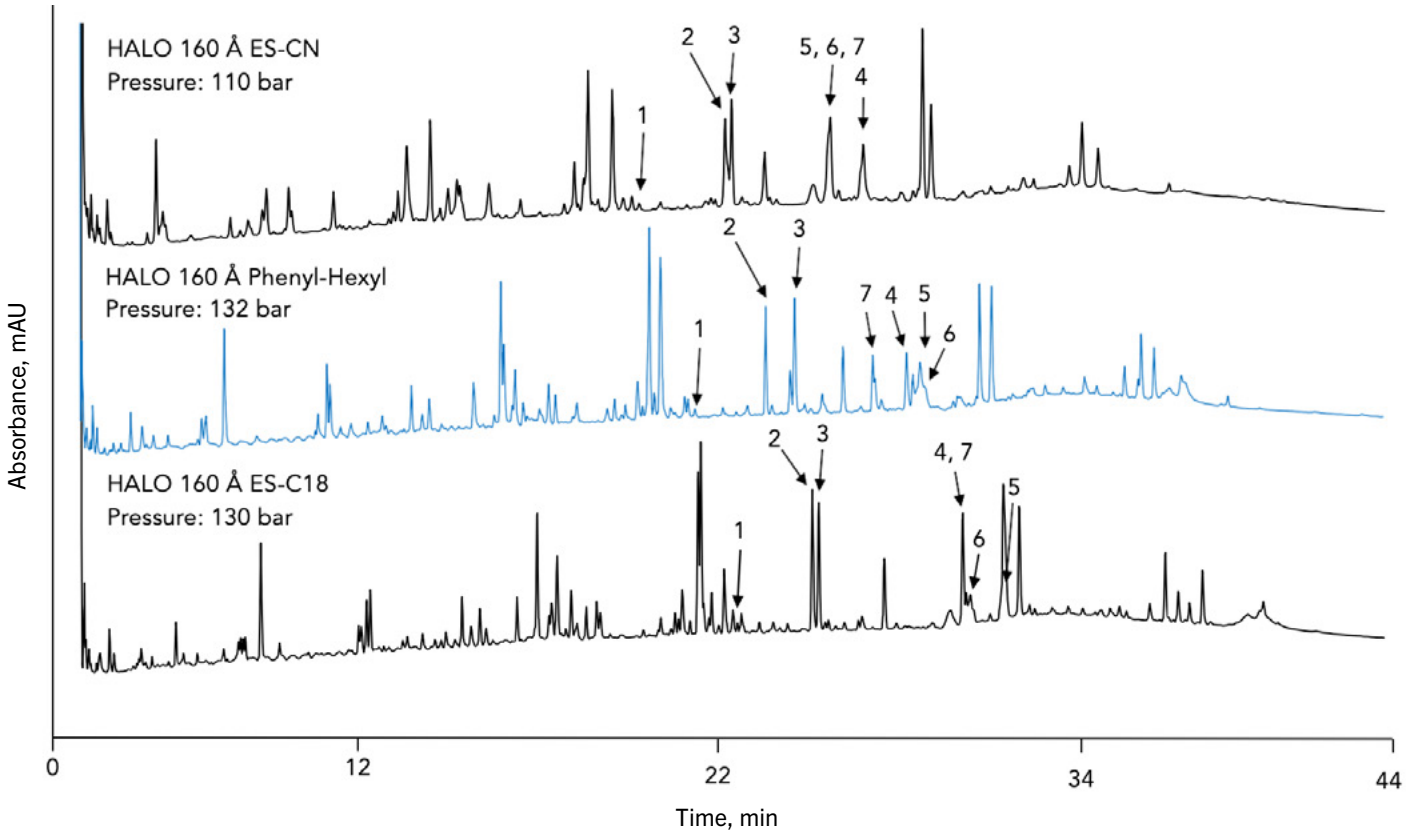
TEST CONDITIONS

Column:	HALO BioClass Peptide ES-CN, 160 Å, 2.7 µm, 2.1 x 100 mm Part Number: 92122-604
	HALO BioClass Peptide Phenyl-Hexyl, 160 Å, 2.7 µm, 2.1 x 100 mm Part Number: 92112-606
	HALO BioClass Peptide ES-C18, 160 Å, 2.7 µm, 2.1 x 100 mm Part Number: 92122-602
Mobile Phase:	A: H_2O + 10 mM Difluoroacetic acid (DFA) B: Acetonitrile + 10 mM Difluoroacetic acid
Flow Rate:	0.3 mL/min
Gradient:	2–50 %B in 60 min
Temperature:	60 °C
Detection:	UV 220 nm, VWD
Injection Volume:	5 µL of 0.2 mg/mL digest
Sample Solvent:	50 mM Tris-HCl/1.5 M Guanidine-HCl with 0.25% Formic acid
Response Time:	0.15 sec
Data Rate:	10 Hz
LC System:	Shimadzu Nexera
Flow Cell:	2.5 µL semi-micro

The HALO 160 Å Phenyl-Hexyl column provided improved resolution between tryptic digest fragments 2 and 3 compared to the 160 Å ES-CN column and the 160 Å ES-C18 column. Peptide identification was accomplished by using MS-MS fragmentation spectra.

ANALYTES (using one-letter amino acid abbreviations):

1. FTISADTSKNTAYLQMNSLR (754 m/z)
2. LScAASGFNIKDTYIHWVR (747 m/z)
3. GFYPSDIAVEWESNGQPENNYK (849 m/z)
4. LLIYSASFLYSGVPSR (592 m/z)
5. SGTASVVcLLNNFYPR (899 m/z)
6. ScDKTHTcPPcPAPELLGGPSVFLFPPKPK (834 m/z)
7. VVSVLTVLHQDWLNGKEYK (1115 m/z)



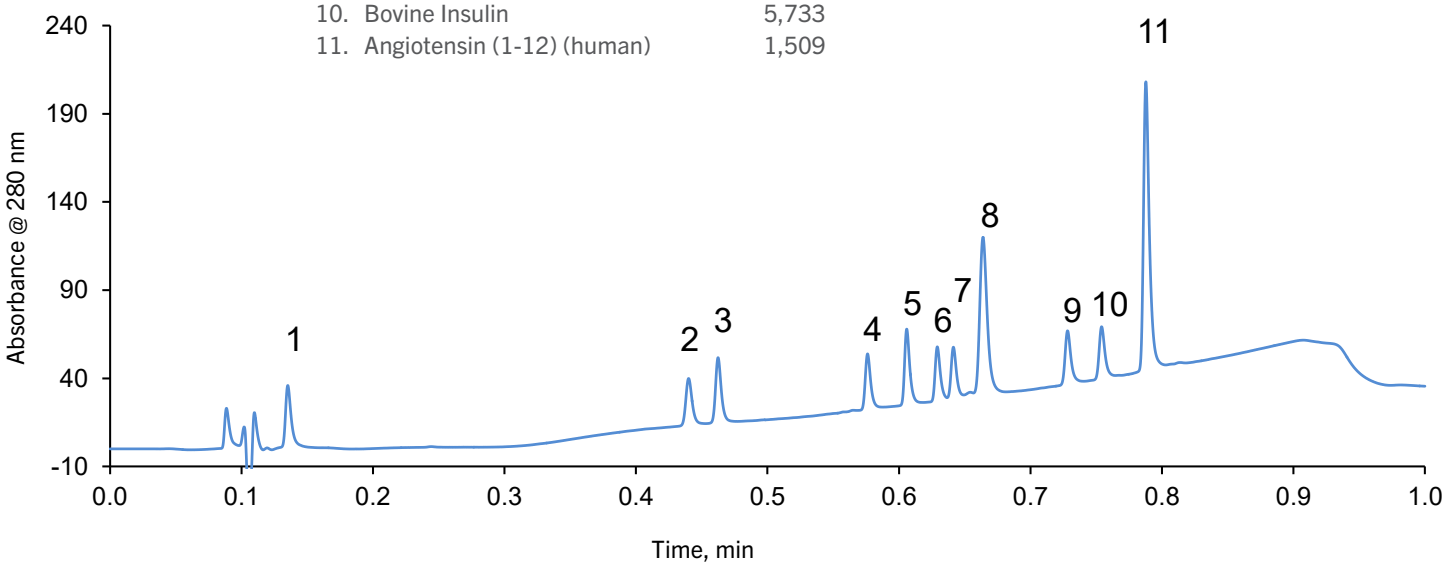
FAST PEPTIDE SEPARATION

TEST CONDITIONS

Column:	HALO BioClass Peptide ES-C18, 160 Å, 2 µm, 3.0 x 50 mm
Part Number:	91123-402
Mobile Phase:	A: 0.1% Trifluoroacetic acid in H_2O B: 0.1% Trifluoroacetic acid in 80/20 Acetonitrile/H_2O
Flow Rate:	2.2 mL/min
Gradient:	Hold at 12.5% B for 0.1 min; 12.5% B to 63% B from 0.1–1.0 min
Initial pressure:	556 bar
Temperature:	60 °C
Detection:	UV 215 nm, PDA
Injection Volume:	0.5 µL
Sample Solvent:	Mobile phase A
Response Time:	0.025 sec
Data Rate:	200 Hz
LC System:	Shimadzu Nexera X2
Flow Cell:	1 µL

A one-minute separation of a mixture of peptides and small proteins is demonstrated on a 2 µm HALO Peptide ES-C18 column. Separations can be run at high flow rate in order to maximize sample throughput.

ANALYTES	MW (g/mol)
1. Gly-Tyr	238
2. Val-Tyr-Val	380
3. Angiotensin 1/2 (1-7) amide	898
4. Met-enkephalin	574
5. Angiotensin 1/2 (1-8) amide	1,045
6. Angiotensin II	1,046
7. Leu-enkephalin	556
8. Ribonuclease A	13,700
9. Angiotensin (1-12) (mouse)	1,573
10. Bovine Insulin	5,733
11. Angiotensin (1-12) (human)	1,509



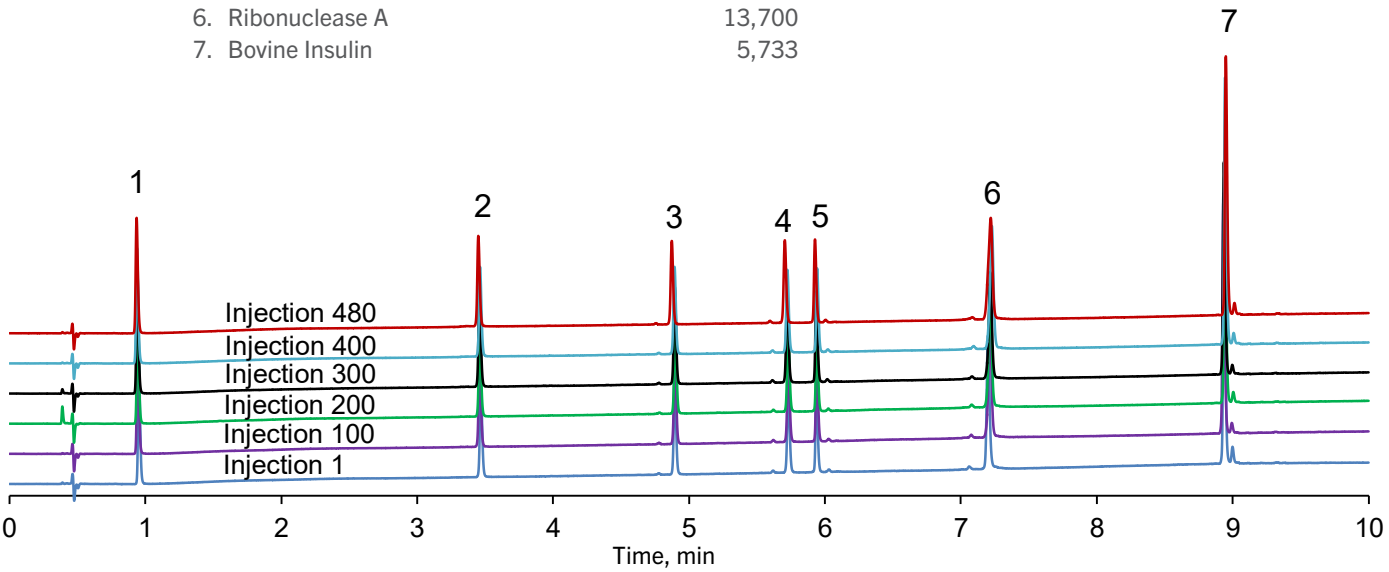
HIGH TEMPERATURE, LOW PH STABILITY WITH A 2 µm PEPTIDE COLUMN

TEST CONDITIONS

Column:	HALO BioClass Peptide ES-C18, 160 Å, 2 µm, 2.1 x 100 mm
Part Number:	91122-602
Mobile Phase:	A: 0.1% Trifluoroacetic acid in H_2O
	B: 0.1% Trifluoroacetic acid in 80/20 Acetonitrile/H_2O
Flow Rate:	0.5 mL/min
Gradient:	6% B–54% B in 10 min
Initial pressure:	395 bar
Maximum pressure:	417 bar
Temperature:	60 °C
Detection:	UV 215 nm, PDA
Injection Volume:	0.5 µL
Sample Solvent:	Mobile phase A
Response Time:	0.025 sec
Data Rate:	40 Hz
LC System:	Shimadzu Nexera X2
Flow Cell:	1 µL

The sterically-protected C18 phase on the 2 µm HALO Peptide column enables high temperature stability with low pH mobile phases. The replicate injections were stopped at injection 480 (15,500 column volumes). The column is expected to have a lifetime of ~ 1000 injections, depending on the type of sample and conditions used.

