Extended length capillary columns for simple peptide mapping and complex proteomic separations

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Mass Spectrometry Laboratory
Department of Chemistry
Stanford University
Stanford California

Long Column Data

Session: Proteomics: Sample Preparation | **Code:** ThPW | **Time Slot/Poster Number:** 469

Ammonium sulfate precipitation as a novel first dimension in the multidimensional analysis of complex proteomes

Allis S. Chien; Andrew W. Guzzetta;
Stanford University, Stanford, CA

Introduction:

We have applied the classic protein purification technique of ammonium sulfate precipitation to the first dimension of a 2−D analysis of the human plasma proteome. This simple offline technique is accessible to all labs and avoids complicated column formats and column switching schemes, minimizes the number of steps to avoid sample loss, and is unlimited by the pI or hydrophobicity of analyte components. In addition, our approach is easily scaleable and allows for multiple subsequent analyses. Extremely hydrophobic proteins as well as small peptides are captured by this technique. The first dimension separation operates at the protein level so that peptides from the same protein remain together for the subsequent dimension of reverse-phase LC−MS peptide analysis.

Long Columns are Better

The Current Big Kahunas of the Long Column

Richard Smith & Yufeng Shen

1: Smith RD, Shen Y, Tang K. Related Articles, Links

Ultrasensitive and quantitative analyses from combined separations-mass spectrometry for the characterization of proteomes.
Acc Chem Res. 2004 Apr;37(4):269-78.
PMID: 15096064 [PubMed - in process]

2: Shen Y, Jacobs JM, Camp DG 2nd, Fang R, Moore RJ, Smith RD, Xiao W, Davis RW, Tompkins RG. Related Articles, Links

Ultra-high-efficiency strong cation exchange LC/RPLC/MS/MS for high dynamic range characterization of the human plasma proteome.
Anal Chem. 2004 Feb 15;76(4):1134-44.
PMID: 14961748 [PubMed - in process]

3: Shen Y, Tolic N, Masselon C, Pasa-Tolic L, Camp DG 2nd, Hixson KK, Zhao R, Anderson GA, Smith RD. Related Articles, Links

Ultrasensitive proteomics using high-efficiency on-line micro-SPE-nanoLC-nanoESI MS and MS/MS.
Anal Chem. 2004 Jan 1;76(1):144-54.
PMID: 14697044 [PubMed - in process]

4: Shen Y, Tolic N, Masselon C, Pasa-Tolic L, Camp DG 2nd, Lipton MS, Anderson GA, Smith RD. Related Articles, Links

Nanoscale proteomics.
Anal Bioanal Chem. 2004 Feb;378(4):1037-45. Epub 2003 Nov 29.
PMID: 14647945 [PubMed - in process]

5: Shen Y, Moore RJ, Zhao R, Blonder J, Auberry DL, Masselon C, Pasa-Tolic L, Hixson KK, Auberry KJ, Smith RD. Related Articles, Links

High-efficiency on-line solid-phase extraction coupling to 15-150-microm-i.d. column liquid chromatography for proteomic analysis.
Anal Chem. 2003 Jul 15;75(14):3264-73.
PMID: 14570215 [PubMed - indexed for MEDLINE]

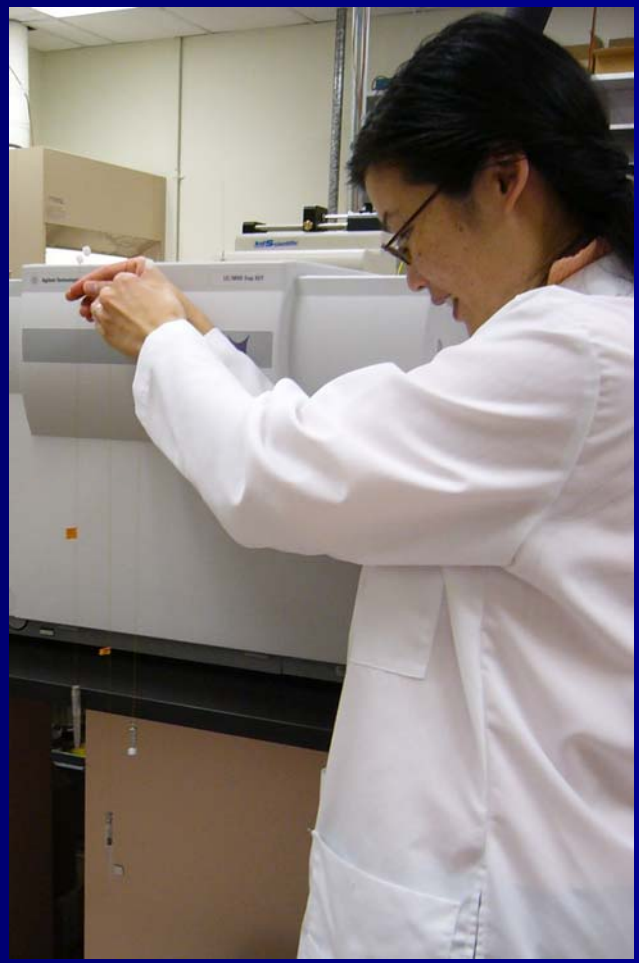
6: Smith RD, Anderson GA, Lipton MS, Masselon C, Pasa-Tolic L, Udseth H, Belov M, Shen Y, Veenstra TD. Related Articles, Links

High-performance separations and mass spectrometric methods for high-throughput proteomics using accurate mass tags.

Old Cap Masters

D. Ishi
C. Horvath
F. Yang
B. Karger
J. Henion
R. Simpson
J-P. Chervet

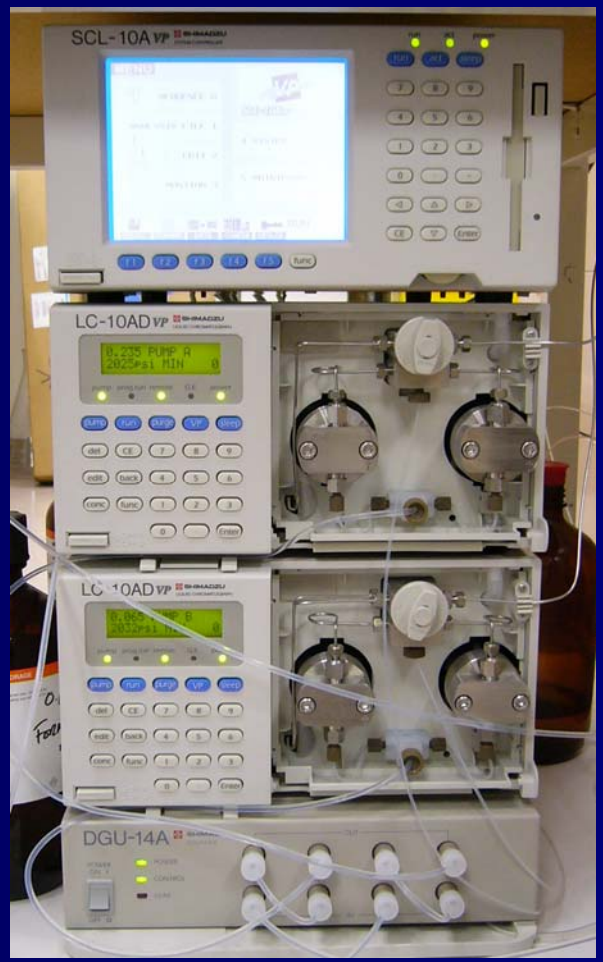
Should We Use Long or Short Columns?

