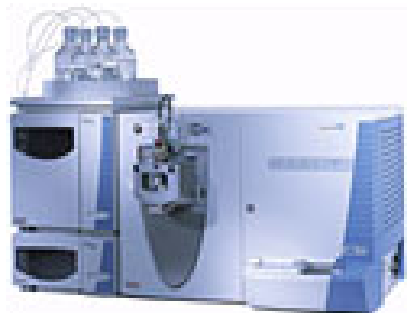


**ThermoFisher**  
S C I E N T I F I C

*The world leader in serving science*

**Targeted Analysis of Proteins in  
Human Serum:  
High-Throughput h-SRM Analysis with  
the TSQ Quantum Ultra**

# Protein Analysis and Biomarker Discovery



LTQ XL Linear Ion Trap

ETD



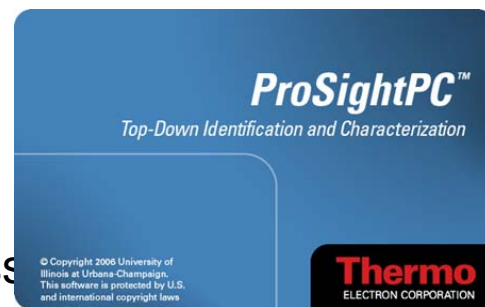
LTQ Orbitrap

- High Res, Accurate Mass
- Ultimate Peptide Machine



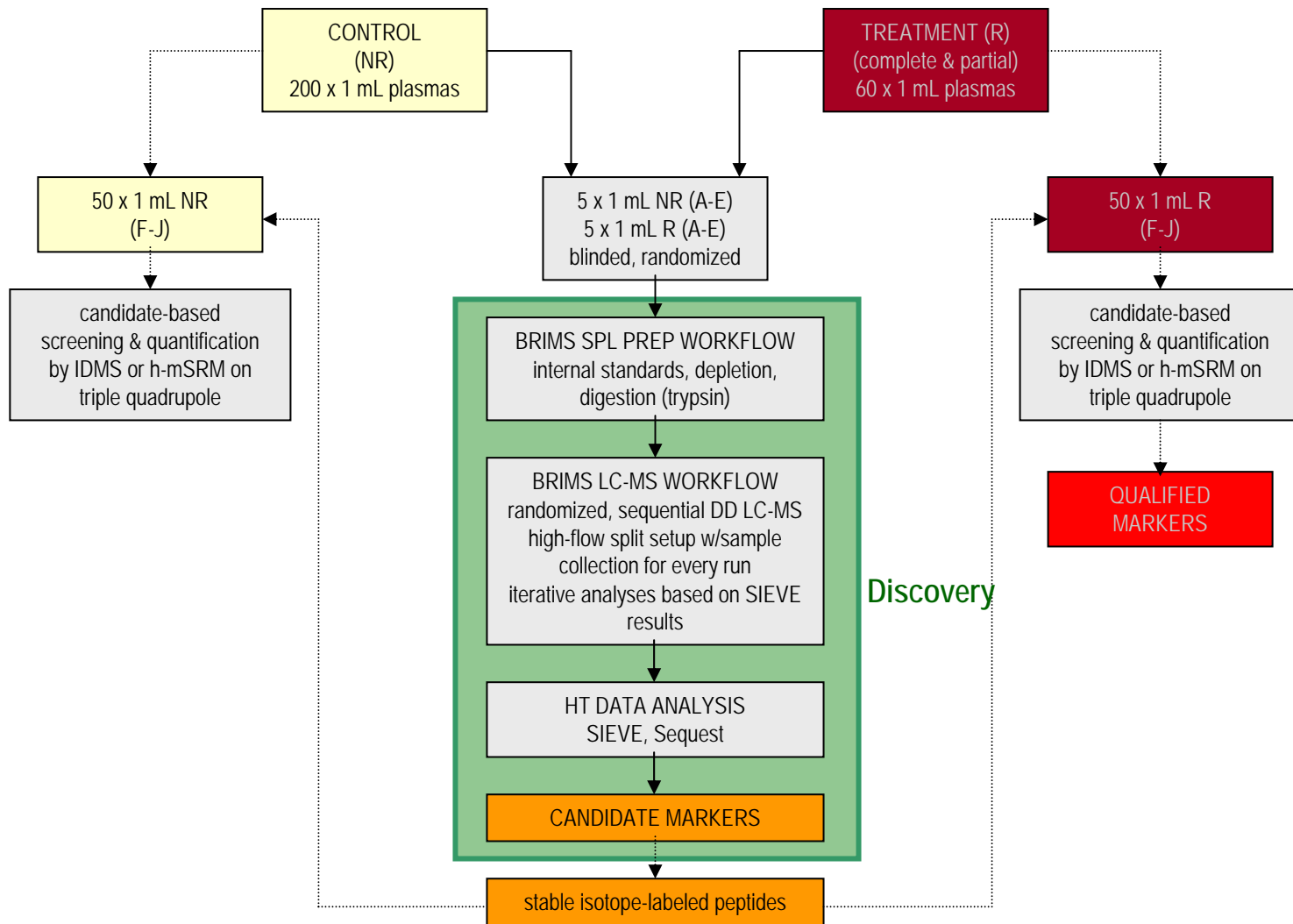
LTQ FT Ultra

- Ultra high res., Accurate Mass
- Top Down, Biomarkers, PTMs



and PEAKS *de novo*

# General Biomarker Discovery & Qualification Strategy: a Split-sample Design and h-SRM Targeted Analysis



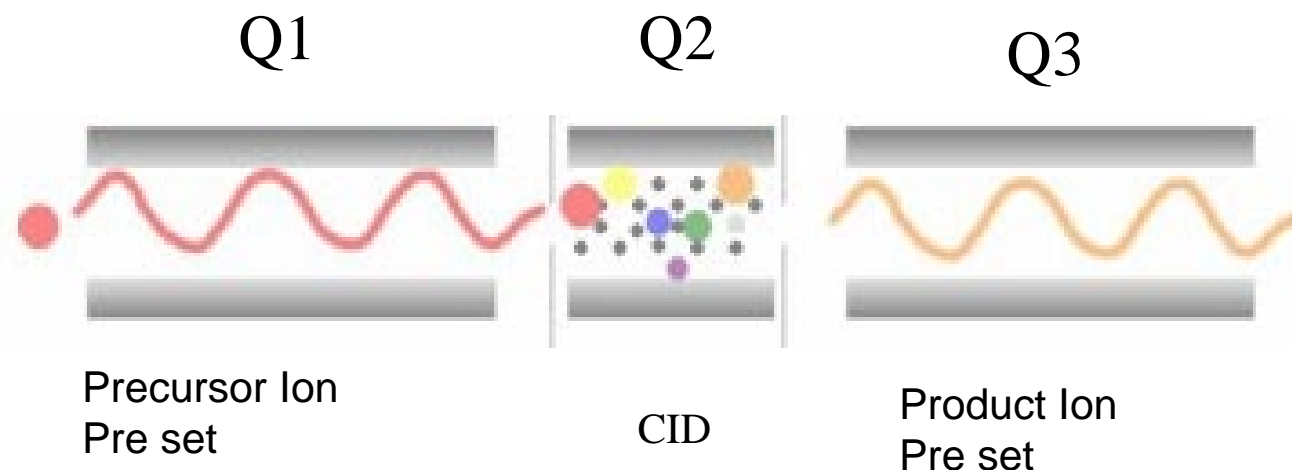
# Marker Verification: Targeted Protein Quantitation



TSQ Quantum Ultra

- Sensitive, Robust, Peptide Assays
- Enhanced Selectivity
  - h-SRM
  - FAIMS
- Hundreds of SRM transitions / second
  - Very short dwell times (2 ms)
  - 1 microscan/transition
- MRM-triggered MS/MS for peptide structure confirmation
- Fast chromatography with NEW Accela UHPLC

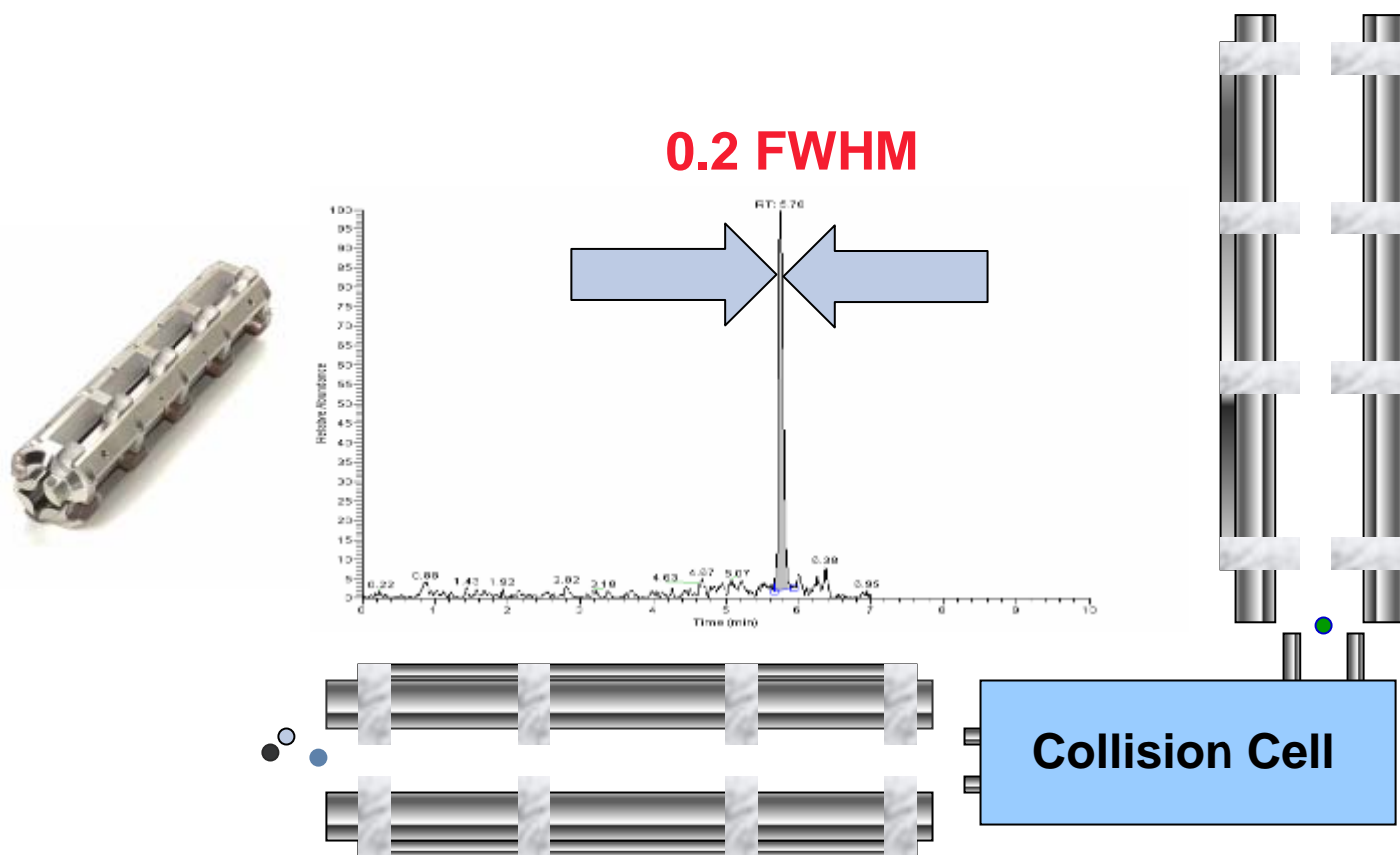
# Schematic of Reaction Monitoring: High Selectivity and Sensitivity