ANALYTES	MW (g/mol)
1. Gly-Tyr	238
2. Val-Tyr-Val	380
3. Met-enkephalin	574
4. Angiotensin II	1,046
5. Leu-enkephalin	556
6. Ribonuclease A	13,700
7. Bovine Insulin	5,733



OXIDATION OF NIST mAb FRAGMENT

TEST CONDITIONS

Column: HALO® Penta-HILIC, 90 Å, 2.7 µm, 0.5 x 150 mm

Part Number: 98215-705

Mobile Phase: A: 50 mM Ammonium formate, pH 4.4
B: 0.1% Formic acid in Acetonitrile

Gradient:

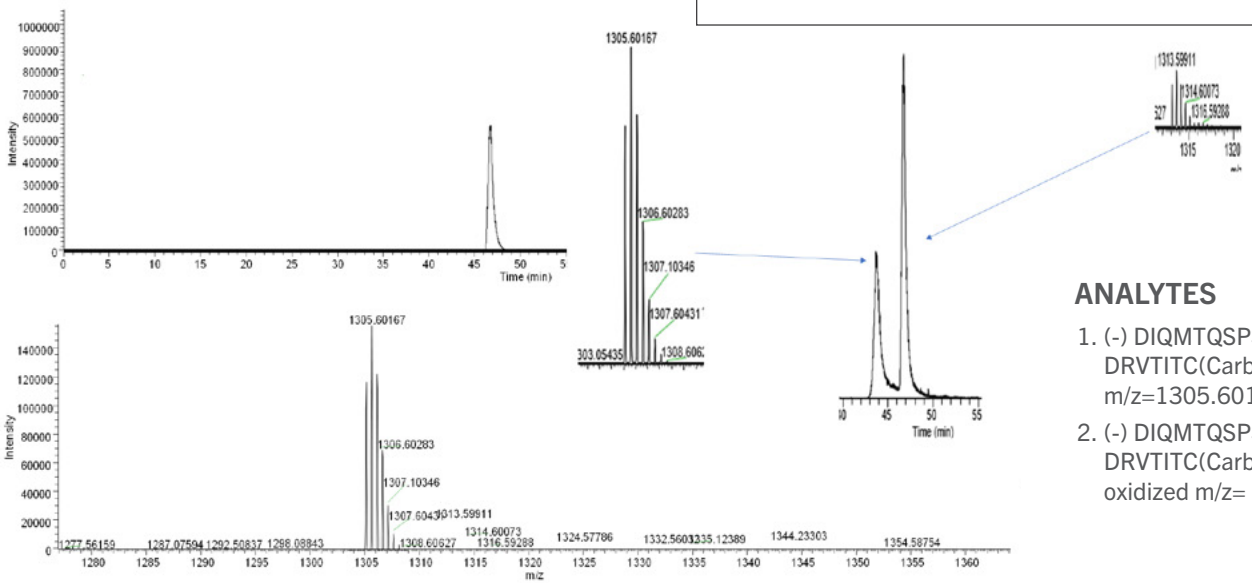
Time (min)	%B
0.0	80
4.0	80
55.0	48
59.0	48
63.0	80
70.0	80

Flow Rate: 50 µL/min
Pressure: 158 bar
Temperature: 60 °C (standard)
80 °C (oxidized)
Detection: +ESI
Injection Volume: 5.0 µL
Sample Solvent: 70% Acetonitrile, 30% H_2O
LC System: Shimadzu Nexera X2
MS System: Thermo LTQ VELOS PRO

MS CONDITIONS

Ion mode: Positive
Aux gas: 2 arbitrary units
Sheath gas: 4 arbitrary units
Sweep gas: 0 arbitrary units
Rf lens: 55 V
Heater temp: 225 °C
Ion transfer tube: 275 °C
Capillary Voltage: 3.5 kV

Post-translational modifications (PTMs), such as oxidation, are a critical variable that must be accounted for during protein analysis. Frequently, the minor mass shifts associated with these modifications are too small to be resolved during intact protein analysis, due to the charge envelope produced by large proteins, such as monoclonal antibodies (mAbs). However, chromatographically, these compounds will have a difference in retention time relative to the native, and can be separated before getting to the detector. Peptide analysis is an important method of characterization for mAbs because, in addition to revealing modifications such as oxidation, it can provide valuable insight into additional post-translational modifications, which may not be evident during intact mass analysis. In this experiment, the digested NIST mAb was exposed to high temperature in order to induce oxidation, and then analyzed using the HALO® Penta-HILIC capillary column, demonstrating it is an ideal choice for peptide oxidation analysis of mAbs.



(A) Extracted ion chromatogram of (-) DIQMTQSPSSLSASVGDRVTITC(Carbamidomethyl)R(A)

(B) Extracted ion chromatogram oxidized (-) DIQMTQSPSSLSASVGDRVTITC(Carbamidomethyl)R(A) showing a mass shift of 8, as expected for an oxidized doubly charged peptide

ANALYTES

1. (-) DIQMTQSPSSLSASVG-DRVTITC(Carbamidomethyl)R(A) m/z=1305.60167
2. (-) DIQMTQSPSSLSASVG-DRVTITC(Carbamidomethyl)R(A) oxidized m/z= 1313.59911

PEPTIDE SIX-COLUMN SCREEN

TEST CONDITIONS

Column:
Avantor® ACE® Excel C18, 100 Å, 2 µm, 3.0 x 100 mm
Part Number: EXL-101-1003U

Avantor® ACE® Excel C18-AR, 100 Å, 2 µm, 3.0 x 100 mm
Part Number: EXL-109-1003U

Avantor® ACE® Excel C18-PFP, 100 Å, 2 µm, 3.0 x 100 mm
Part Number: EXL-1010-1003U

Avantor® ACE® Excel SuperC18, 90 Å, 2 µm, 3.0 x 100 mm
Part Number: EXL-1011-1003U

Avantor® ACE® Excel C18-Amide, 100 Å, 2 µm, 3.0 x 100 mm
Part Number: EXL-1012-1003U

Avantor® ACE® Excel CN-ES, 100 Å, 2 µm, 3.0 x 100 mm
Part Number: EXL-1013-1003U

Mobile Phase:
A: 0.05% TFA in H_2O
B: 0.05% TFA in Acetonitrile

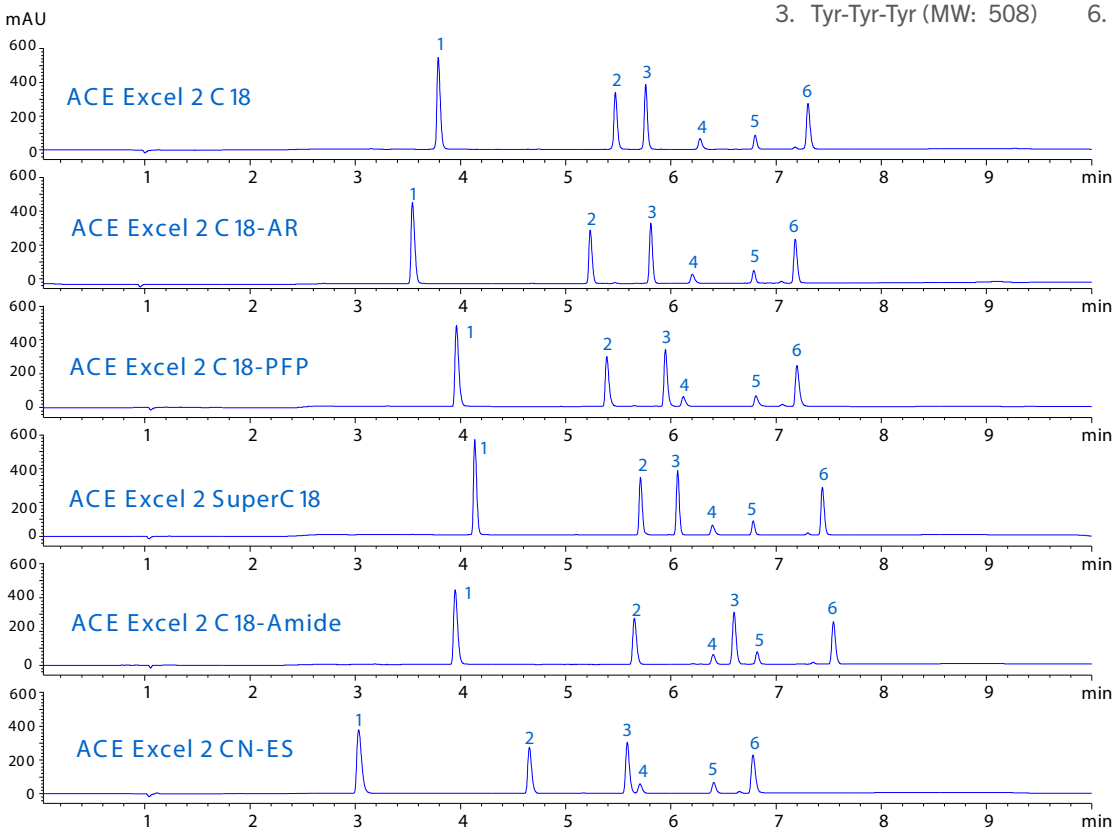
Gradient:

Time (min)	%B
0.0	5
1.0	5
8.0	40
9.0	40
9.5	5
15.0	5

Flow Rate: 0.6 mL/min
Temperature: 22 °C
Injection Volume: 2 µL
Detection: UV, 220 nm

ANALYTES

1. Gly-Tyr (MW: 238)
2. Val-Tyr-Val (MW: 380)
3. Tyr-Tyr-Tyr (MW: 508)
4. Oxytocin (MW: 1007)
5. Angiotensin II (MW: 1046)
6. Leucine Enkephalin (MW: 556)



PEPTIDE TEST MIX

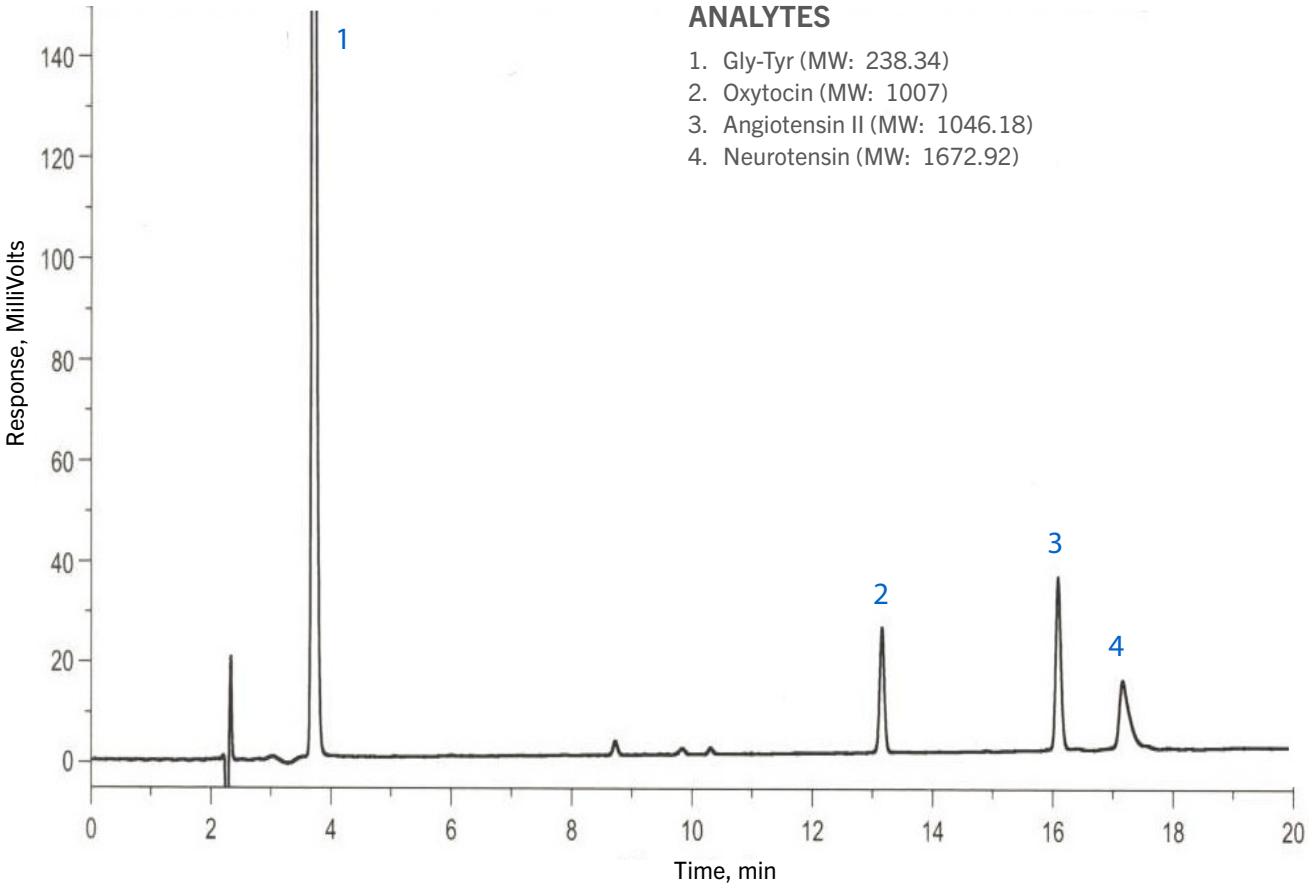
TEST CONDITIONS

Column: Avantor® ACE® C18-300, 5 µm, 4.6 x 250 mm
Part Number: ACE-221-2546
Mobile Phase: A: 0.1% Trifluoroacetic acid in H_2O
B: 0.1% Trifluoroacetic acid in Acetonitrile

Gradient:

Time (min)	%B
0	10
25	40

Flow Rate: 2 mL/min
Temperature: Ambient
Injection Volume: 5 µL
Detection: UV, 220 nm