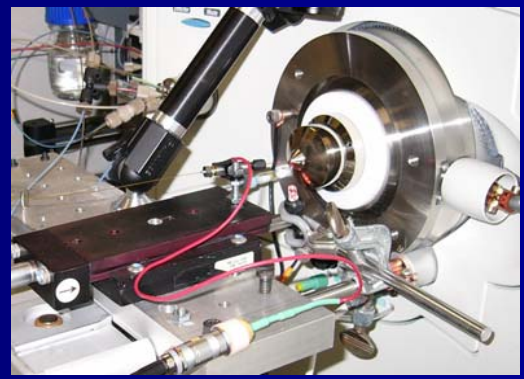


Core Facility at Stanford

- Gel spot analysis
- Detailed peptide mapping
- Proteomics

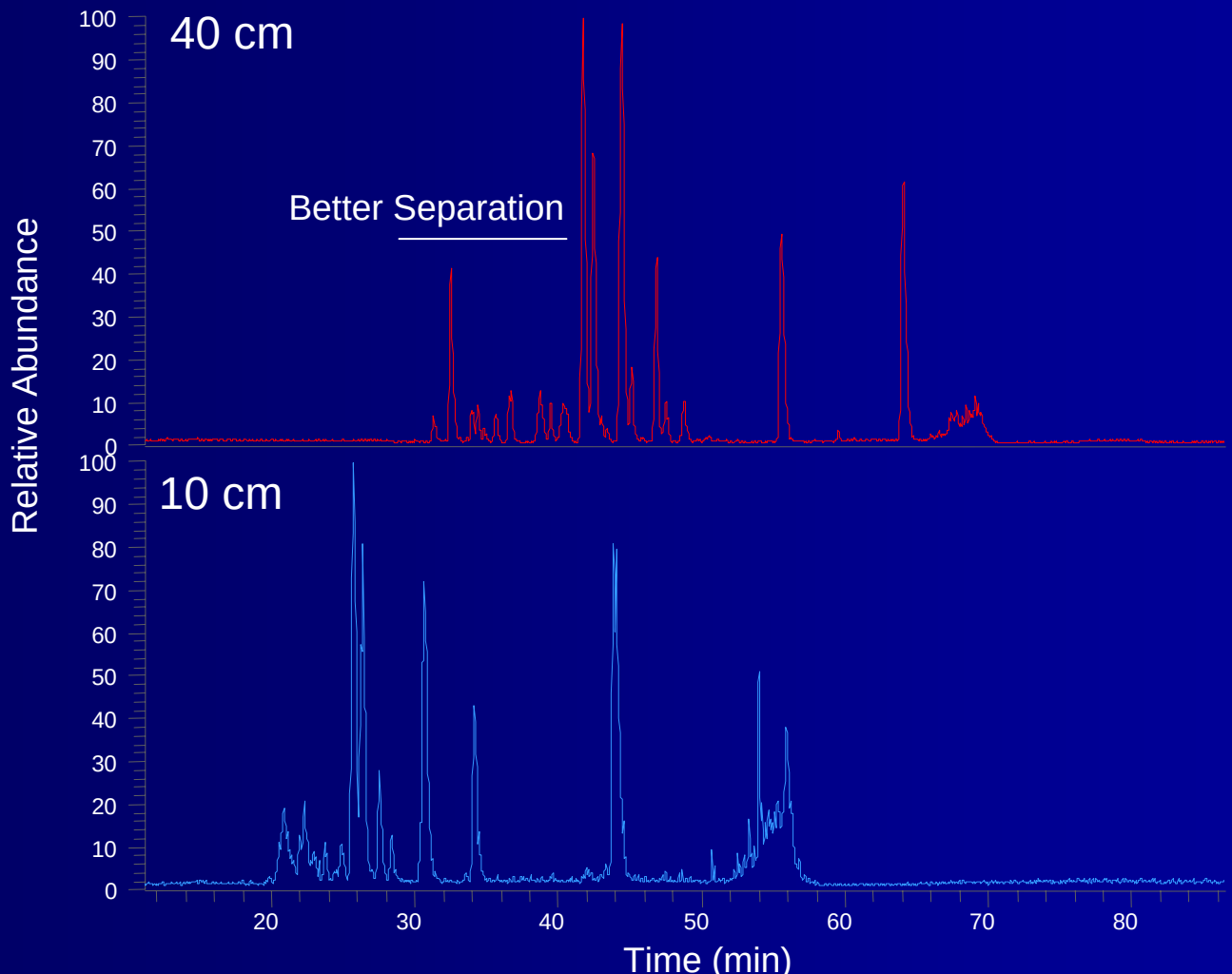
Simple Peptide Mapping



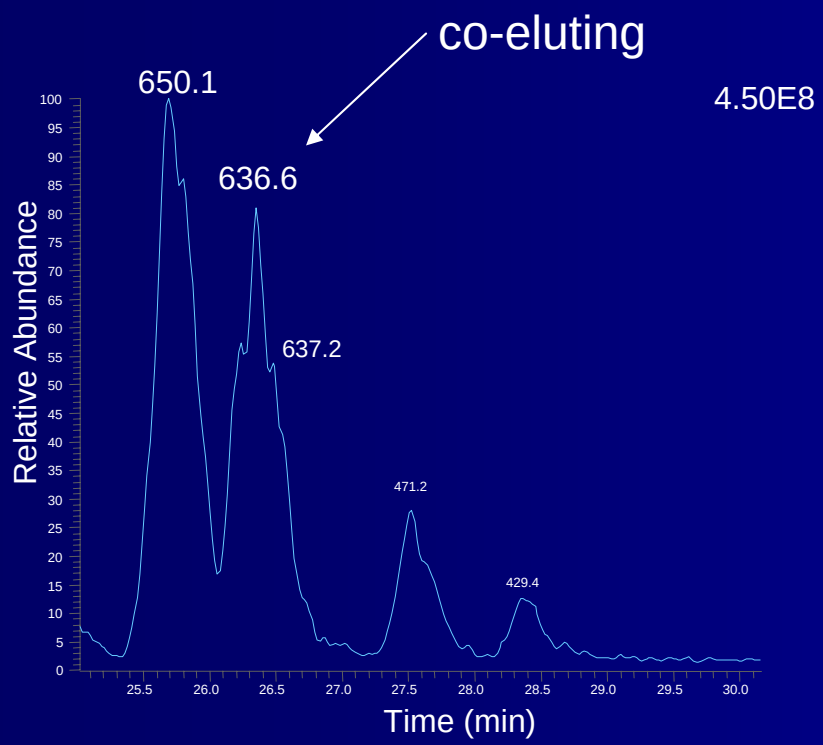


Column: 400 or 100 mm in Length, 150 µm ID
Stationary Phase: Vydac C18, 300A, 5µm
Temperature: Ambient
Flow Rate: 1 µ l /min
Mobile Phase: A:H20, **B**:ACN, 0.1%FA
Trapping Column:
Poros 10, 10um, 10 X 0.15 mm, 10ul/min, 0%B
Mass Spectrometer: XP plus , IonTrap
HPLC: Shimadzu 10ADvp

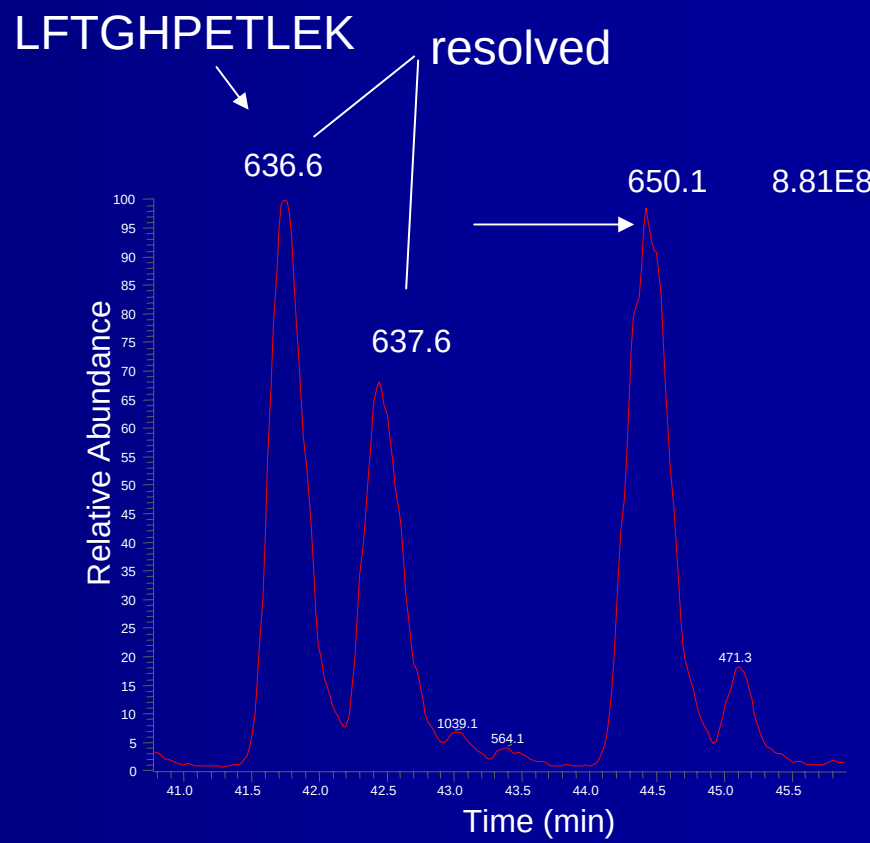
Myoglobin Tryptic Digest
400 mm vs. 100mm