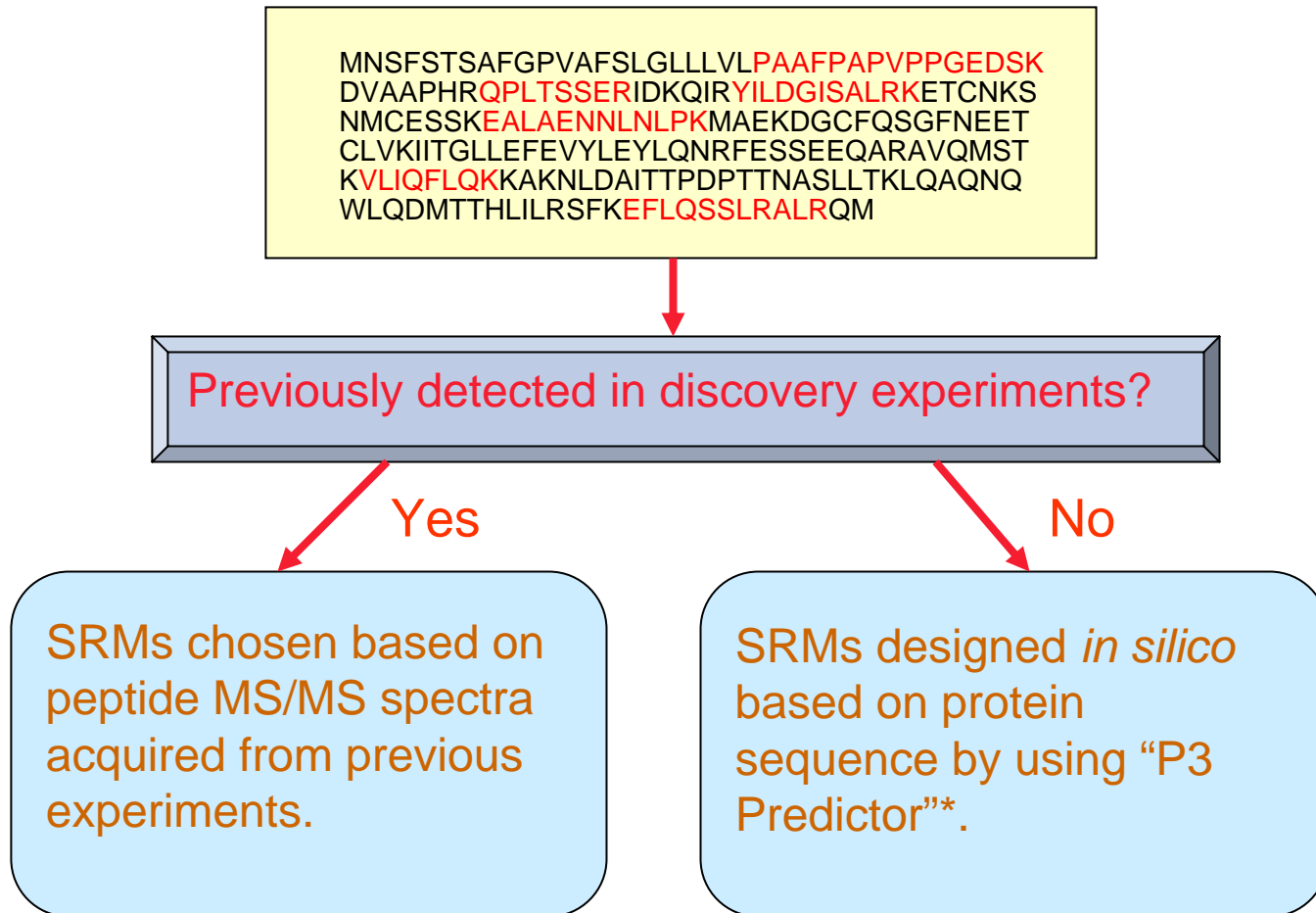
A “proteotypic” peptide is selected as being representative of a targeted protein. Q1 is set to transmit only the parent  $m/z$  of the selected peptide and Q3 is set to transmit only a selected fragment ion. Quantum has a very fast SRM dwell time (2 ms), so that multiple SRM (MRM) analyses can be carried out during each scan of an LC/MS/MS run. It is practical to monitor up to 300 SRM transitions/sec.

# Hyperbolic INVAR Alloy Rod Design: Unmatched Resolution to Enhance S/N

- ✓ *Increased bioanalytical method robustness*
- ✓ *Eliminate matrix interferences*



# From Protein...to SRM



\*Use of P3 courtesy of Mike MacCoss,  
University of Washington

# Peptide Selection and SRM design

- Protein detected in previous LC/MS/MS experiments:
  - peptide was seen many times
  - unique for targeted protein
  - contain no Cys, Met or other commonly modified aa residues
  - 600 – 2000 MW
  - SRM transition chosen based on MS/MS spectrum
- If no HPLC/MS/MS data, use P3 Predictor SRM prediction tool
  - provide amino acid sequences of targeted proteins
  - *in silico* trypsin digestion
    - Tryptic peptides which contain no Cys, Met, His, NxS(T) modification, or R-P or K-P
    - user defined peptide length
  - User selects one or multiple candidate peptides from the list and P3 Predictor provides Q1 and Q3 SRM transitions automatically with proper collision energies
  - Output .csv file which Quantum can accept directly
- Generally, multiple fragment ions for each selected peptide will be used to maximize specificity (reduce possibility of interferences).

## Quantitative Mass Spectrometric Multiple Reaction Monitoring Assays for Major Plasma Proteins\*

Leigh Anderson<sup>‡§</sup> and Christie L. Hunter<sup>¶</sup>

Quantitative LC-MS/MS assays were designed for tryptic peptides representing 53 high and medium abundance proteins in human plasma using a multiplexed multiple reaction monitoring (MRM) approach. Of these, 47 produced acceptable quantitative data, demonstrating within-run coefficients of variation (CVs) ( $n = 10$ ) of 2–22% (78% of assays had  $CV < 10\%$ ). A number of peptides gave CVs in the range 2–7% in five experiments (10 replicate runs each) continuously measuring 137 MRMs, demonstrating the precision achievable in complex digests. Depletion of six high abundance proteins by immunosubtraction significantly improved CVs compared with whole plasma, but analytes could be detected in both sample types. Replicate digest and depletion/digest runs yielded correlation coefficients ( $R^2$ ) of 0.995 and 0.989, respectively. Absolute analyte specificity for each peptide was demonstrated using MRM-triggered MS/MS scans. Reliable detection of L-selectin (measured at  $0.67 \mu\text{g/ml}$ ) indicates that proteins down to the  $\mu\text{g/ml}$  level can be quantitated in plasma with minimal sample preparation, yielding a dynamic range of 4.5 orders of magnitude in a single experiment. Peptide MRM measurements in plasma digests thus provide a rapid and specific assay platform for biomarker validation, one that can be extended to lower abundance proteins by enrichment of specific target peptides (stable isotope standards and capture by anti-peptide antibodies (SISCAPA)). *Molecular & Cellular Proteomics* 5:573–588, 2006.

53 targeted proteins

MRM assay

137 SRM transitions

Add IL-6 to determine  
Sensitivity  
Linear dynamic range

Assay performance

Peptide confirmation by MS/MS

# Sample Preparation

- Whole Human Serum (Sigma).
- Diluted 40X with 6M Guanidine
- IL-6 exogenous protein standard curve
- 1 mL of diluted human serum sample was reduced with 1M dithiothreitol and S-carboxymethylated with 1M iodoacetic acid.
- Reduced and S-carboxymethylated human serum sample was exchanged into 100mM ammonium bicarbonate buffer and digested with trypsin.
- The digest mixture was dried by a Speed-Vac and reconstituted with 200 $\mu$ L water containing 0.1% TFA.

