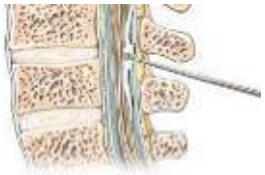
Peptide Quantitation With An Agilent 6410 QQQ System

Agilent Proteomics Biomarker Workflow

SAMPLE1

SAMPLE2

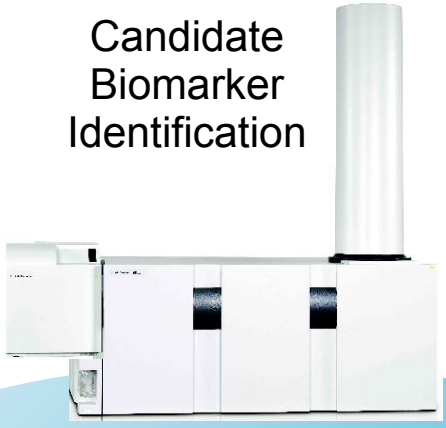
Extraction



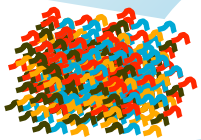
Depletion

Fractionation

Candidate Biomarker Identification



6520 QTOF



Proteolytic Digest

6410 QQQ

Validation

DATA

Candidates

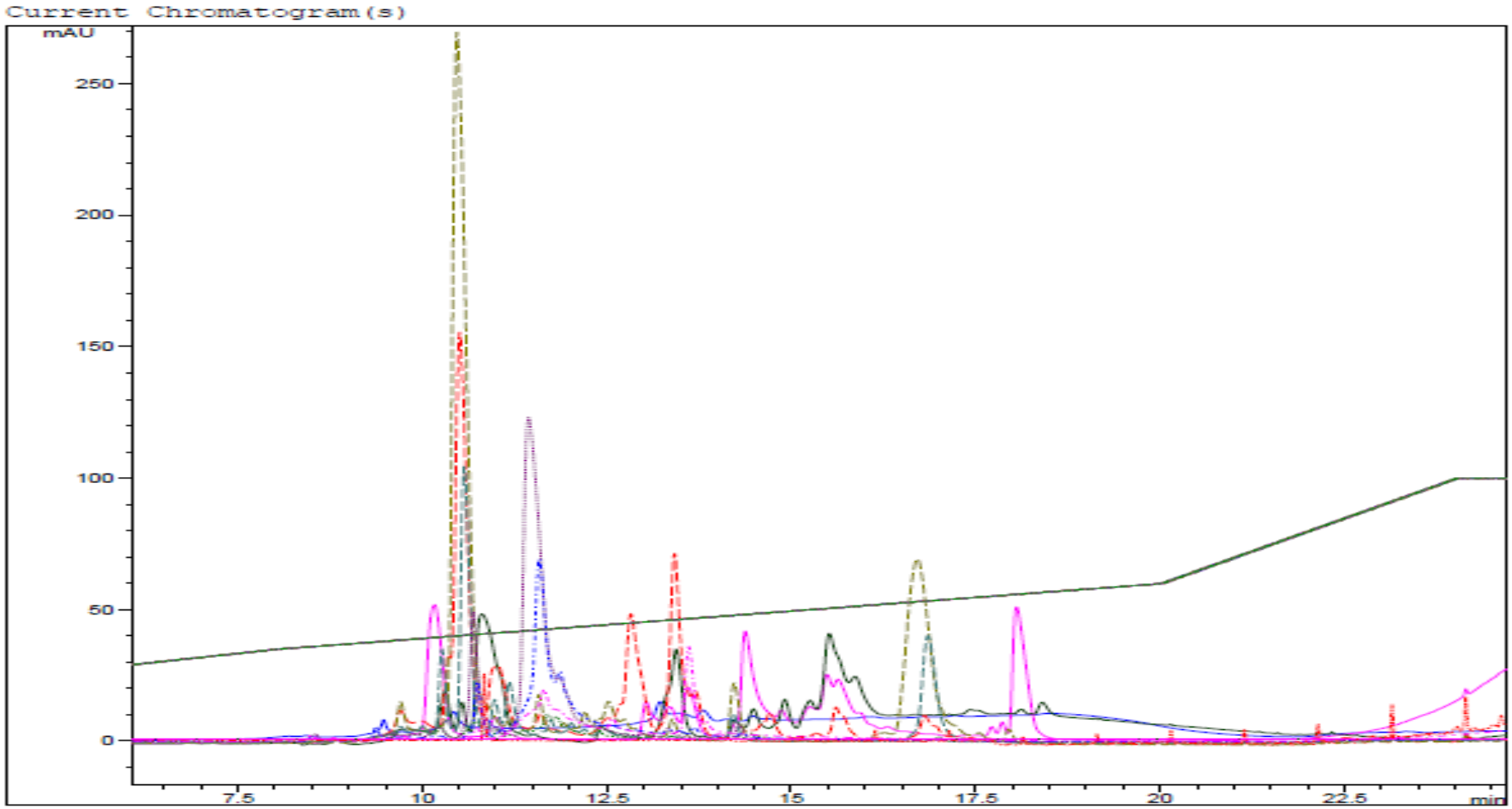
Extraction

Identification

Discovery, Validation, Verification - Biomarker

Discovery	Validation	Verification
100s of proteins 50s of samples Technical and Biological replicates Intense data analysis	20s to 50s of proteins 100s of samples Technical and Biological triplicates Intense data analysis	< 10s to 20s of proteins 1000s of samples Technical and Biological replicates Intense data analysis
Identify differences Between the samples	Statistical validation of Candidate proteins	Statistical validation of Candidate proteins
ID of proteins	Quantitative analysis	Quantitative analysis
Selection of a few Candidate proteins	Internal global standards	Internal global standards
Internal standards		

Off-gel and mRP
Fraction from off-gel IEF run on mRP



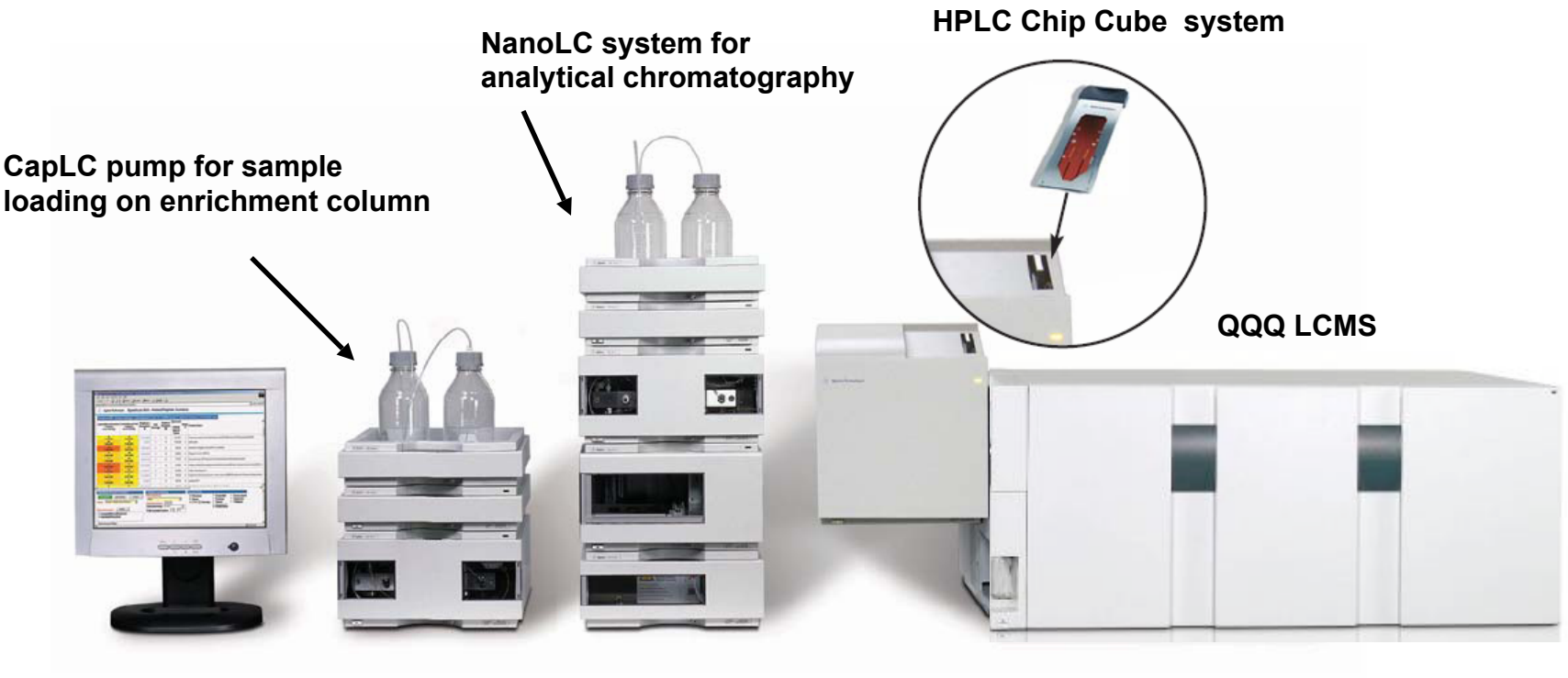
Biomarker Validation – Jump From Research Lab to Clinical Lab

	Research	Clinical
# of samples	<100	Hundreds - thousands
# of proteins	50-500	1-20
Time	Months - years	minutes - hours
Cost	$100k-1M	$10-100
CV	20-50%	3-5%

- Reduce cost
- Improve CV
- Reduce the time needed for testing
- Increase throughput

New HPLC-Chip/QQQ LCMS Technology

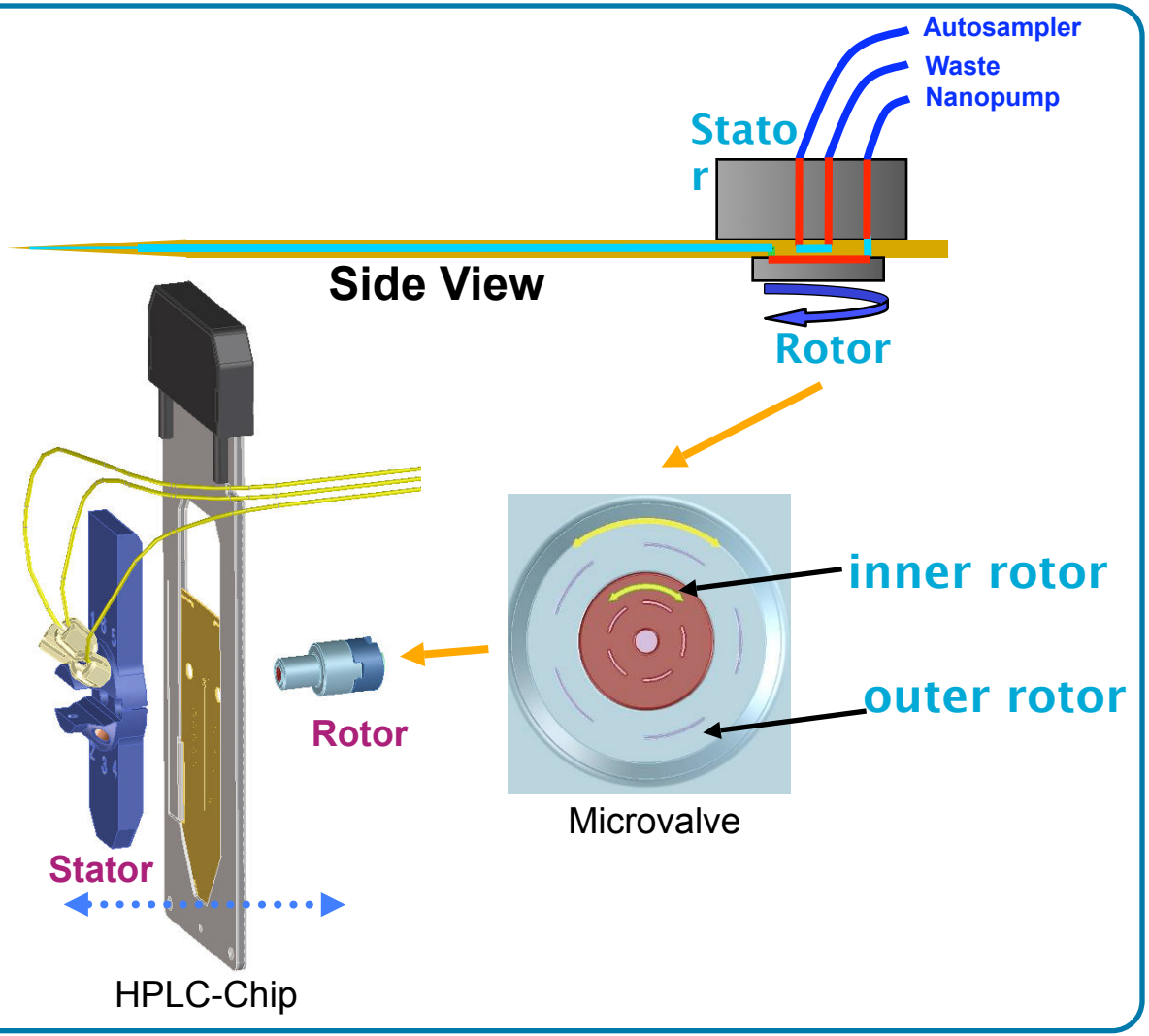
Nanospray chip configuration brings new era in high sensitivity quantitation