ANALYTES

1. Gly-Tyr (MW: 238.34)
2. Oxytocin (MW: 1007)
3. Angiotensin II (MW: 1046.18)
4. Neurotensin (MW: 1672.92)

PEPTIDES - SELECTIVITY CHANGES WITH BONDED PHASE AND MOBILE PHASE

TEST CONDITIONS

Column:
Avantor® ACE® C18-300, 5 µm, 4.6 x 250 mm
Part Number: ACE-221-2546

Avantor® ACE® C8-300, 5 µm, 4.6 x 250 mm
Part Number ACE-222-2546

Avantor® ACE® C4-300, 5 µm, 4.6 x 250 mm
Part Number ACE-223-2546

Avantor® ACE® Phenyl-300, 5 µm, 4.6 x 250 mm
Part Number ACE-225-2546

Avantor® ACE® CN-300, 5 µm, 4.6 x 250 mm
Part Number ACE-224-2546

Mobile Phase:
A: 0.1% TFA or 0.1% Formic acid in H_2O
B: Acetonitrile

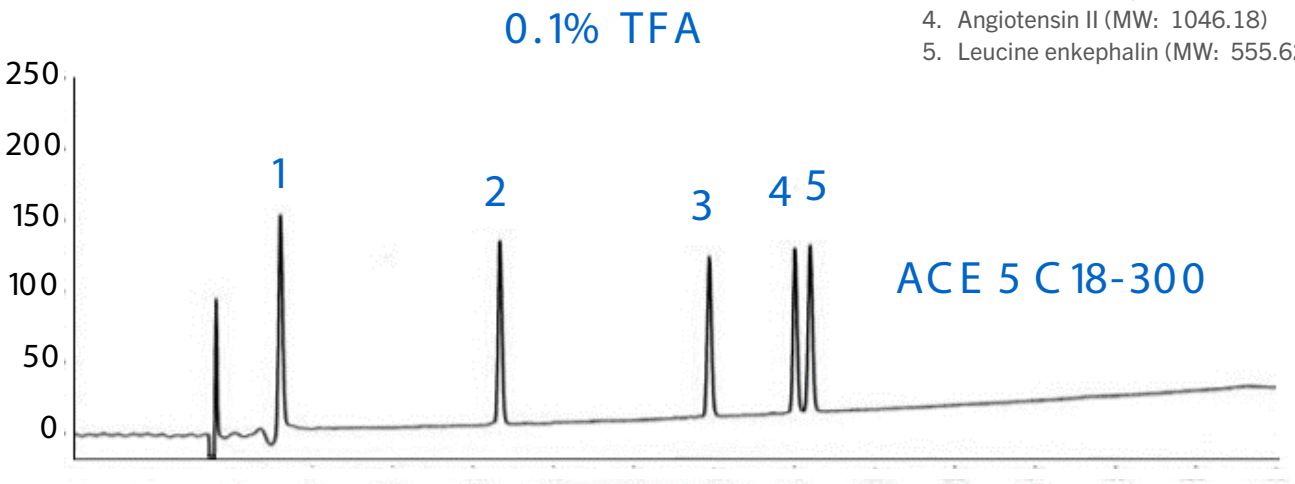
Gradient:

Time (min)	%B
0	10
25	40

Flow Rate: 1 mL/min
Temperature: Ambient
Detection: UV, 220 nm

ANALYTES

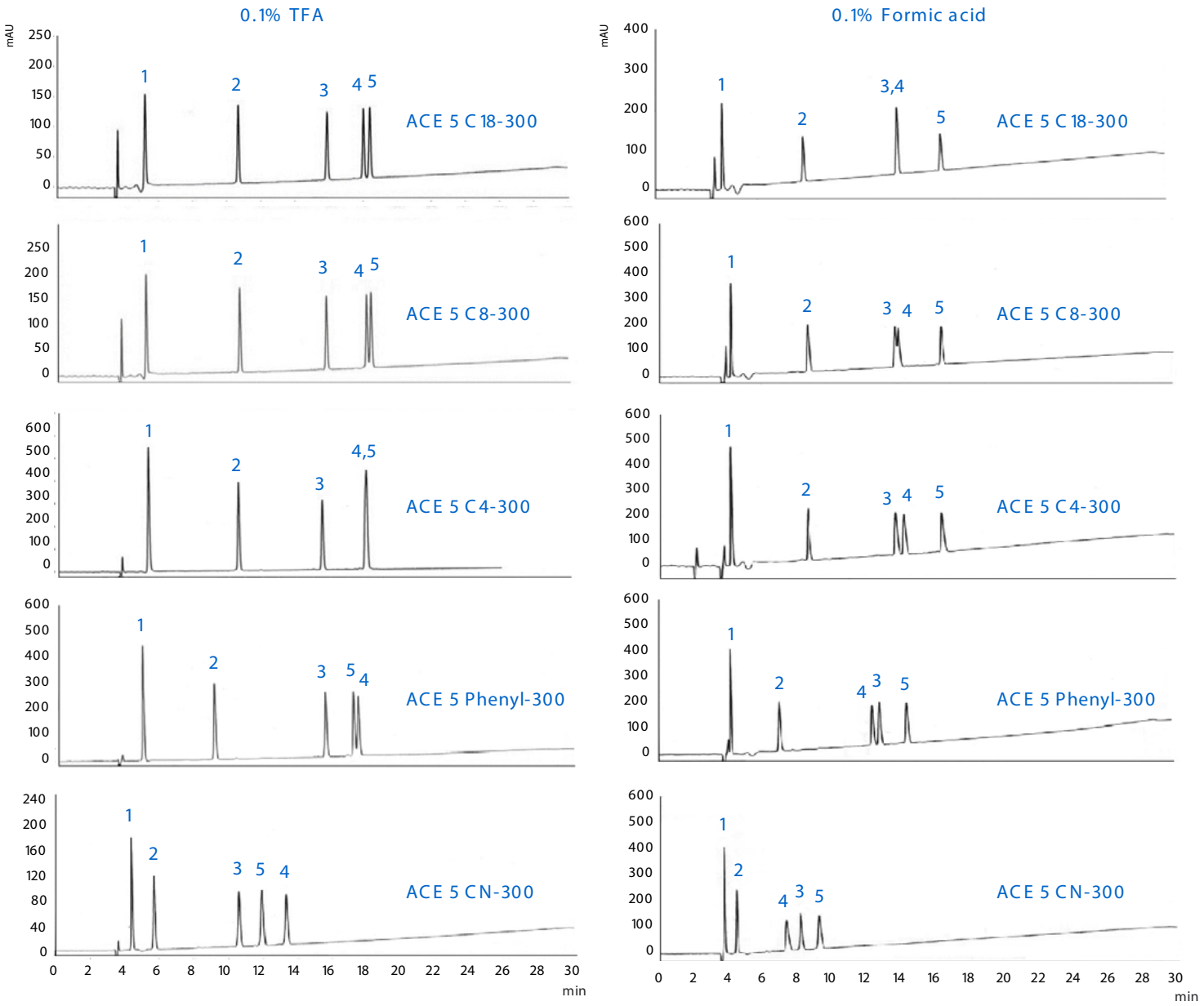
1. Gly-Tyr (MW: 238.34)
2. Val-Tyr-Val (MW: 379.45)
3. Methionine enkephalin (MW: 573.67)
4. Angiotensin II (MW: 1046.18)
5. Leucine enkephalin (MW: 555.62)



PEPTIDES - SELECTIVITY CHANGES WITH BONDED PHASE AND MOBILE PHASE *(continued)*

ANALYTES

1. Gly-Tyr (MW: 238.34)
2. Val-Tyr-Val (MW: 379.45)
3. Methionine enkephalin (MW: 573.67)
4. Angiotensin II (MW: 1046.18)
5. Leucine enkephalin (MW: 555.62)



PEPTIDES - VARYING PH WITH PHOSPHATE BUFFERS

TEST CONDITIONS

Column: Avantor® ACE® C18-300, 5 µm, 4.6 x 250 mm
Part Number: ACE-221-2546
Mobile Phase: A: 20 mM KH_2PO_4 in H_2O
B: Acetonitrile

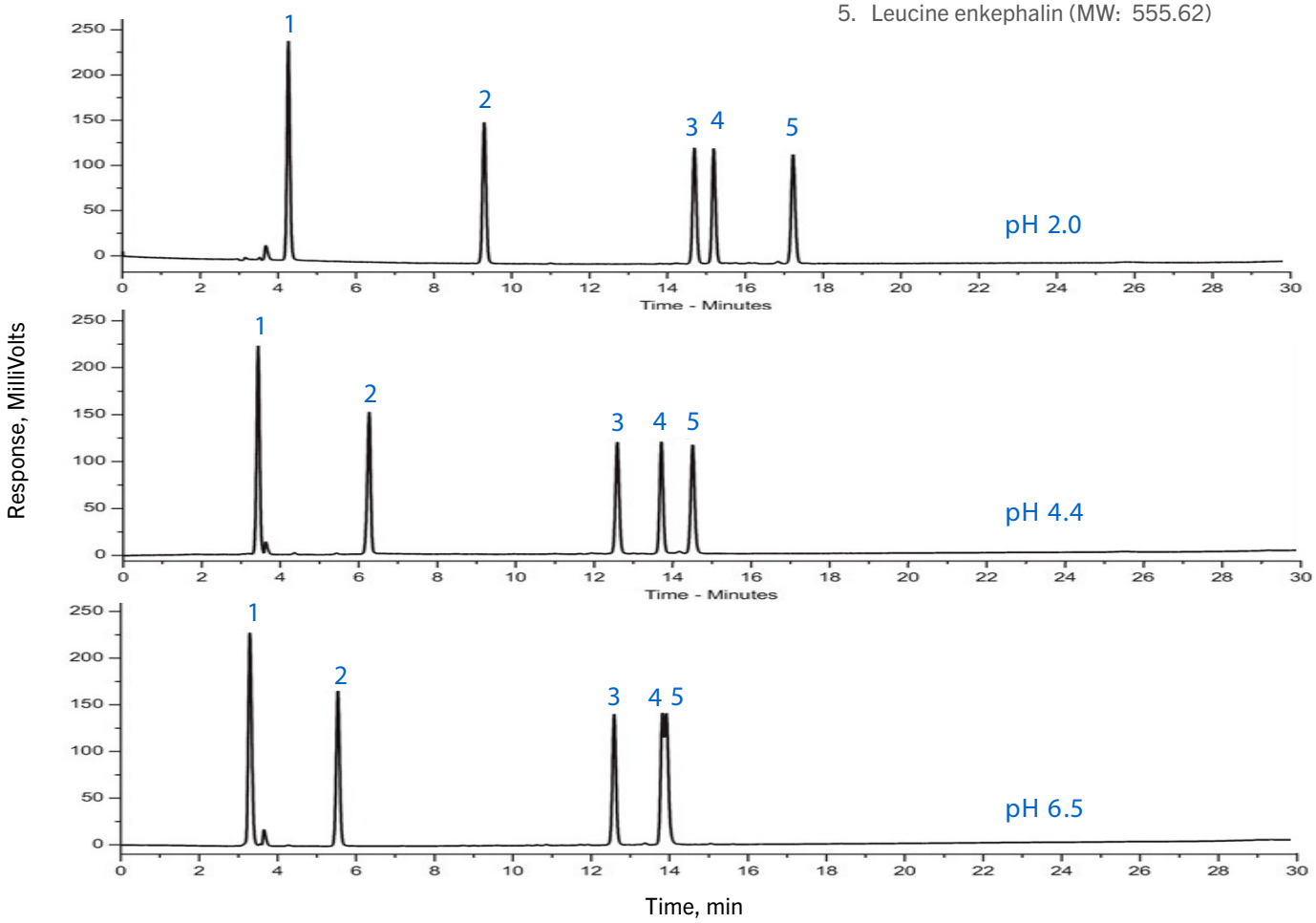
Gradient:

Time (min)	%B
0	10
25	40

Flow Rate: 1 mL/min
Temperature: Ambient
Detection: UV, 220 nm

ANALYTES

1. Gly-Tyr (MW: 238.34)
2. Val-Tyr-Val (MW: 379.45)
3. Methionine enkephalin (MW: 573.67)
4. Angiotensin II (MW: 1046.18)
5. Leucine enkephalin (MW: 555.62)



RAPID SEPARATION OF PEPTIDES

TEST CONDITIONS

Column: Avantor® ACE® Excel C18, 100 Å, 1.7 µm, 3.0 x 50 mm
Part Number: EXL-171-0503U
Mobile Phase: A: 0.05% TFA in H_2O
B: 0.05% TFA in Acetonitrile

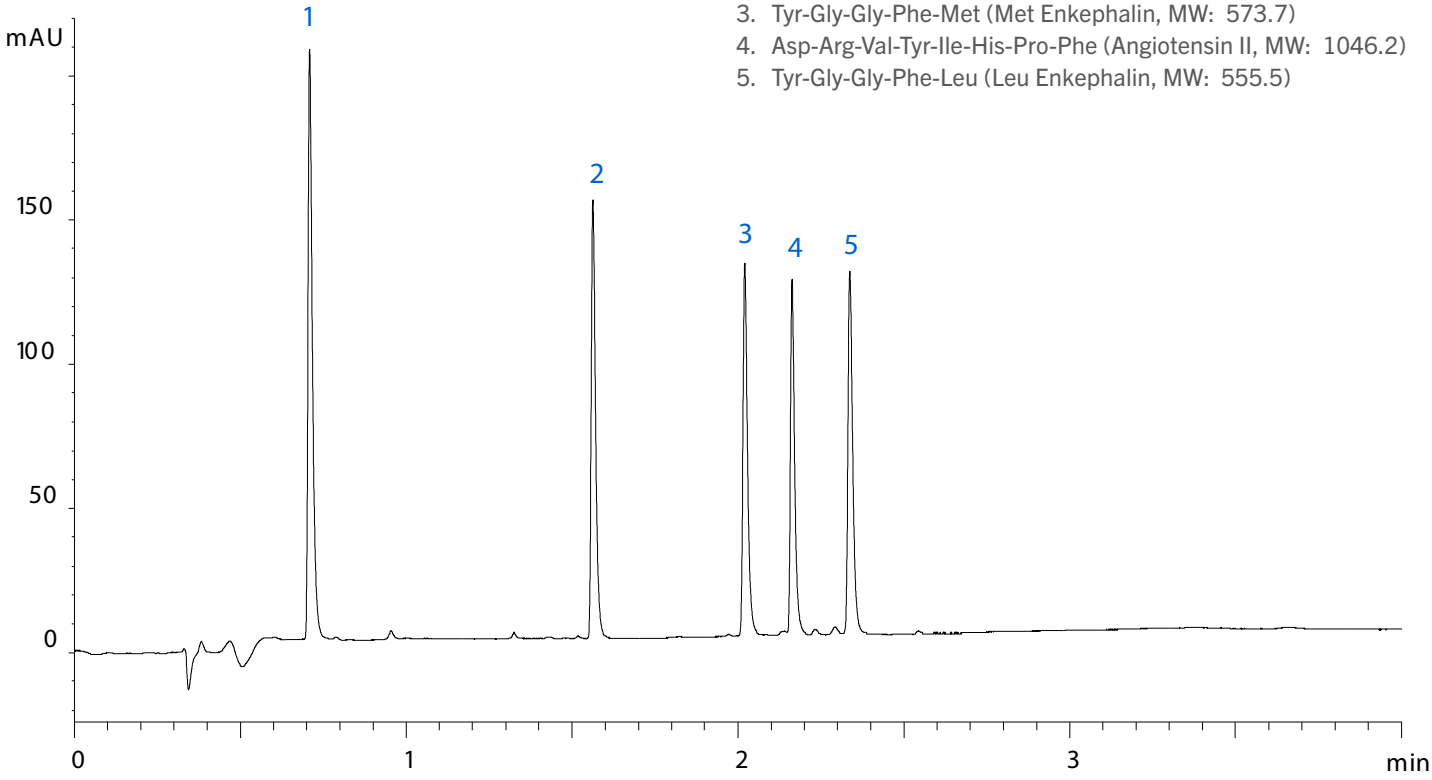
Gradient:

Time (min)	%B
0.0	5
3.0	40
4.0	90
5.5	90
10.5	5

Flow Rate: 0.8 mL/min
Temperature: 60 °C
Injection Volume: 1 µL
Detection: UV, 220 nm

ANALYTES

1. Gly-Tyr (MW: 238.2)
2. Val-Tyr-Val (MW: 379.5)
3. Tyr-Gly-Gly-Phe-Met (Met Enkephalin, MW: 573.7)
4. Asp-Arg-Val-Tyr-Ile-His-Pro-Phe (Angiotensin II, MW: 1046.2)
5. Tyr-Gly-Gly-Phe-Leu (Leu Enkephalin, MW: 555.5)



TRYPTIC DIGEST OF IgG

TEST CONDITIONS

Column: Avantor® ACE® UltraCore BIO C18, 300 Å, 3.5 µm, 3.0 x 100 mm
Part Number: BIO-350-1030

Avantor® ACE® UltraCore BIO C4, 300 Å, 3.5 µm, 3.0 x 100 mm
Part Number: BIO-351-1030

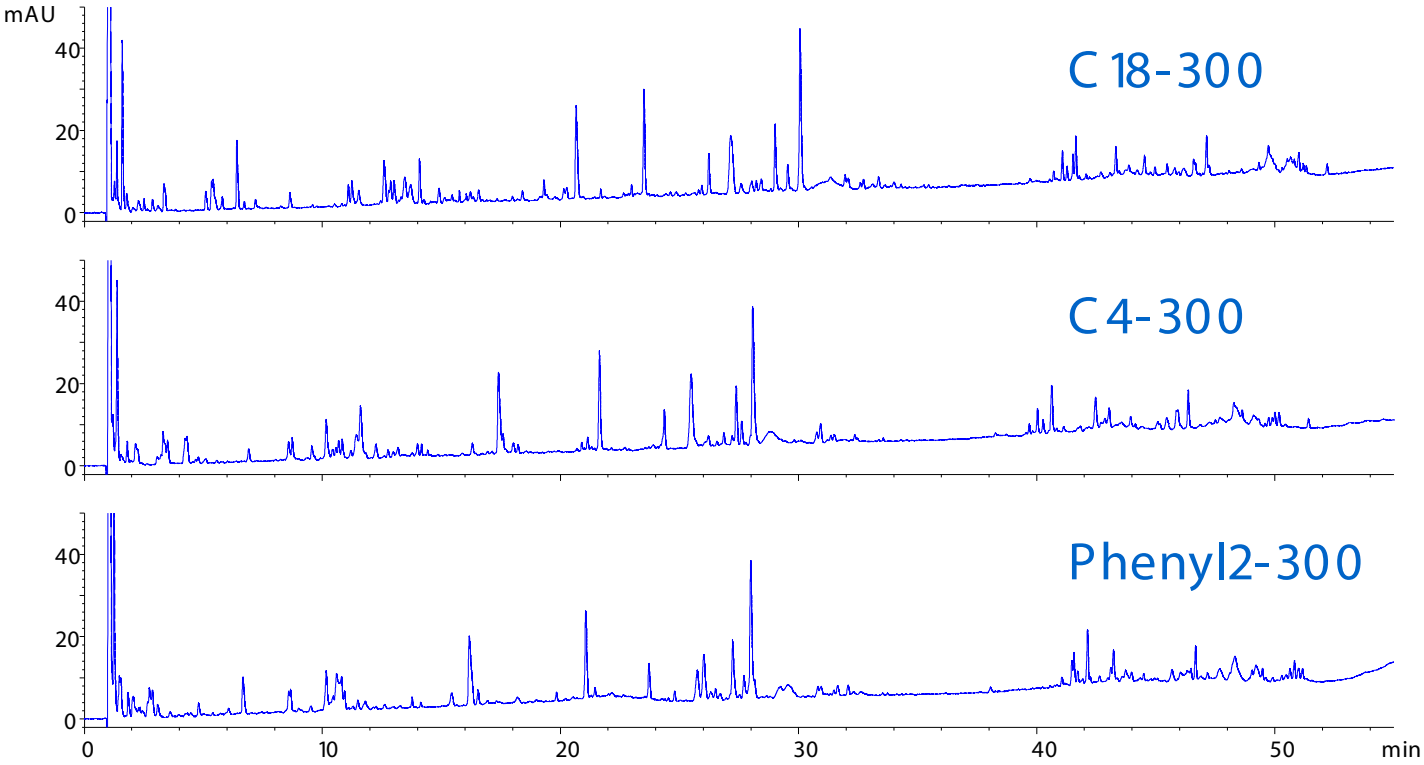
Avantor® ACE® UltraCore BIO Phenyl2, 300 Å, 3.5 µm, 3.0 x 100 mm
Part Number: BIO-352-1030

Mobile Phase: A: 0.1% TFA in H_2O
B: 0.1% TFA in Acetonitrile

Gradient:

Time (min)	%B
0	2
60	40
61	95
64	95
65	2
75	2

Flow Rate: 0.43 mL/min
Temperature: 60 °C
Injection Volume: 20 µL
Detection: UV, 214 nm
System Dwell Volume: 525 µL



TRYPTIC DIGEST OF IgG USING 500 Å PORE COLUMNS

TEST CONDITIONS

Column: Avantor® ACE® UltraCore C18, 500 Å, 2.5 µm, 3.0 x 100 mm
Part Number: BIO-250-1030

Avantor® ACE® UltraCore BIO C4, 500 Å, 2.5 µm, 3.0 x 100 mm
Part Number: BIO-251-1030

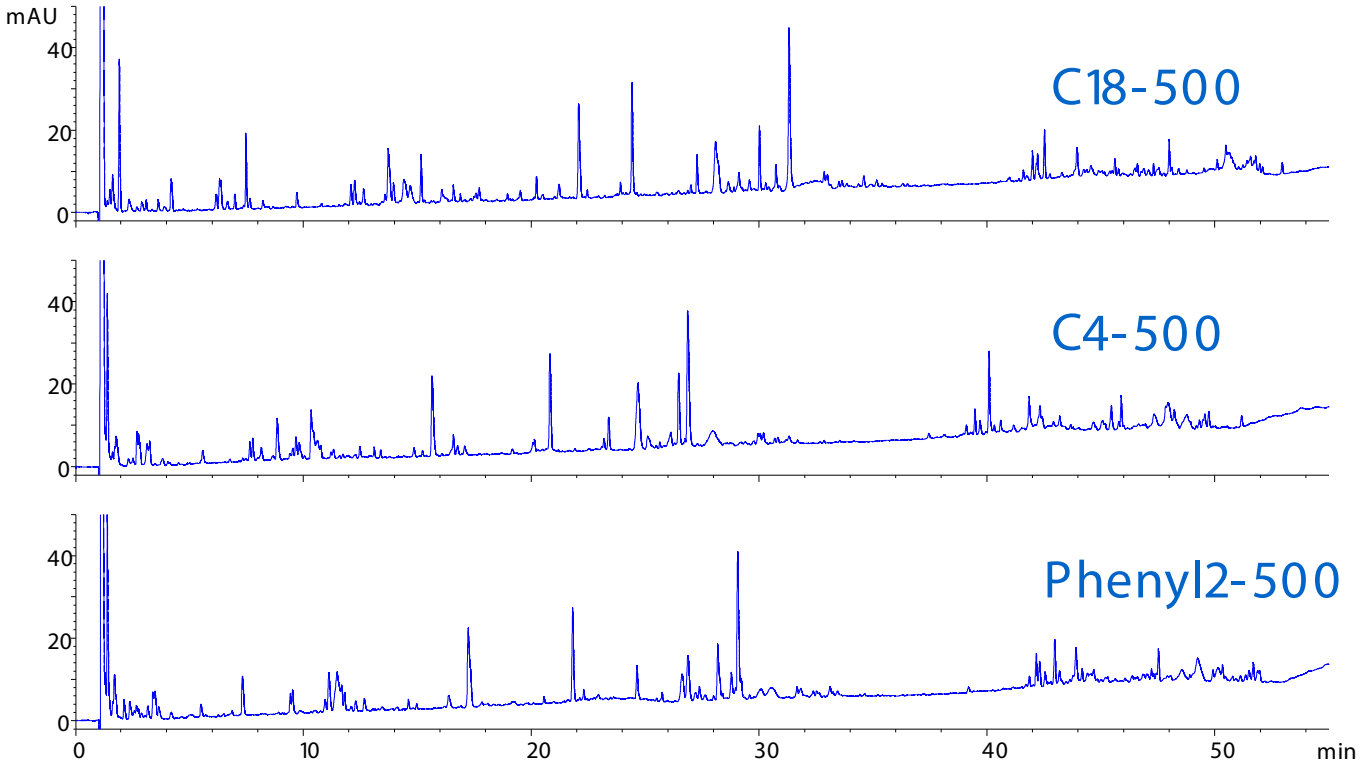
Avantor® ACE® UltraCore BIO Phenyl2, 500 Å, 2.5 µm, 3.0 x 100 mm
Part Number: BIO-252-1030

Mobile Phase: A: 0.1% TFA in H_2O
B: 0.1% TFA in Acetonitrile

Gradient:

Time (min)	%B
0	2
60	40
61	95
64	95
65	2
75	2

Flow Rate: 0.43 mL/min
Temperature: 60 °C
Injection Volume: 10 µL
Detection: UV, 214 nm
System Dwell Volume: 525 µL



TRYPTIC DIGEST OF LYSOZYME

TEST CONDITIONS

Column: Avantor® ACE® UltraCore BIO C4, 300 Å, 3.5 µm, 3.0 x 100 mm
Part Number: BIO-351-1030

Avantor® ACE® UltraCore BIO C18, 300 Å, 3.5 µm, 3.0 x 100 mm
Part Number: BIO-350-1030

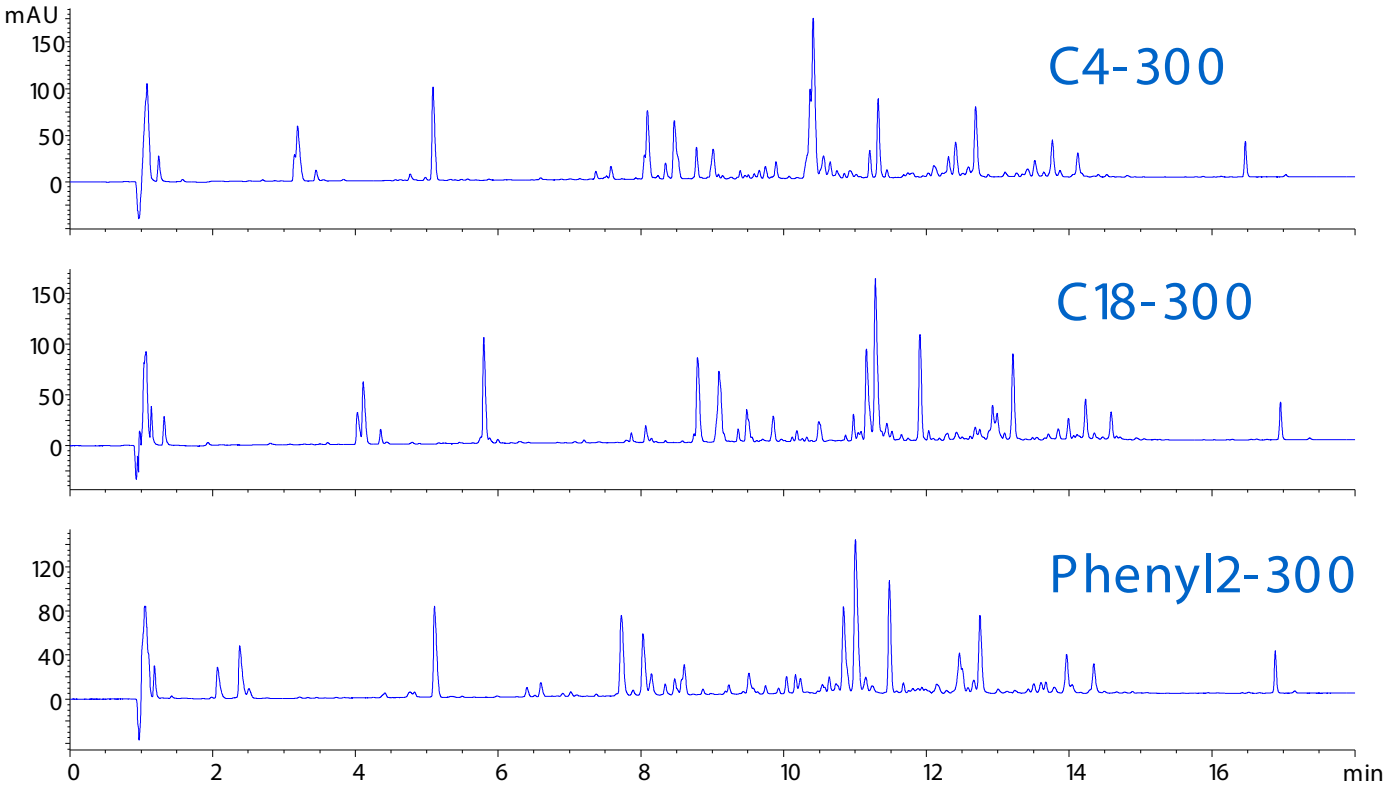
Avantor® ACE® UltraCore BIO Phenyl2, 300 Å, 3.5 µm, 3.0 x 100 mm
Part Number: BIO-352-1030

Mobile Phase: A: 0.1% TFA in H_2O
B: 0.1% TFA in Acetonitrile

Gradient:

Time (min)	%B
0.0	5
20.0	45
21.0	95
23.0	95
23.5	5
33.5	5

Flow Rate: 0.43 mL/min
Temperature: 60 °C
Injection Volume: 10 µL
Detection: UV, 214 nm
System Dwell Volume: 525 µL