# Peptide Selection and SRM transition design

- 53 high and medium abundance proteins (same as Anderson) were analyzed.
- One representative tryptic peptide for each protein was selected based on several criteria defined by Anderson

*50 peptides - multiple fragment ions were chosen*

*3 peptides - single fragment ion*

- Total of 103 SRM transitions

# P3 Predictor Software

### Paste Protein Sequence

```
MNSFSTSAFGPVAFSLGILLVLPAAFPAPVPPGEDSKDVAAPHROPLTSSERIDKQ
IRYILDGISALRKETCNKSNMCESSKEALAENNLNLPKMAEKDGCFOSGFNEETCL
VKIITGLLEFEVYLEYLQNRFEESSEEQARAVQMSTKVLIQFLQKKAKNLDAITTPD
PTTNASLLTKIAQNQWLQDMTTHLILRSFKFLQSSLRALRQM
```

### Peptide Features

Min Peptide Length:

Max Peptide Length:

Use Monoisotopic Precursor Ions

Use Monoisotopic Product Ions

**Exclude Peptides Containing:**

Cys

Met

His

N-X/S/T motif

R-P or K-P

### Protein Features

Exclude Potential Ragged Ends

Eliminate first  AAs

Don't Digest

Check Library:

**Digest Protein**

**Reset**

### Peptide Sequences: 7

```
FPAPVPPGEDSK
QPLTSSER
YILDGISALR
EALAENNLNLPK
FESSEEQAR
VLIQFLQK
EFLQSSLR
```

### Precursor Info

Peptide: EFLQSSLR

Monoisotopic Mass  
M = 978.513  
M+H = 979.521  
(M+2H)/2 = 490.265  
(M+3H)/3 = 327.179

Average Mass  
M = 979.101  
M+H = 980.109  
(M+2H)/2 = 490.559  
(M+3H)/3 = 327.375

Collision Energy (+1) = 36.6  
Collision Energy (+2) = 20  
Collision Energy (+3) = 14.4

Hydrophobicity Retention Factor = 25.4

### Product Ion Info

Seq #	B-Ion	#	Y-ion
E 1:	130.05	8:	979.52
F 2:	277.12	7:	850.48
L 3:	390.2	6:	703.41
Q 4:	518.26	5:	590.33
S 5:	605.29	4:	462.27
S 6:	692.33	3:	375.24
L 7:	805.41	2:	288.2
R 8:	961.51	1:	175.12

### Predicted SRM Transitions

```
490.26, 590.33, 20
490.26, 703.41, 20
```

```
459.24, 579.27, 18.9
459.24, 789.41, 18.9
490.26, 590.33, 20
490.26, 703.41, 20
494.81, 535.32, 20.1
494.81, 663.38, 20.1
541.74, 632.3, 21.7
541.74, 719.33, 21.7
560.82, 616.38, 22.4
560.82, 731.41, 22.4
620.81, 729.34, 24.4
620.81, 925.46, 24.4
663.36, 698.42, 25.9
663.36, 812.46, 25.9
```

**Add**

**Remove**

**Output CSV**

# HPLC

- PicoFrit C18 column from New Objective (75 $\mu$ m x 100mm)
- Surveyor MS pump; MicroAS autosampler
  - Buffer A: 0.1%FA/2%ACN/98%H<sub>2</sub>O
  - Buffer B: 0.1%FA/100%ACN
  - Post-split flow rate was 300nL/min.
  - Gradient 2% B to 50% B in 85 min.
- The sample loading flow rate was 5 $\mu$ L/min and loading time was 15 min.

TSQ Quantum Ultra with IonMax source equipped with a column adapter for nanoflow (New Objective).

For SRM:

SRM 1: Q1, 0.7 FWHM; Q3, 0.7 FWHM

SRM 2: Q1, 0.4 FWHM; Q3, 0.7 FWHM

SRM 3 (h-SRM): Q1, 0.2 FWHM; Q3, 0.7 FWHM

Q2, 1.5 mTorr (Ar)

Scan width, 0.002 m/z

Scan time, 20 ms and 2 ms

**Compare 3 Q1  
Resolution Settings**

**Compare 2  
Q2 Scan Times**

SRM-triggered MS/MS:

Scan Event 1:

Q1 & Q3: 0.7 FWHM; Q2: 1.5 mTorr; Scan width, 0.002m/z; Scan time, 20ms

Scan Event 2:

DD precursor mass from Scan Event 1; Q1, 0.7 FWHM; signal threshold

30,000 counts; Q2: 1.5 mTorr, CE: 0.034 x precursor mass m/z + 3.134;

Dynamic exclusion settings: repeat, 1; duration, 30s; exclusion time, 30s;

exclusion list size, 50.

# Fast SRM Scan Time (2 ms available)

Scan Editor | Syringe Pump | Divert Valve | Accurate Mass | Method Summary

Calibration Correction Method

Run Settings  
MS Acquire Time (min): 55.00      Segments: 1      Current Segment: 1

To display a chromatogram here, use Quantum/Open Raw File...

Segment 1 Settings  
Segment Time (min): 55.00      Tune Method: C:\McHale\Peptide Analysis SMP\angiotensin\_60uLmin.TSQTune  
Scan Events: 1      Chrom Filter Peak Width (s):  10      Collision Gas Pressure (mTorr):  1.5  
Current Scan Event: 1

Scan Event 1

Full Scan | SIM | SRM

Same value for all SRMs  
Scan Width (m/z):  0.002  
**Scan Time (s):  0.020**  
Coll. Energy (V):  10  
Peak Width  
Q1 (FWHM):  0.70  
Q3 (FWHM):  0.70  
Use Tuned Tube Lens Value:

	Parent Mass	Product Mass	Collision Energy
1	407.750	672.400	18
2	408.260	586.500	18
3	437.270	689.400	20
4	448.730	662.300	20
5	495.260	674.300	22
6	495.260	773.300	22
7	507.300	586.300	23
8	598.850	754.400	27
9	602.790	675.100	27
10	612.790	706.400	28
11	612.800	547.500	28
12	626.340	752.400	28
13	644.350	745.500	29

Polarity:  Positive     Negative  
Data Type:  Centroid     Profile  
Skimmer Offset:  
Skimmer Offset (V):  7  
Accurate Mass Mode: Off  
Micro Scans: 1  
Copy ScanEvent    Paste ScanEvent  
Help    Tune

# Unique High Resolution For Q1 & Q3

The screenshot displays the 'Instrument Setup' window for a method named 'humanserum\_Q1\_Q2.meth'. The interface includes a sidebar with instrument icons (TSQ Quantum, Surveyor MS Pump Plus, Micro AS) and a main configuration area. The 'Scan Editor' tab is active, showing 'Run Settings' with 'MS Acquire Time (min): 130.00' and 'Segments: 1'. A retention time axis at the bottom shows 'Segment 1' from 0 to 130 minutes. Below this, 'Segment 1 Settings' are shown, including 'Segment Time (min): 130.00', 'Tune Method: C:\xcalibur\data\Ecol\method\09\_14\_06\_NSI.TSQTune', 'Scan Events: 1', 'Chrom Filter Peak Width (s): 8', and 'Collision Gas Pressure (mTorr): 1.5'. The 'Scan Event 1' section is expanded to show 'Full Scan' settings. A table lists SRM parameters:

	Parent Mass	Product Mass	Collision
1	362.900	645.400	16
2	362.900	744.400	16
3	405.900	664.400	17
4	405.900	777.500	17
5	409.500	533.300	17
6	409.500	670.400	17
7	437.200	704.300	18
8	437.200	803.400	18
9	438.300	502.300	18
10	438.300	615.400	18
11	442.300	685.400	18
12	442.300	784.500	18
13	444.300	729.400	18

In the 'Peak Width' section, 'Q1 (FWHM)' is set to 0.20 and 'Q3 (FWHM)' is set to 0.25. A red circle highlights these two settings, with a red arrow pointing to a text box below that reads 'You get high resolution with one click'. Other settings include 'Scan Width (m/z): 0.002', 'Scan Time (s): 0.020', 'Collision Energy (V): 1', 'Polarity: Positive', 'Data Type: Centroid', 'Skimmer Offset (V): 8', and 'Accurate Mass Mode: Off'. The 'Micro Scans' are set to 1. The status bar at the bottom left shows 'Ready'.

# “MRM-Triggered” Data Dependent MS/MS

Segment 1

Retention Time (min)

Segment 1 Settings

Segment Time (min): 22.00    Tune Method: C:\Peteman\090606\bradykinin.TSQ.Tune

Scan Events: 2    Chrom Filter Peak Width (s): 5    Collision Gas Pressure (mTorr): 1.2

Current Scan Event: 1

Scan Event 1

Scan Event 2

Scan Event 1

Full Scan | SIM | SRM

Same value for all SRMs

Scan Width (m/z): 0.002

Scan Time (s): 0.010

Coll. Energy (V): 10

Peak Width

Q1 (FWHM): 0.70

Q3 (FWHM): 0.70

Use Tuned Tube Lens Value:

	Parent Mass	Product Mass	Collision
1	280.060	347.190	13
2	280.060	418.300	13
3	286.660	343.210	16
4	286.660	414.250	16
5	330.190	361.210	15
6	330.190	474.290	15
7	337.660	349.170	15
8	337.660	463.220	15
9	337.660	560.270	15
10	367.220	423.260	16
11	367.220	520.310	16
12	378.740	401.290	16
13	378.740	529.350	16

Polarity:  Positive     Negative

Data Type:  Centroid     Profile

Skimmer Offset: Skimmer Offset (V): 7

Accurate Mass Mode: Off

Micro Scans: 1

Copy ScanEvent    Paste ScanEvent

Help    Tune

# “MRM-Triggered” Data Dependent MS/MS

Segment 1

Retention Time (min)

Segment 1 Settings

Segment Time (min): 22.00

Tune Method: C:\Peteman\090606\bradykinin.TSQTune

Scan Events: 2

Chrom Filter Peak Width (s): 5

Collision Gas Pressure (mTorr): 1.2

Current Scan Event: 2

Scan Event 1

Scan Event 2

Scan Event 2

Full Scan | SIM | SRM | Dependent Scan

**Data Dependent Settings**

Scan Selection

Mass determined from scan event: [dropdown]

Nth Most Intense Ion: 1

Signal Threshold (10<sup>4</sup> counts): 0.1

From Scan  From Parent List

If no acceptable parent found convert to most intense from scan

Weighting Factor: 0.0

Scan Parameters

Scan Time (s): 1.000

Charge State: 2

Source Delta(m/z): 1.000

Collision Energy (V): 3

CE grad((V per m/z): 0.0340

DD Delta(m/z): 1.000

Q1 Peak Width (FWHM): 0.70

Q3 Peak Width (FWHM): 0.70

Energy Ramp (V): 0

Advanced Data Dependent Settings And Activation

Dynamic Exclusion  Isotope Ratio  Specify mass lists in sequence row (Global Setting)

Advanced Settings...