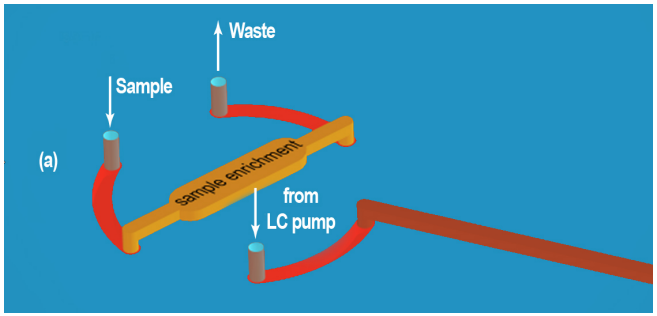


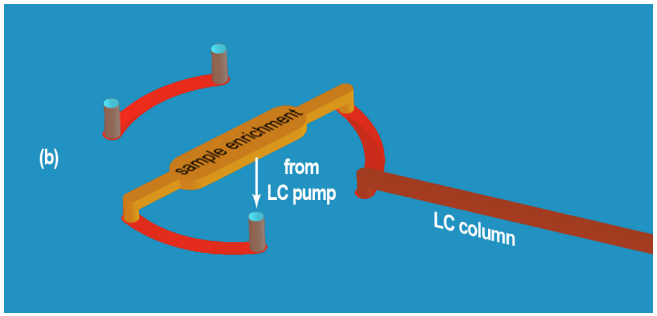
Sensitivity: low -mid amol

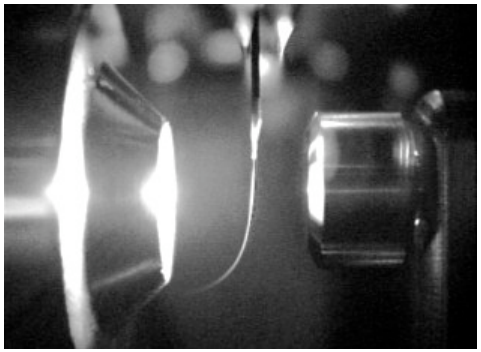
Dynamic range: 10^3 -10^5

HPLC-Chip/MS Interface: Fluid Connections to the HPLC-Chip

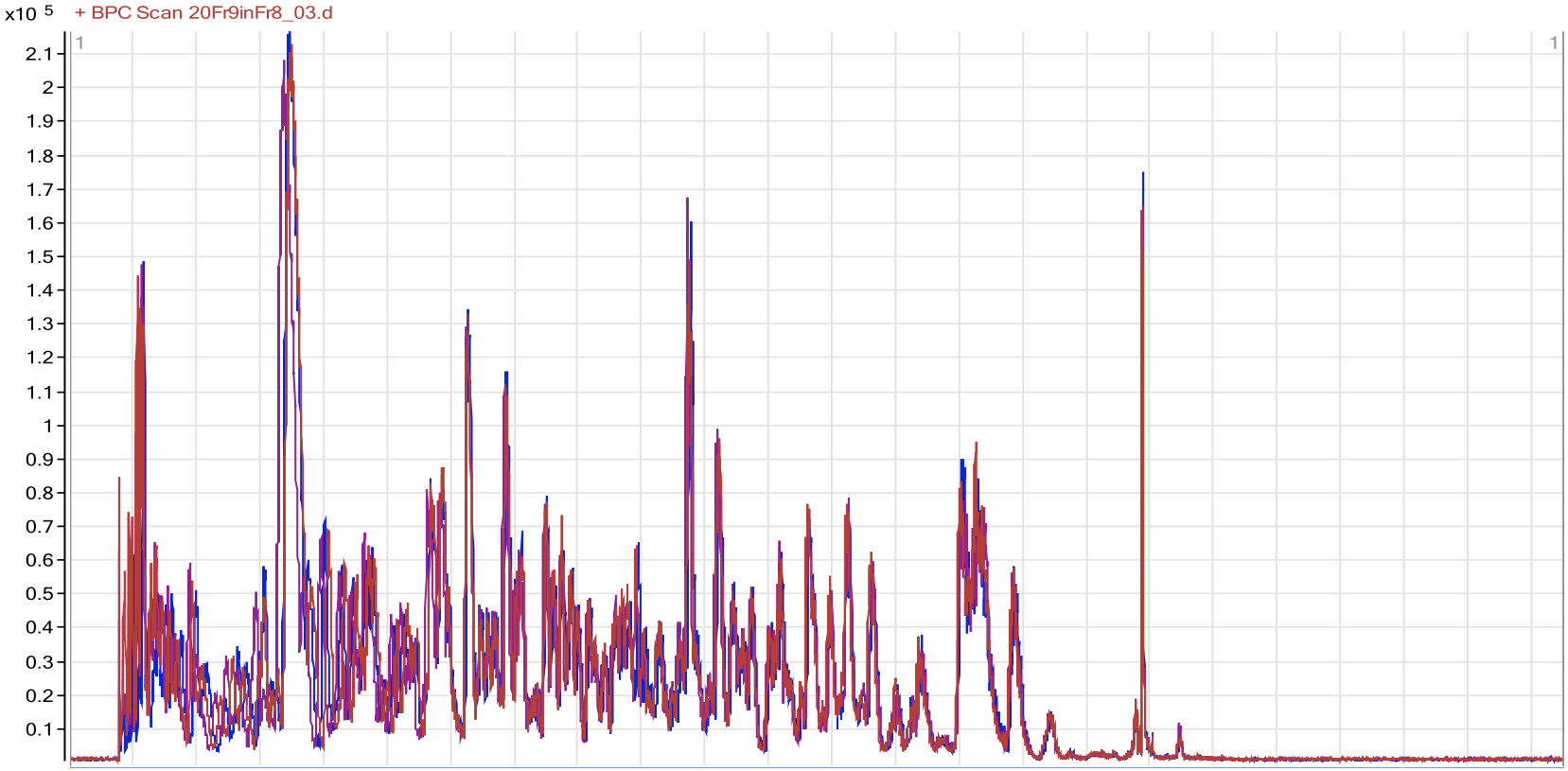






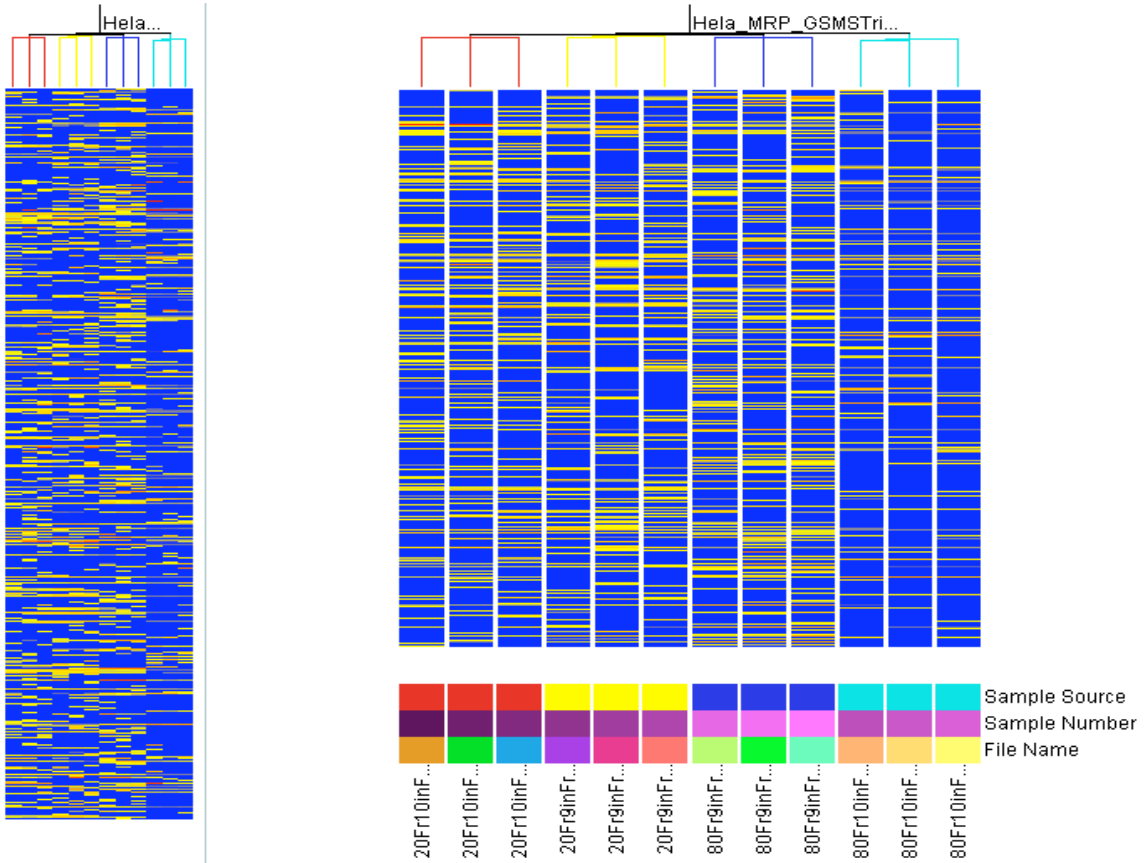


LC-MS Reproducibility



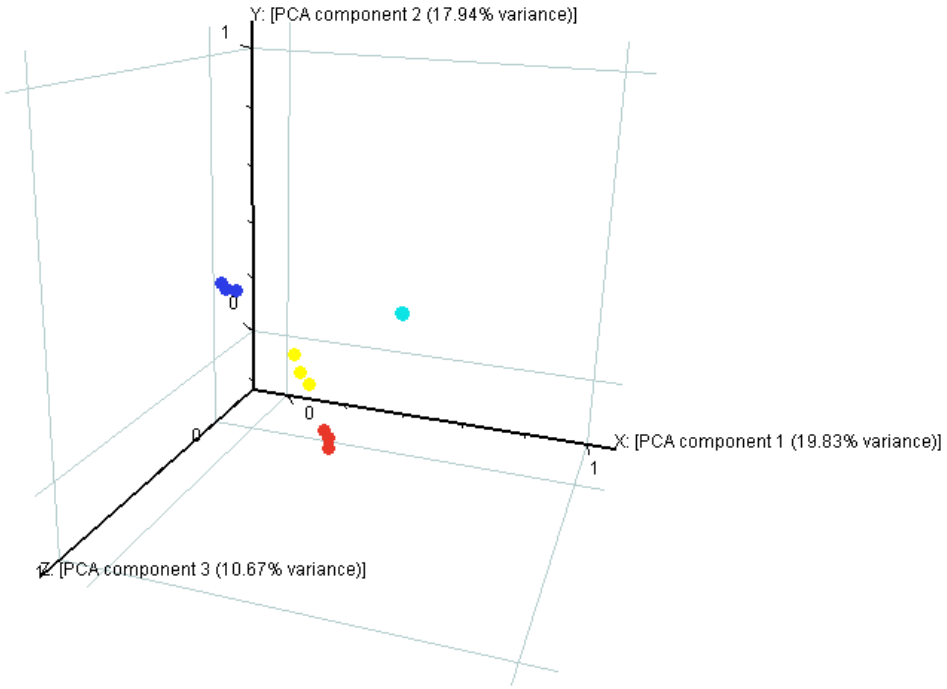
HeLa cell lysate, mRP-subfraction, tryptic digest, run on 15 cm C18 LC-chip (AGILENT) and analysed by MS only on a QTOF (AGILENT 6520)

Clustering



Selected Condition Tree:	Hela_MRP_GSMSTrial (All Samples)	Colored by:	Hela_MRP_GSMSTrial, Default Interpretation
Branch color parameter:	Sample Source	Mass List:	all Masses (21545), 1392.7022 selected

Principal Component Analysis



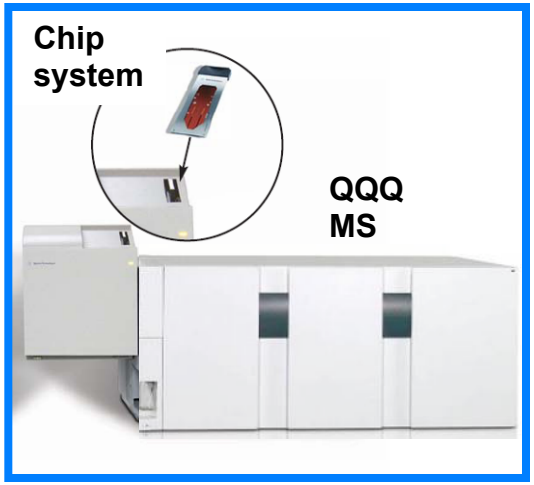
X-axis: [PCA component 1 (19.83% variance)]
Y-axis: [PCA component 2 (17.94% variance)]
Z-axis: [PCA component 3 (10.67% variance)]

Conditions: Hela_MRP_GSMSTrial, Default Interpretation
Colored by: Parameter Sample Source

Triple Quadrupole Mass Spectrometer
Extending Outstanding Performance

6400 Series – Triple Quad – NEW Functionality

- ☑ Compliance (CFR 21 Part 11 Support)
- ☑ Chip Cube (Ultimate sensitivity)
- ☑ Extended mass range (2000 amu) Upgrade Kit
- ☑ Additional autotune support (Chip Cube)

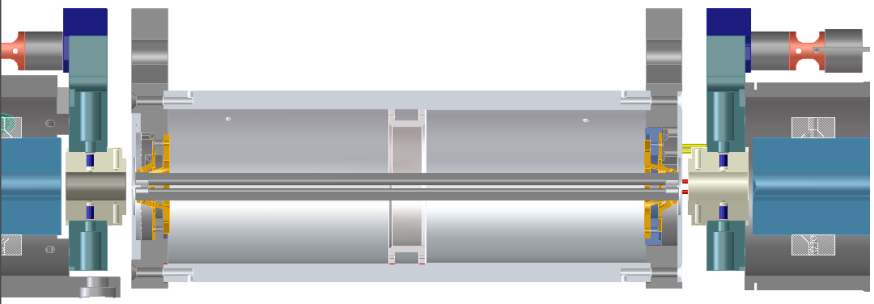


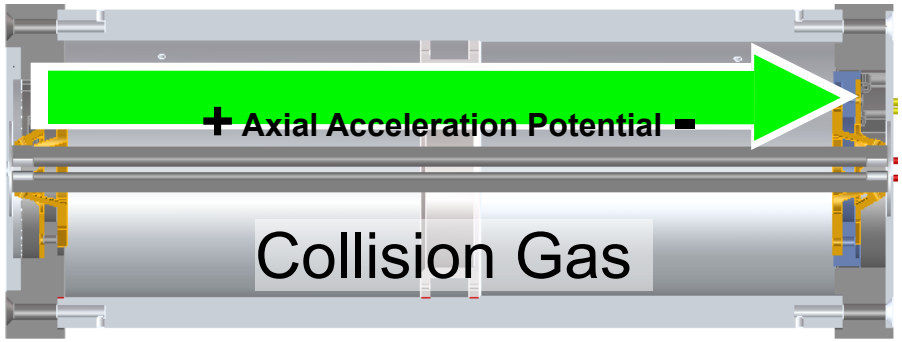
Agilent's New *Axial Acceleration* Collision Cell

Overcomes memory or cross-talk effects!
- using high speed ion transport

Maximum sensitivity
- using wide mass range hexapole design

Simple to operate
- no complicated wave forms





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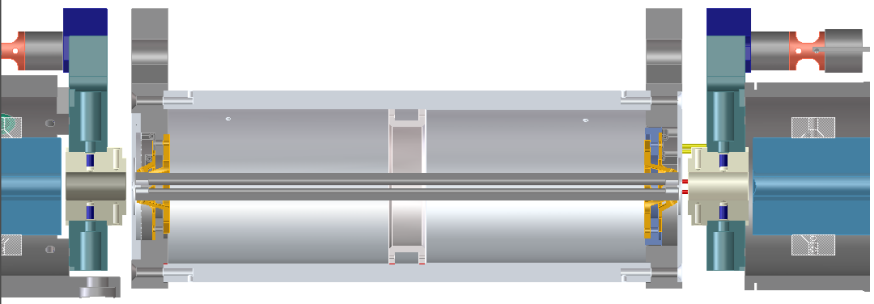
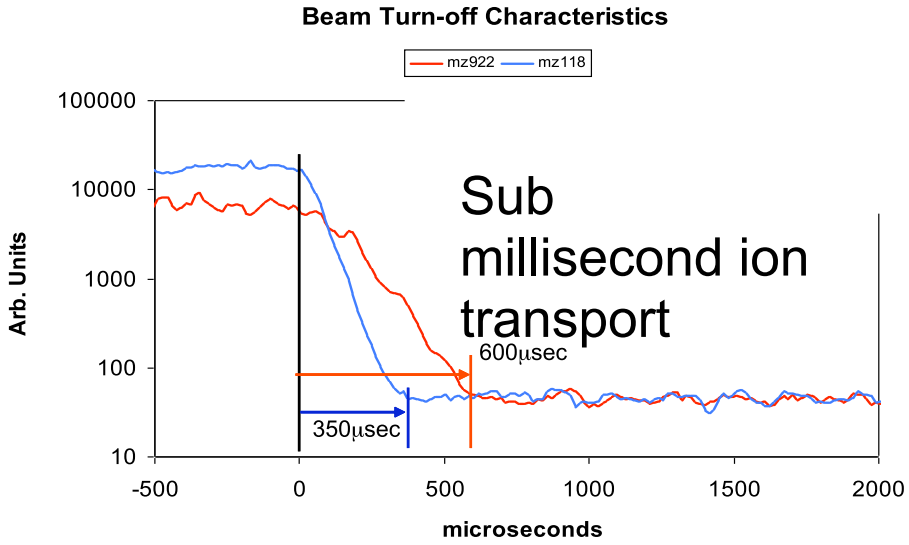
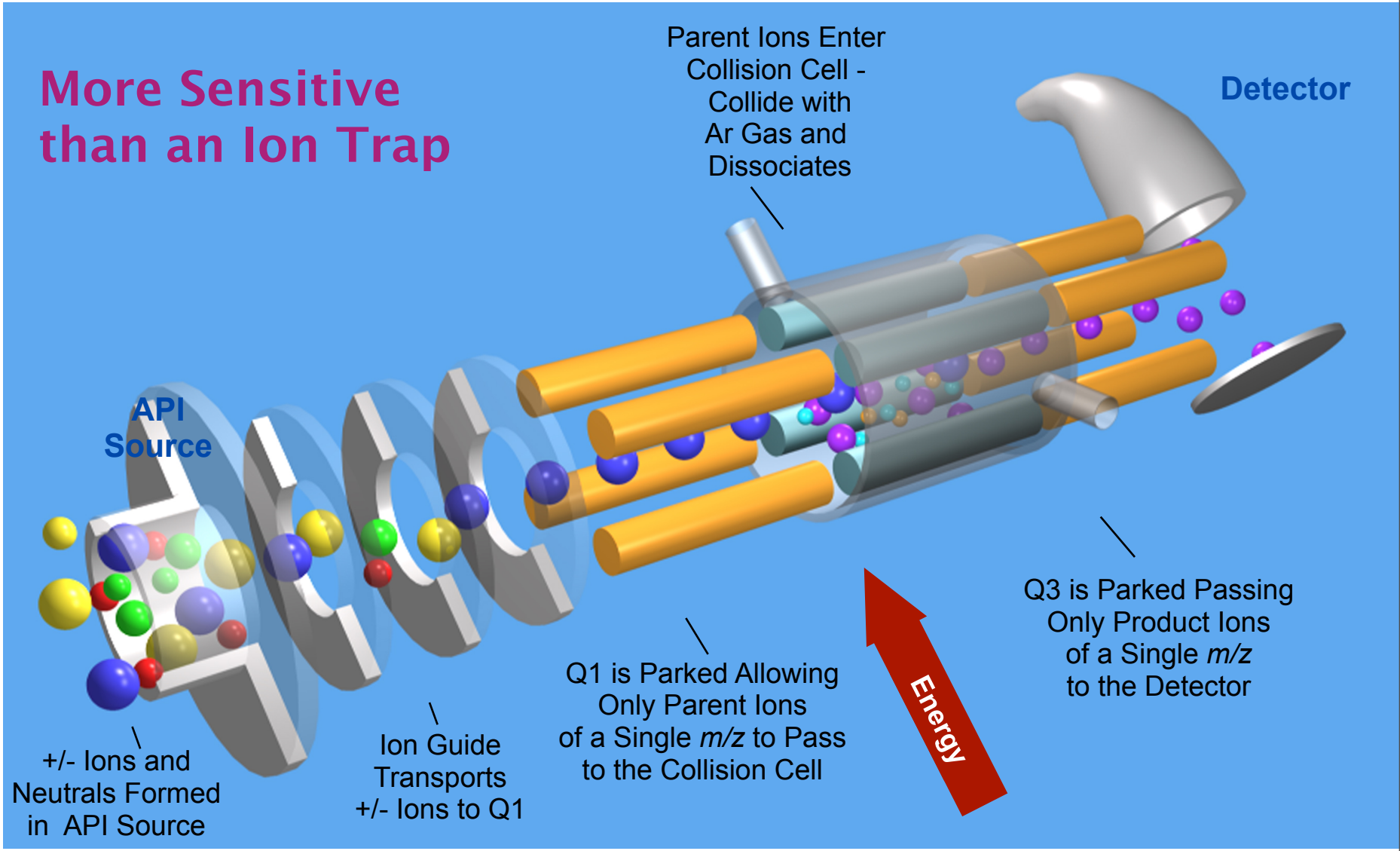