GRADIENT SEPARATION OF PEPTIDES AND PROTEINS

TEST CONDITIONS

Column: Avantor® ACE® C4-300, 3 µm, 2.1 x 150 mm
Part Number: ACE-213-1502
Mobile Phase: A: 0.1% Trifluoroacetic acid in H_2O
B: 0.1% Trifluoroacetic acid in Acetonitrile:H_2O (80:20 v/v)

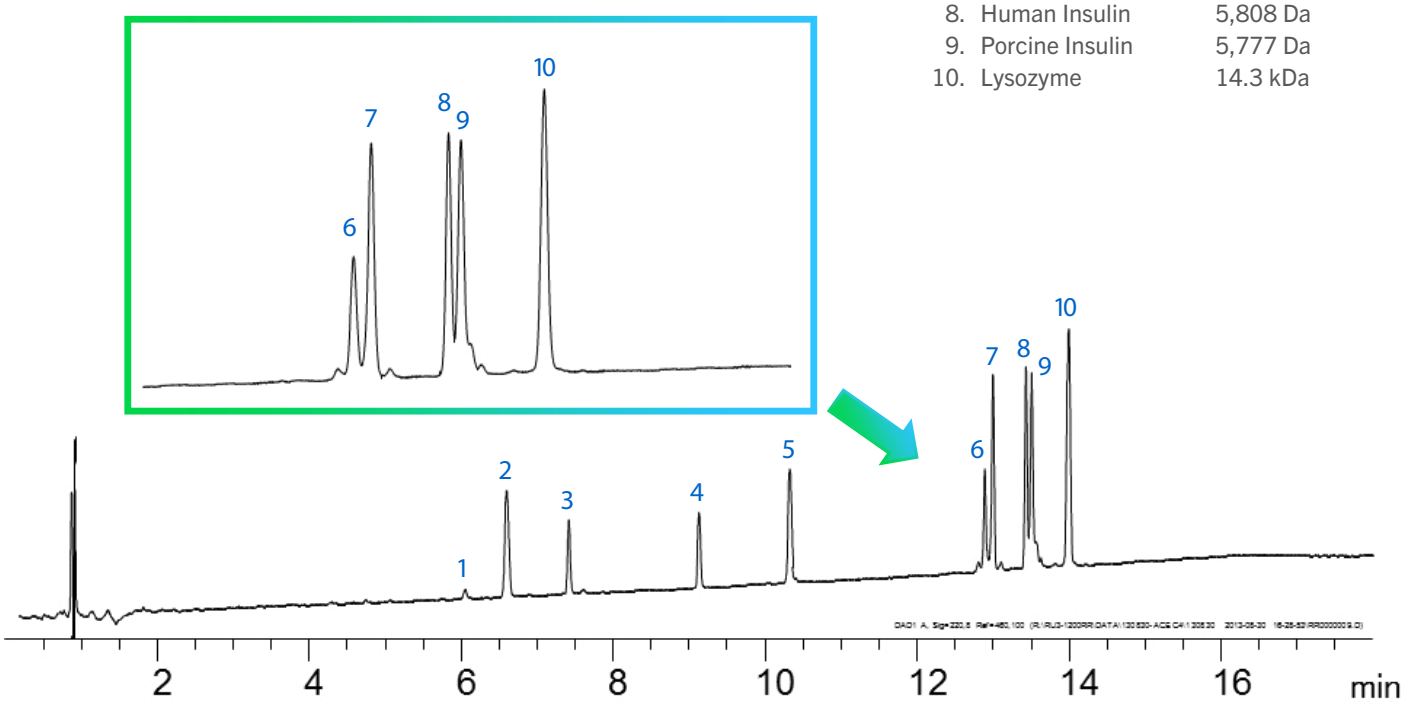
Gradient:

Time (min)	%B
0.0	10
15.0	50
17.5	50
18.0	10

Flow Rate: 0.5 mL/min
Temperature: 60 °C
Injection Volume: 3 µL
Detection: UV, 220 nm

ANALYTES

1. Oxytocin 1,007 Da
2. Bradykinin 1,060 Da
3. Angiotensin II 1,046 Da
4. Angiotensin I 1,296 Da
5. Ribonuclease A 13.7 kDa
6. Cytochrome C 12.3 kDa
7. Bovine Insulin 5,733 Da
8. Human Insulin 5,808 Da
9. Porcine Insulin 5,777 Da
10. Lysozyme 14.3 kDa



PEPTIDE AND PROTEIN MIX

TEST CONDITIONS

Column: HALO BioClass Protein ES-C18, 400 Å, 3.4 µm, 2.1 x 150 mm

Part Number: 93412-702

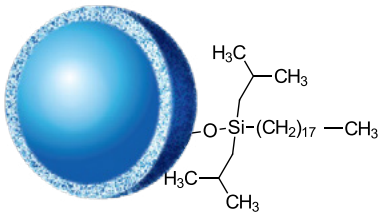
Mobile Phase: A: H_2O + 0.1% DFA
B: 80/20 Acetonitrile/H_2O + 0.1% DFA

Gradient:

Time (min)	%B
0.0	0
15.0	60
16.0	60
16.1	0
20.0	0

Flow Rate: 0.5 mL/min
Initial Pressure: 165 bar
Temp erature: 60 °C
Detection: UV 220 nm, PDA
Injection Volume: 1.5 µL
Sample Solvent: H_2O
Data Rate: 40 Hz
Response Time: 0.025 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

STRUCTURE:

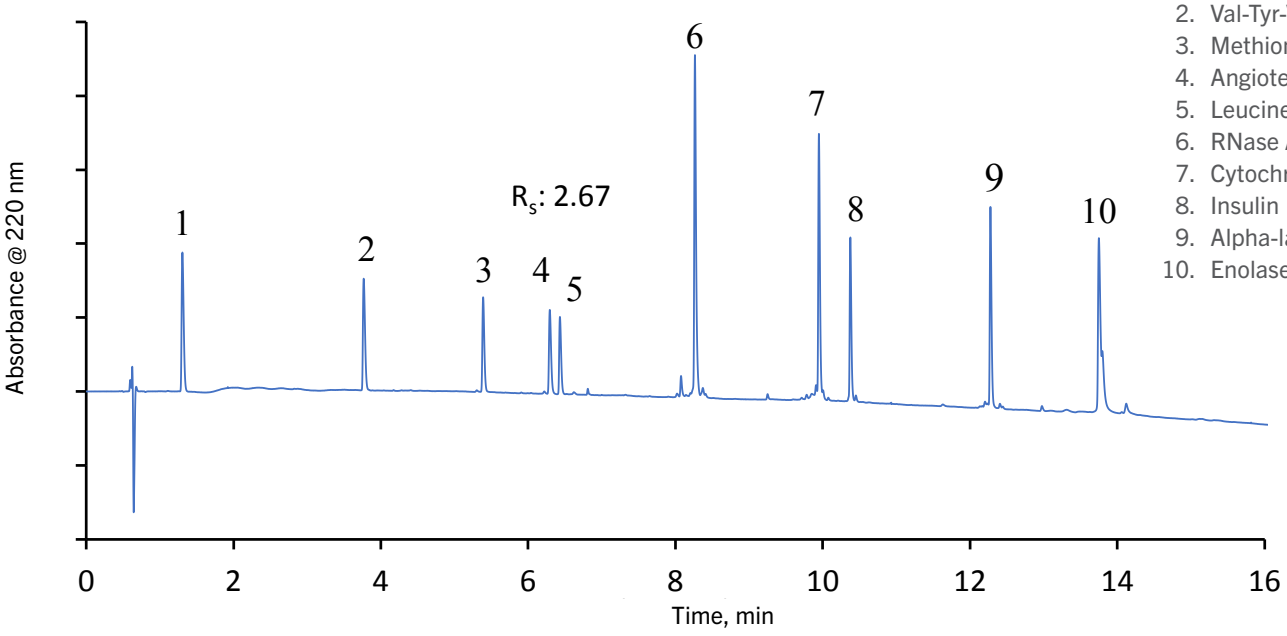


HALO 400 Å ES-C18, 3.4 µm

A mix of peptides and proteins was separated with excellent resolution and peak shape using the HALO 400 Å ES-C18. The steric protection of this phase makes it particularly ideal for the high temperature and low pH conditions often required for peptide and protein separations. Because of its smaller pore size compared to the 1000 Å ES-C18, the 400 Å ES-C18 easily separates mixtures of peptides and smaller proteins such as cytochrome C, alpha-lactalbumin, and enolase.

ANALYTES

1. Gly-Tyr
2. Val-Tyr-Val
3. Methionine Enkephalin
4. Angiotensin II
5. Leucine Enkephalin
6. RNase A
7. Cytochrome C
8. Insulin
9. Alpha-lactalbumin
10. Enolase



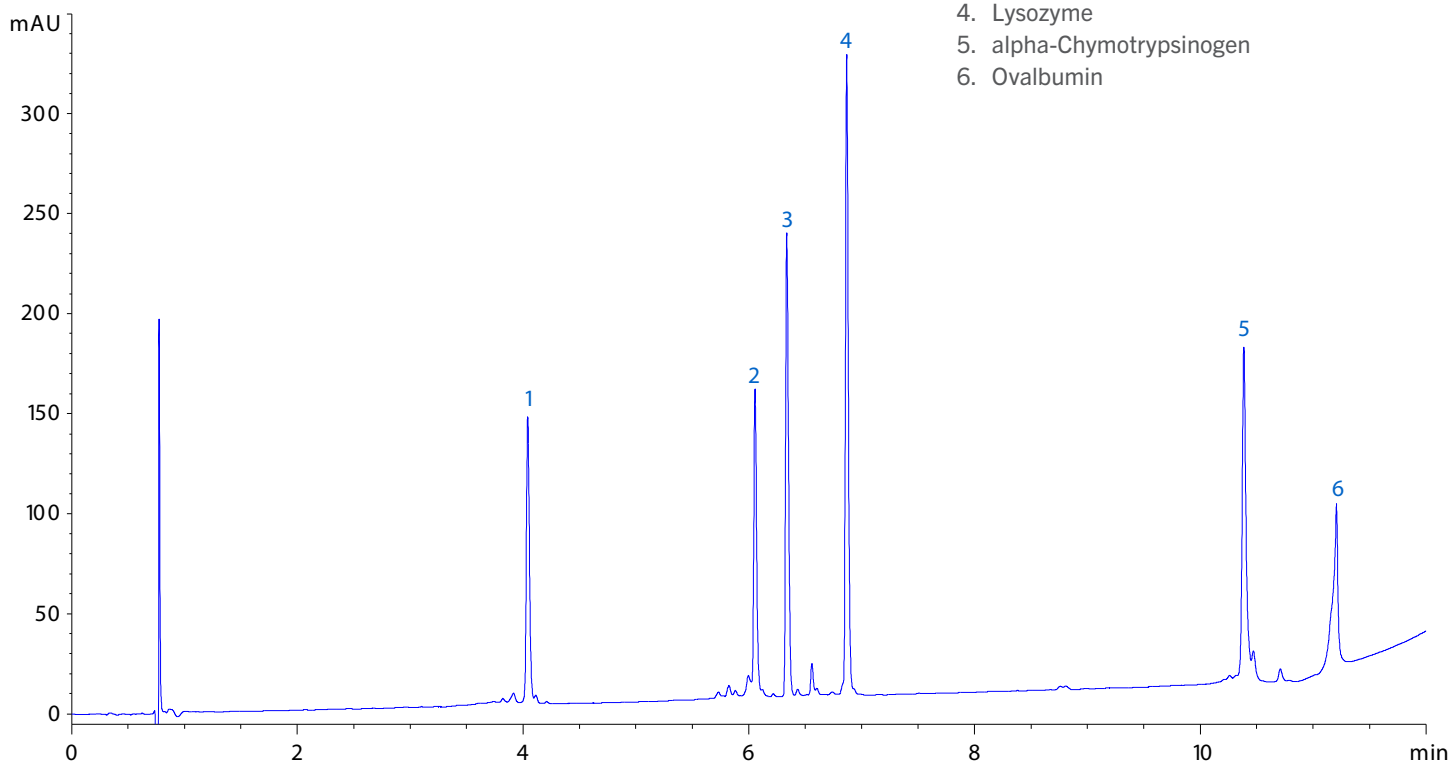
PEPTIDES AND PROTEINS

TEST CONDITIONS

Column:	Avantor® ACE® UltraCore BIO C4, 500 Å, 2.5 µm, 3.0 x 100 mm
Part Number:	BIO-251-1030
Mobile Phase:	A: 0.1% TFA in H_2O B: 0.1% TFA in Acetonitrile/H_2O 90:10 v/v
Flow Rate:	0.6 mL/min
Temperature:	60 °C
Injection Volume:	5 µL
Detection:	UV, 220 nm

ANALYTES

1. Ribonuclease A
2. Cytochrome C
3. Insulin (human)
4. Lysozyme
5. alpha-Chymotrypsinogen
6. Ovalbumin



EFFECT OF PORE SIZE ON PROTEIN PEAK SHAPE AND WIDTH

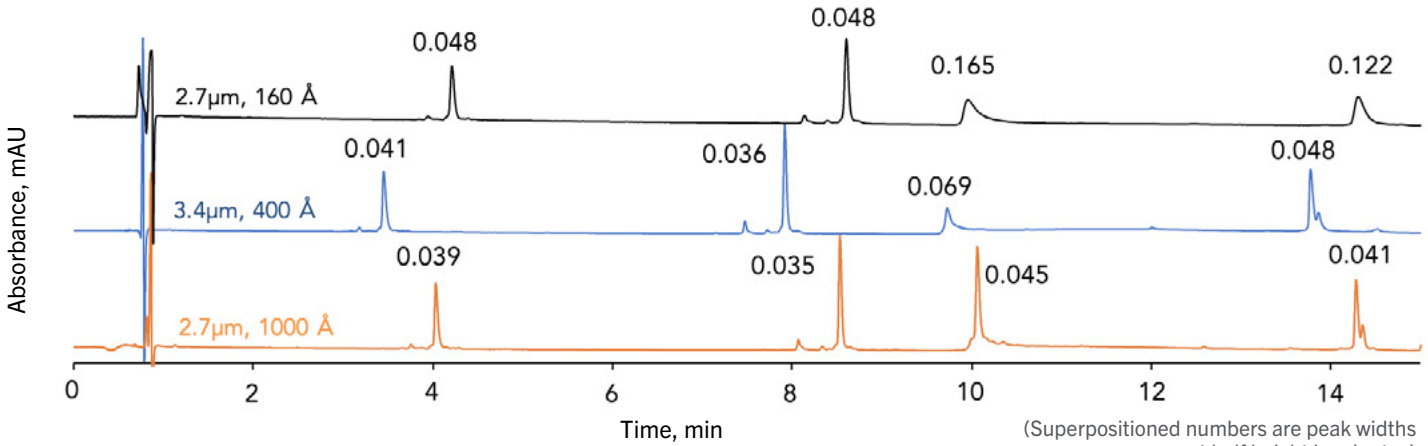
TEST CONDITIONS

Column:	HALO BioClass ES-C18, 2.1 x 150 mm
Part Number:	92122-702 (160 Å) 93412-702 (400 Å) 92712-702 (1000 Å)
Mobile Phase:	A: H_2O (0.1% TFA) B: 80/20 Acetonitrile/ H_2O (0.085% TFA)
Gradient:	27–60% B in 15 minutes
Flow Rate:	0.4 mL/min
Temperature:	60 °C
Detection:	UV 280 nm, PDA
Injection Volume:	4 µL
Sample Solvent:	H_2O (0.1% TFA)
Data Rate:	40 Hz
Response Time:	0.025 sec
Flow Cell:	1 µL
LC System:	Shimadzu Nexera X2

Pore size can play an important part in your HPLC separations. A range of proteins and a monoclonal antibody are separated on HALO ES-C18 160 Å, 400 Å, and 1000 Å columns. Peak widths decrease as the column packing's pore size becomes larger, especially for the monoclonal antibody. The 160 Å pore size is recommended for molecules in the range of 100 Da to 15 kDa. The 400 Å pore size is recommended for molecules between 2 kDa to 500 kDa. The 1000 Å pore size is used for molecules over 50 kDa.