Polarity:  Positive  Negative

Data Type:  Centroid  Profile

Skimmer Offset: Skimmer Offset (V): 7

Accurate Mass Mode: Off

Micro Scans: 1

Copy ScanEvent Paste ScanEvent

Help Tune

**Normalized Collision Energy Settings**

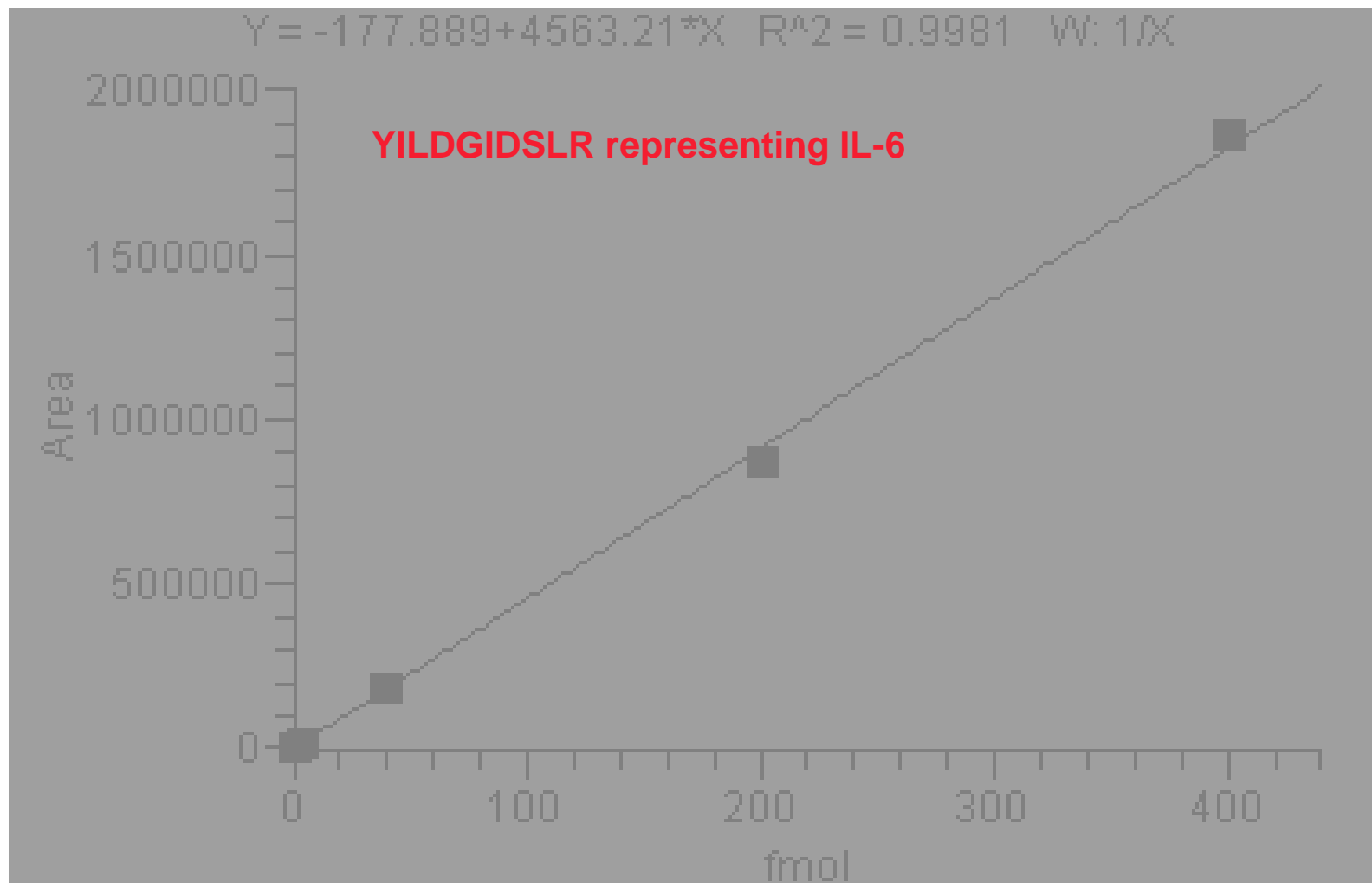
# Excellent sensitivity: 20 amol Detection Limit for IL-6

RT: 29.99 - 36.11 SM: 7G



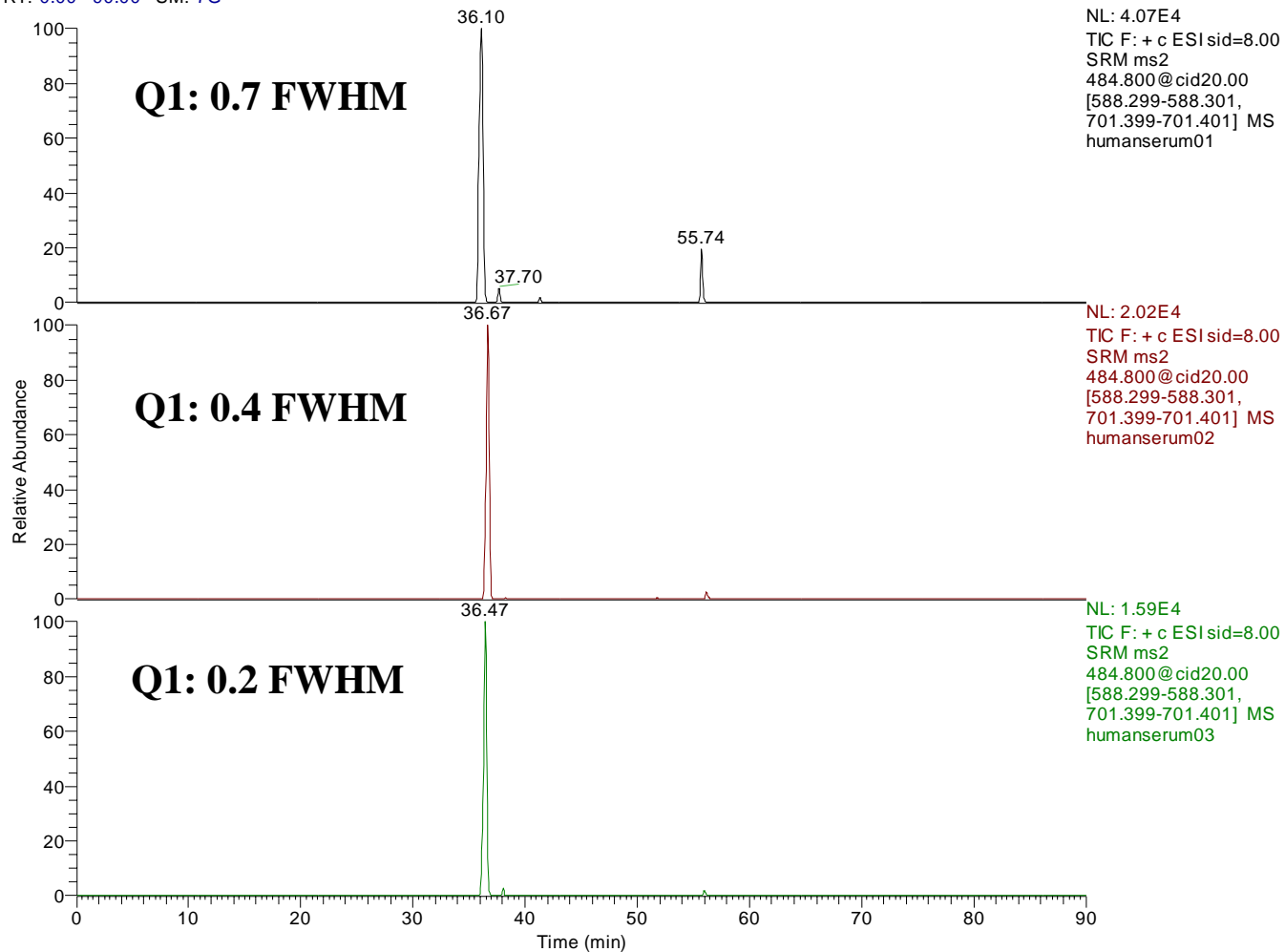
NL: 1.15E1  
m/z= 844.39-844.59 F: + c ESI  
sid=8.00 SRM ms2  
560.820@cid22.00  
[616.379-616.381, 731.409-731.411,  
844.489-844.491] MS Genesis  
IL\_6\_20amol