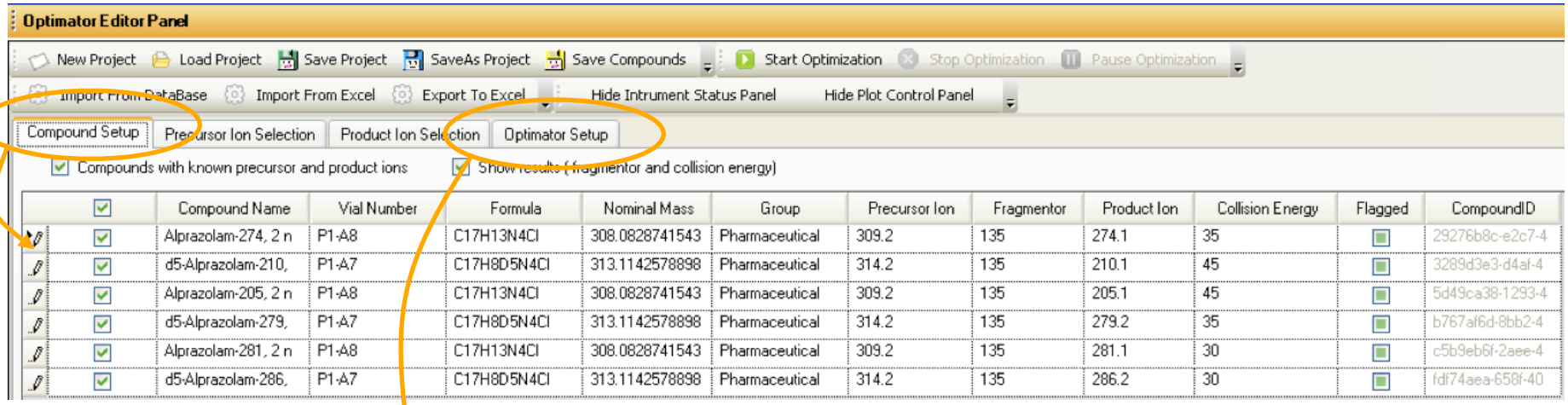


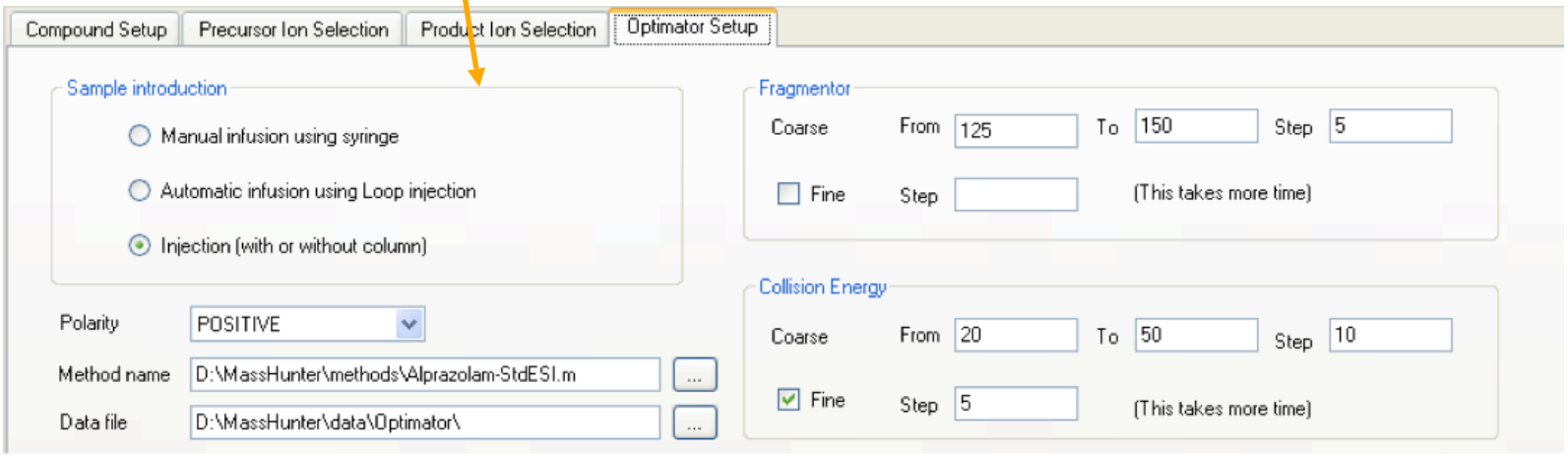
Triple Quadrupole: SRM



Mass Hunter Optimizer

Compound Entry & Method Setup





MassHunter MS/MS Method Optimizer
Basic Steps

Run 1: The precursor adducts (H+, Na+, etc) and corresponding Fragmentor value are optimized. Based on user criteria, the optimum precursor mass and Fragmentor value are set.

Run 2: (optional) fine adjustment of the Fragmentor value.

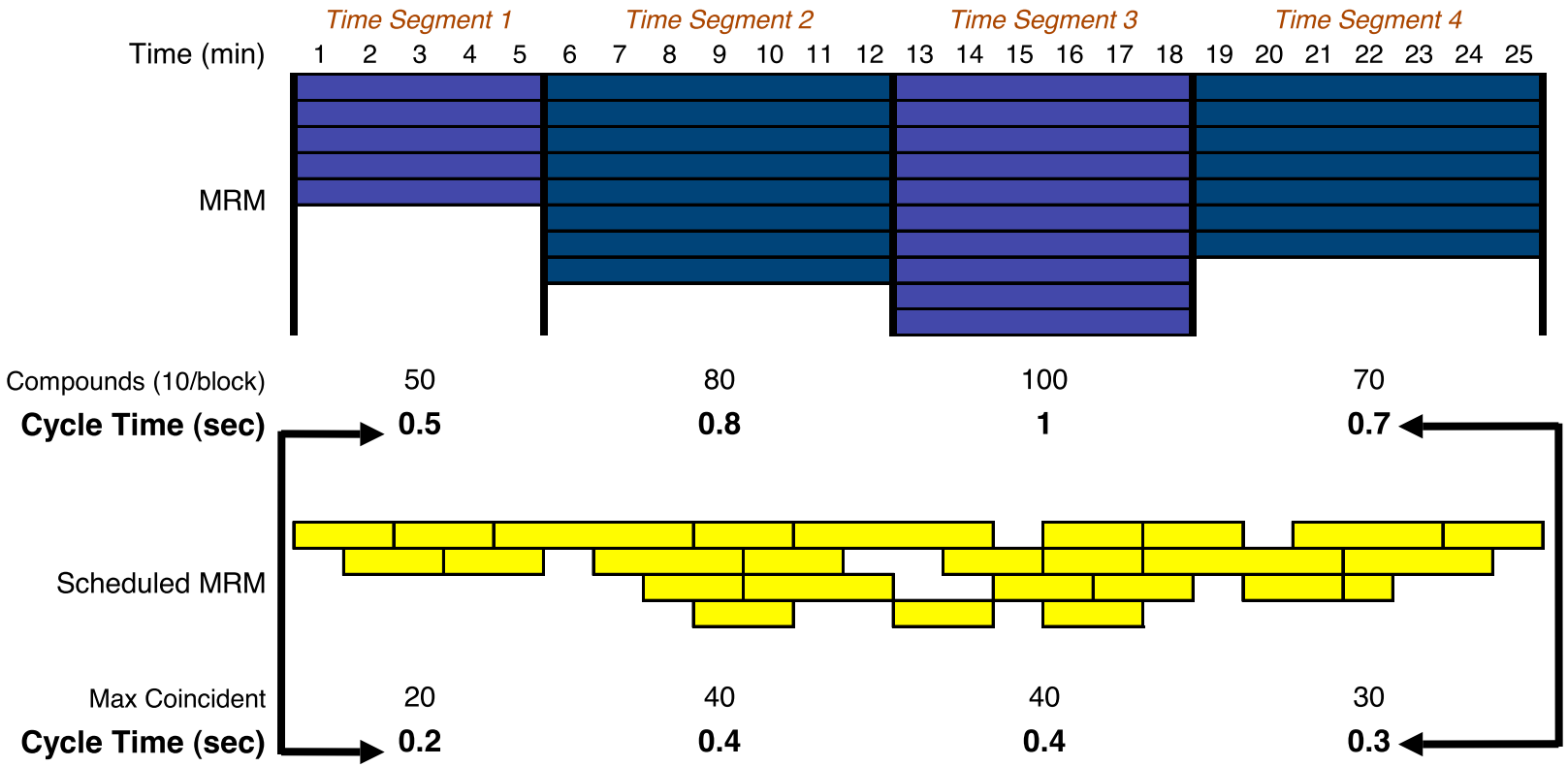
Run 3: Coarse product ion scan finds the largest 4 product ions with corresponding Collision Energy. As a default 0, 10, 20, 30, 40 volt CE are surveyed.

Run 4: Fine adjustment (profile) of each product ion to establish precise mass value.

Run 5: (optional) fine adjustment of the Collision Energy.

Scheduled MRM
Increased Utility and Performance

A simple comparison of MRM and Scheduled MRM

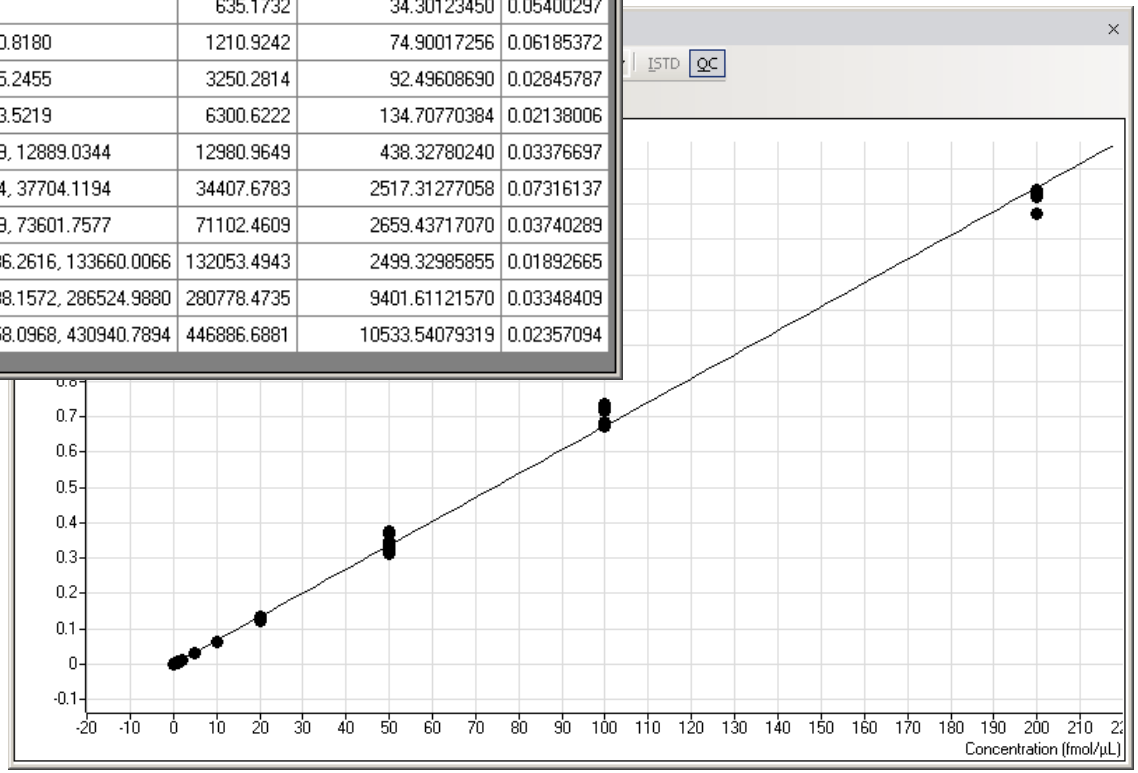


2 x shorter cycle times supports narrow chromatographic peaks, more analytes or longer dwell per analyte.

Reproducibility of MRM response of HSA Peptide from 10 amol to 200 fmol: (n=6)

Calibration Response RSD

Level Name	Responses	Average	Standard Deviation	RSD
01	18.9525, 20.8175, 18.3310, 18.0205, 15.5350, 24.2350	19.3153	2.95051515	0.15275573
02	31.0000, 32.6240, 23.9240, 27.0300, 25.1665	27.9489	3.74004406	0.13381722
03	34.2699, 35.7310, 29.5165, 34.1775, 41.3235, 41.9440	36.1604	4.73157795	0.13084969
04	69.2860, 61.8290, 78.9180, 68.6645, 68.6650, 64.6260	68.6648	5.80610139	0.08455723
05	118.6870, 103.1530, 113.0950, 115.2695, 108.4340, 115.5800	112.3698	5.64953978	0.05027634
06	265.0285, 280.5620, 286.1540, 306.0395, 288.6410, 310.7000	289.5208	16.81468530	0.05807764
07	581.9415, 616.4265, 630.1005, 656.1970, 645.0130, 681.3605	635.1732	34.30123450	0.05400297
08	1313.3305, 1251.1835, 1119.7660, 1159.2205, 1161.2268, 1260.8180	1210.9242	74.90017256	0.06185372
09	3339.4015, 3121.2870, 3212.6375, 3288.4455, 3184.6715, 3355.2455	3250.2814	92.49608690	0.02845787
10	6548.3065, 6159.3390, 6304.4050, 6323.6760, 6234.4850, 6233.5219	6300.6222	134.70770384	0.02138006
11	12736.6533, 13629.5363, 13343.9445, 12406.5650, 12880.0559, 12889.0344	12980.9649	438.32780240	0.03376697
12	31088.2466, 32526.2448, 33591.0696, 34795.9507, 36740.4384, 37704.1194	34407.6783	2517.31277058	0.07316137
13	68472.6433, 67181.4113, 71693.2472, 73327.0359, 72338.6699, 73601.7577	71102.4609	2659.43717070	0.03740289
14	127253.4288, 133378.7599, 131800.4635, 133992.0454, 132236.2616, 133660.0066	132053.4943	2499.32985855	0.01892665
15	272565.1274, 283791.4292, 285408.1916, 265992.9477, 290388.1572, 286524.9880	280778.4735	9401.61121570	0.03348409
16	440772.4250, 450591.4181, 443733.2413, 455624.1579, 459658.0968, 430940.7894	446886.6881	10533.54079319	0.02357094



Limite of quantitation in the low amol range
Peroxidase spiked into human serum

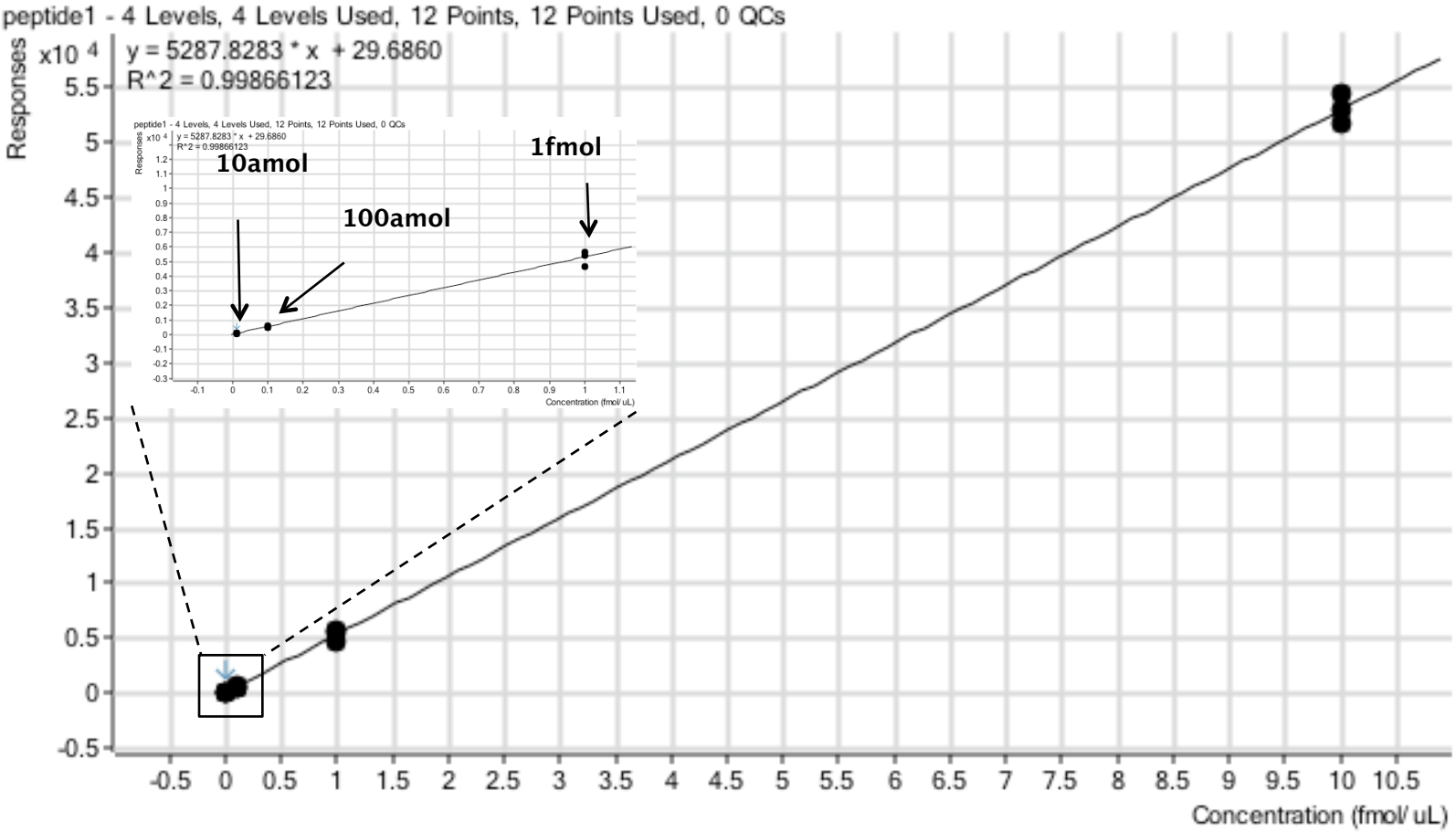
10 amol peroxidase in 1ug serum

100 amol peroxidase in 1ug serum

1 fmol peroxidase in 1ug serum

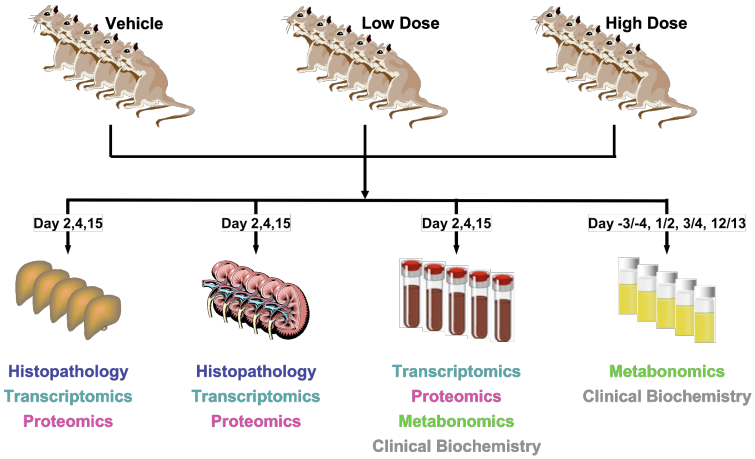
10 fmol peroxidase in 1ug serum

External Quantitation Curve of Peroxidase Peptide DTIVNELR From 10 amol to 10 fmol Spiked into Human Serum