Separation of Near Isobars and Added Selectivity

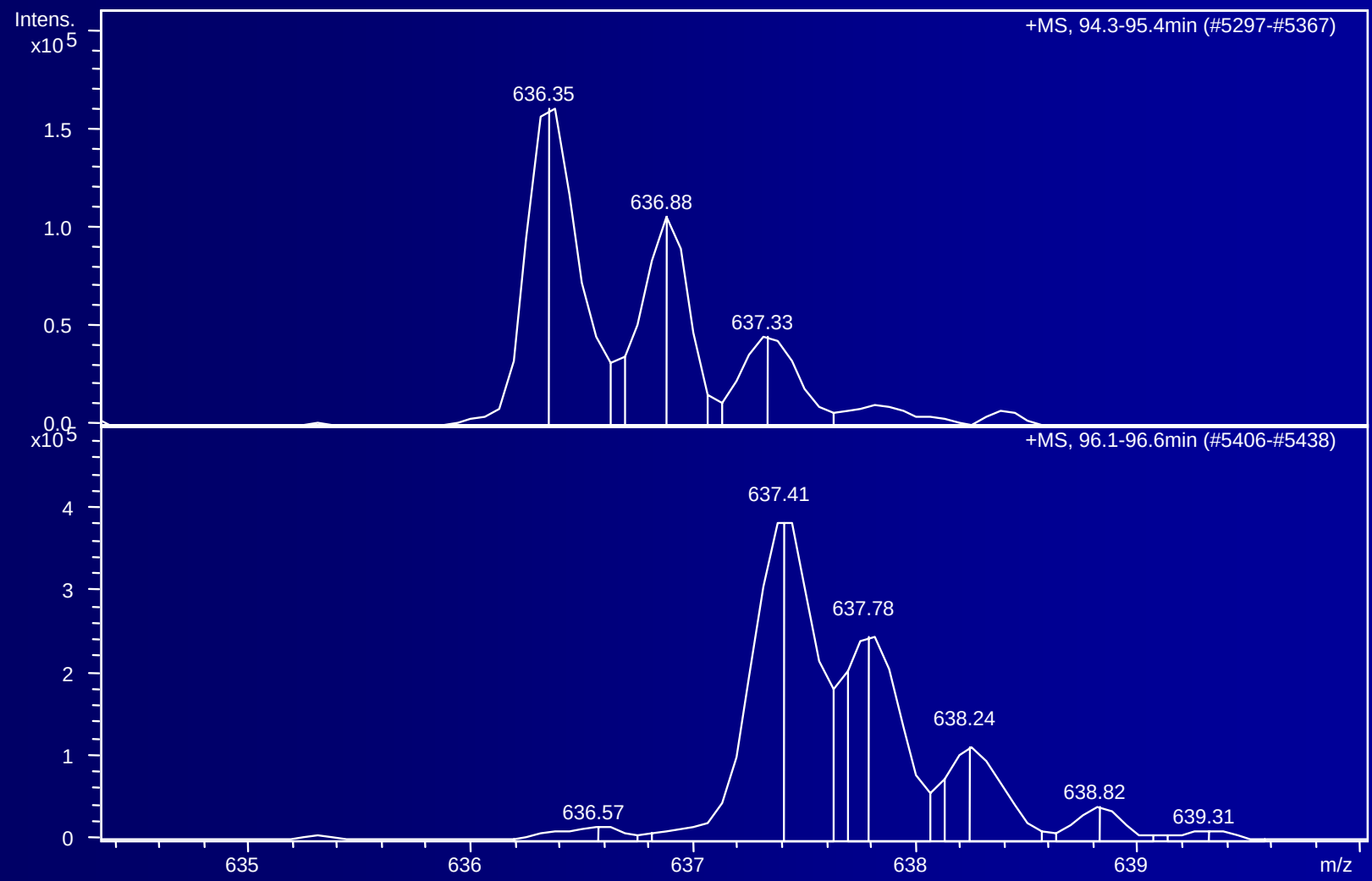


100 mm



400 mm

Isotopic data on the separated near isobars



Long vs. Short Columns and Proteomics Development

or

Optimizing for Complex Peptide Mixtures

Serum Proteomics Method

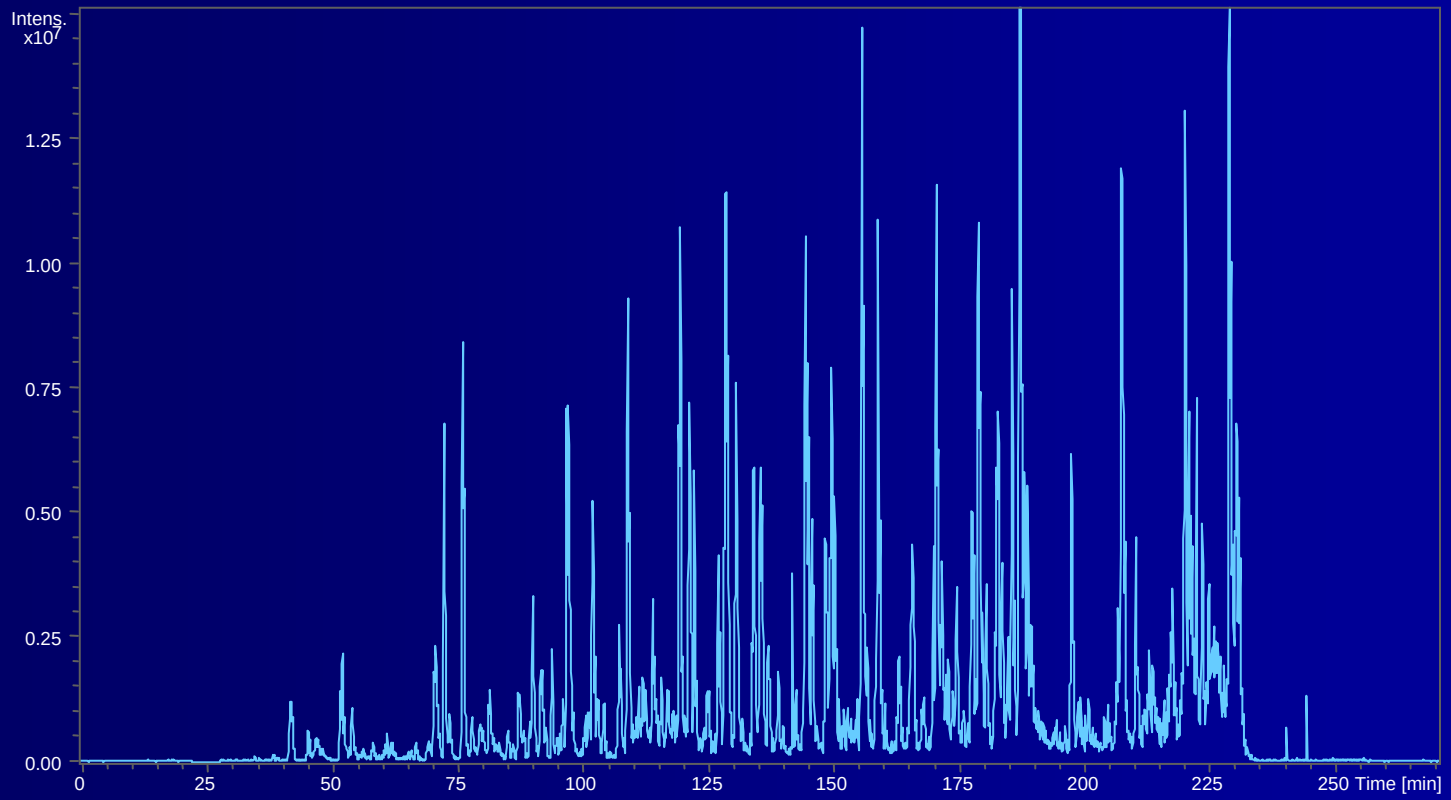
Column: 60 or 10 cm in Length, 75 µm ID
Stationary Phase: Vydac C18, 300A, 5µm
Temperature: Ambient
Flow Rate: 200 nl/min
Mobile Phase: A:H20, **B**:ACN, 0.1%FA
Gradient:

T	%B
0	5
4	5
184	30
200	80
210	80
211	5
270	5

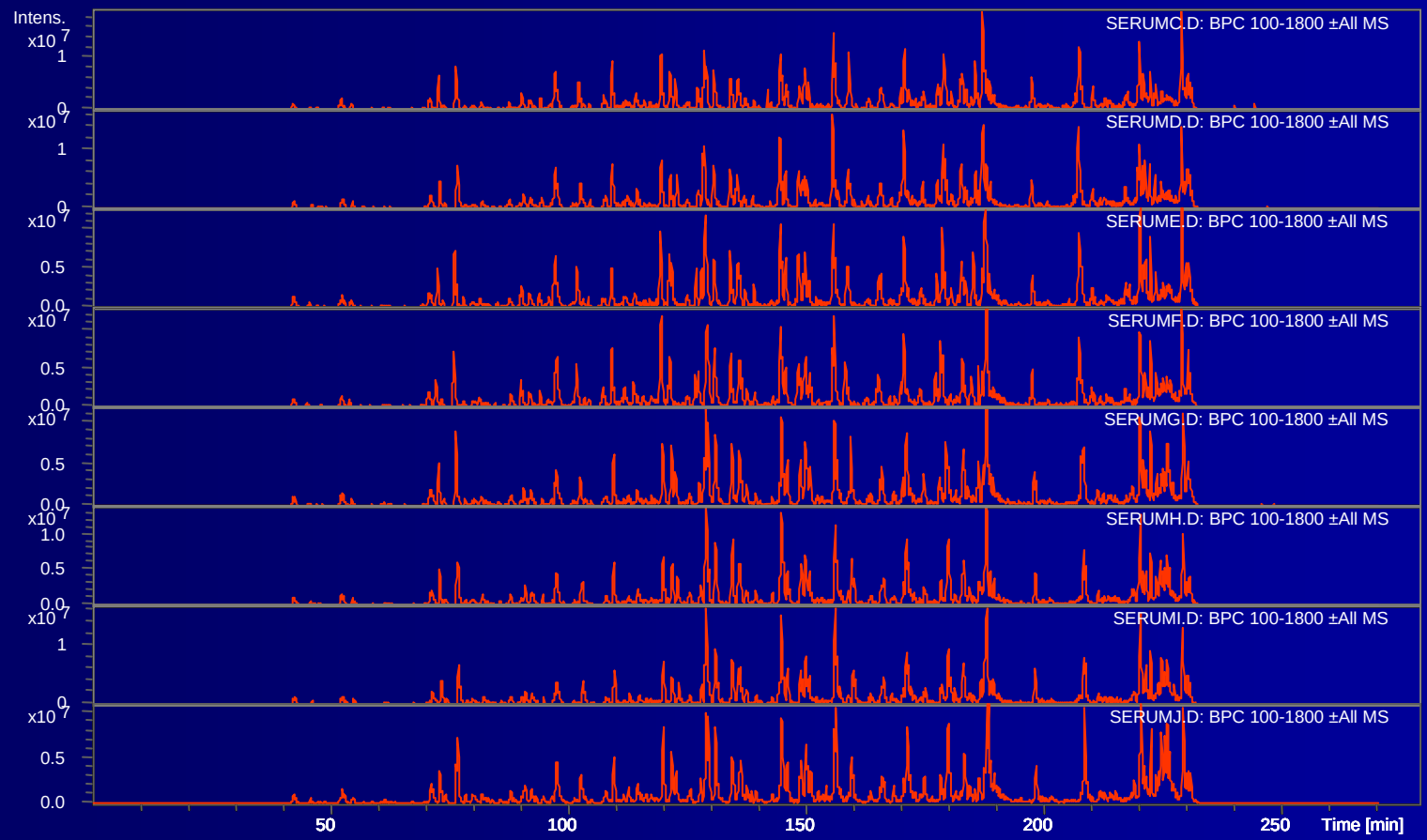
Trapping Column:
Zorbax 300 SB, 5um, 5 X 0.3 mm, 10ul/min, 0%B
Flow Rate: 10ul/min

Mass Spectrometer: Agilent XCT, IonTrap
HPLC: Agilent Capillary HPLC
Database Searching: SpectrumMill