# Wide Linear Dynamic Range: Over 4 Orders of Magnitude



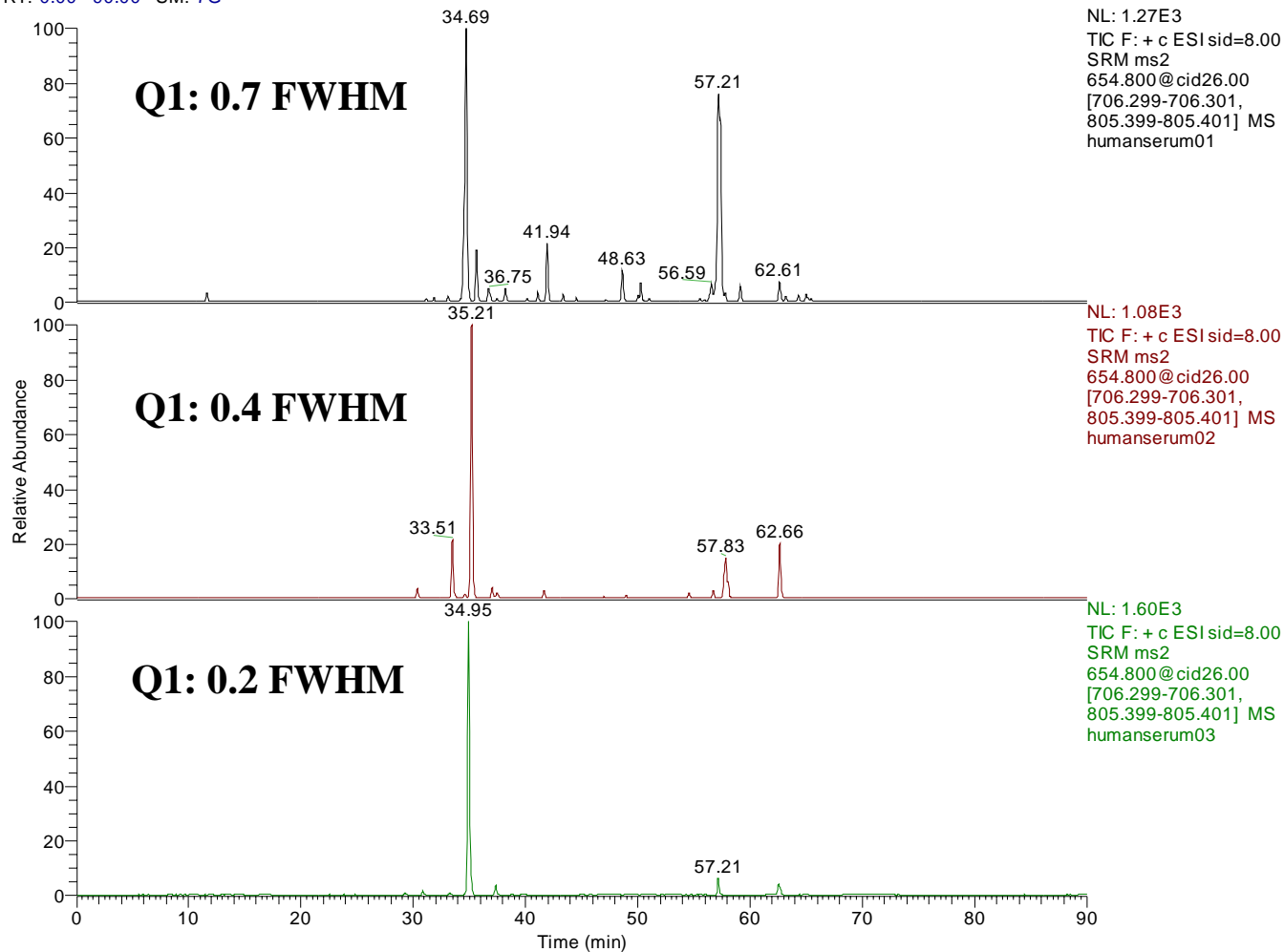
# LGPLVEQGR (Apolipoprotein E)

RT: 0.00 - 90.00 SM: 7G



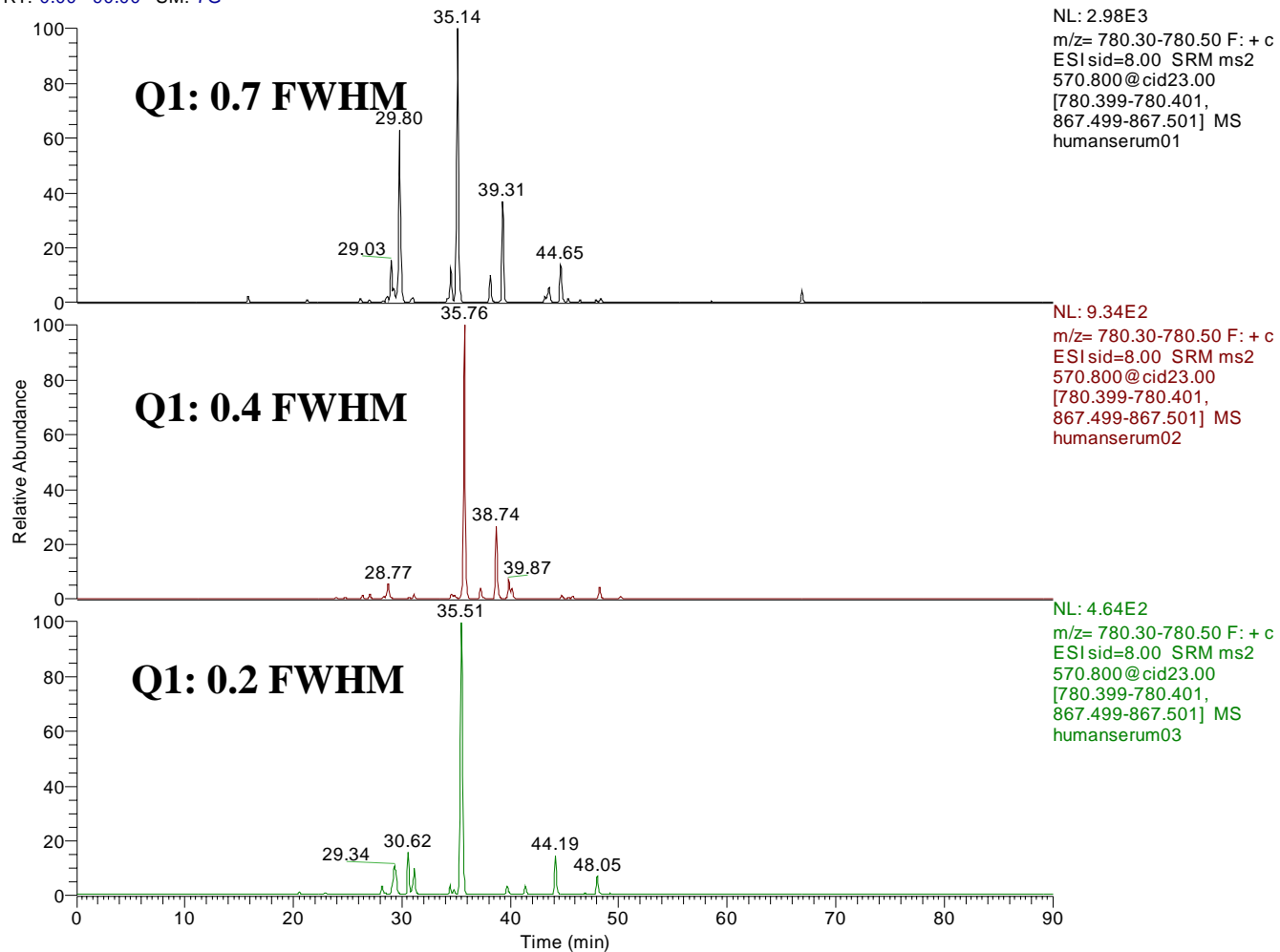
# QGFGNVATNTDGK (Fibrinogen beta chain)

RT: 0.00 - 90.00 SM: 7G



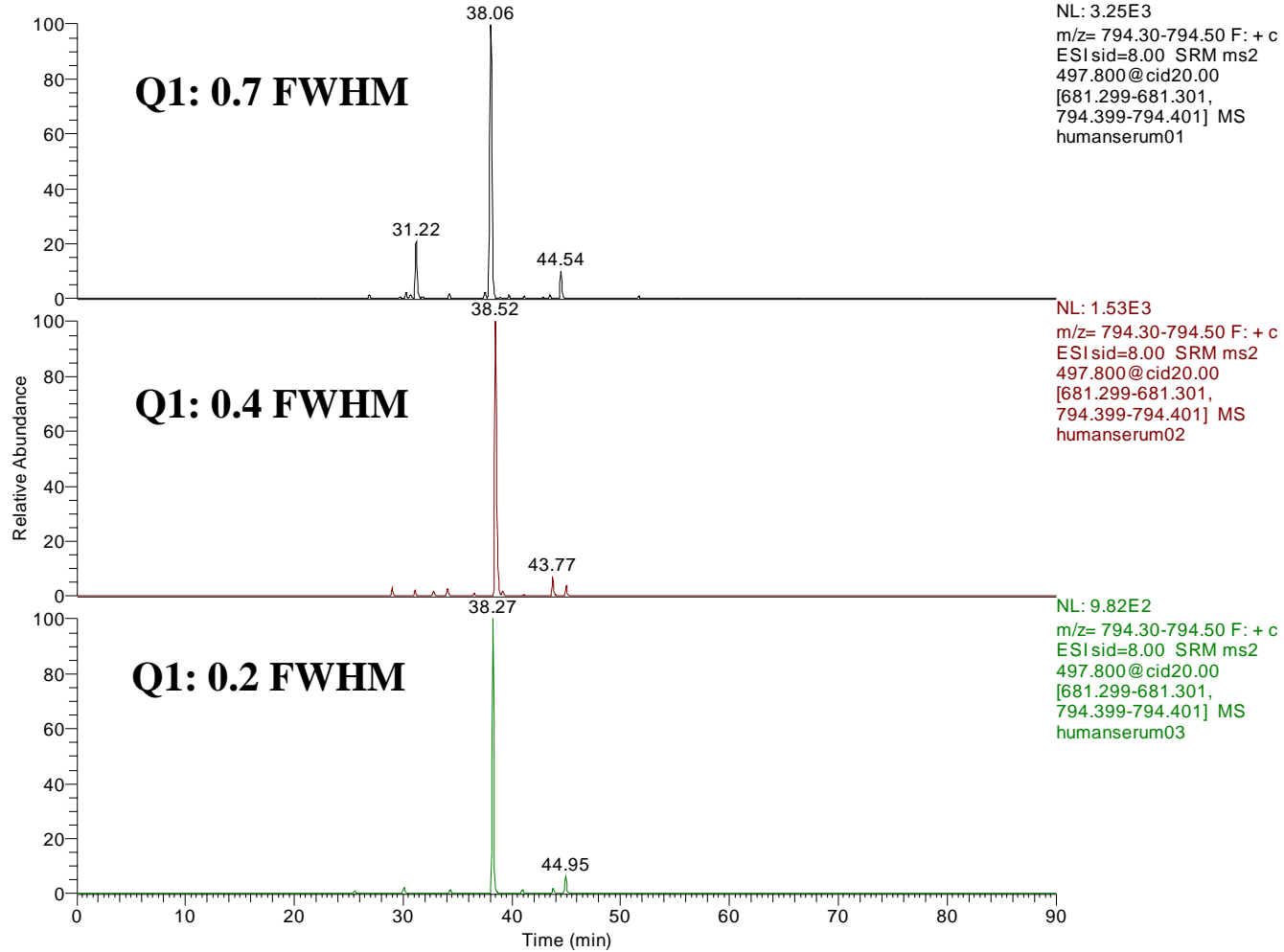
# TVIGPDGHK (Fibrinogen alpha chain)