Absolute Protein Quantification in the Context of Non-clinical Drug Safety Evaluation

UCD Conway Institute
University college Dublin
And
Agilent Technologies



Collins B. C. et al. ASMS 2008 MPQ 477

Experimental Design

Catalase was selected based on previous 2D-DIGE data

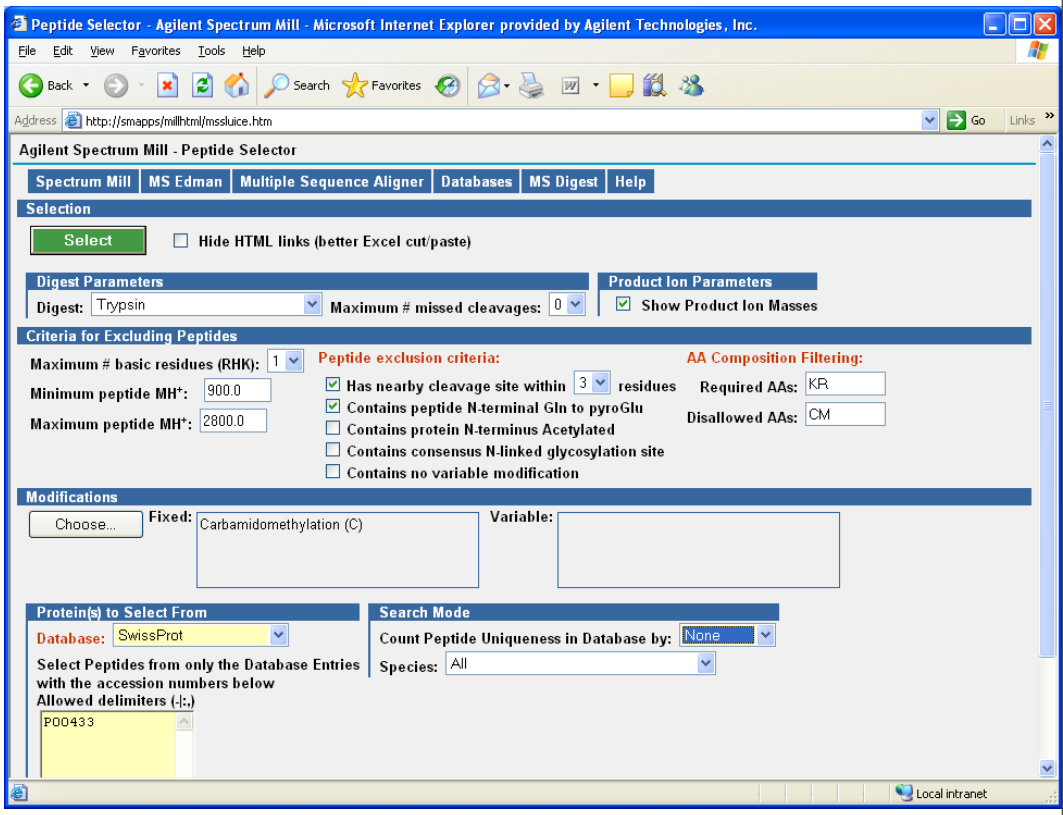
Peptides and MRM transitions were selected using Peptide Selector in Spectrum Mill and 13C, 15N labeled peptides were synthesized

Rat liver lysate were prepared from rats treated with troglitazone or vehicle control

1 mg of soluble protein extract was reduced, alkylated, acetone precipitated and trypsin digested

The liver digest were spiked with the isotope-labeled peptides and analyzed by Agilent 6410 QQQ system

Using Spectrum Mill Peptide Selector for Optimising MRM Transitions



Using Spectrum Mill Peptide Selector for Optimising MRM Transitions

Chemically reactive residues

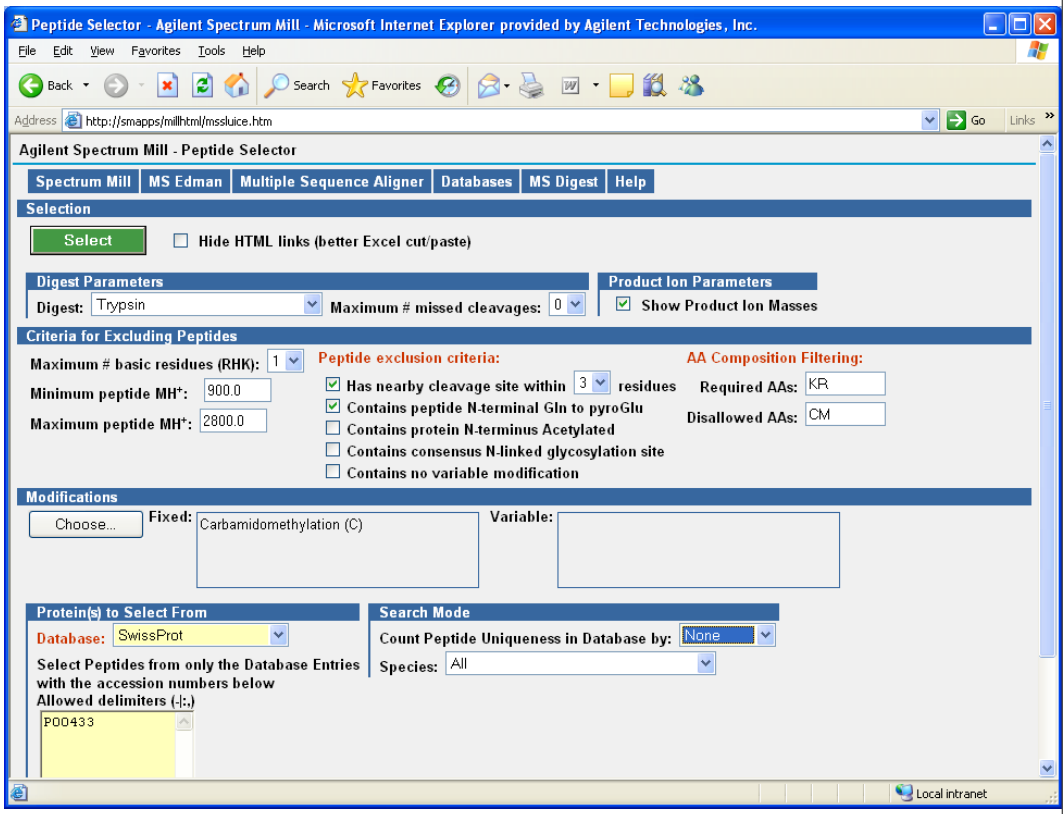
(Cys = C, Met = M, Trp = W)

Peptides adjacent to multiple cleavage site

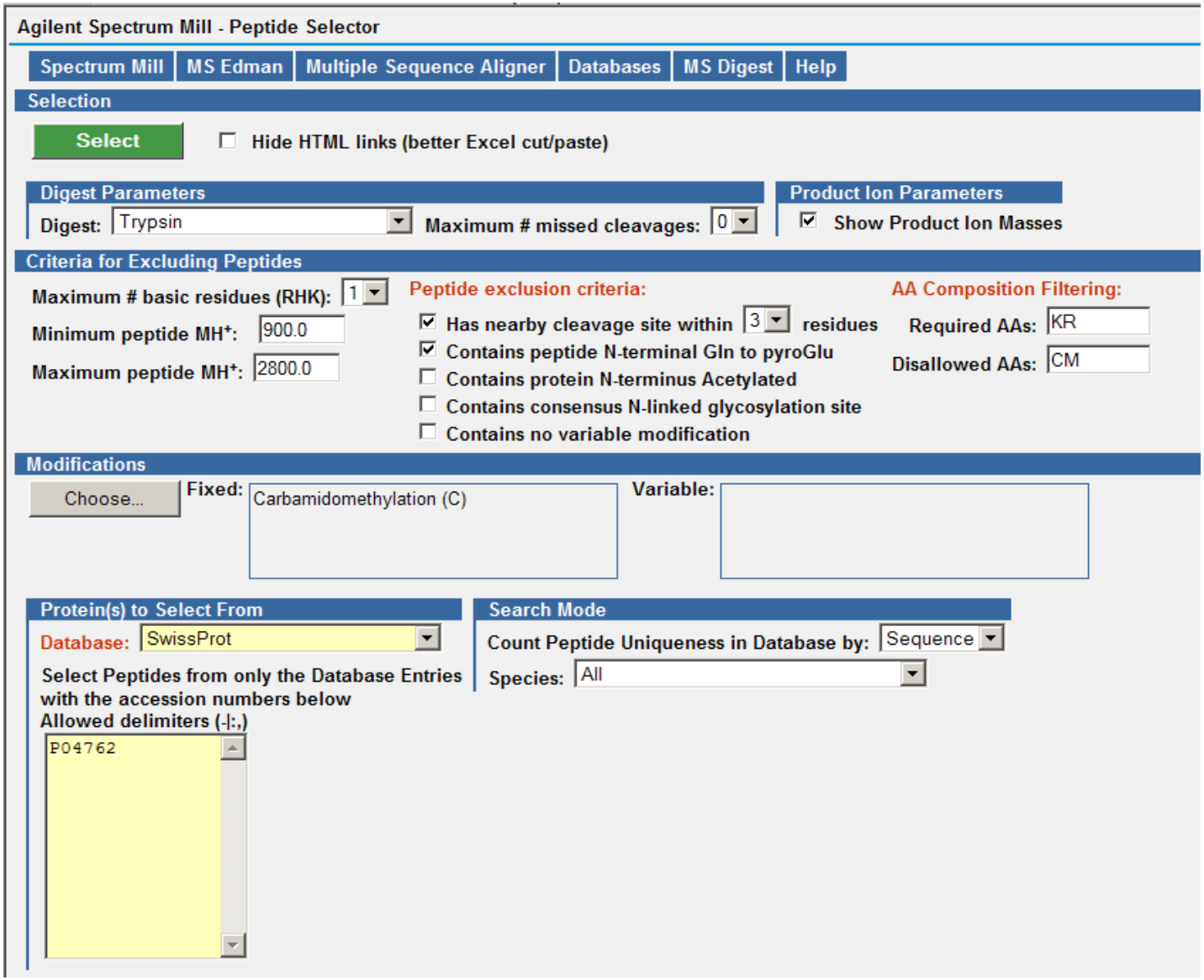
Chemically unstable residues (Asp-Gly = D-G; Asn-Gly = N-G; N-term Glu = E; N-term Asn = N)

Eliminate "LC-incompatible" peptides

Uniqueness



Spectrum Mill – Peptide Selector



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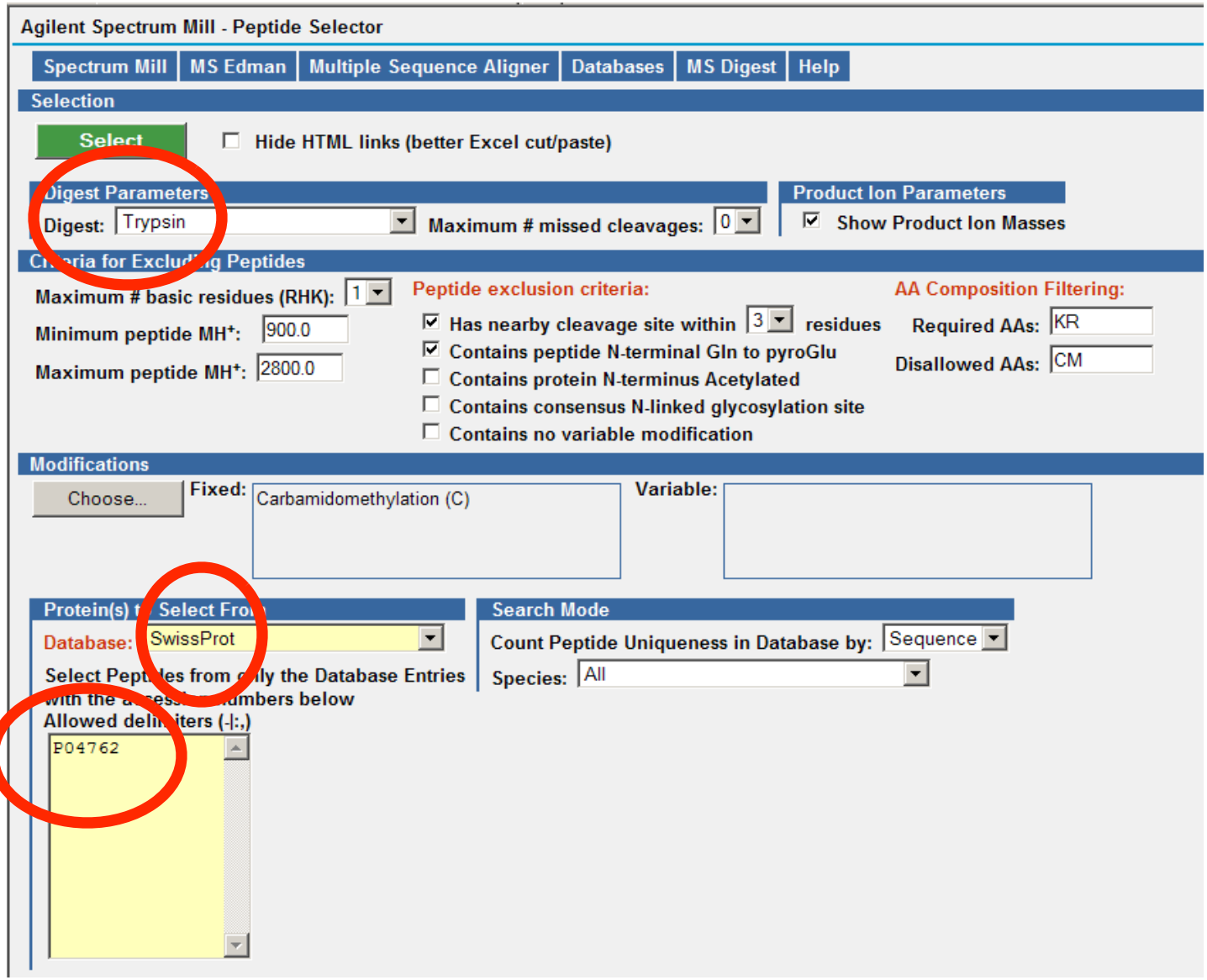