Human Serum$_{\text{albumin}}$ Proteomics



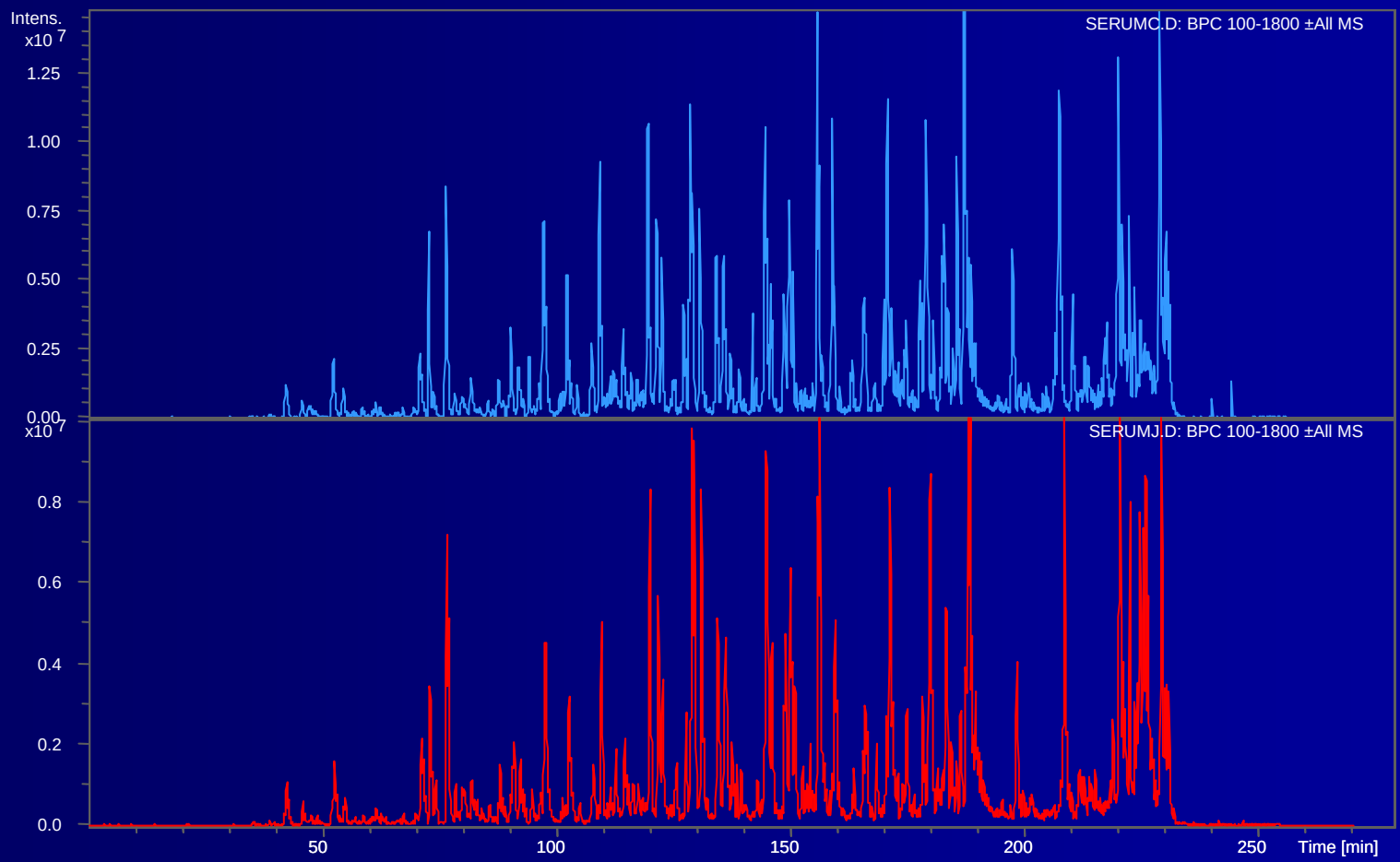
Chromatographic Stability



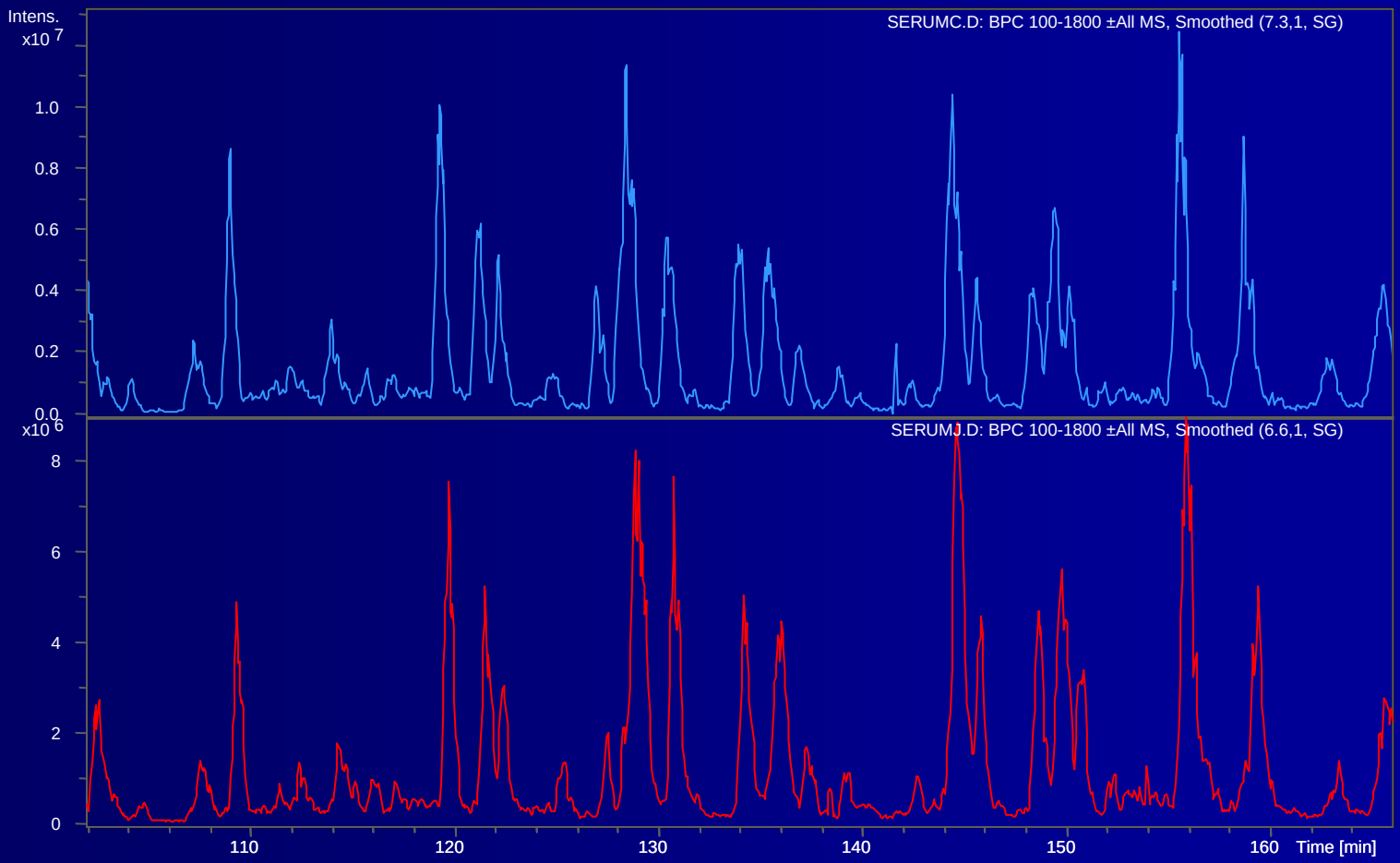
Eight Serum Digest Replicates

Chromatographic Stability

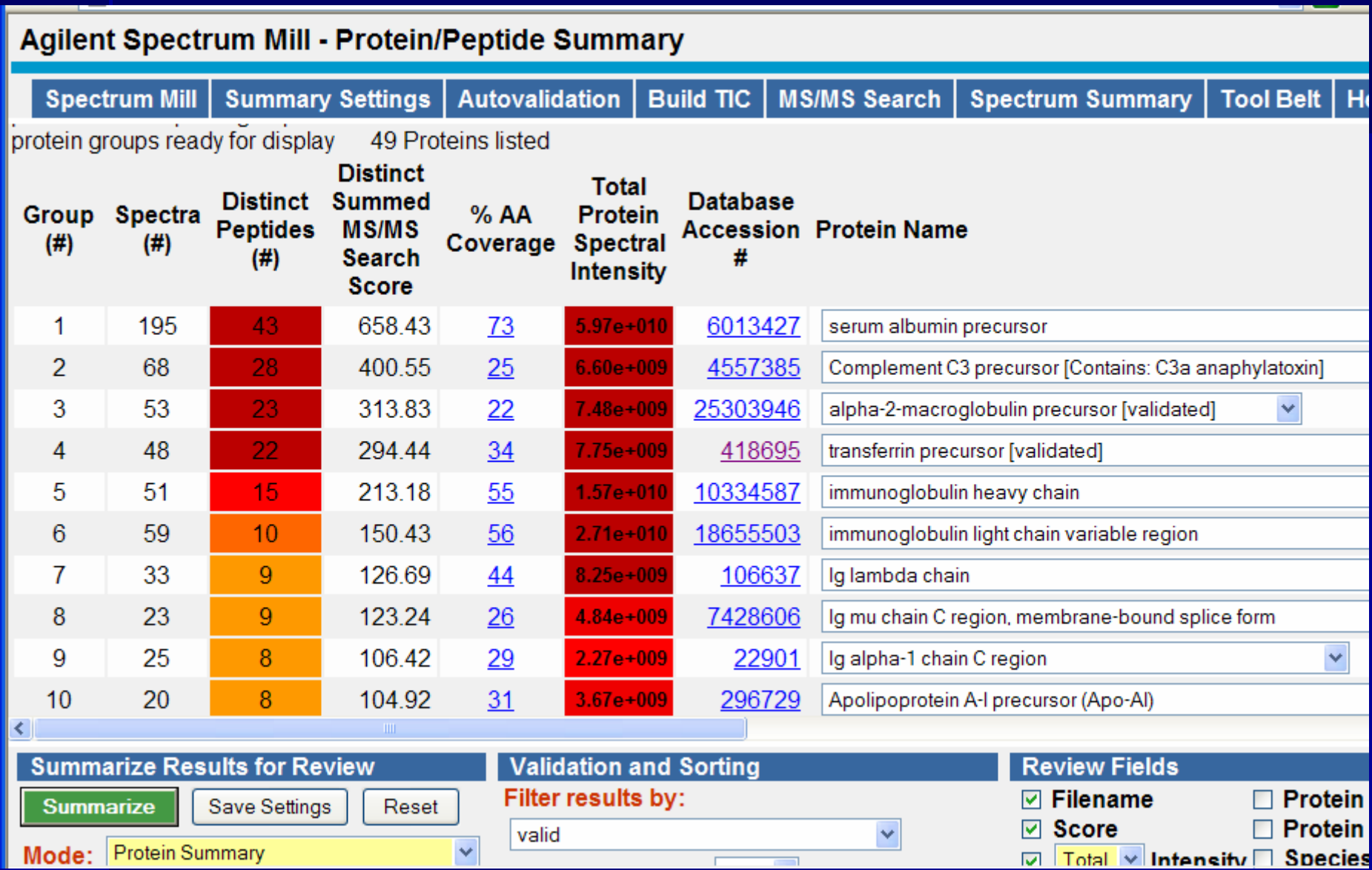
Same sample separated by 40hrs of continuous running