RT: 0.00 - 90.00 SM: 7G



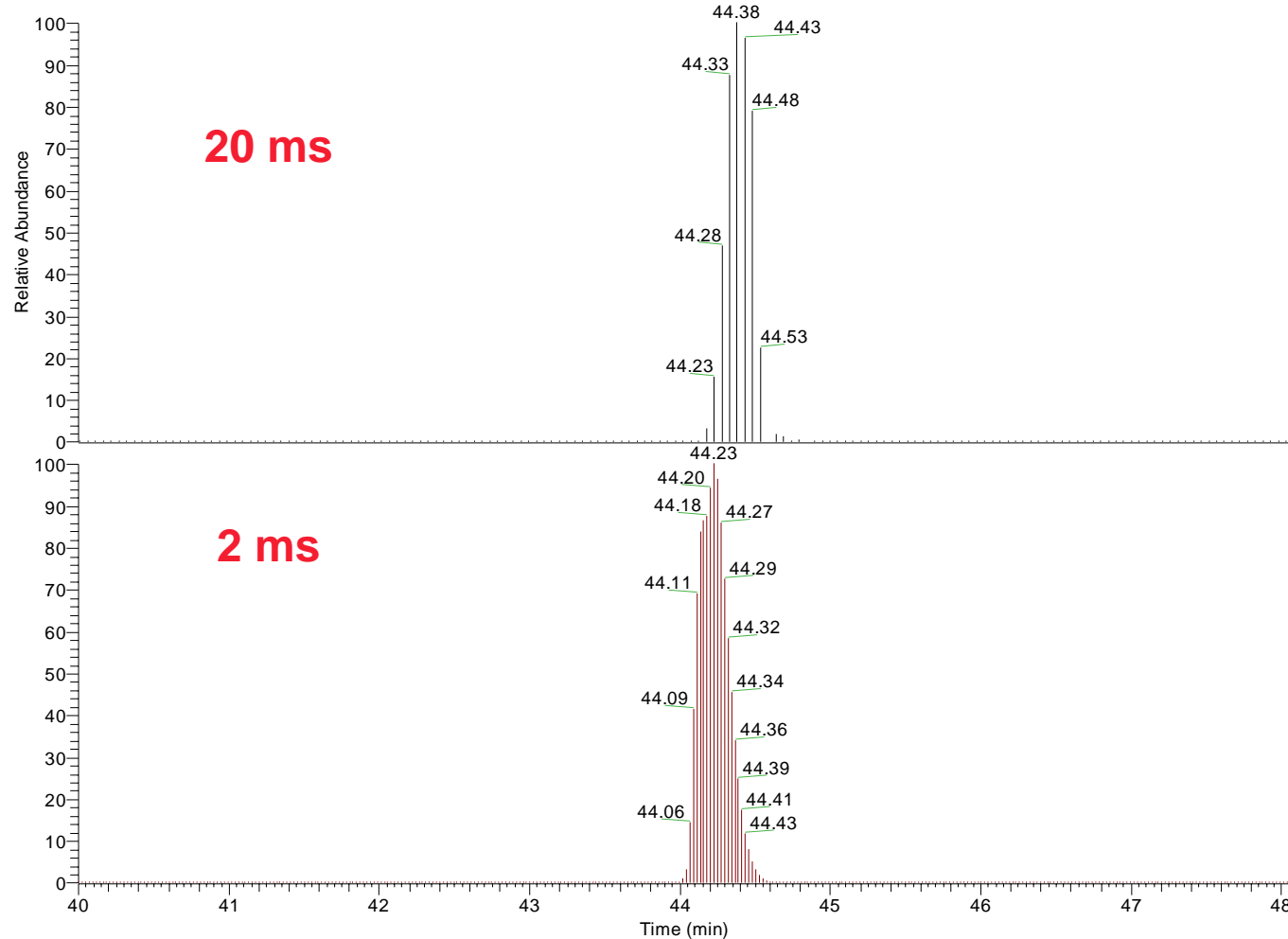
# AEIEYLEK (L-Selectin)

RT: 0.00 - 90.00 SM: 7G



# VVGGLVALR with Different MRM Scan Times

RT: 39.99 - 48.05

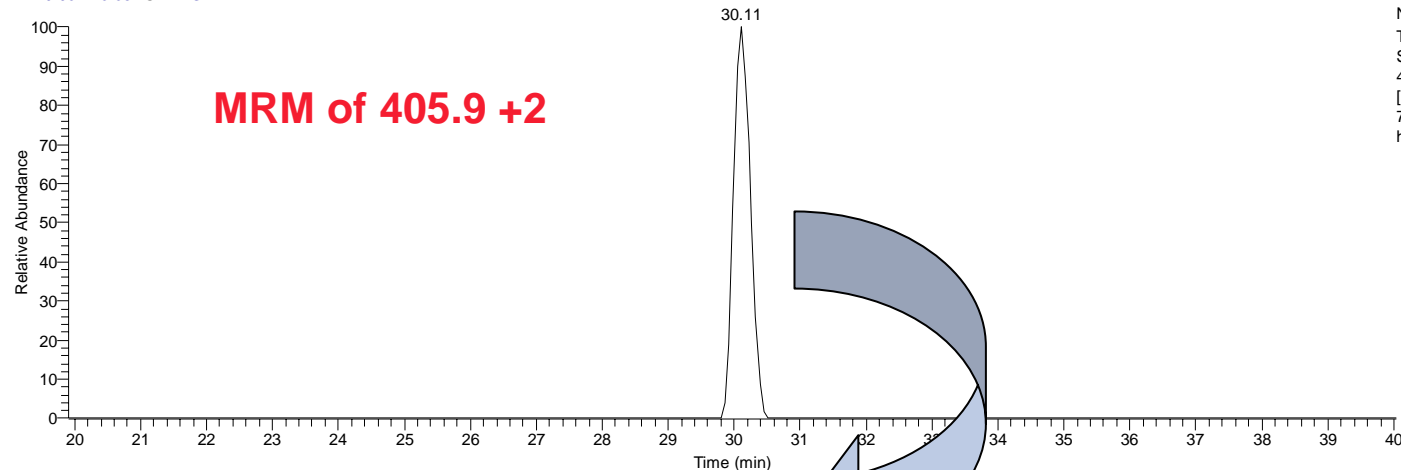


NL: 6.15E4  
TIC F: + c ESI sid=8.00  
SRM ms2  
442.300@cid18.00  
[685.399-685.401,  
784.499-784.501] MS  
humanserum01

NL: 4.02E3  
TIC F: + c ESI sid=8.00  
SRM ms2  
442.300@cid18.00  
[685.399-685.401,  
784.499-784.501] MS  
serum\_2msdwelltime

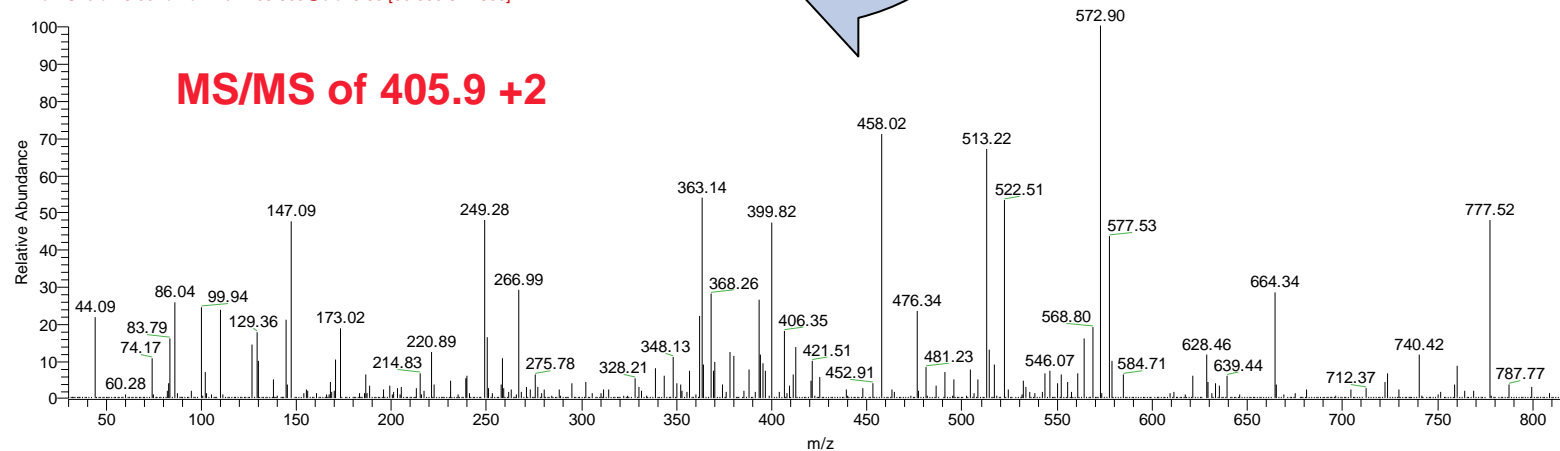
# MRM-Triggered MS/MS: ATEHSTLSEK (Apolipoprotein A-I)