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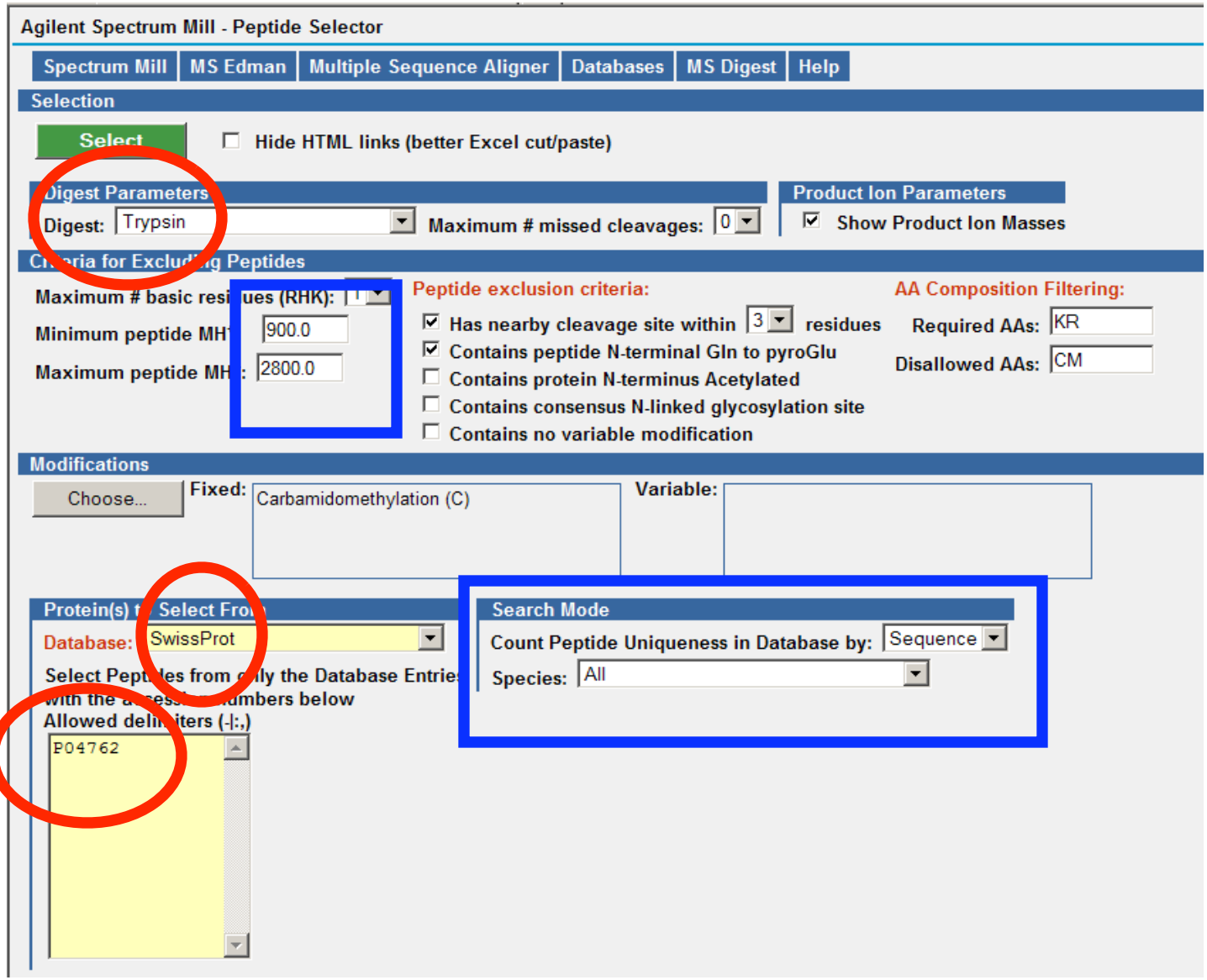

Peptide Selector – Catalase Results

Peptide Selector Results

Number of database entries:**211104**
Database: **SwissProt**
Exclusion criteria: | **Peptide N-terminal Gln to pyroGlu** |
Required AA's: **KR** Disallowed AA's: **CM**
Exclude if Nearby Cleavage Sites within **3** residues

Digest Used	Max. # Missed Cleavages	Fixed Mods	Peptide N terminus	Peptide C terminus	Masses are	Uniqueness Count by
Trypsin	**0**	**carbamidomethylation**	**Hydrogen (H)**	**Free Acid (O H)**	**Monoisotopic**	**Sequence**

Protein Name: **Catalase (EC 1.11.1.6)**
Species: **RAT**
SwissProt Accession #: **P04762**
MS-Digest Index #: **18675**
pI of Protein: **7.15**
Protein MW: **59626.3** Da
Amino Acid Composition: **A42 C6 D36 E25 F31 G35 H21 I20 K30 L31 M12 N33 P39 Q26 R30 S24 T25 V33 W6 Y21**

Protein Name	Acc #	RP-RT	MH+	m/2	# DB peps	Start AA	End AA	Prev.	Sequence	Next	b_2	y_n - y_1 C-side of Asp, Glu N-side of Pro							
Catalase (EC 1.11.1.6)	P04762	13.63	984.5109	492.7591	1	243	251	(GIK)	NLPVEEAGR	(LAQ)	**228.14**	870.47	757.38	660.33	561.26	432.22	303.18	232.14	175.1
Catalase (EC 1.11.1.6)	P04762	18.97	1001.5666	501.2869	6	306	314	(PHK)	DYPLIPVGK	(LVL)	**279.10**	886.54	723.48	626.42	513.34	400.26	303.20	204.13	147.1
Catalase (EC 1.11.1.6)	P04762	19.22	1276.6168	638.8120	2	252	262	(AGR)	LAQEDPDYGLR	(DLF)	**185.13**	1163.53	1092.50	964.44	835.39	720.37	623.31	508.29	345.2
Catalase (EC 1.11.1.6)	P04762	22.02	1655.7952	828.4012	2	287	300	(TFK)	EAETFPFNPFDLTK	(VWP)	**201.09**	1526.75	1455.72	1326.67	1225.63	1078.56	981.50	834.44	720.3
Catalase (EC 1.11.1.6)	P04762	26.89	2518.2038	1259.6055	7	135	155	(AVK)	FYTEDGNWDLVGNNTPIFFIR	(DAM)	**311.14**	2371.14	2208.07	2107.02	1977.98	1862.95	1805.93	1691.89	1505.8
Catalase (EC 1.11.1.6)	P04762				9														

```
1   ADSRDPASDQ MKQWKEQRAP QKPDVLTTGG GNPIGDKLNI MTAGPRGPLL VQDVVFTDEM AHFDRERIPE RVVHAKGAGA 80
81  FGYFEVTHDI TRYSKAKVFE HIGKRTPIAV RFSTVAGESG SADTVRDPRG FAVKFYTEDG NWDLVGNNTP IFFIRDAMLF 160
161 PSFIHSQKRN PQTHLKDPDM VWDFWSLCPE SLHQVTFLFS DRGIPDGHRH MNGYGSHTFK LVNANGEAVY CKFHYKTDQG 240
241 IKNLPVEEAG RLAQEDPDYG LRDLFNAIAS GNYPSWTFYI QVMTFKEAET FPFNPFDLTK VWPHKDYPLI PVGKLVLNRN 320
321 PANYFAEVEQ MAFDPSNMPP GIEPSPDKML QGRLFAYPDT HRHRLGPNYL QIPVNCPYRA RVANYQRDGP MCMHDNQGGA 400
401 PNYYPNSFSA PEQQGSALEH HSQCSADVKR FNSANEDNVT QVRTFYTKVL NEEERKRLCE NIANHLKDAQ LFIQRKAVKN 480
481 FTDVHPDYGA RVQALLDQYN SQKPKNAIHT YVQAGSHIAA KGKANL                                   526
```

The matched peptides cover **12%** (64/526 AA's) of the protein.

303.18	232.14	175.12						
303.20	204.13	147.11						
623.31	508.29	345.22	288.20	175.12				
981.50	834.44	720.39	623.34	476.27	361.24	248.16	147.11	
1805.93	1691.89	1505.81	1390.78	1277.70	1178.63	1121.61	1007.57	

Peptide Selector - Agilent Spectrum Mill Rev. 3.3.078

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Catalase (EC 1.11.1.6)	P04762	13.63	984.5109	492.7591	1	243	251	(GIK)	NLPVEEAGR	(LAQ)	**228.14**	870.47	757.38	660.33	561.26	432.22	303.18	232.14	175.1
Catalase (EC 1.11.1.6)	P04762	18.97	1001.5666	501.2869	6	306	314	(PHK)	DYPLIPVGK	(LVL)	**279.10**	886.54	723.48	626.42	513.34	400.26	303.20	204.13	147.1
Catalase (EC 1.11.1.6)	P04762	19.22	1276.6168	638.8120	2	252	262	(AGR)	LAQEDPDYGLR	(DLF)	**185.13**	1163.53	1092.50	964.44	835.39	720.37	623.31	508.29	345.2
Catalase (EC 1.11.1.6)	P04762	22.02	1655.7952	828.4012	2	287	300	(TFK)	EAETFPFNPFDLTK	(VWP)	**201.09**	1526.75	1455.72	1326.67	1225.63	1078.56	981.50	834.44	720.3
Catalase (EC 1.11.1.6)	P04762	26.89	2518.2038	1259.6055	7	135	155	(AVK)	FYTEDGNWDLVGNNTPIFFIR	(DAM)	**311.14**	2371.14	2208.07	2107.02	1977.98	1862.95	1805.93	1691.89	1505.8
Catalase (EC 1.11.1.6)	P04762				9														

```
1   ADSRDPASDQ MKQWKEQRAP QKPDVLTTGG GNPIGDKLNI MTAGPRGPLL VQDVVFTDEM AHFDRERIPE RVVHAKGAGA 80
81  FGYFEVTHDI TRYSKAKVFE HIGKRTPIAV RFSTVAGESG SADTVRDPRG FAVKFYTEDG NWDLVGNNTP IFFIRDAMLF 160
161 PSFIHSQKRN PQTHLKDPDM VWDFWSLCPE SLHQVTFLFS DRGIPDGHRH MNGYGSHTFK LVNANGEAVY CKFHYKTDQG 240
241 IKNLPVEEAG RLAQEDPDYG LRDLFNAIAS GNYPSWTFYI QVMTFKEAET FPFNPFDLTK VWPHKDYPLI PVGKLVLNRN 320
321 PANYFAEVEQ MAFDPSNMPP GIEPSPDKML QGRLFAYPDT HRHRLGPNYL QIPVNCPYRA RVANYQRDGP MCMHDNQGGA 400
401 PNYYPNSFSA PEQQGSALEH HSQCSADVKR FNSANEDNVT QVRTFYTKVL NEEERKRLCE NIANHLKDAQ LFIQRKAVKN 480
481 FTDVHPDYGA RVQALLDQYN SQKPKNAIHT YVQAGSHIAA KGKANL                                   526
```

The matched peptides cover **12%** (64/526 AA's) of the protein.

303.18	232.14	175.12						
303.20	204.13	147.11						
623.31	508.29	345.22	288.20	175.12				
981.50	834.44	720.39	623.34	476.27	361.24	248.16	147.11	
1805.93	1691.89	1505.81	1390.78	1277.70	1178.63	1121.61	1007.57	

Peptide Selector - Agilent Spectrum Mill Rev. 3.3.078

Peptide Selector – Catalase Results

Peptide Selector Results

Number of database entries: **211104**
Database: **SwissProt**
Exclusion criteria: | **Peptide N-terminal Gln to pyroGlu** |
Required AA's: **KR** Disallowed AA's: **CM**
Exclude if Nearby Cleavage Sites within **3** residues

Digest Used	Max. # Missed Cleavages	Fixed Mods	Peptide N terminus	Peptide C terminus	Masses are	Uniqueness Count by
Trypsin	**0**	**carbamidomethylation**	**Hydrogen (H)**	**Free Acid (O H)**	**Monoisotopic**	**Sequence**

Protein Name: **Catalase (EC 1.11.1.6)**
Species: **RAT**
SwissProt Accession #: **P04762**
MS-Digest Index #: **18675**
pI of Protein: **7.15**
Protein MW: **59626.3** Da
Amino Acid Composition: **A42 C0 D36 E25 F31 G35 H21 I20 K30 L31 M12 N33 P35 Q26 R30 S24 T25 V33 W6 Y21**

Protein Name	Acc #	RP-RT	MH+	m/2	# DB peps	Start AA	End AA	Prev.	Sequence	Next	b_2	$y_n - y_1$ C-side of Asp, Glu N-side of Pro							
Catalase (EC 1.11.1.6)	P04762	13.63	984.5109	492.7591	1	243	251	(GIK)	NLPVEEAGR	(LAQ)	**228.14**	870.47	757.38	660.33	561.26	432.22	303.18	232.14	175.1
Catalase (EC 1.11.1.6)	P04762	18.97	1001.5666	501.2869	6	306	314	(PHK)	DYPLIPVGK	(LVL)	**279.10**	886.54	723.48	626.42	513.34	400.26	303.20	204.13	147.1
Catalase (EC 1.11.1.6)	P04762	19.22	1276.6168	638.8120	2	252	262	(AGR)	LAQEDPDYGLR	(DLF)	**185.13**	1163.53	1092.50	964.44	835.39	720.37	623.31	508.29	345.2
Catalase (EC 1.11.1.6)	P04762	22.02	1655.7952	828.4012	2	287	300	(TFK)	EAETFPFN[illegible]EDLTK	(VWP)	**201.09**	1526.75	1455.72	1326.67	1225.63	1078.56	981.50	834.44	720.3
Catalase (EC 1.11.1.6)	P04762	26.89	2518.2038	1259.6055	7	135	155	(AVK)	FYTEDGNWD[illegible]VGNNTPIFFIR	(DAM)	**311.14**	2371.14	2208.07	2107.02	1977.98	1862.95	1805.93	1691.89	1505.8
Catalase (EC 1.11.1.6)	P04762				9														

```
1   ADSRDPASDQ MKQWKEQRAP QKPDVLTTGG GNPIGDKLNI MTAGPRGPLL VQDVVFTDEM AHFDRERIPE RVVHAKGAGA 80
81  FGYFEVTHDI TRYSKAKVFE HIGKRTPIAV RFSTVAGESG SADTVRDPRG FAVKFYTEDG NWDLVGNNTP IFFIRDAMLF 160
161 PSFIHSQKRN PQTHLKDPDM VWDFWSLCPE SLHQVTFLFS DRGIPDGHRH MNGYGSHTFK LVNANGEAVY CKFHYKTDQG 240
241 IKNLPVEEAG RLAQEDPDYG LRDLFNAIAS GNYPSWTFYI QVMTFKEAET FPFNPFDLTK VWPHKDYPLI PVGKLVLNRN 320
321 PANYFAEVEQ MAFDPSNMPP GIEPSPDKML QGRLFAYPDT HRHRLGPNYL QIPVNCPYRA RVANYQRDGP MCMHDNQGGA 400
401 PNYYPNSFSA PEQQGSALEH HSQCSADVKR FNSANEDNVT QVRTFYTKVL NEEERKRLCE NIANHLKDAQ LFIQRKAVKN 480
481 FTDVHPDYGA RVQALLDQYN SQKPKNAIHT YVQAGSHIAA KGKANL                                  526
```

The matched peptides cover **12%** (64/526 AA's) of the protein.

303.18	232.14	175.12						
303.20	204.13	147.11						
623.31	508.29	345.22	288.20	175.12				
981.50	834.44	720.39	623.34	476.27	361.24	248.16	147.11	
1805.93	1691.89	1505.81	1390.78	1277.70	1178.63	1121.61	1007.57	

Peptide Selector - Agilent Spectrum Mill Rev. 3.3.078

Catalase Peptide LAQ – Peptide Selector

Peptide Sequence: **LAQEDPDYGLR**
Peptide Mass MH+(Average): **1277.38**
Peptide Mass MH+(Monoisotopic): **1276.6168**
Elemental Compositon: C55 H86 N15 O20
Amino Acid Composition: **A1 D2 E1 G1 L2 P1 Q1 R1 Y1**

Fixed Modifications: **Carbamidomethylation**
Variable Modifications: **PTM - KMQSTY**
All fragment ion masses below are calculated as: **Monoisotopic** masses

$(MH)^{+2}$(Average): 639.1939
$(MH)^{+2}$(Monoisotopic): 638.8120

N-terminal ions

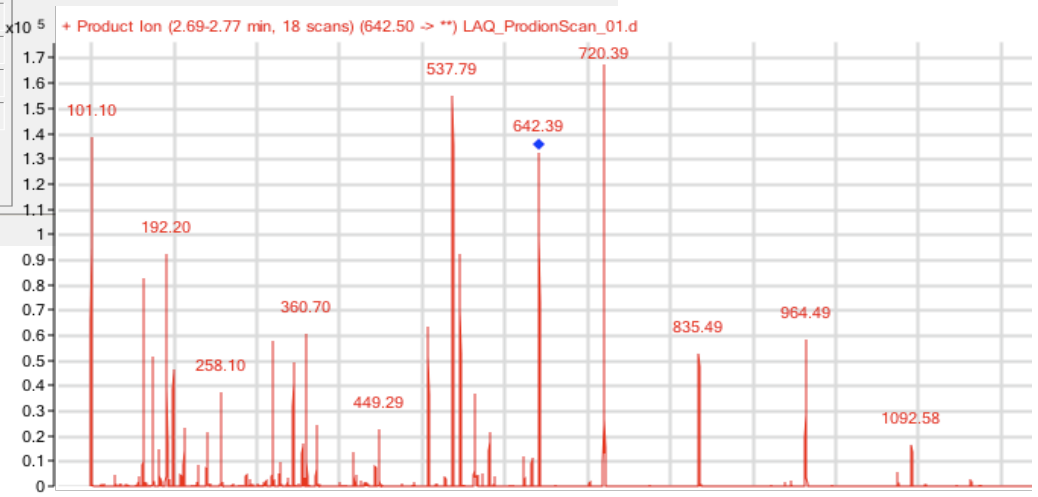
b ions	114.0924	185.1296	313.1881	442.2307	557.2577	654.3104	769.3374	932.4007	989.4222	1102.5062
	---	---	157.0977^{+2}	221.6190^{+2}	279.1325^{+2}	327.6589^{+2}	385.1723^{+2}	466.7040^{+2}	495.2147^{+2}	551.7568^{+2}
	1	2	3	4	5	6	7	8	9	10	11
H -	L	A	Q	E	D	P	D	Y	G	L	R	- O H
	11	10	9	8	7	6	5	4	3	2	1	