Chromatographic Stability



Database Searching Program, Spectrum Mill



Agilent Spectrum Mill - Protein/Peptide Summary

Spectrum Mill | Summary Settings | Autovalidation | Build TIC | MS/MS Search | Spectrum Summary | Tool Belt | H

protein groups ready for display 49 Proteins listed

Group (#)	Spectra (#)	Distinct Peptides (#)	Distinct Summed MS/MS Search Score	% AA Coverage	Total Protein Spectral Intensity	Database Accession #	Protein Name
1	195	43	658.43	73	5.97e+010	6013427	serum albumin precursor
2	68	28	400.55	25	6.60e+009	4557385	Complement C3 precursor [Contains: C3a anaphylatoxin]
3	53	23	313.83	22	7.48e+009	25303946	alpha-2-macroglobulin precursor [validated]
4	48	22	294.44	34	7.75e+009	418695	transferrin precursor [validated]
5	51	15	213.18	55	1.57e+010	10334587	immunoglobulin heavy chain
6	59	10	150.43	56	2.71e+010	18655503	immunoglobulin light chain variable region
7	33	9	126.69	44	8.25e+009	106637	Ig lambda chain
8	23	9	123.24	26	4.84e+009	7428606	Ig mu chain C region, membrane-bound splice form
9	25	8	106.42	29	2.27e+009	22901	Ig alpha-1 chain C region
10	20	8	104.92	31	3.67e+009	296729	Apolipoprotein A-I precursor (Apo-AI)

Summarize Results for Review

Summarize | Save Settings | Reset

Mode: Protein Summary

Validation and Sorting

Filter results by:

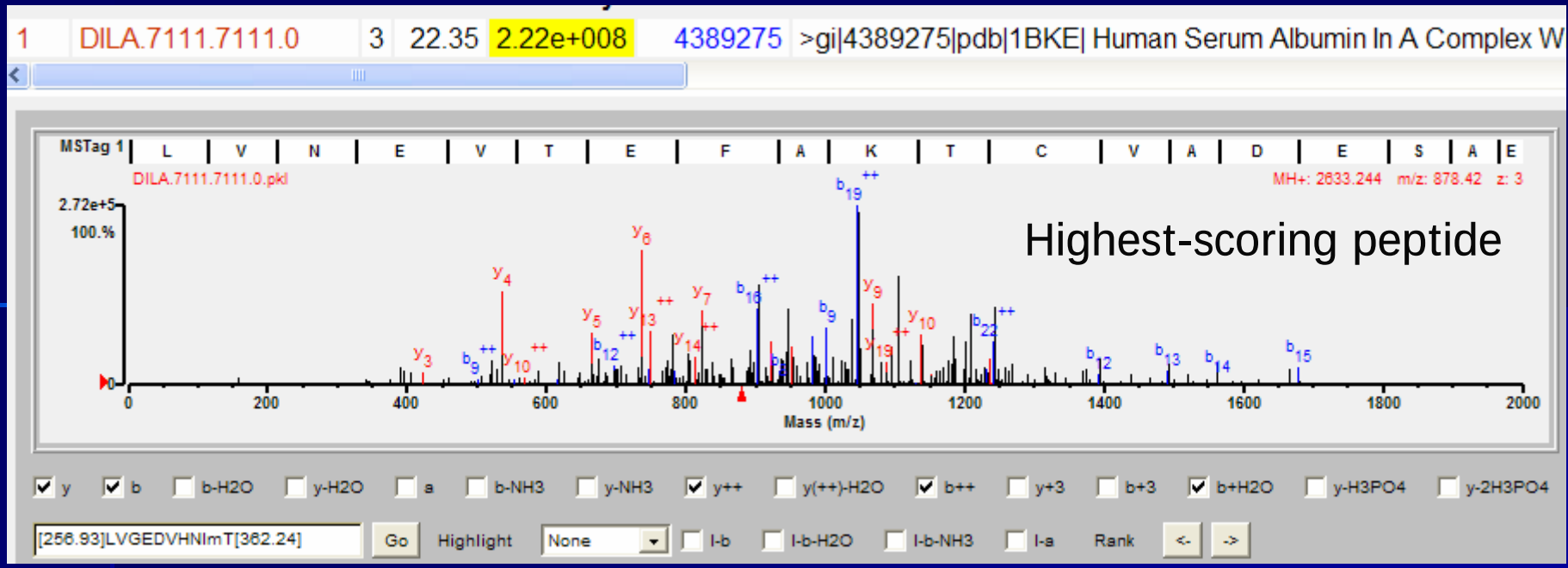
valid

Review Fields

☑ Filename ☐ Protein

☑ Score ☐ Protein

☑ Total Intensity ☐ Species

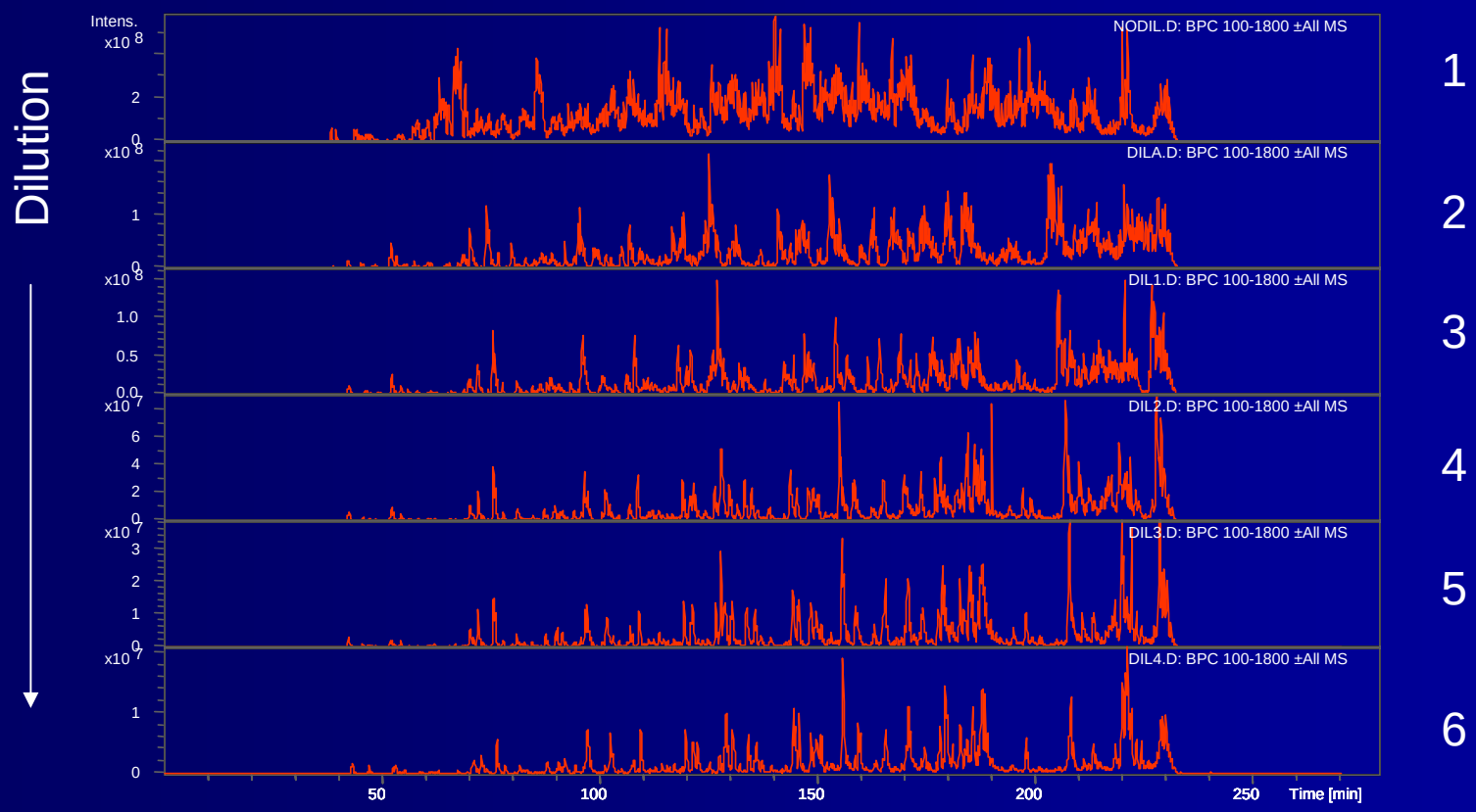

1 DILA.7111.7111.0 3 22.35 2.22e+008 4389275 >gi|4389275|pdb|1BKE| Human Serum Albumin In A Complex W
MSTag 1 L V N E V T E F A K T C V A D E S A E
DILA.7111.7111.0.pkl
MH+: 2633.244 m/z: 878.42 z: 3
2.72e+5
100.%
Highest-scoring peptide
Mass (m/z)
y b b-H2O y-H2O a b-NH3 y-NH3 y++ y(++)-H2O b++ y+3 b+3 b+H2O y-H3PO4 y-2H3PO4
[256.93]LVGEDVHNImT[362.24]
Go
Highlight
None
I-b I-b-H2O I-b-NH3 I-a
Rank
<- ->
811 DIL1.10707.10724.0 3 10.01 2.67e+007 4557485 Ceruloplasmin precursor (Ferroxidase)
MSTag 1 T T I E K P V W L G F L G P I I K
DIL1.10707.10724.0.pkl
MH+: 1912.254 m/z: 638.09 z: 3
2.34e+5
100.%
Lowest-scoring peptide
Mass (m/z)
y b b-H2O y-H2O a b-NH3 y-NH3 y++ y(++)-H2O b++ y+3 b+3 b+H2O y-H3PO4 y-2H3PO4
[256.93]LVGEDVHNImT[362.24]
Go
Highlight
None
I-b I-b-H2O I-b-NH3 I-a
Rank
<- ->
0 200 400 600 800 1000 1200 1400 1600 1800 2000