RT: 19.90 - 40.03 SM: 7G

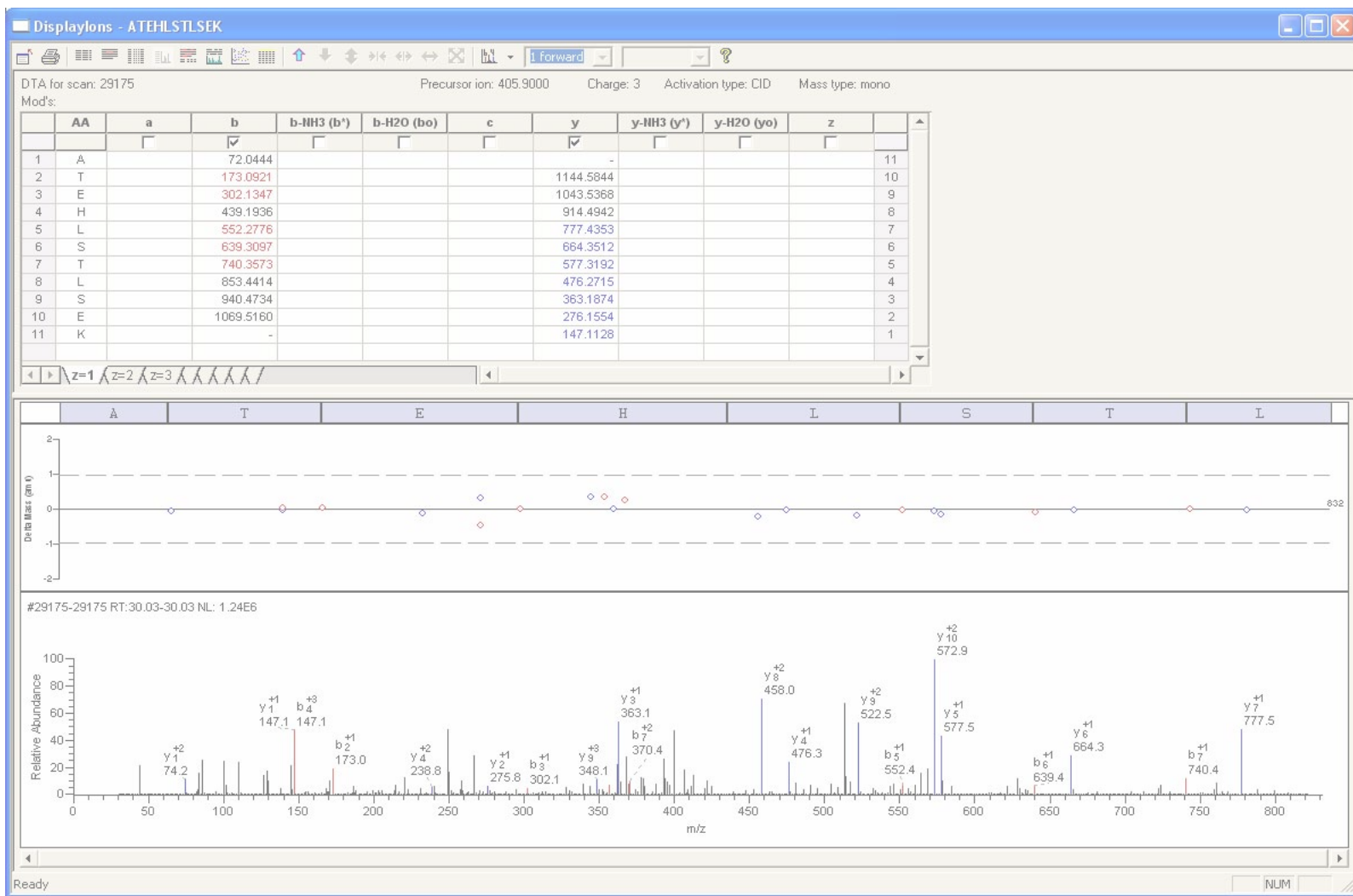


NL: 5.91E5  
TIC F: + c ESI sid=8.00  
SRM ms2  
405.900@cid17.00  
[664.399-664.401,  
777.499-777.501] MS  
humanserum\_MSMS

humanserum\_MSMS #29175 RT: 30.03 AV: 1 NL: 1.24E6  
F: + c ESI sid=8.00 d Full ms2 405.900@cid16.80 [30.000-821.800]



# SEQUEST Database Search Match



# Summary Table: MRM-triggered MS/MS

	A	B	C	D	E	F	G	H
1		Scan(s)	Peptide	MH+	z	XC	DeltaCn	Sp
2	1	Apolipoprotein A-I precursor						
3		29175	K.ATEHLSTLSEK.A	1215.62156	3	2.84	0.44	1055.7
4	2	Similar to Complement C3 precursor						
5		33813	K.TGLQEVEVK.A	1002.54660	2	1.99	0.23	503.1
6	3	Serotransferrin precursor						
7		50892	K.EDPQTFYYAVAVK.K	1629.81590	2	1.81	0.28	593.6
8	4	Apolipoprotein C-III precursor						
9		36836	K.DALSSVQESQVAQQAR.G	1716.85112	2	3.03	0.65	967.3
10	5	Prothrombin precursor						
11		41678	R.ETAASLLQAGYK.G	1251.65794	2	2.57	0.57	741.6
12	6	Alpha-1-antitrypsin precursor						
13		39698	K.DTEEDFDHVDQVTTVK.V	1891.85559	3	2.46	0.29	1257.7
14	7	Ceruloplasmin precursor						
15		32091	R.EYTDASFTNR.K	1203.52766	2	1.91	0.48	560.3
16	8	Alpha-1B-glycoprotein precursor						
17		53494	R.LETPDFQLFK.N	1237.64632	2	1.84	0.41	375.4
18	9	haptoglobin						
19		36783	R.VGYVSGWGR.N	980.49484	2	1.76	0.50	822.6
20	10	C4b-binding protein alpha chain precursor						
21		52557	K.LSLEIEQLELQR.D	1470.81623	2	1.54	0.43	294.1
22	12	Beta-2-glycoprotein I precursor						
23		27718	K.ATVYQGER.V	1022.52653	2	1.46	0.32	479.6
24	13	Alpha-1-antichymotrypsin precursor						
25		45013	R.EIGELYLPK.F	1061.58774	2	1.46	0.27	625.5
26	14	Complement factor H						
27		36260	K.SPDVINGSPISQK.I	1341.70087	2	1.45	0.43	275.5
28	15	Hemopexin precursor						
29		46055	K.NFPSPVDAAFR.Q	1220.60585	2	1.38	0.21	232.1
30	16	Plasma retinol-binding protein precursor						
31		53651	K.YWGVASFQK.G	1198.62552	2	1.35	0.17	189.4
32	17	Gelsolin						
33		33082	K.TGAQELLR.V	887.49451	2	1.32	0.41	201.7