C-terminal ions

1163.5327	1092.4956	964.4371	835.3945	720.3675	623.3148	508.2878	345.2245	288.2030	175.1190	y ions
582.2700^{+2}	546.7515^{+2}	482.7222^{+2}	418.2009^{+2}	360.6874^{+2}	312.1610^{+2}	254.6475^{+2}	173.1159^{+2}	144.6051^{+2}	88.0631^{+2}	

y_1^{+2}	88.0631^{+2}	y_4^{+2}	254.6475^{+2}	b_7^{+2}	385.1723^{+2}	b_{10}^{+2}	551.7568^{+2}	b_8	932.4007
b_1	114.0924	b_5^{+2}	279.1325^{+2}	y_7^{+2}	418.2009^{+2}	b_5	557.2577	y_8	964.4371
y_2^{+2}	144.6051^{+2}	y_2	288.2030	b_4	442.2307	y_{10}^{+2}	582.2700^{+2}	b_9	989.4222
b_3^{+2}	157.0977^{+2}	y_5^{+2}	312.1610^{+2}	b_8^{+2}	466.7040^{+2}	y_5	623.3148	y_9	1092.4956
y_3^{+2}	173.1159^{+2}	b_3	313.1881	y_8^{+2}	482.7222^{+2}	b_6	654.3104	b_{10}	1102.5062
y_1	175.1190	b_6^{+2}	327.6589^{+2}	b_9^{+2}	495.2147^{+2}	y_6	720.3675	y_{10}	1163.5327
b_2	185.1296	y_3	345.2245	y_4	508.2878	b_7	769.3374		
b_4^{+2}	221.6190^{+2}	y_6^{+2}	360.6874^{+2}	y_9^{+2}	546.7515^{+2}	y_7	835.3945		

MS Product - Agilent Spectrum Mill Rev. 3.3.078



Catalase Peptide LAQ – Peptide Selector

Peptide Sequence: **LAQEDPDYGLR**
Peptide Mass MH+(Average): **1277.38**
Peptide Mass MH+(Monoisotopic): **1276.6168**
Elemental Compositon: C55 H86 N15 O20
Amino Acid Composition: **A1 D2 E1 G1 L2 P1 Q1 R1 Y1**

Fixed Modifications: **Carbamidomethylation**
Variable Modifications: **PTM - KMQSTY**
All fragment ion masses below are calculated as: **Monoisotopic** masses

$(MH)^{+2}$(Average): 639.1939
$(MH)^{+2}$(Monoisotopic): 638.8120

N-terminal ions

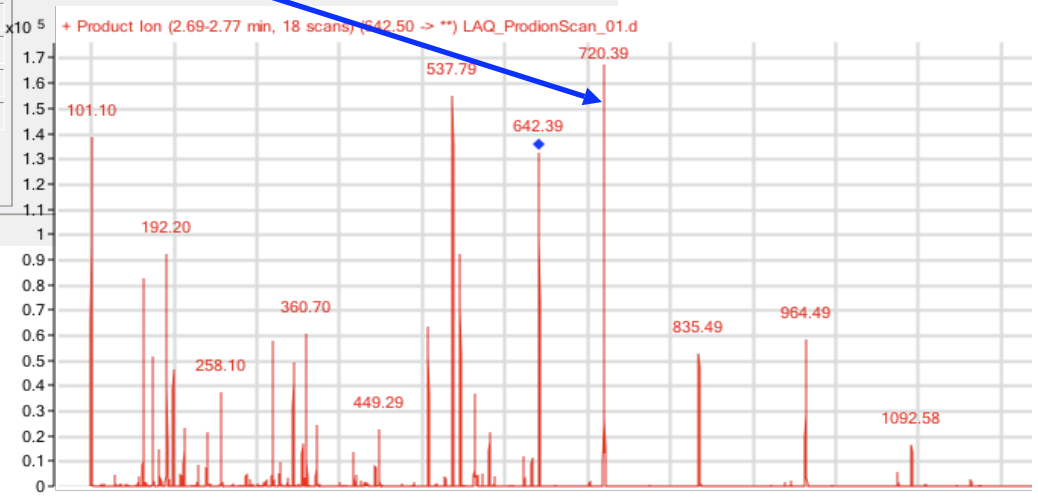
b ions	114.0924	185.1296	313.1881	442.2307	557.2577	654.3104	769.3374	932.4007	989.4222	1102.5062
	---	---	157.0977^{+2}	221.6190^{+2}	279.1325^{+2}	327.6589^{+2}	385.1723^{+2}	466.7040^{+2}	495.2147^{+2}	551.7568^{+2}
	1	2	3	4	5	6	7	8	9	10	11
H -	L	A	Q	E	D	P	D	Y	G	L	R	- O H
	11	10	9	8	7	6	5	4	3	2	1	

C-terminal ions

	1163.5327	1092.4956	964.4371	835.3945	720.3675	623.3148	508.2878	345.2245	288.2030	175.1190	y ions
	582.2700^{+2}	546.7515^{+2}	482.7222^{+2}	418.2009^{+2}	360.6874^{+2}	312.1610^{+2}	254.6475^{+2}	173.1159^{+2}	144.6051^{+2}	88.0631^{+2}	

y_1^{+2}	88.0631^{+2}	y_4^{+2}	254.6475^{+2}	b_7^{+2}	385.1723^{+2}	b_{10}^{+2}	551.7568^{+2}	b_8	932.4007
b_1	114.0924	b_5^{+2}	279.1325^{+2}	y_7^{+2}	418.2009^{+2}	b_5	557.2577	y_8	964.4371
y_2^{+2}	144.6051^{+2}	y_2	288.2030	b_4	442.2307	y_{10}^{+2}	582.2700^{+2}	b_9	989.4222
b_3^{+2}	157.0977^{+2}	y_5^{+2}	312.1610^{+2}	b_8^{+2}	466.7040^{+2}	y_5	623.3148	y_9	1092.4956
y_3^{+2}	173.1159^{+2}	b_3	313.1881	y_8^{+2}	482.7222^{+2}	b_6	654.3104	b_{10}	1102.5062
y_1	175.1190	b_6^{+2}	327.6589^{+2}	b_9^{+2}	495.2147^{+2}	y_6	720.3675	y_{10}	1163.5327
b_2	185.1296	y_3	345.2245	y_4	508.2878	b_7	769.3374		
b_4^{+2}	221.6190^{+2}	y_6^{+2}	360.6874^{+2}	y_9^{+2}	546.7515^{+2}	y_7	835.3945		

MS Product - Agilent Spectrum Mill Rev. 3.3.078



Catalase Peptide LAQ – Peptide Selector

Peptide Sequence: **LAQEDPDYGLR**
Peptide Mass MH+(Average): **1277.38**
Peptide Mass MH+(Monoisotopic): **1276.6168**
Elemental Compositon: C55 H86 N15 O20
Amino Acid Composition: **A1 D2 E1 G1 L2 P1 Q1 R1 Y1**

Fixed Modifications: **Carbamidomethylation**
Variable Modifications: **PTM - KMQSTY**
All fragment ion masses below are calculated as: **Monoisotopic** masses

$(MH)^{+2}$(Average): 639.1939
$(MH)^{+2}$(Monoisotopic): 638.8120

N-terminal ions

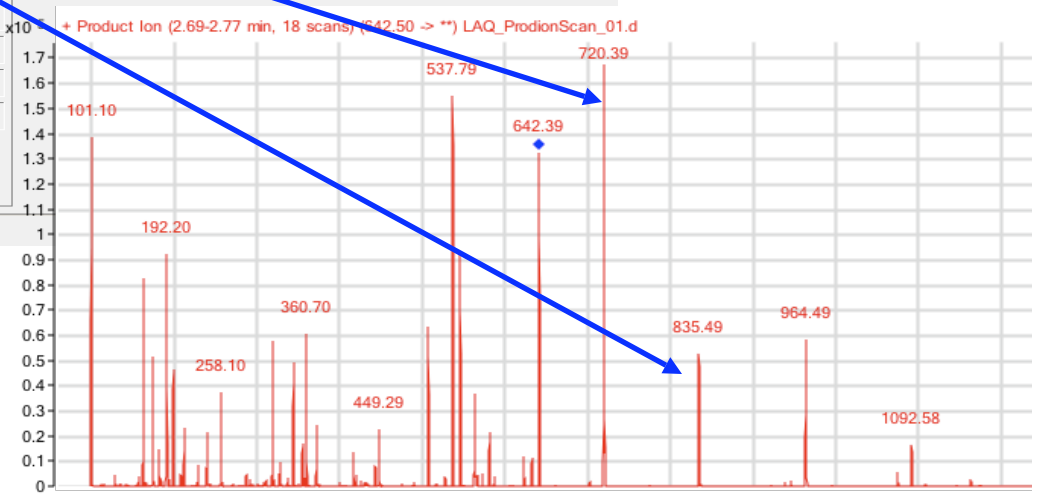
b ions	114.0924	185.1296	313.1881	442.2307	557.2577	654.3104	769.3374	932.4007	989.4222	1102.5062
	---	---	157.0977^{+2}	221.6190^{+2}	279.1325^{+2}	327.6589^{+2}	385.1723^{+2}	466.7040^{+2}	495.2147^{+2}	551.7568^{+2}
	1	2	3	4	5	6	7	8	9	10	11
H -	L	A	Q	E	D	P	D	Y	G	L	R	- O H
	11	10	9	8	7	6	5	4	3	2	1	

C-terminal ions

1163.5327	1092.4956	964.4371	835.3945	720.3675	623.3148	508.2878	345.2245	288.2030	175.1190	y ions
582.2700^{+2}	546.7515^{+2}	482.7222^{+2}	418.2009^{+2}	360.6874^{+2}	312.1610^{+2}	254.6475^{+2}	173.1159^{+2}	144.6051^{+2}	88.0631^{+2}	

y_1^{+2}	88.0631^{+2}	y_4^{+2}	254.6475^{+2}	b_7^{+2}	385.1723^{+2}	b_{10}^{+2}	551.7568^{+2}	b_8	932.4007
b_1	114.0924	b_5^{+2}	279.1325^{+2}	y_7^{+2}	418.2009^{+2}	b_5	557.2577	y_8	964.4371
y_2^{+2}	144.6051^{+2}	y_2	288.2030	b_4	442.2307	y_{10}^{+2}	582.2700^{+2}	b_9	989.4222
b_3^{+2}	157.0977^{+2}	y_5^{+2}	312.1610^{+2}	b_8^{+2}	466.7040^{+2}	y_5	623.3148	y_9	1092.4956
y_3^{+2}	173.1159^{+2}	b_3	313.1881	y_8^{+2}	482.7222^{+2}	b_6	654.3104	b_{10}	1102.5062
y_1	175.1190	b_6^{+2}	327.6589^{+2}	b_9^{+2}	495.2147^{+2}	y_6	720.3675	y_{10}	1163.5327
b_2	185.1296	y_3	345.2245	y_4	508.2878	b_7	769.3374		
b_4^{+2}	221.6190^{+2}	y_6^{+2}	360.6874^{+2}	y_9^{+2}	546.7515^{+2}	y_7	835.3945		

MS Product - Agilent Spectrum Mill Rev. 3.3.078



Catalase Peptide LAQ – Peptide Selector

Peptide Sequence: **LAQEDPDYGLR**
Peptide Mass MH+(Average): **1277.38**
Peptide Mass MH+(Monoisotopic): **1276.6168**
Elemental Compositon: C55 H86 N15 O20
Amino Acid Composition: **A1 D2 E1 G1 L2 P1 Q1 R1 Y1**

Fixed Modifications: **Carbamidomethylation**
Variable Modifications: **PTM - KMQSTY**
All fragment ion masses below are calculated as: **Monoisotopic** masses

$(MH)^{+2}$(Average): 639.1939
$(MH)^{+2}$(Monoisotopic): 638.8120

N-terminal ions

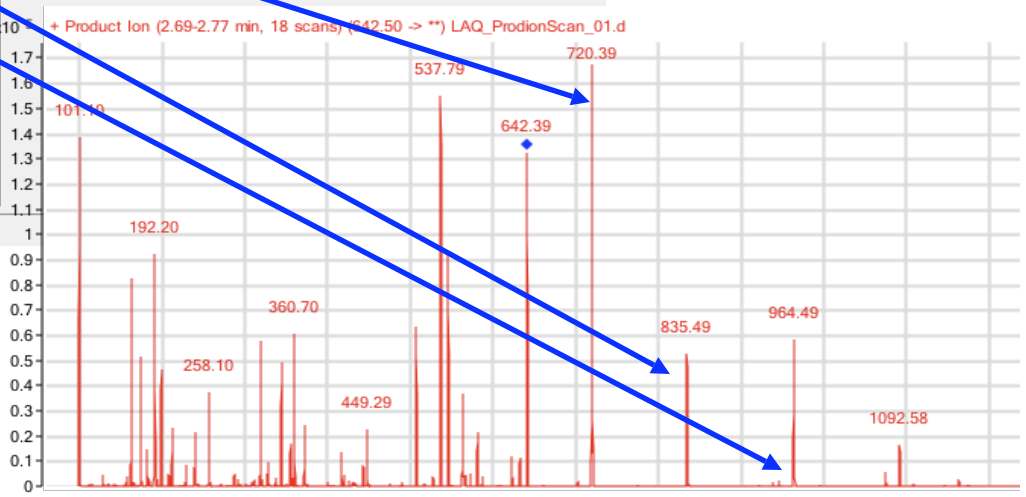
b ions	114.0924	185.1296	313.1881	442.2307	557.2577	654.3104	769.3374	932.4007	989.4222	1102.5062
	---	---	157.0977^{+2}	221.6190^{+2}	279.1325^{+2}	327.6589^{+2}	385.1723^{+2}	466.7040^{+2}	495.2147^{+2}	551.7568^{+2}
	1	2	3	4	5	6	7	8	9	10	11
H -	L	A	Q	E	D	P	D	Y	G	L	R	- O H
	11	10	9	8	7	6	5	4	3	2	1	

C-terminal ions

	1163.5327	1092.4956	964.4371	835.3945	720.3675	623.3148	508.2878	345.2245	288.2030	175.1190	y ions
	582.2700^{+2}	546.7515^{+2}	482.7222^{+2}	418.2009^{+2}	360.6874^{+2}	312.1610^{+2}	254.6475^{+2}	173.1159^{+2}	144.6051^{+2}	88.0631^{+2}	

y_1^{+2}	88.0631^{+2}	y_4^{+2}	254.6475^{+2}	b_7^{+2}	385.1723^{+2}	b_{10}^{+2}	551.7568^{+2}	b_8	932.4007
b_1	114.0924	b_5^{+2}	279.1325^{+2}	y_7^{+2}	418.2009^{+2}	b_5	557.2577	y_8	964.4371
y_2^{+2}	144.6051^{+2}	y_2	288.2030	b_4	442.2307	y_{10}^{+2}	582.2700^{+2}	b_9	989.4222
b_3^{+2}	157.0977^{+2}	y_5^{+2}	312.1610^{+2}	b_8^{+2}	466.7040^{+2}	y_5	623.3148	y_9	1092.4956
y_3^{+2}	173.1159^{+2}	b_3	313.1881	y_8^{+2}	482.7222^{+2}	b_6	654.3104	b_{10}	1102.5062
y_1	175.1190	b_6^{+2}	327.6589^{+2}	b_9^{+2}	495.2147^{+2}	y_6	720.3675	y_{10}	1163.5327
b_2	185.1296	y_3	345.2245	y_4	508.2878	b_7	769.3374		
b_4^{+2}	221.6190^{+2}	y_6^{+2}	360.6874^{+2}	y_9^{+2}	546.7515^{+2}	y_7	835.3945		

MS Product - Agilent Spectrum Mill Rev. 3.3.078



Catalase Peptide LAQ – Peptide Selector

Peptide Sequence: **LAQEDPDYGLR**
Peptide Mass MH+(Average): **1277.38**
Peptide Mass MH+(Monoisotopic): **1276.6168**
Elemental Compositon: C55 H86 N15 O20
Amino Acid Composition: **A1 D2 E1 G1 L2 P1 Q1 R1 Y1**

Fixed Modifications: **Carbamidomethylation**
Variable Modifications: **PTM - KMQSTY**
All fragment ion masses below are calculated as: **Monoisotopic** masses

$(MH)^{+2}$(Average): 639.1939
$(MH)^{+2}$(Monoisotopic): 638.8120

N-terminal ions

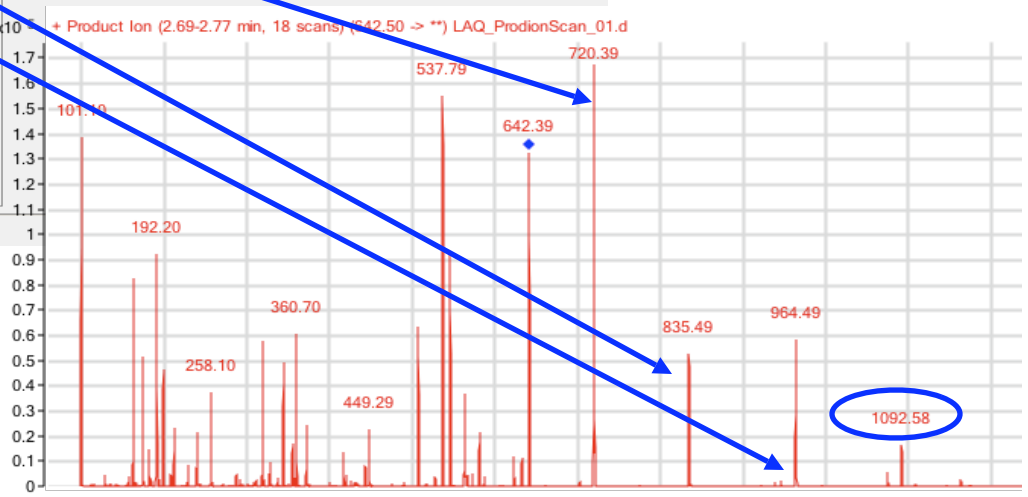
b ions	114.0924	185.1296	313.1881	442.2307	557.2577	654.3104	769.3374	932.4007	989.4222	1102.5062
	---	---	157.0977^{+2}	221.6190^{+2}	279.1325^{+2}	327.6589^{+2}	385.1723^{+2}	466.7040^{+2}	495.2147^{+2}	551.7568^{+2}
	1	2	3	4	5	6	7	8	9	10	11
H -	L	A	Q	E	D	P	D	Y	G	L	R	- O H
	11	10	9	8	7	6	5	4	3	2	1	