ANALYTES (in order)

1. Ribonuclease A (13.8 kDa)
2. Lysozyme (14.4kDa)
3. SILu™ Lite SigmaMAb Antibody (~150 kDa)
4. Enolase (46.7 kDa)



(Superpositioned numbers are peak widths at half height in minutes)

EFFECT OF SILICA PORE SIZE ON PROTEIN SEPARATIONS

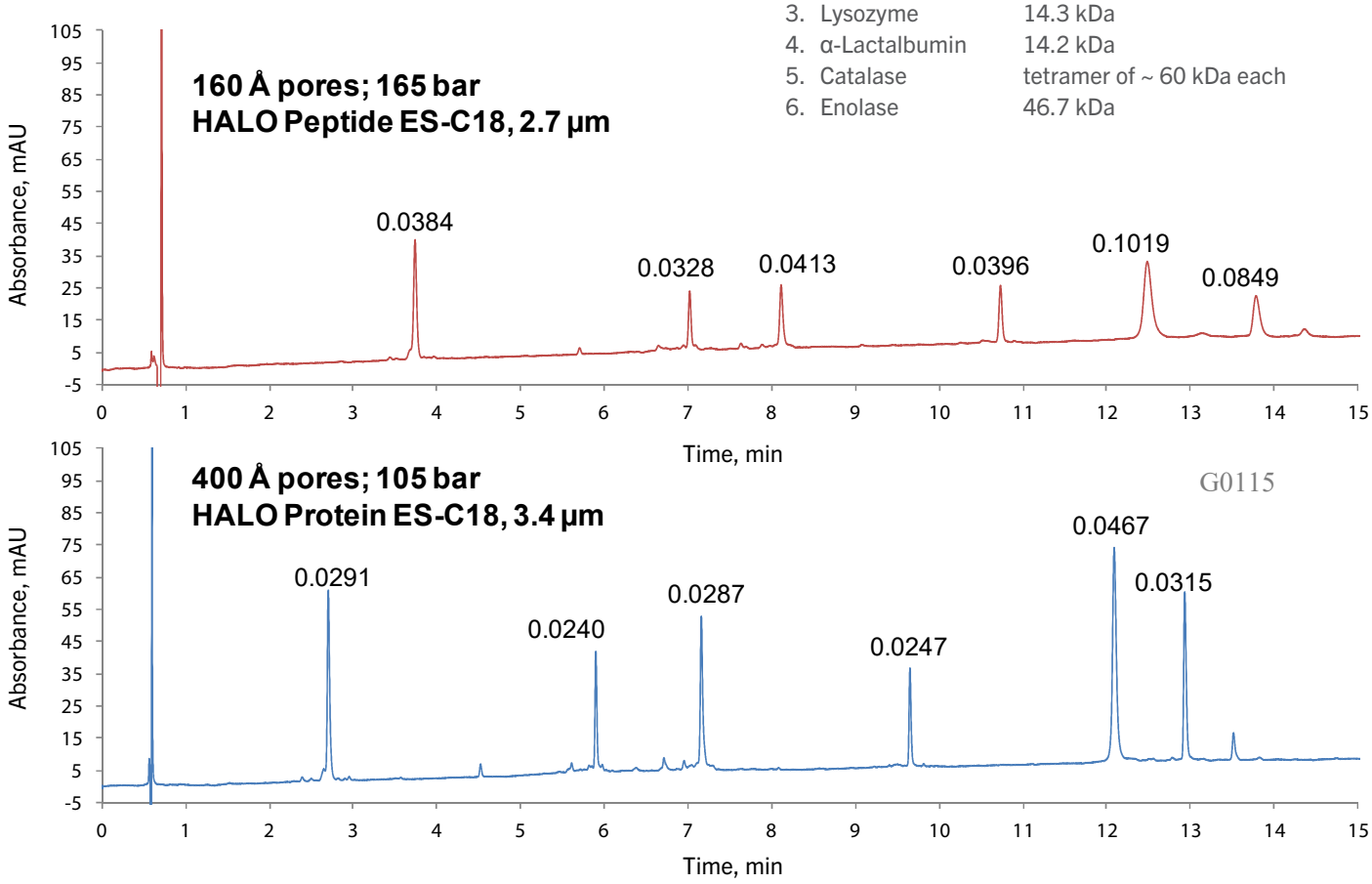
TEST CONDITIONS

Column:	HALO BioClass Peptide ES-C18, 160 Å, 2.7 µm, 4.6 x 100 mm Part Number: 92124-602
	HALO BioClass Protein ES-C18, 400 Å, 3.4 µm, 4.6 x 100 mm Part Number: 93414-602
Mobile Phase:	A: 0.1% Trifluoroacetic acid in H_2O B: 0.1% Trifluoroacetic acid in Acetonitrile
Flow Rate:	1.5 mL/min
Gradient:	23% B–50% B in 15 minutes
Starting pressure:	As indicated on chromatograms
Temperature:	60 °C
Detection:	UV 215 nm, VWD
Injection Volume:	5 µL
Sample Solvent:	Mobile phase A
Response Time:	0.12 sec
Data Rate:	14 Hz
LC System:	Agilent 1100 Quaternary
Flow Cell:	5 µL semi-micro

Sharper, taller peaks are observed using the HALO 400 Å Protein ES-C18 column because the larger pore size allows unrestricted diffusion for these biomolecules into and out of the porous shell. The half height peak widths above each protein peak are significantly smaller with the HALO Protein column despite the larger particle size of the packing material, emphasizing the importance of larger pores when separating proteins.

ANALYTES

1.	Ribonuclease A	13.7 kDa
2.	Cytochrome c	12.4 kDa
3.	Lysozyme	14.3 kDa
4.	α-Lactalbumin	14.2 kDa
5.	Catalase	tetramer of ~ 60 kDa each
6.	Enolase	46.7 kDa



EFFECT OF TEMPERATURE ON THE SEPARATION OF PROTEINS

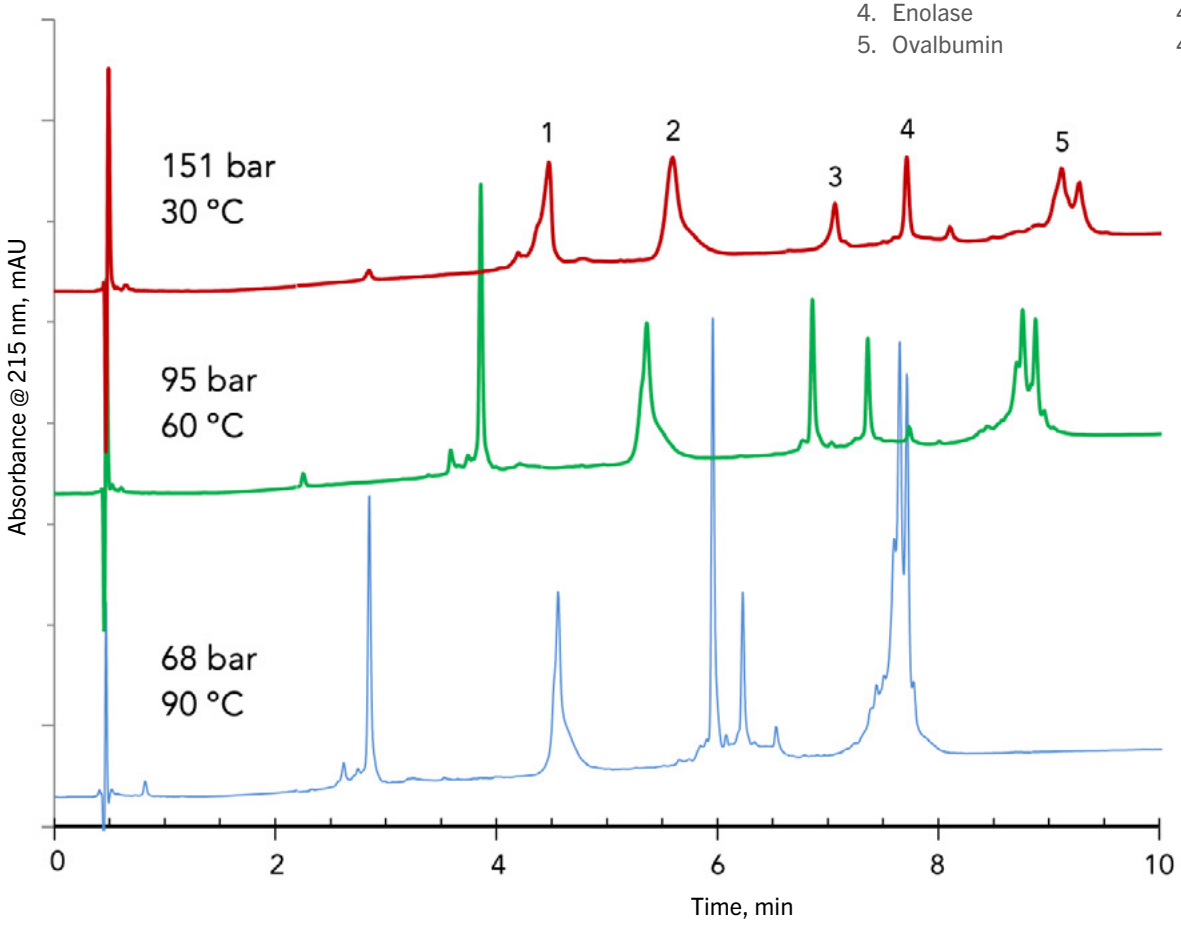
TEST CONDITIONS

Column:	HALO BioClass Protein C4, 400 Å, 3.4 µm, 2.1 x 100 mm
Part Number:	93412-614
Mobile Phase:	72/28: A/B A: 0.1% Trifluoroacetic acid in H_2O B: 0.1% Trifluoroacetic acid in Acetonitrile
Gradient:	28% B–58% B in 10 minutes
Flow Rate:	0.45 mL/min
Pressures:	See chromatograms
Temperatures:	See chromatograms
Detection:	UV 215 nm, PDA
Injection Volume:	2 µL
Sample Solvent:	Mobile phase A
Response Time:	1 sec
Flow Cell:	2 µL micro cell
LC System:	Agilent 1200 SL
Gradient delay volume:	~ 250 µL

These separations demonstrate the effect of elevated temperatures on the efficiency of protein separations done under reversed-phase conditions on a HALO Protein C4, 3.4 µm, 400 Å pore column. One observes taller and narrower peaks as the temperature increases. The HALO C4 phase has been shown to be very stable even at these elevated temperatures.