Serum Proteins

- Serum Albumin 35-45 mg ml
- Ceruloplasmin 0.3 mg/ml

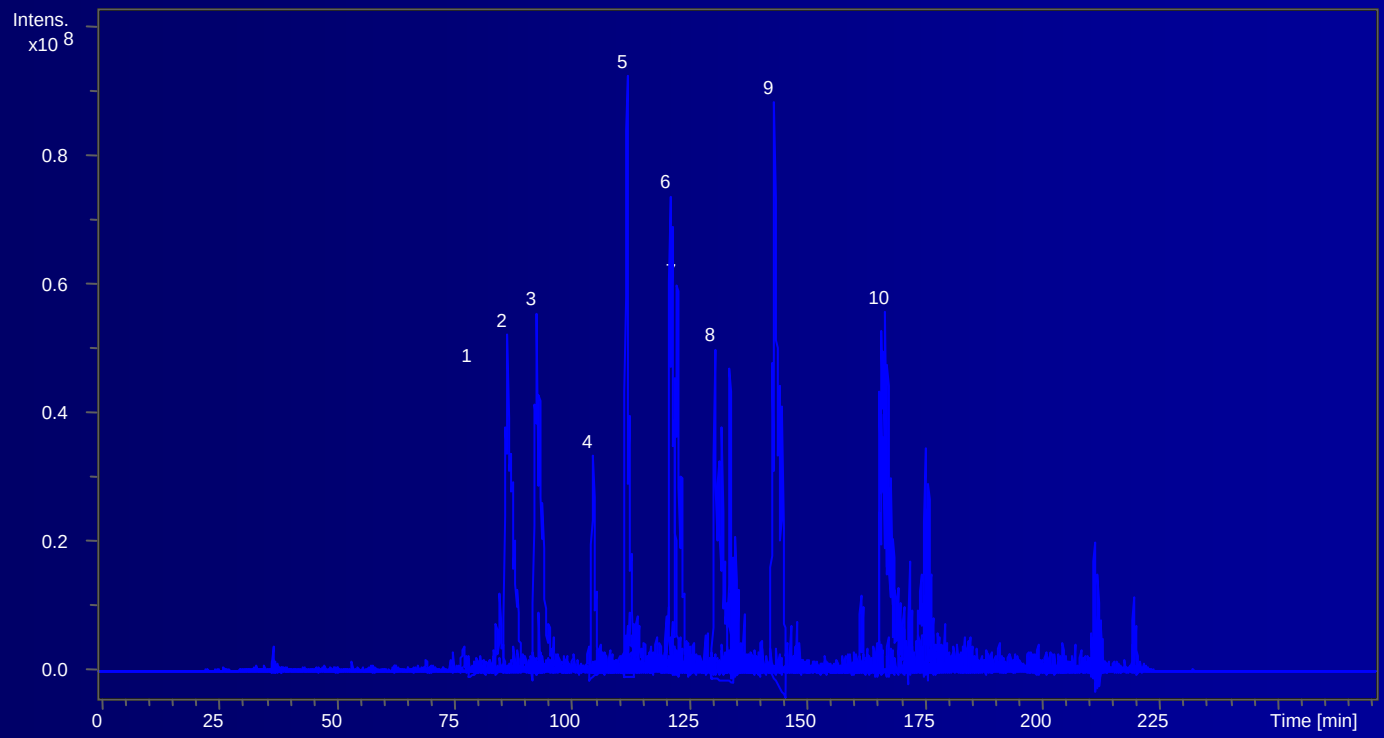
Column Load Range Finding Experiment

Range Finding

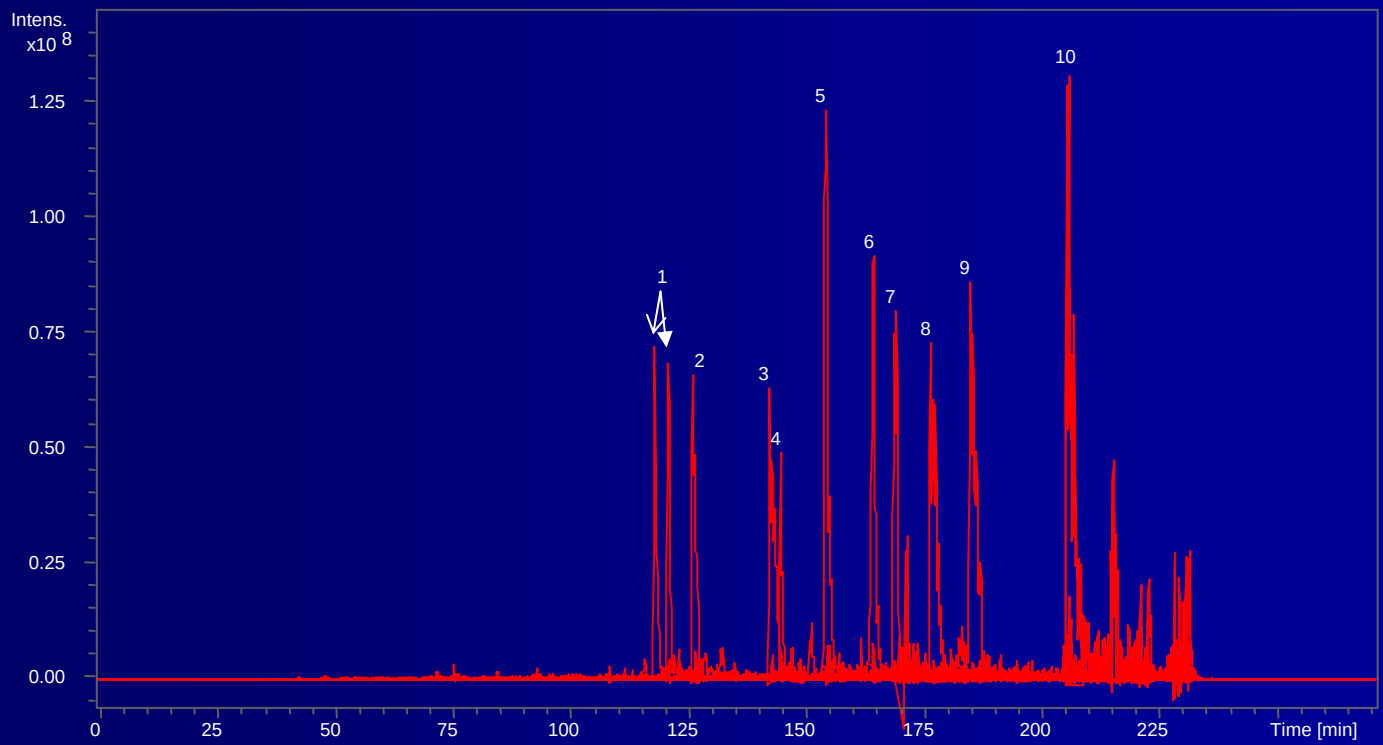


Vydac, 300A, 5um C18 0.075ID X 600 mm length

100 X 0.075 mm column