C-terminal ions

1163.5327	1092.4956	964.4371	835.3945	720.3675	623.3148	508.2878	345.2245	288.2030	175.1190	y ions
582.2700^{+2}	546.7515^{+2}	482.7222^{+2}	418.2009^{+2}	360.6874^{+2}	312.1610^{+2}	254.6475^{+2}	173.1159^{+2}	144.6051^{+2}	88.0631^{+2}	

y_1^{+2}	88.0631^{+2}	y_4^{+2}	254.6475^{+2}	b_7^{+2}	385.1723^{+2}	b_{10}^{+2}	551.7568^{+2}	b_8	932.4007
b_1	114.0924	b_5^{+2}	279.1325^{+2}	y_7^{+2}	418.2009^{+2}	b_5	557.2577	y_8	964.4371
y_2^{+2}	144.6051^{+2}	y_2	288.2030	b_4	442.2307	y_{10}^{+2}	582.2700^{+2}	b_9	989.4222
b_3^{+2}	157.0977^{+2}	y_5^{+2}	312.1610^{+2}	b_8^{+2}	466.7040^{+2}	y_5	623.3148	y_9	1092.4956
y_3^{+2}	173.1159^{+2}	b_3	313.1881	y_8^{+2}	482.7222^{+2}	b_6	654.3104	b_{10}	1102.5062
y_1	175.1190	b_6^{+2}	327.6589^{+2}	b_9^{+2}	495.2147^{+2}	y_6	720.3675	y_{10}	1163.5327
b_2	185.1296	y_3	345.2245	y_4	508.2878	b_7	769.3374		
b_4^{+2}	221.6190^{+2}	y_6^{+2}	360.6874^{+2}	y_9^{+2}	546.7515^{+2}	y_7	835.3945		

MS Product - Agilent Spectrum Mill Rev. 3.3.078



Catalase Peptide LAQ – Peptide Selector

Peptide Sequence: **LAQEDPDYGLR**
Peptide Mass MH+(Average): **1277.38**
Peptide Mass MH+(Monoisotopic): **1276.6168**
Elemental Compositon: C55 H86 N15 O20
Amino Acid Composition: **A1 D2 E1 G1 L2 P1 Q1 R1 Y1**

Fixed Modifications: **Carbamidomethylation**
Variable Modifications: **PTM - KMQSTY**
All fragment ion masses below are calculated as: **Monoisotopic** masses

$(MH)^{+2}$(Average): 639.1939
$(MH)^{+2}$(Monoisotopic): 638.8120

N-terminal ions

b ions	114.0924	185.1296	313.1881	442.2307	557.2577	654.3104	769.3374	932.4007	989.4222	1102.5062
	---	---	157.0977^{+2}	221.6190^{+2}	279.1325^{+2}	327.6589^{+2}	385.1723^{+2}	466.7040^{+2}	495.2147^{+2}	551.7568^{+2}

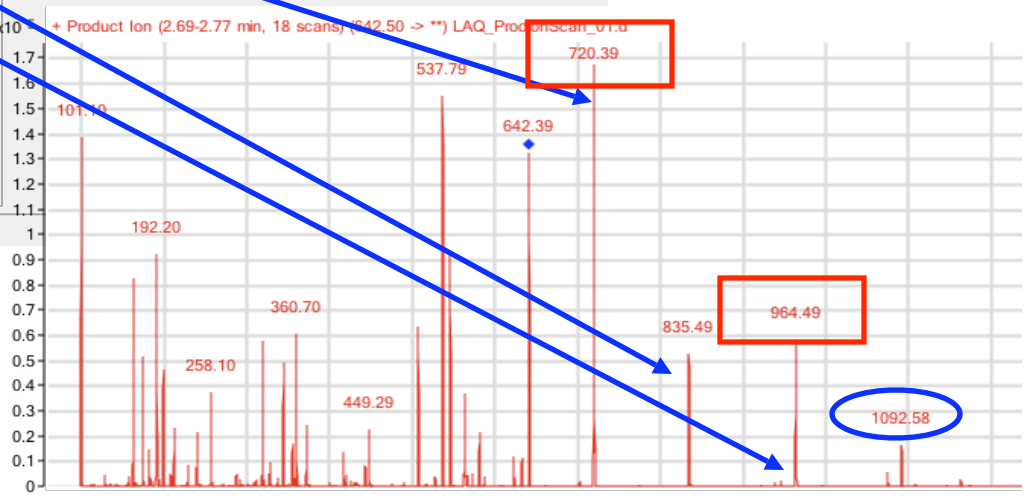
	1	2	3	4	5	6	7	8	9	10	11	
H -	L	A	Q	E	D	P	D	Y	G	L	R	- O H
	11	10	9	8	7	6	5	4	3	2	1	

C-terminal ions

1163.5327	1092.4956	964.4371	835.3945	720.3675	623.3148	508.2878	345.2245	288.2030	175.1190	y ions
582.2700^{+2}	546.7515^{+2}	482.7222^{+2}	418.2009^{+2}	360.6874^{+2}	312.1610^{+2}	254.6475^{+2}	173.1159^{+2}	144.6051^{+2}	88.0631^{+2}	

y_1^{+2}	88.0631^{+2}	y_4^{+2}	254.6475^{+2}	b_7^{+2}	385.1723^{+2}	b_{10}^{+2}	551.7568^{+2}	b_8	932.4007
b_1	114.0924	b_5^{+2}	279.1325^{+2}	y_7^{+2}	418.2009^{+2}	b_5	557.2577	y_8	964.4371
y_2^{+2}	144.6051^{+2}	y_2	288.2030	b_4	442.2307	y_{10}^{+2}	582.2700^{+2}	b_9	989.4222
b_3^{+2}	157.0977^{+2}	y_5^{+2}	312.1610^{+2}	b_8^{+2}	466.7040^{+2}	y_5	623.3148	y_9	1092.4956
y_3^{+2}	173.1159^{+2}	b_3	313.1881	y_8^{+2}	482.7222^{+2}	b_6	654.3104	b_{10}	1102.5062
y_1	175.1190	b_6^{+2}	327.6589^{+2}	b_9^{+2}	495.2147^{+2}	y_6	720.3675	y_{10}	1163.5327
b_2	185.1296	y_3	345.2245	y_4	508.2878	b_7	769.3374		
b_4^{+2}	221.6190^{+2}	y_6^{+2}	360.6874^{+2}	y_9^{+2}	546.7515^{+2}	y_7	835.3945		

MS Product - Agilent Spectrum Mill Rev. 3.3.078



Catalase Peptide EAE – Peptide Selector

Peptide Sequence: **EAETFPFNPFDLTK**
Peptide Mass MH^+(Average): **1656.84**
Peptide Mass MH^+(Monoisotopic): **1655.7952**
Elemental Compositon: C78 H111 N16 O24
Amino Acid Composition: **A1 D1 E2 F3 K1 L1 N1 P2 T2**

Fixed Modifications: **Carbamidomethylation**
Variable Modifications: **PTM - KMQSTY**
All fragment ion masses below are calculated as: **Monoisotopic** masses

$(MH)^{+2}$(Average): 828.9225
$(MH)^{+2}$(Monoisotopic): 828.4012

N-terminal ions

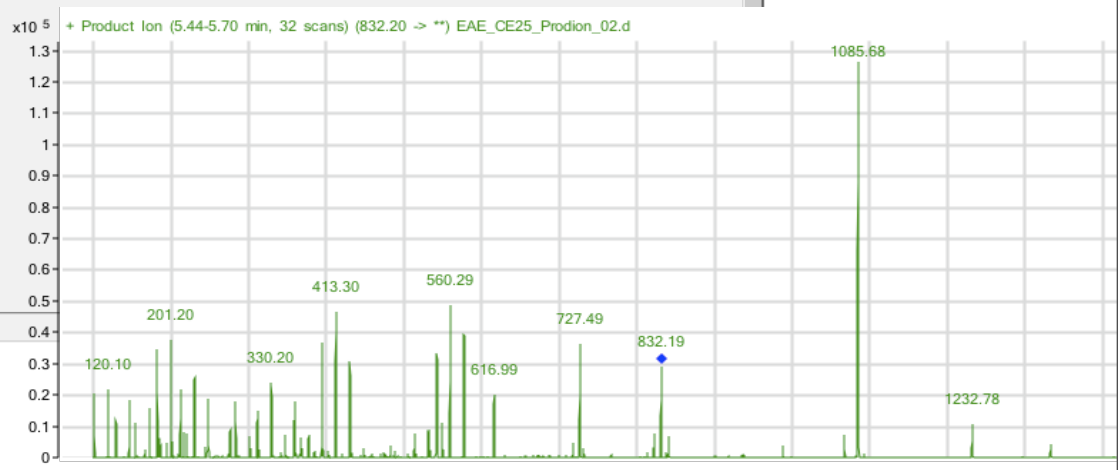
b ions	130.0510	201.0881	330.1307	431.1784	578.2468	675.2995	822.3679	936.4109	1033.4636	1180.5320	1295.5590	1408.6431	1509.6907
	---	---	---	---	---	---	---	468.7091^{+2}	517.2355^{+2}	590.7697^{+2}	648.2831^{+2}	704.8252^{+2}	755.3490^{+2}
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
H -	E	A	E	T	F	P	F	N	P	F	D	L	T	K	- O H
	14	13	12	11	10	9	8	7	6	5	4	3	2	1	

C-terminal ions

1526.7526	1455.7155	1326.6729	1225.6252	1078.5568	981.5040	834.4356	720.3927	623.3399	476.2715	361.2445	248.1605	147.1128	y ions
763.8799^{+2}	728.3614^{+2}	663.8401^{+2}	613.3162^{+2}	539.7820^{+2}	491.2556^{+2}	417.7214^{+2}	360.7000^{+2}	312.1736^{+2}	238.6394^{+2}	181.1259^{+2}	124.5839^{+2}	74.0600^{+2}	

y_1^{+2}	74.0600^{+2}	b_3	330.1307	y_9^{+2}	539.7820^{+2}	y_6	720.3927	y_9	1078.5568
y_2^{+2}	124.5839^{+2}	y_6^{+2}	360.7000^{+2}	b_5	578.2468	y_{12}^{+2}	728.3614^{+2}	b_{10}	1180.5320
b_1	130.0510	y_3	361.2445	b_{10}^{+2}	590.7697^{+2}	b_{13}^{+2}	755.3490^{+2}	y_{10}	1225.6252
y_1	147.1128	y_7^{+2}	417.7214^{+2}	y_{10}^{+2}	613.3162^{+2}	y_{13}^{+2}	763.8799^{+2}	b_{11}	1295.5590
y_3^{+2}	181.1259^{+2}	b_4	431.1784	y_5	623.3399	b_7	822.3679	y_{11}	1326.6729
b_2	201.0881	b_8^{+2}	468.7091^{+2}	b_{11}^{+2}	648.2831^{+2}	y_7	834.4356	b_{12}	1408.6431
y_4^{+2}	238.6394^{+2}	y_4	476.2715	y_{11}^{+2}	663.8401^{+2}	b_8	936.4109	y_{12}	1455.7155
y_2	248.1605	y_8^{+2}	491.2556^{+2}	b_6	675.2995	y_8	981.5040	b_{13}	1509.6907
y_5^{+2}	312.1736^{+2}	b_9^{+2}	517.2355^{+2}	b_{12}^{+2}	704.8252^{+2}	b_9	1033.4636	y_{13}	1526.7526

MS Product - Agilent Spectrum Mill Rev. 3.3.078



Catalase Peptide EAE – Peptide Selector

Peptide Sequence: **EAETFPFNPFDLTK**
Peptide Mass MH^+(Average): **1656.84**
Peptide Mass MH^+(Monoisotopic): **1655.7952**
Elemental Compositon: C78 H111 N16 O24
Amino Acid Composition: **A1 D1 E2 F3 K1 L1 N1 P2 T2**

Fixed Modifications: **Carbamidomethylation**
Variable Modifications: **PTM - KMQSTY**
All fragment ion masses below are calculated as: **Monoisotopic** masses

$(MH)^{+2}$(Average): 828.9225
$(MH)^{+2}$(Monoisotopic): 828.4012

N-terminal ions

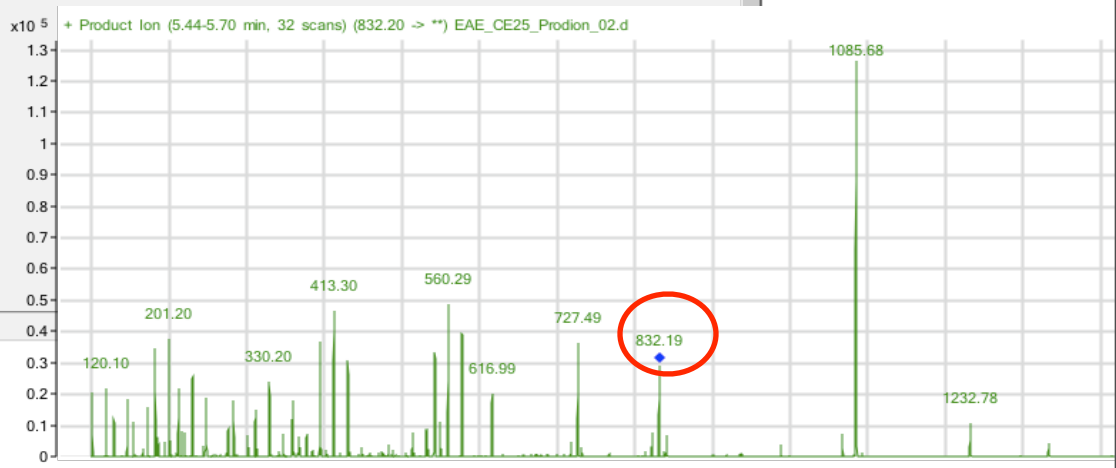
b ions	130.0510	201.0881	330.1307	431.1784	578.2468	675.2995	822.3679	936.4109	1033.4636	1180.5320	1295.5590	1408.6431	1509.6907
	---	---	---	---	---	---	---	468.7091^{+2}	517.2355^{+2}	590.7697^{+2}	648.2831^{+2}	704.8252^{+2}	755.3490^{+2}
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
H -	E	A	E	T	F	P	F	N	P	F	D	L	T	K	- O H
	14	13	12	11	10	9	8	7	6	5	4	3	2	1	

C-terminal ions

	1526.7526	1455.7155	1326.6729	1225.6252	1078.5568	981.5040	834.4356	720.3927	623.3399	476.2715	361.2445	248.1605	147.1128	y ions
	763.8799^{+2}	728.3614^{+2}	663.8401^{+2}	613.3162^{+2}	539.7820^{+2}	491.2556^{+2}	417.7214^{+2}	360.7000^{+2}	312.1736^{+2}	238.6394^{+2}	181.1259^{+2}	124.5839^{+2}	74.0600^{+2}	

y_1^{+2}	74.0600^{+2}	b_3	330.1307	y_9^{+2}	539.7820^{+2}	y_6	720.3927	y_9	1078.5568
y_2^{+2}	124.5839^{+2}	y_6^{+2}	360.7000^{+2}	b_5	578.2468	y_{12}^{+2}	728.3614^{+2}	b_{10}	1180.5320
b_1	130.0510	y_3	361.2445	b_{10}^{+2}	590.7697^{+2}	b_{13}^{+2}	755.3490^{+2}	y_{10}	1225.6252
y_1	147.1128	y_7^{+2}	417.7214^{+2}	y_{10}^{+2}	613.3162^{+2}	y_{13}^{+2}	763.8799^{+2}	b_{11}	1295.5590
y_3^{+2}	181.1259^{+2}	b_4	431.1784	y_5	623.3399	b_7	822.3679	y_{11}	1326.6729
b_2	201.0881	b_8^{+2}	468.7091^{+2}	b_{11}^{+2}	648.2831^{+2}	y_7	834.4356	b_{12}	1408.6431
y_4^{+2}	238.6394^{+2}	y_4	476.2715	y_{11}^{+2}	663.8401^{+2}	b_8	936.4109	y_{12}	1455.7155
y_2	248.1605	y_8^{+2}	491.2556^{+2}	b_6	675.2995	y_8	981.5040	b_{13}	1509.6907
y_5^{+2}	312.1736^{+2}	b_9^{+2}	517.2355^{+2}	b_{12}^{+2}	704.8252^{+2}	b_9	1033.4636	y_{13}	1526.7526

MS Product - Agilent Spectrum Mill Rev. 3.3.078



Catalase Peptide EAE – Peptide Selector

Peptide Sequence: **EAETFPFNPFDLTK**
Peptide Mass MH^+(Average): **1656.84**
Peptide Mass MH^+(Monoisotopic): **1655.7952**
Elemental Compositon: C78 H111 N16 O24
Amino Acid Composition: **A1 D1 E2 F3 K1 L1 N1 P2 T2**

Fixed Modifications: **Carbamidomethylation**
Variable Modifications: **PTM - KMQSTY**
All fragment ion masses below are calculated as: **Monoisotopic** masses