ANALYTES

1.	Lysozyme	14.3 kDa
2.	Bovine serum albumin	66.4 kDa
3.	α-Chymotrypsinogen A	25.0 kDa
4.	Enolase	46.7 kDa
5.	Ovalbumin	44.0 kDa



IMPROVED PROTEIN SEPARATIONS WITH HALO PROTEIN C4 COMPARED TO TOTALLY POROUS C4

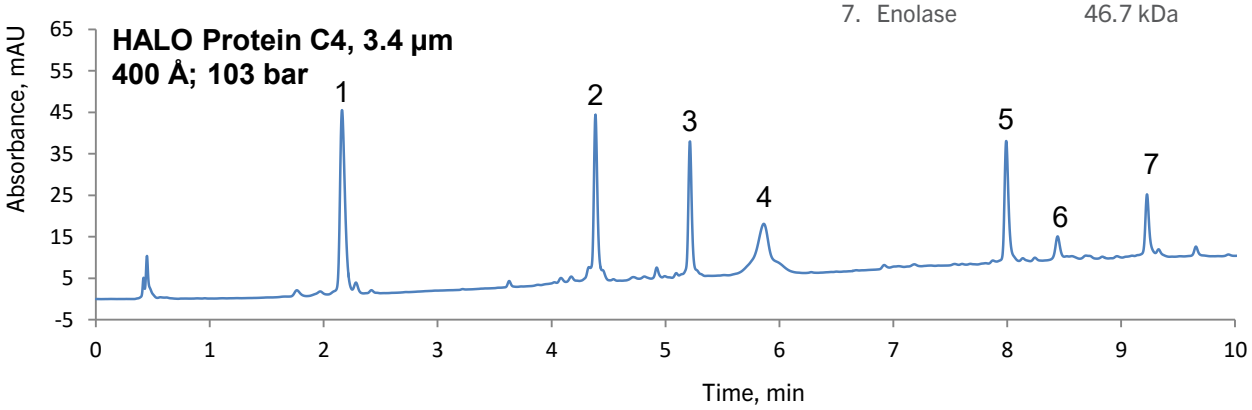
TEST CONDITIONS

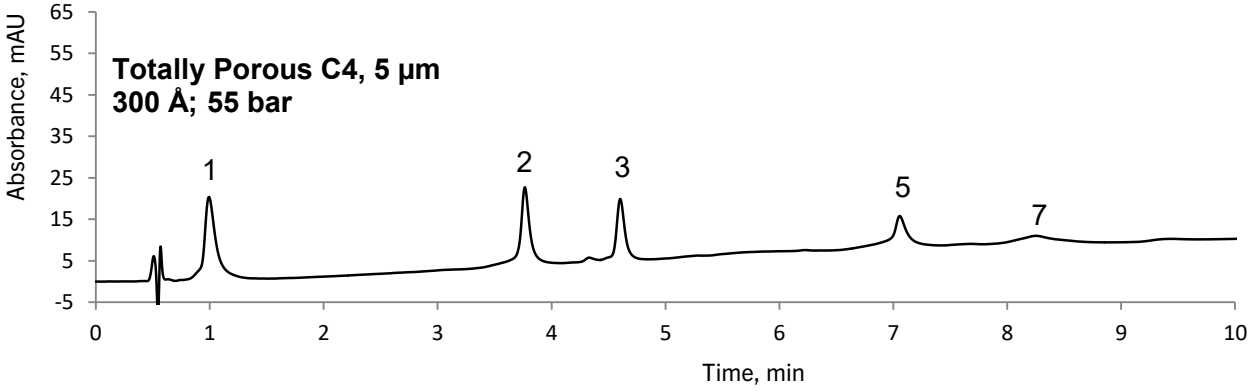
Column:	HALO BioClass Protein C4, 400 Å, 3.4 µm, 2.1 x 100 mm
Part Number:	93412-614
Column 2:	Totally Porous C4, 2.1 x 100 mm, 5 µm
Mobile Phase:	A: H_2O/0.1% TFA B: Acetonitrile/0.1% TFA
Flow Rate:	0.5 mL/min
Gradient:	25% B to 52% B in 10 minutes
Starting pressure:	As indicated on chromatograms
Temperature:	60 °C
Injection Volume:	1 µL
Sample Solvent:	Mobile phase A
Detection:	UV 215 nm, PDA
Data Rate:	5 Hz
Response Time:	1 sec
Flow Cell:	2 µL micro cell
LC System:	Agilent 1200 SL

Sharper, taller peaks are observed using the HALO Protein C4 column compared to a conventional totally porous C4 column. Additionally, the HALO Protein C4 column provides improved recoveries for holotransferrin, apomyoglobin, catalase, and enolase.

ANALYTES

1.	Ribonuclease A	13.7 kDa
2.	Cytochrome c	12.4 kDa
3.	Lysozyme	14.3 kDa
4.	Holotransferrin	77 kDa
5.	Apomyoglobin	17 kDa
6.	Catalase	tetramer of ~ 60 kDa each
7.	Enolase	46.7 kDa





INSULIN ANALOGS IN CLINICAL AND POST-MORTEM ANALYSES

TEST CONDITIONS

Column: Avantor® ACE® C18-300, 5 µm, 2.1 x 50 mm
Part Number: ACE-221-0502

Mobile Phase: A: 0.1% Acetic acid in H_2O
B: 0.1% Acetic acid in IPA/Acetonitrile (25:75 v/v)

Gradient:

Time (min)	%B
0.0	22
0.5	22
1.0	34
3.0	36
4.0	98
6.2	98
6.3	22

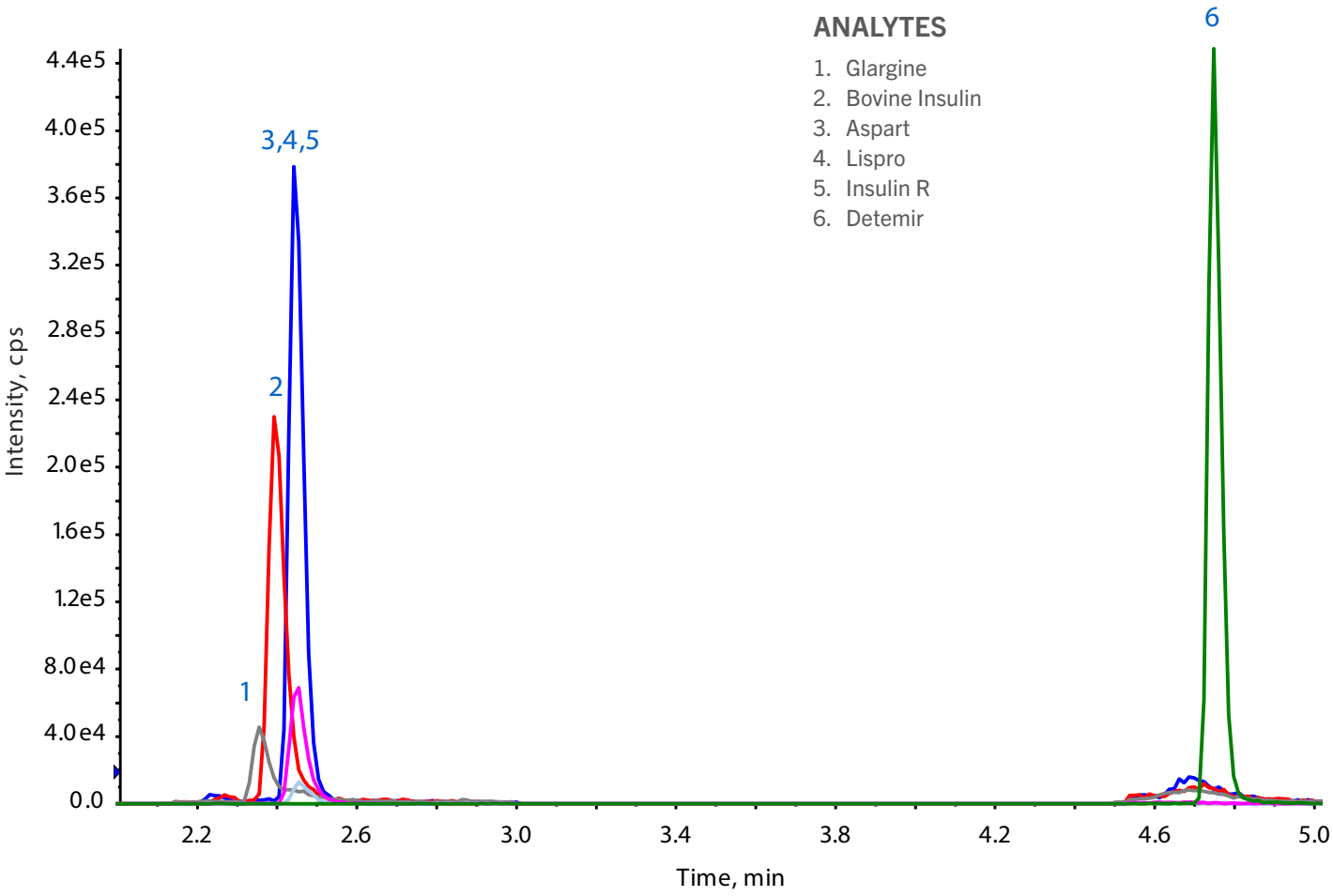
Flow Rate: 0.55 mL/min
Injection Volume: 40 µL

Detection: ESI positive ion mode
Ion spray voltage: 5500 V
Temperature: 600 °C
MS Instrument: AB Sciex QTRAP 5500

Sample: 100 µg/mL insulin analogs in steroid-free serum

ANALYTES

1. Glargine
2. Bovine Insulin
3. Aspart
4. Lispro
5. Insulin R
6. Detemir



MEDIUM MOLECULAR WEIGHT PROTEINS

TEST CONDITIONS

Column: Avantor® ACE® C8-300, 3 µm, 2.1 x 150 mm
Part Number: ACE-212-1502
Mobile Phase: A: 0.1% Trifluoroacetic acid in H_2O
B: 0.1% Trifluoroacetic acid in Acetonitrile

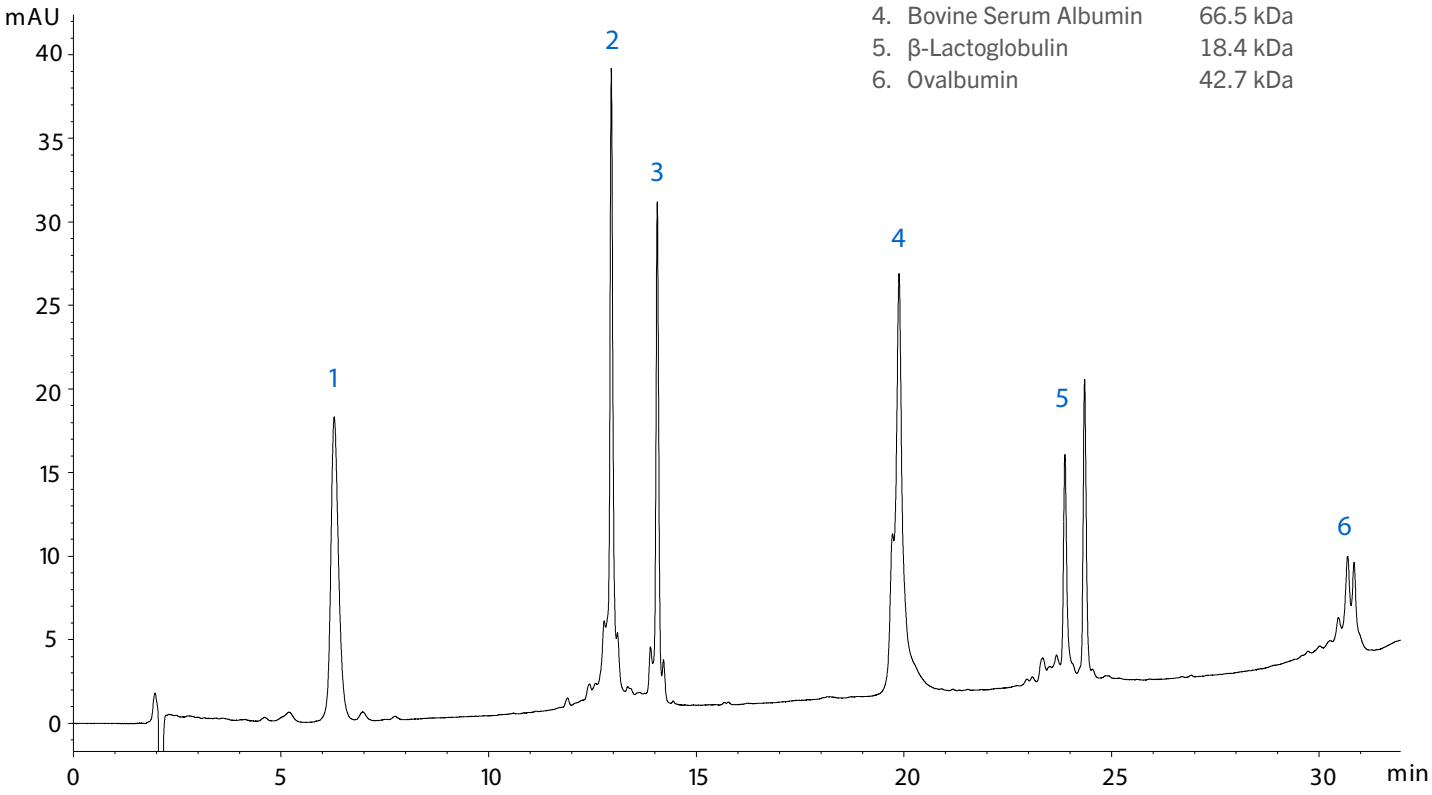
Gradient:

Time (min)	%B
0	25
25	50
28	80
30	80
31	25
45	25

Flow Rate: 0.21 mL/min
Temperature: 60 °C
Injection Volume: 5 µL
Detection: UV, 220 nm

ANALYTES

1. Ribonuclease A 13.7 kDa
2. Cytochrome C (Equine) 12.4 kDa
3. Cytochrome C (Bovine) 12.3 kDa
4. Bovine Serum Albumin 66.5 kDa
5. β-Lactoglobulin 18.4 kDa
6. Ovalbumin 42.7 kDa



PROTEIN SEPARATION ON 1000 Å COLUMN

TEST CONDITIONS

Column: HALO BioClass Protein ES-C18, 1000 Å, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-702
Mobile Phase: A: H_2O, 0.1% TFA
B: 80/20 Acetonitrile/ H_2O, 0.085% TFA

Gradient:

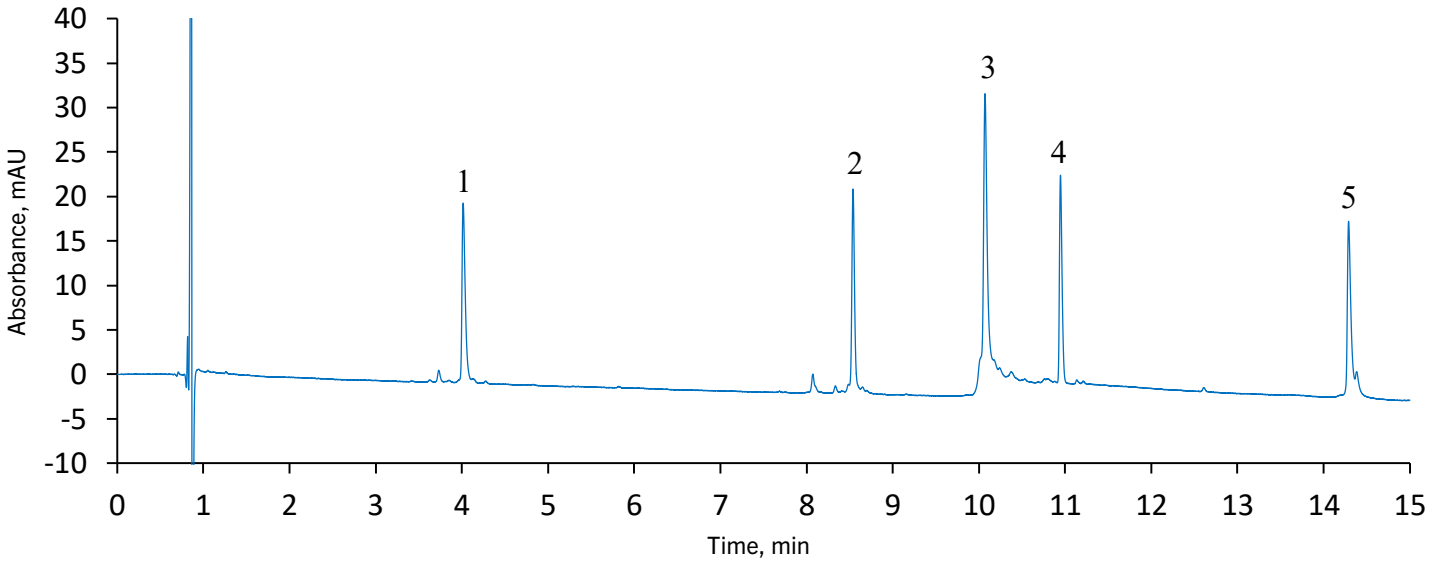
Time (min)	%B
0.0	27
15.0	60

Flow Rate: 0.4 mL/min
Pressure: 268 bar
Temperature: 60 °C
Injection Volume: 2 µL
Sample Solvent: H_2O/ 0.1 %TFA
Detection: UV 280 nm, PDA
Data Rate: 12.5Hz
Response Time: 0.05 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

This mix of proteins with a wide range of molecular weights is separated with high efficiency on a HALO 1000 Å ES-C18 column. Because it provides improved access to the particle surface, the 1000 Å pore size enables large biomolecule analysis with excellent peak shape and high resolution.