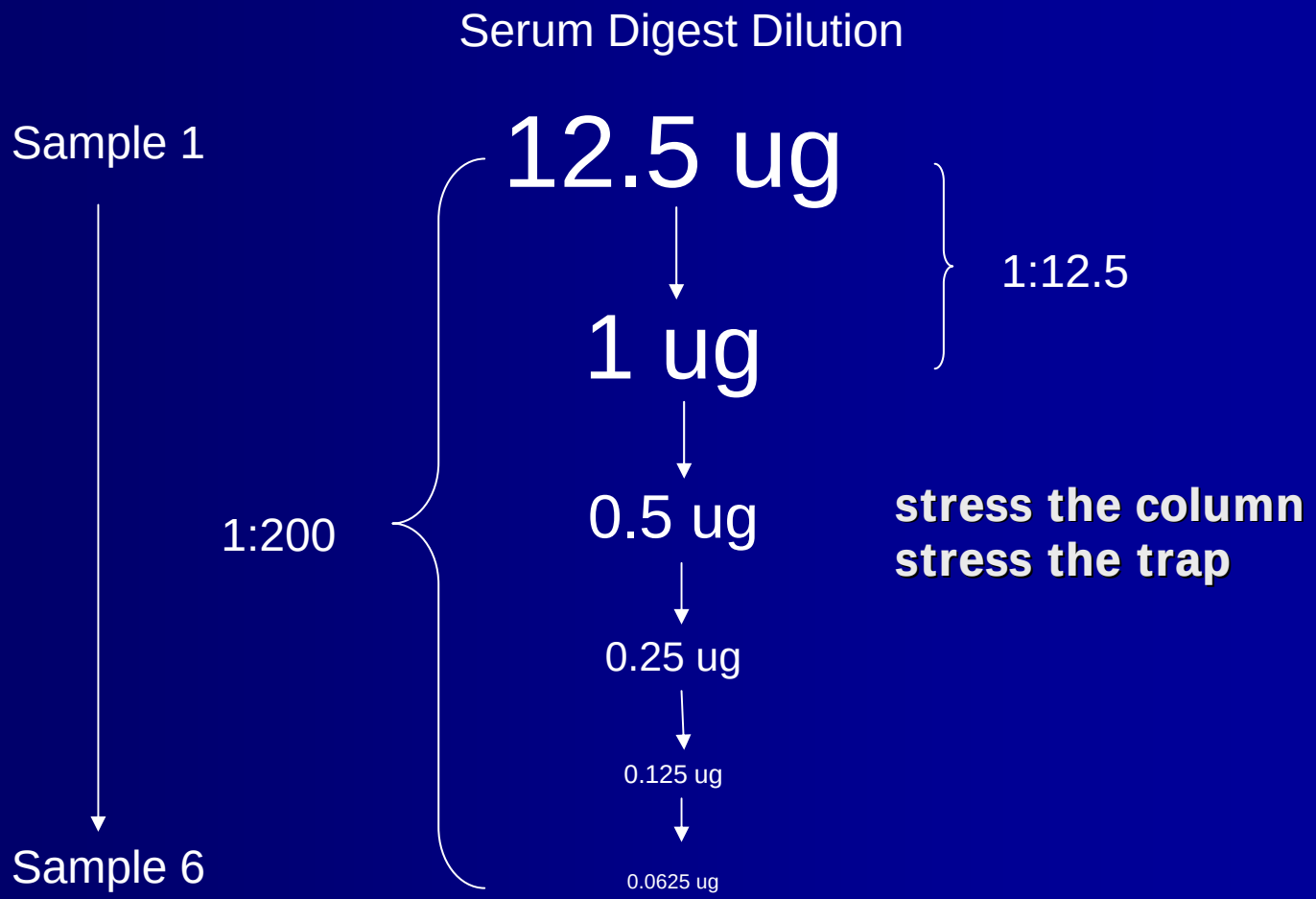


600 X 0.075 mm column

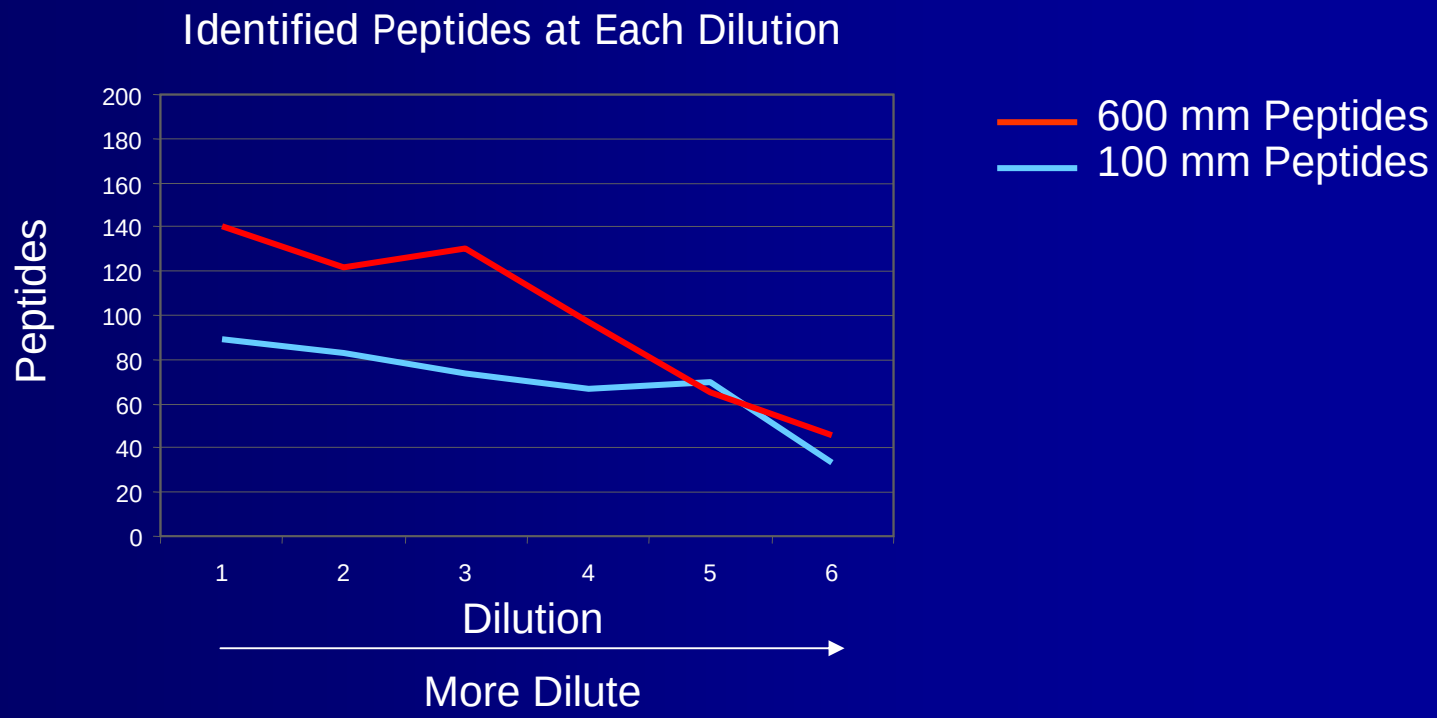


Column comparison in the context of

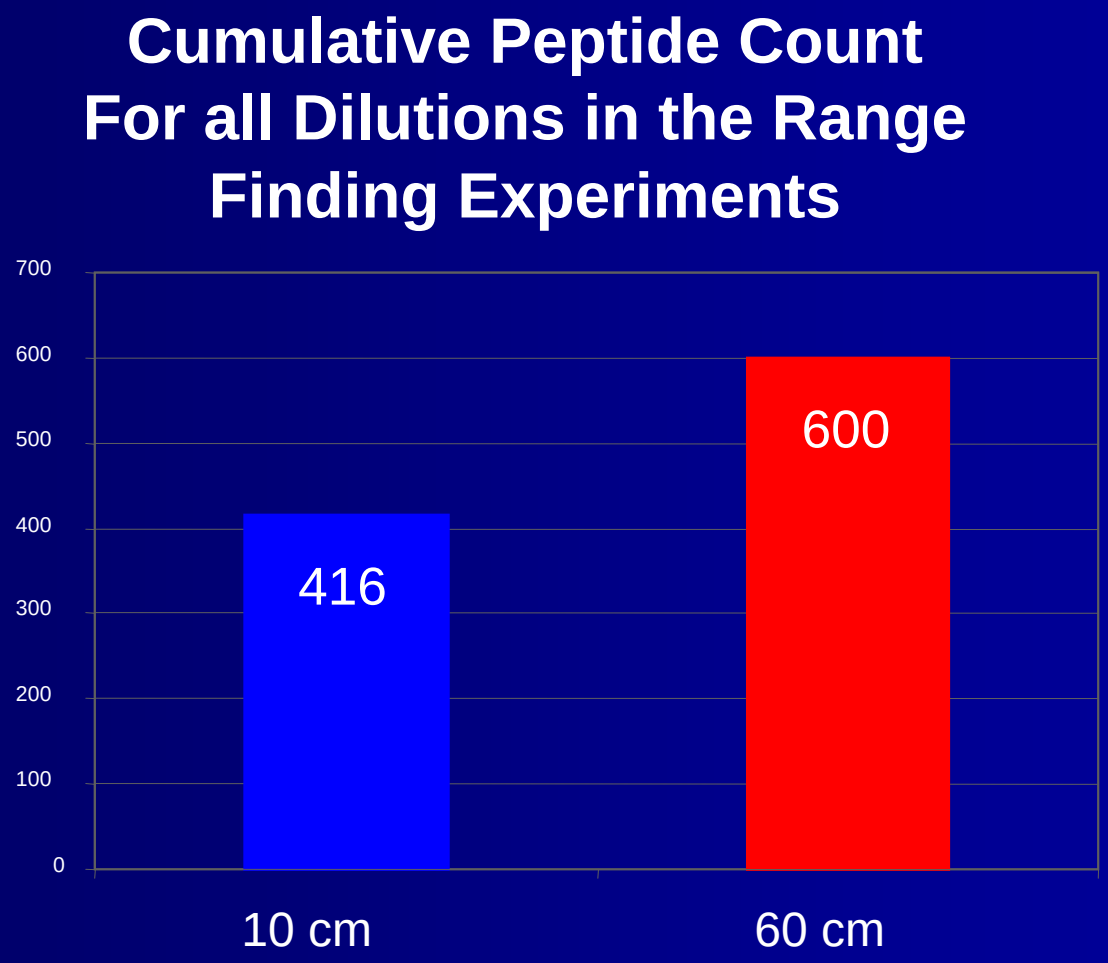
Range Finding



Identified Peptides at Each Dilution in the 100 and 600mm Range Finding Experiments