# Assay Precision

Protein	Peptide Sequence	C		D	E	F	G	H	I	J	K	L	M
		Q1	Q3	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Ave	Std	CV (%)	
		Area of total fragment ions											
Afamin	DADPDTFFAK	563.8	825.4	460361	343876	453639	375549	358701	449877	407001	53231	13.1	
		563.8	940.4										
Alpha-1-acid glycoprotein 1	NWGLSVYADKPETTK	570.3	575.3	3363991	3149662	4087824	3888870	3181300	3630443	3550348	385063	10.8	
		570.3	1052.5										
Alpha-1-antichymotrypsin	EIGELYLPK	531.3	633.4	3570363	3212687	3963132	3359817	3064500	3544359	3452476	316139	9.2	
		531.3	819.5										
Alpha-1B- glycoprotein	LETPDFQLFK	619.4	995.5	3815348	3411667	4013810	3945637	3965385	3791761	3823935	219975	5.8	
		619.4	894.5										
Alpha-2-antiplasmin	LGNQEPGGQTALK	656.8	771.4	1063920	804714	1221469	1097863	812108	1013693	1002295	165111	16.5	
		656.8	900.5										
Alpha-1-antitrypsin	DTEEEDFHVDQVTTVK	631.3	790.4	2103352	2725101	1671968	1594758	1806790	182299	1680711	841187	50	
		631.3	889.5										
Alpha-2- macroglobulin	LLIYAVLPT	923	1172.6	9115043	7565135	6513412	9040774	8044853	7233501	7918786	1027185	13	
		923	1059.5										
Angiotensinogen	PKDPTFIPAPIQAK	508.3	556.4	157986	222990	226592	226163	163494	209165	201065	31923	15.9	
		508.3	724.4										
Antithrombin-III	DDLIVSDAFHK	437.2	803.4	208027	274147	172331	143431	167764	147782	185580	49084	26.4	
		437.2	704.3										
Apolipoprotein A-I	ATEHLSTLSEK	405.9	664.4	8310093	7404901	8758816	7389413	8732920	8181190	8129556	611263	7.5	
		405.9	777.5										
Apolipoprotein A-II precursor	SPELQAEAK	486.8	546.4	14682243	13161798	16449486	15604447	15516303	15091455	15084289	1111632	7.4	
		486.8	659.4										
Apolipoprotein A-IV	SLAPYAQDTQEK	675.8	982.4	882356	1040338	943183	1086796	1066425	978521	999603	78869	7.9	
		675.8	1079.5										
Apolipoprotein B-100	FPEVDVLTK	524.3	803.5	815385	751581	822726	844726	590265	666462	748524	101015	13.5	
		524.3	674.4										
Apolipoprotein C I liprotein	TPDVSSALDK	516.8	719.3	3775528	3585466	4138996	3724512	3750057	4077021	3841930	217222	5.7	
		516.8	620.3										
Apolipoprotein C II liprotein	STAAM*STYTGIFTDQVLSVLK	750.4	1149.7	6787	13319	47439	20992	46405	37616	28760	17437	60.6	
		750.4	1002.6										
Apolipoprotein C III liprotein	DALSSVQESQVAQQAR	858.9	1144.6	1508129	1271980	1643745	1482811	1323109	1294646	1420737	147500	10.4	
		858.9	1144.6										
Apolipoprotein E	LGPLVEQGR	484.8	588.3	1219946	1091977	1272193	1028347	1144104	1103460	1143338	89358	7.8	
		484.8	701.4										
Beta-2- glycoprotein I	ATVYYQGER	511.8	751.4	9889076	8814814	10796000	9447598	10202994	10095120	9874267	679873	6.9	
		511.8	652.3										
C4b-binding protein alpha chain	LSLEIEQLELQR	735.9	915.5	1350409	1340155	963933	1775467	1643076	1614620	1447944	292755	20.2	
		735.9	915.5										
Ceruloplasmin	EYTDASFTR	602.3	695.3	1856971	1680288	1787627	2014591	2157404	1910802	1901281	168670	8.9	
		602.3	624.3										
Clusterin	LFSDSPITVTPVEVSR	937.5	1296.7	2395112	2520417	2382234	2690174	2636590	2777964	2566915	161392	6.3	
		937.5	686.4										
Coagulation factor V	DPPSDLLLLK	555.8	898.6	2957516	3484642	4611308	2649725	3077363	3572073	3392105	687963	20.3	
		555.8	898.6										
Coagulation factor XIIIa heavy chain	VVGGVLVALR	442.3	784.5	668570	618838	826962	698422	898715	692279	733964	106135	14.5	
		442.3	685.4										
Complement C3	TGLQEVEVK	501.8	731.4	4566881	4737491	5720918	5120795	5411222	5561088	5186399	461795	8.9	
		501.8	603.3										
Complement C4 gamma chain	LTQVLHFTK	362.9	645.4	111095	112564	100589	159819	133237	128646	124325	21141	17	
		362.9	744.4										
Complement C4 beta chain	VGDTLNLNLR	557.8	843.5	2302932	2038312	1821734	2460076	1632647	2100302	2059334	303503	14.7	
		557.8	629.4										
Complement factor C9	AIEDYINEFSVR	728.5	1271.6	185702	212040	174997	161374	230387	205447	194991	25570	13.1	
		728.5	1027.5										

# Assay Precision

Protein	Peptide Sequence	C		D	E	F	G	H	I	J	K	L	M
		Q1	Q3	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Ave	Std	CV (%)	
		Area of total fragment ions											
Complement factor B	EELLPAQDIK	578.4	671.4	2011175	2312117	2128099	2011175	2447949	2128099	2173102	173987		8
		578.4	784.5										
Complement factor H	SPDVINGSPISQK	671.4	830.4	330940	425920	267351	267545	427823	267351	331155	78120		23.6
		671.4	572.3										
Fibrinogen alpha chain	GSESGIFTNTK	570.8	780.4	110715	94678	137387	116792	138690	138164	122738	18299		14.9
		570.8	867.5										
Fibrinogen beta chain	QGFGNVATNTDGK	654.8	706.3	64139	61523	76351	87158	91996	88959	78354	13156		16.8
		654.8	805.4										
Fibrinogen gamma chain	DTVQIHDITGK	409.5	670.4	980969	1127408	1211579	1290416	1105767	1122725	1139811	104564		9.2
		409.5	533.3										
Fibronectin	DLQFVEVTDVK	647.3	789.4	148897	79771	95323	118265	113737	124585	113433	23945		21.1
		647.3	690.4										
Gelsolin, isoform 1	TGAQELLR	444.3	786.5	422213	430807	504372	465432	559462	569154	491907	63210		12.9
		444.3	729.4										
Haptoglobin beta chain	VGYYVSGWGR	490.8	562.3	36340479	38692289	43475501	35250502	44157166	46000993	40652822	4482765		11
		490.8	661.3										
Hemopexin	NFPSPVDAAFR	610.8	959.6	19718474	17178303	21208304	18586954	17250682	21933002	19312620	1997910		10.3
		610.8	775.3										
Heparin cofactor II	TLEAQLTPR	514.8	814.4	1176390	1501771	1764117	1165217	1594067	1725999	1487927	262912		17.7
		514.8	685.4										
Histidine-rich glycoprotein	DSPVLIDFFEDTER	841.9	1171.5	1249897	1304182	1606963	1714889	1660153	1811153	1557873	228422		14.7
		841.9	1058.4										
Inter-alpha-trypsin inhibitor heavy chain	AAISGENAGLVR	579.4	629.4	2118520	2104894	2392357	2244207	2302923	2245588	2234748	109605		4.9
		579.4	902.5										
Inter-alpha-trypsin inhibitor light	AFIQLWAFDAVK	704.9	836.4	1316403	1797675	1834854	1347062	1395663	1588799	1546743	229564		14.8
		704.9	949.5										
Kininogen	TVGSDTFYSFK	626.3	1051.4	2601441	2998187	3752582	2872619	2383236	3727717	3055964	571426		18.7
		626.3	994.5										
L-selectin	AEIEYLEK	497.8	794.4	77482	105168	91786	96749	102618	97221	95171	9864		10.4
		497.8	681.3										
Plasma retinol-binding protein precursor	YWGVASFLQK	599.8	849.5	2178485	1964456	2480949	2284090	2047641	2444580	2233367	208950		9.4
Plasminogen	LSSPAVITDK	438.3	615.4	2221967	1958030	2432750	2097034	1719680	2008048	2072918	242677		11.7
		438.3	502.3										
Prothrombin	ETAASLLQAGYK	626.3	879.5	1543698	1348273	1748347	1559187	1602953	1570716	1562196	128452		8.2
		626.3	679.4										
Serum albumin	LVNEVTEFAK	575.4	937.4	580114007	506210531	661792006	512338571	503622280	583128349	557867624	62637353		11.2
		575.4	694.4										
Serum amyloid P-component	VGEYSLVIGR	578.8	1057.5	605914	504502	508405	563431	552861	554938	548342	37744		6.9
		578.8	871.5										
Transferrin	EDPQTFYYAVAVVK	815.4	1160.6	8844379	7043347	9137074	8080055	8450470	8322479	8312967	727562		8.8
		815.4	1288.7										
Transthyretin	AADDTWEPFASGK	697.8	921.4	6443753	6682339	7274622	6840174	5414265	7032782	6614656	653601		9.9
		697.8	606.4										
Vitamin D-binding protein	THLPEVFLSK	585.8	819.5	3237009	3218221	4293045	3497041	3206544	3713418	3527546	425348		12.1
		585.8	932.5										
vitamin K-dependent protein C	WELDLDIK	516.3	716.4	168787	145466	258455	158618	205679	200416	189570	41128		21.7
		516.3	603.3										
Vitronectin	FEDGVLDPDYPR	711.9	875.4	1924890	1666834	1919119	1972180	1809208	2005032	1882877	124962		6.6
		711.9	1031.5										
Zinc-alpha-2-glycoprotein	EIPAWVPFDPAAQITK	891.9	1087.7	2062315	2009293	2619535	2236890	1758158	2666391	2225430	358207		16.1
		891.9	728.4										

# Results comparison

Anderson et. al.

*Sensitivity:*

Two proteins with relatively low normal concentrations in plasma were clearly detected among the MRMs tested:  
L-selectin and  
Fibronectin

Current Study

*Sensitivity:*

Two proteins with relatively low normal concentrations in serum were clearly detected among the MRMs tested:  
L-selectin and  
Fibronectin

# Results comparison

## Anderson et. al.

*Maximum working dynamic range: >4 orders of magnitude*

(from 1E+04 cutoff, corresponding to a S/N of 10, to the highest peak area of 1E+08 for an albumin peptide in whole plasma digest samples)

## Current Study

*Maximum working dynamic range: >4 orders of magnitude*

(from lowest peak areas of 6E+04 from Fibrinogen beta chain to the highest peak areas of 6E+08 from albumin peptide in whole serum digest samples)

# Results comparison

Anderson et. al.

*Six proteins were not reliably observed:*

coagulation factor V

vitamin K-dependent protein C

C4b-binding protein

inter-alpha trypsin inhibitor light chain

apolipoprotein C-II

alpha1-antitrypsin

Current Study

*Two proteins were not reliably observed:*

apolipoprotein C-II  
(CV: 60%)

alpha1-antitrypsin  
(CV: 50%)

# Results comparison

Anderson et. al.

*Assay Precision:*

Undepleted plasma:

CVs (n=10) were from 3-56%  
(50% of MRMs had CV<10%)

Depleted plasma:

CVs (n=10) were 2-22%  
(78% of MRMs had CV<10%)

Current Study

*Assay Precision:*

Undepleted serum:

CVs (n=6) were from 5-26%  
(50% of MRMs had CV≤10%)

# Conclusions

- h-mSRM assays on TSQ Quantum Ultra provide robust targeted protein quantitation in serum or plasma
  - Biomarker verification
  - Targeted discovery
- 2 ms scan times allow hundreds of SRMs/sec
- h-SRM reduces matrix interference without significant signal loss
  - improves assay sensitivity and robustness
  - More proteins reliably quantified
- “MRM-triggered” MS/MS for peptide confirmation
- Demonstrated excellent analytical performance
  - 51/53 proteins quantified
  - CVs from 5-26% (with more than half less than 10%)
  - Most peptides confirmed with MRM-triggered MS/MS
  - Assay linear over 4 logs
  - Sensitivity 20 amol on column

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