ANALYTES

1. Ribonuclease A 13.7 kDa
2. Lysozyme 14.3 kDa
3. SigmaMAb ~150 kDa
4. α-Lactalbumin 14.2 kDa
5. Enolase 46 kDa monomer



PROTEIN TEST MIX

TEST CONDITIONS

Column: Avantor® ACE® C18-300, 5 µm, 4.6 x 250 mm
Part Number: ACE-221-2546
Mobile Phase: A: 0.1% TFA in H_2O
B: 0.1% TFA in Acetonitrile

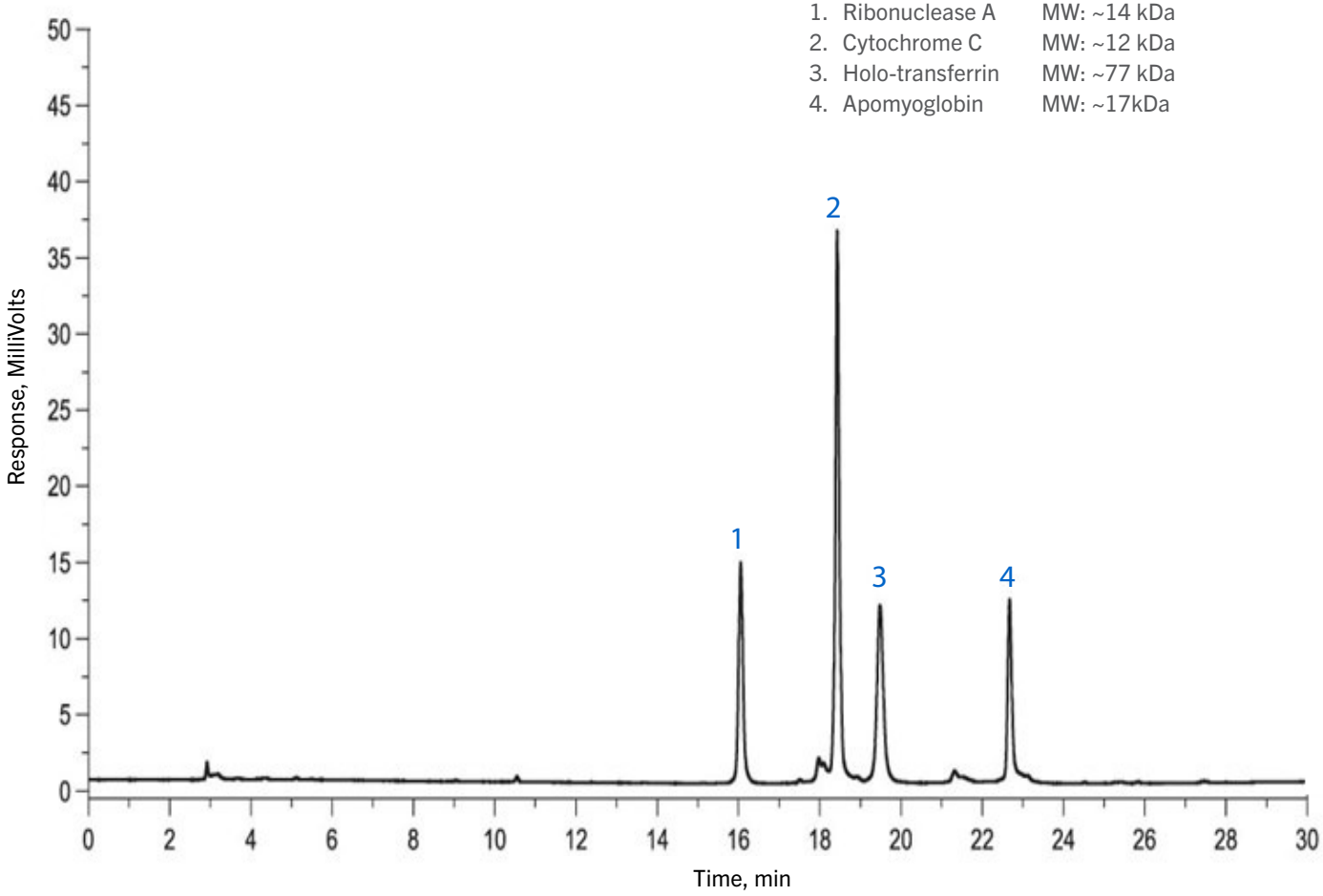
Gradient:

Time (min)	%B
0	5
30	70

Flow Rate: 1.0 mL/min
Temperature: Ambient
Detection: UV, 280 nm

ANALYTES

1. Ribonuclease A MW: ~14 kDa
2. Cytochrome C MW: ~12 kDa
3. Holo-transferrin MW: ~77 kDa
4. Apomyoglobin MW: ~17kDa



WHEY PROTEINS FROM WHOLE MILK

TEST CONDITIONS

Column: Avantor® ACE® C4-300, 3 µm, 2.1 x 150 mm
Part Number: ACE-213-1502
Mobile Phase: A: 0.5% Formic acid in H_2O
B: 0.5% Formic acid in Acetonitrile

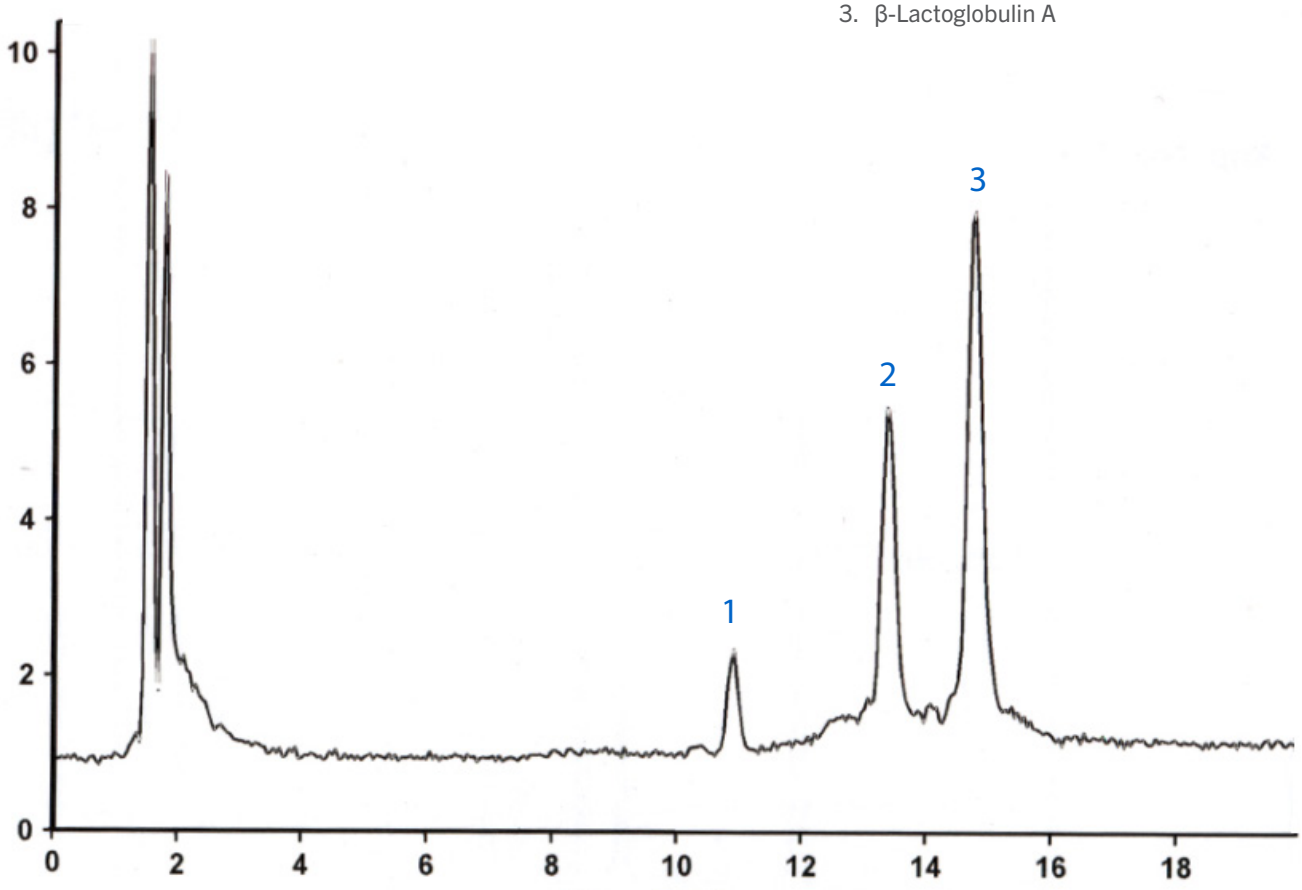
Gradient:

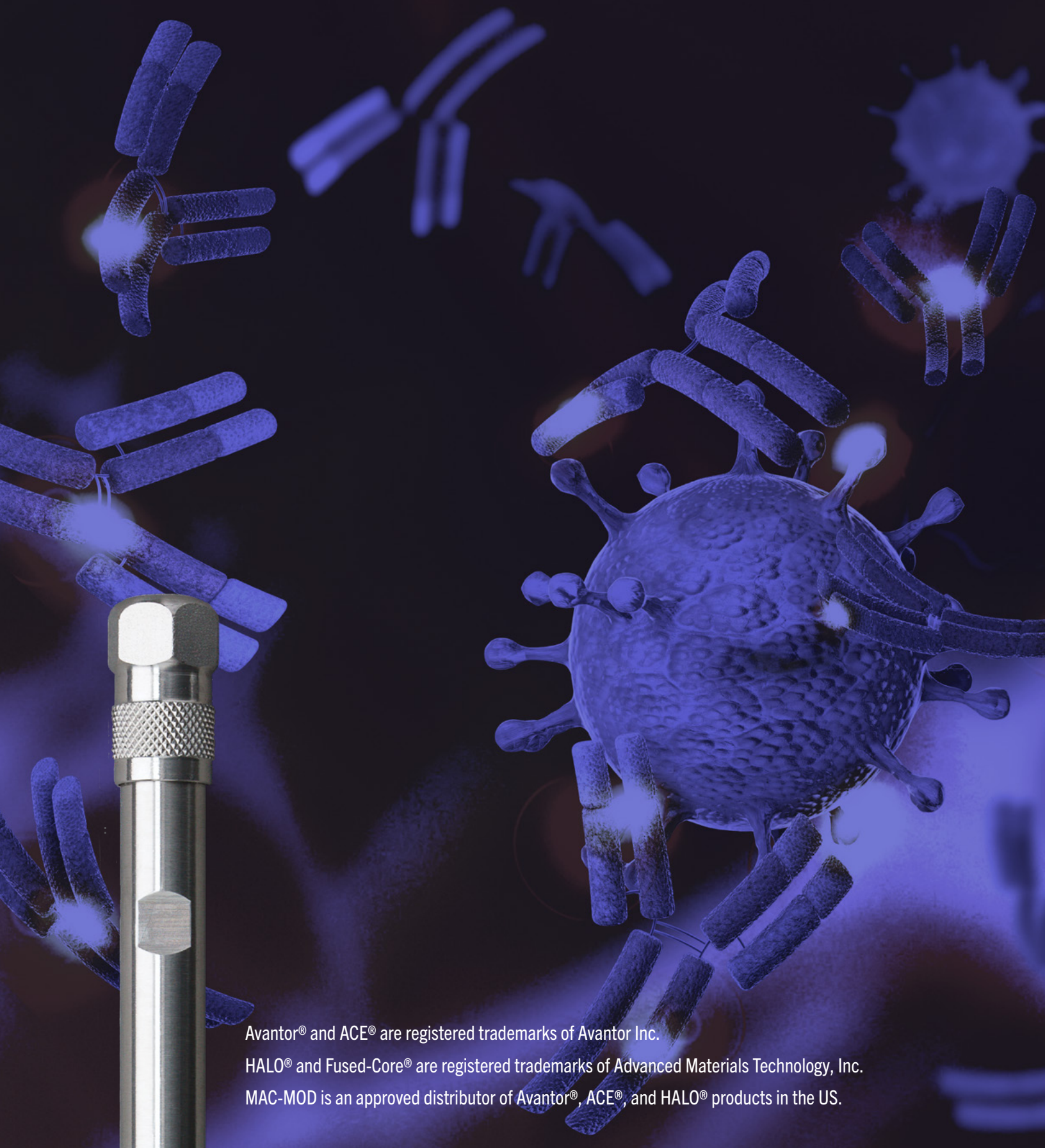
Time (min)	%B
0	35
16	43
17	80
20	80
21	35
31	35

Flow Rate: 0.4 mL/min
Temperature: 40 °C
Injection Volume: 10 µL
Detection: ESI-MS (+ve)

ANALYTES

1. α-Lactalbumin
2. β-Lactoglobulin B
3. β-Lactoglobulin A





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