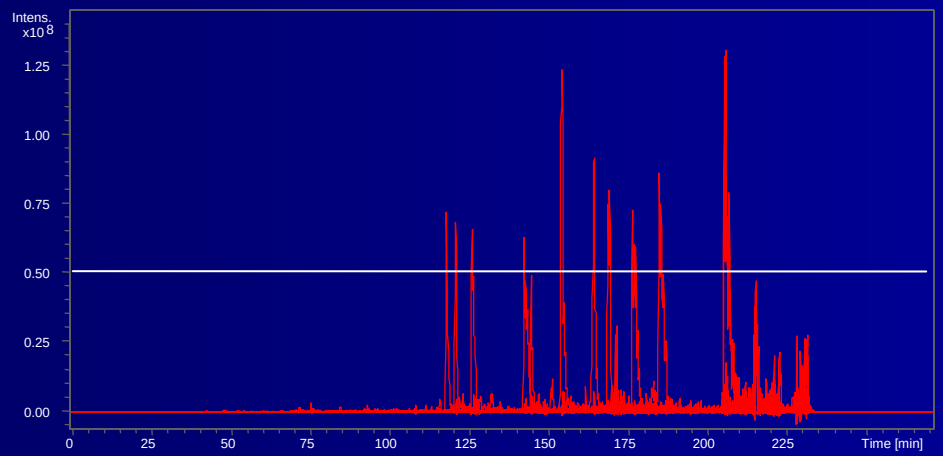
Pick The Right Column Length



10 extracted peaks

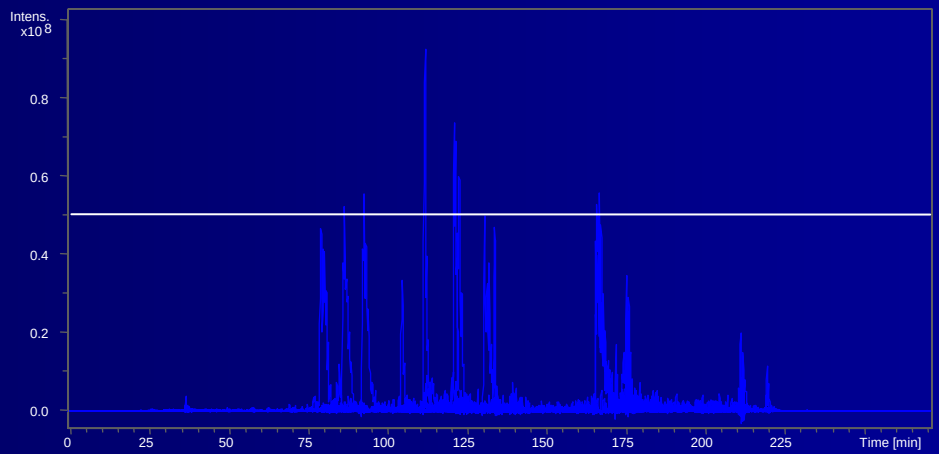
Why is the long column winning?

600 mm



5E7 counts

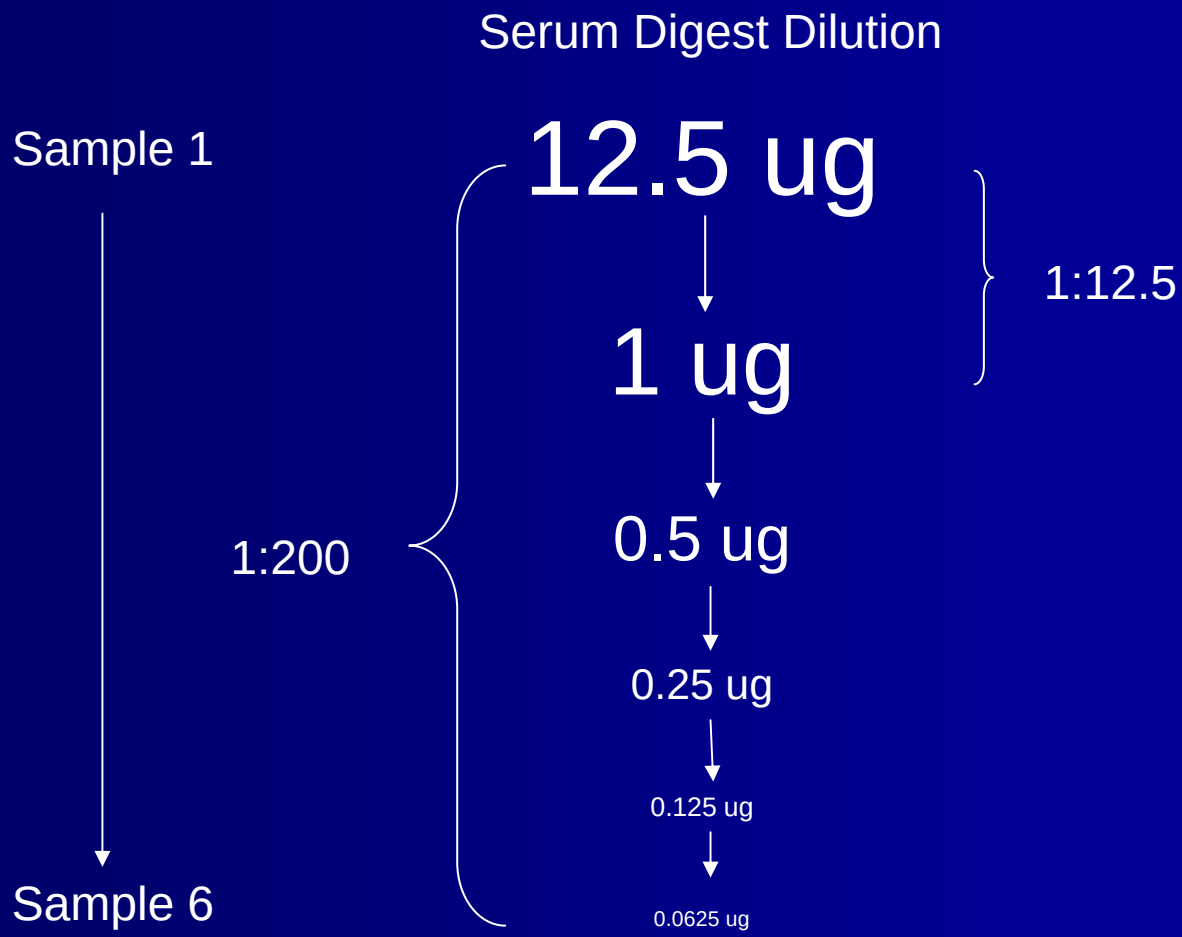
100 mm



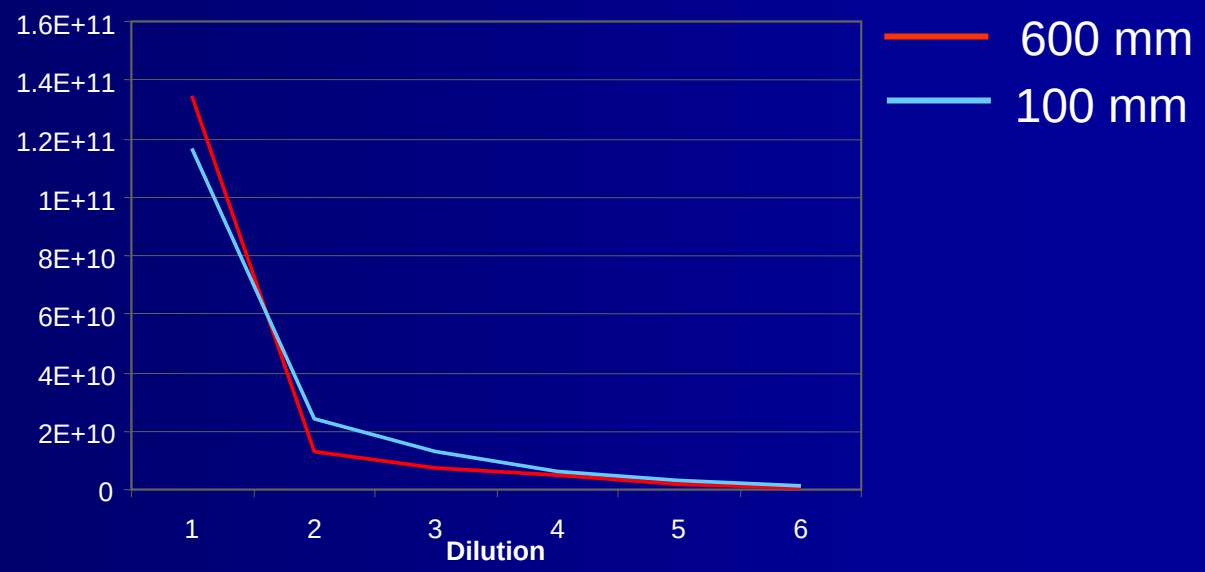
5E7

Range Finding

Why is short column failing, is it capacity?

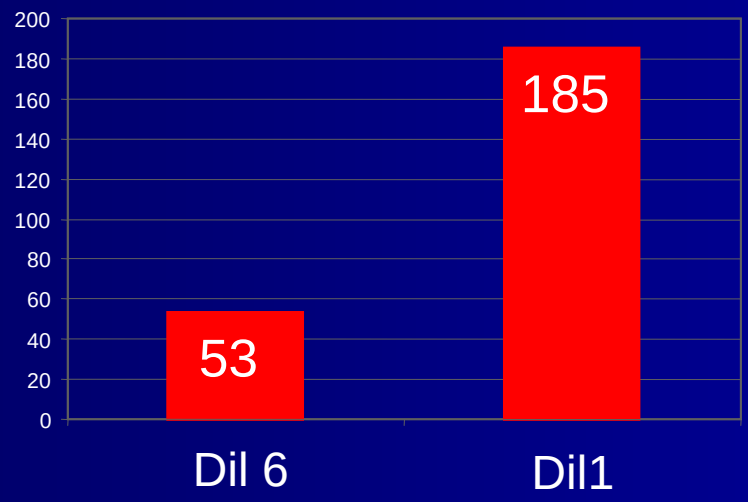


Cumulative Extracted Area Counts



Pick The Right Load

Peptide Count in Range Finding Experiment



Conclusion

- Long columns are better for peptide mapping and more complex mixtures
- Method development is essential
- Data analysis is the bottle neck in the study of complex mixtures
- Do better

Acknowledgements

- The Vincent & Stella Coates Foundation
- Agilent
- ThermoFinnigan