$(MH)^{+2}$(Average): 828.9225
$(MH)^{+2}$(Monoisotopic): 828.4012

N-terminal ions														
b ions	130.0510	201.0881	330.1307	431.1784	578.2468	675.2995	822.3679	936.4109	1033.4636	1180.5320	1295.5590	1408.6431	1509.6907	
	---	---	---	---	---	---	---	468.7091^{+2}	517.2355^{+2}	590.7697^{+2}	648.2831^{+2}	704.8252^{+2}	755.3490^{+2}	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	H - E	A	E	T	F	P	F	N	P	F	D	L	T	K - O H
	14	13	12	11	10	9	8	7	6	5	4	3	2	1
C-terminal ions														
	1526.7526	1455.7155	1326.6729	1225.6252	1078.5568	981.5040	834.4356	720.3927	623.3399	476.2715	361.2445	248.1605	147.1128	y ions
	763.8799^{+2}	728.3614^{+2}	663.8401^{+2}	613.3162^{+2}	539.7820^{+2}	491.2556^{+2}	417.7214^{+2}	360.7000^{+2}	312.1736^{+2}	238.6394^{+2}	181.1259^{+2}	124.5839^{+2}	74.0600^{+2}	

y_1^{+2}	74.0600^{+2}	b_3	330.1307	y_9^{+2}	539.7820^{+2}	y_6	720.3927	y_9	1078.5568
y_2^{+2}	124.5839^{+2}	y_6^{+2}	360.7000^{+2}	b_5	578.2468	y_{12}^{+2}	728.3614^{+2}	b_{10}	1180.5320
b_1	130.0510	y_3	361.2445	b_{10}^{+2}	590.7697^{+2}	b_{13}^{+2}	755.3490^{+2}	y_{10}	1225.6252
y_1	147.1128	y_7^{+2}	417.7214^{+2}	y_{10}^{+2}	613.3162^{+2}	y_{13}^{+2}	763.8799^{+2}	b_{11}	1295.5590
y_3^{+2}	181.1259^{+2}	b_4	431.1784	y_5	623.3399	b_7	822.3679	y_{11}	1326.6729
b_2	201.0881	b_8^{+2}	468.7091^{+2}	b_{11}^{+2}	648.2831^{+2}	y_7	834.4356	b_{12}	1408.6431
y_4^{+2}	238.6394^{+2}	y_4	476.2715	y_{11}^{+2}	663.8401^{+2}	b_8	936.4109	y_{12}	1455.7155
y_2	248.1605	y_8^{+2}	491.2556^{+2}	b_6	675.2995	y_8	981.5040	b_{13}	1509.6907
y_5^{+2}	312.1736^{+2}	b_9^{+2}	517.2355^{+2}	b_{12}^{+2}	704.8252^{+2}	b_9	1033.4636	y_{13}	1526.7526

MS Product - Agilent Spectrum Mill Rev. 3.3.078

Catalase Peptide EAE – Peptide Selector

Peptide Sequence: **EAETFPFNPFDLTK**
Peptide Mass MH^+(Average): **1656.84**
Peptide Mass MH^+(Monoisotopic): **1655.7952**
Elemental Compositon: C78 H111 N16 O24
Amino Acid Composition: **A1 D1 E2 F3 K1 L1 N1 P2 T2**

Fixed Modifications: **Carbamidomethylation**
Variable Modifications: **PTM - KMQSTY**
All fragment ion masses below are calculated as: **Monoisotopic** masses

$(MH)^{+2}$(Average): 828.9225
$(MH)^{+2}$(Monoisotopic): 828.4012

N-terminal ions

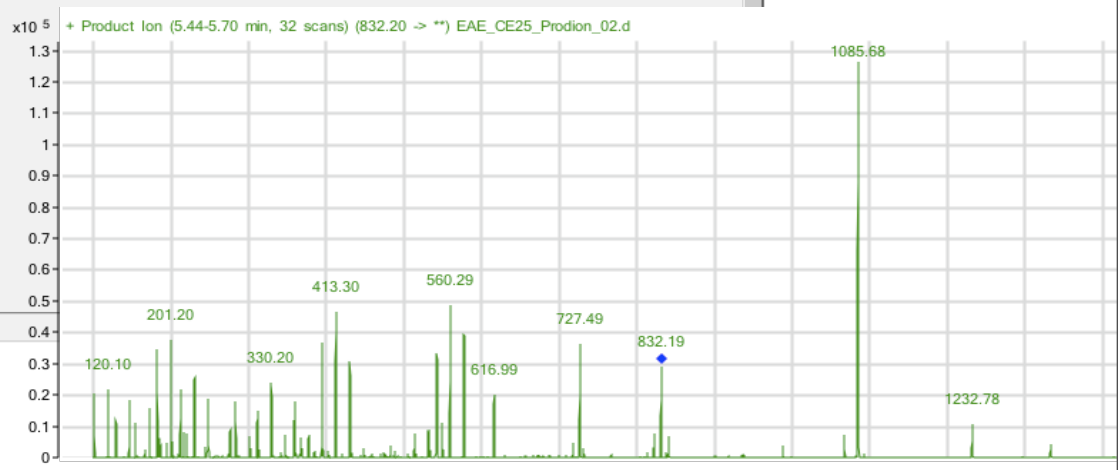
b ions	130.0510	201.0881	330.1307	431.1784	578.2468	675.2995	822.3679	936.4109	1033.4636	1180.5320	1295.5590	1408.6431	1509.6907
	---	---	---	---	---	---	---	468.7091^{+2}	517.2355^{+2}	590.7697^{+2}	648.2831^{+2}	704.8252^{+2}	755.3490^{+2}
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
H -	E	A	E	T	F	P	F	N	P	F	D	L	T	K	- O H
	14	13	12	11	10	9	8	7	6	5	4	3	2	1	

C-terminal ions

1526.7526	1455.7155	1326.6729	1225.6252	1078.5568	981.5040	834.4356	720.3927	623.3399	476.2715	361.2445	248.1605	147.1128	y ions
763.8799^{+2}	728.3614^{+2}	663.8401^{+2}	613.3162^{+2}	539.7820^{+2}	491.2556^{+2}	417.7214^{+2}	360.7000^{+2}	312.1736^{+2}	238.6394^{+2}	181.1259^{+2}	124.5839^{+2}	74.0600^{+2}	

y_1^{+2}	74.0600^{+2}	b_3	330.1307	y_9^{+2}	539.7820^{+2}	y_6	720.3927	y_9	1078.5568
y_2^{+2}	124.5839^{+2}	y_6^{+2}	360.7000^{+2}	b_5	578.2468	y_{12}^{+2}	728.3614^{+2}	b_{10}	1180.5320
b_1	130.0510	y_3	361.2445	b_{10}^{+2}	590.7697^{+2}	b_{13}^{+2}	755.3490^{+2}	y_{10}	1225.6252
y_1	147.1128	y_7^{+2}	417.7214^{+2}	y_{10}^{+2}	613.3162^{+2}	y_{13}^{+2}	763.8799^{+2}	b_{11}	1295.5590
y_3^{+2}	181.1259^{+2}	b_4	431.1784	y_5	623.3399	b_7	822.3679	y_{11}	1326.6729
b_2	201.0881	b_8^{+2}	468.7091^{+2}	b_{11}^{+2}	648.2831^{+2}	y_7	834.4356	b_{12}	1408.6431
y_4^{+2}	238.6394^{+2}	y_4	476.2715	y_{11}^{+2}	663.8401^{+2}	b_8	936.4109	y_{12}	1455.7155
y_2	248.1605	y_8^{+2}	491.2556^{+2}	b_6	675.2995	y_8	981.5040	b_{13}	1509.6907
y_5^{+2}	312.1736^{+2}	b_9^{+2}	517.2355^{+2}	b_{12}^{+2}	704.8252^{+2}	b_9	1033.4636	y_{13}	1526.7526

MS Product - Agilent Spectrum Mill Rev. 3.3.078



Catalase Peptide EAE – Peptide Selector

Peptide Sequence: **EAETFPFNPFDLTK**
Peptide Mass MH^+(Average): **1656.84**
Peptide Mass MH^+(Monoisotopic): **1655.7952**
Elemental Compositon: C78 H111 N16 O24
Amino Acid Composition: **A1 D1 E2 F3 K1 L1 N1 P2 T2**

Fixed Modifications: **Carbamidomethylation**
Variable Modifications: **PTM - KMQSTY**
All fragment ion masses below are calculated as: **Monoisotopic** masses

$(MH)^{+2}$(Average): 828.9225
$(MH)^{+2}$(Monoisotopic): 828.4012

N-terminal ions

b ions	130.0510	201.0881	330.1307	431.1784	578.2468	675.2995	822.3679	936.4109	1033.4636	1180.5320	1295.5590	1408.6431	1509.6907
	---	---	---	---	---	---	---	468.7091^{+2}	517.2355^{+2}	590.7697^{+2}	648.2831^{+2}	704.8252^{+2}	755.3490^{+2}
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
H -	E	A	E	T	F	P	F	N	P	F	D	L	T	K	- O H
	14	13	12	11	10	9	8	7	6	5	4	3	2	1	

C-terminal ions

1526.7526	1455.7155	1326.6729	1225.6252	1078.5568	981.5040	834.4356	720.3927	623.3399	476.2715	361.2445	248.1605	147.1128	y ions
763.8799^{+2}	728.3614^{+2}	663.8401^{+2}	613.3162^{+2}	539.7820^{+2}	491.2556^{+2}	417.7214^{+2}	360.7000^{+2}	312.1736^{+2}	238.6394^{+2}	181.1259^{+2}	124.5839^{+2}	74.0600^{+2}	

y_1^{+2}	74.0600^{+2}	b_3	330.1307	y_9^{+2}	539.7820^{+2}	y_6	720.3927	y_9	1078.5568
y_2^{+2}	124.5839^{+2}	y_6^{+2}	360.7000^{+2}	b_5	578.2468	y_{12}^{+2}	728.3614^{+2}	b_{10}	1180.5320
b_1	130.0510	y_3	361.2445	b_{10}^{+2}	590.7697^{+2}	b_{13}^{+2}	755.3490^{+2}	y_{10}	1225.6252
y_1	147.1128	y_7^{+2}	417.7214^{+2}	y_{10}^{+2}	613.3162^{+2}	y_{13}^{+2}	763.8799^{+2}	b_{11}	1295.5590
y_3^{+2}	181.1259^{+2}	b_4	431.1784	y_5	623.3399	b_7	822.3679	y_{11}	1326.6729
b_2	201.0881	b_8^{+2}	468.7091^{+2}	b_{11}^{+2}	648.2831^{+2}	y_7	834.4356	b_{12}	1408.6431
y_4^{+2}	238.6394^{+2}	y_4	476.2715	y_{11}^{+2}	663.8401^{+2}	b_8	936.4109	y_{12}	1455.7155
y_2	248.1605	y_8^{+2}	491.2556^{+2}	b_6	675.2995	y_8	981.5040	b_{13}	1509.6907
y_5^{+2}	312.1736^{+2}	b_9^{+2}	517.2355^{+2}	b_{12}^{+2}	704.8252^{+2}	b_9	1033.4636	y_{13}	1526.7526

MS Product - Agilent Spectrum Mill Rev. 3.3.078

Catalase Peptide EAE – Peptide Selector

Peptide Sequence: **EAETFPFNPFDLTK**
Peptide Mass MH^+(Average): **1656.84**
Peptide Mass MH^+(Monoisotopic): **1655.7952**
Elemental Compositon: C78 H111 N16 O24
Amino Acid Composition: **A1 D1 E2 F3 K1 L1 N1 P2 T2**

Fixed Modifications: **Carbamidomethylation**
Variable Modifications: **PTM - KMQSTY**
All fragment ion masses below are calculated as: **Monoisotopic** masses

$(MH)^{+2}$(Average): 828.9225
$(MH)^{+2}$(Monoisotopic): 828.4012

N-terminal ions

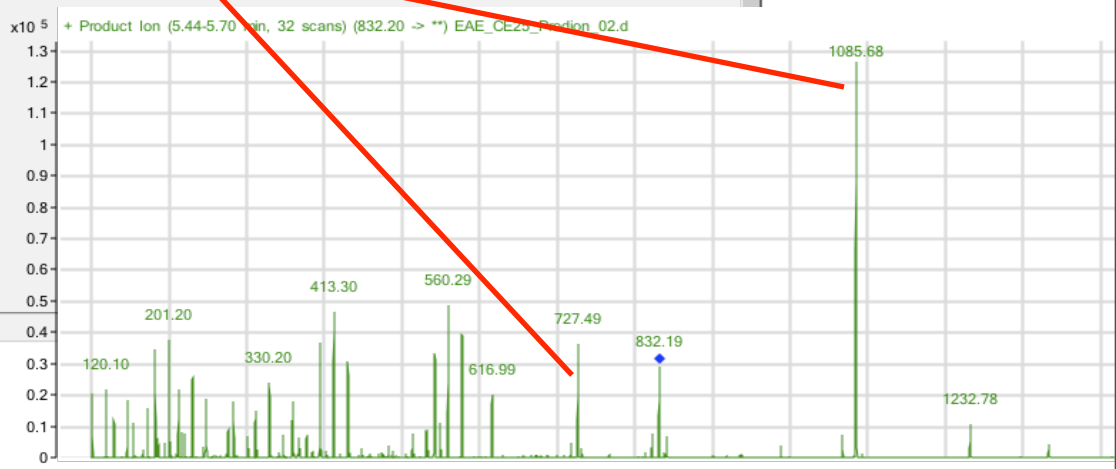
b ions	130.0510	201.0881	330.1307	431.1784	578.2468	675.2995	822.3679	936.4109	1033.4636	1180.5320	1295.5590	1408.6431	1509.6907
	---	---	---	---	---	---	---	468.7091^{+2}	517.2355^{+2}	590.7697^{+2}	648.2831^{+2}	704.8252^{+2}	755.3490^{+2}
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	H - E	A	E	T	F	P	F	N	P	F	D	L	T	K - O H
	14	13	12	11	10	9	8	7	6	5	4	3	2	1

C-terminal ions

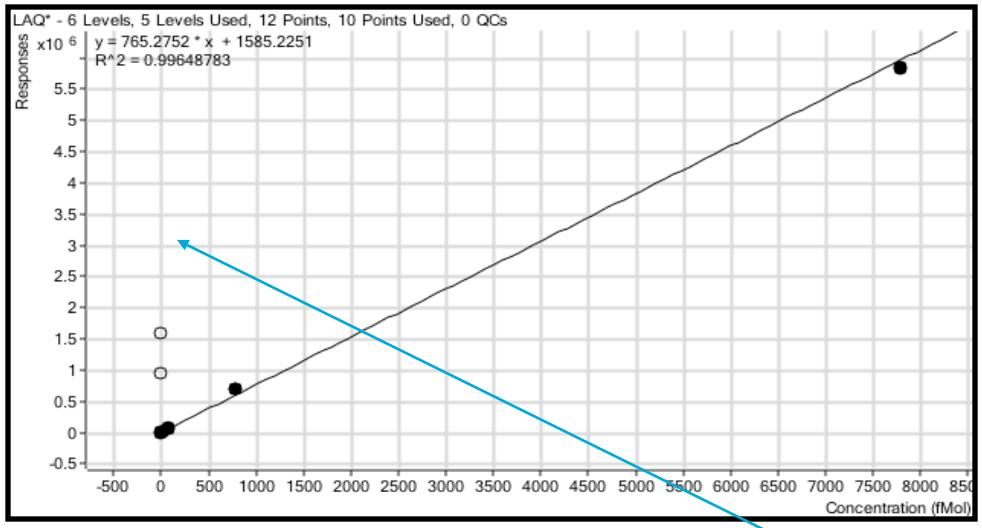
	1526.7526	1455.7155	1326.6729	1225.6252	1078.5568	981.5040	834.4356	720.3927	623.3399	476.2715	361.2445	248.1605	147.1128	y ions
	763.8799^{+2}	728.3614^{+2}	663.8401^{+2}	613.3162^{+2}	539.7820^{+2}	491.2556^{+2}	417.7214^{+2}	360.7000^{+2}	312.1736^{+2}	238.6394^{+2}	181.1259^{+2}	124.5839^{+2}	74.0600^{+2}	

y_1^{+2}	74.0600^{+2}	b_3	330.1307	y_9^{+2}	539.7820^{+2}	y_6	720.3927	y_9	1078.5568
y_2^{+2}	124.5839^{+2}	y_6^{+2}	360.7000^{+2}	b_5	578.2468	y_{12}^{+2}	728.3614^{+2}	b_{10}	1180.5320
b_1	130.0510	y_3	361.2445	b_{10}^{+2}	590.7697^{+2}	b_{13}^{+2}	755.3490^{+2}	y_{10}	1225.6252
y_1	147.1128	y_7^{+2}	417.7214^{+2}	y_{10}^{+2}	613.3162^{+2}	y_{13}^{+2}	763.8799^{+2}	b_{11}	1295.5590
y_3^{+2}	181.1259^{+2}	b_4	431.1784	y_5	623.3399	b_7	822.3679	y_{11}	1326.6729
b_2	201.0881	b_8^{+2}	468.7091^{+2}	b_{11}^{+2}	648.2831^{+2}	y_7	834.4356	b_{12}	1408.6431
y_4^{+2}	238.6394^{+2}	y_4	476.2715	y_{11}^{+2}	663.8401^{+2}	b_8	936.4109	y_{12}	1455.7155
y_2	248.1605	y_8^{+2}	491.2556^{+2}	b_6	675.2995	y_8	981.5040	b_{13}	1509.6907
y_5^{+2}	312.1736^{+2}	b_9^{+2}	517.2355^{+2}	b_{12}^{+2}	704.8252^{+2}	b_9	1033.4636	y_{13}	1526.7526

MS Product - Agilent Spectrum Mill Rev. 3.3.078



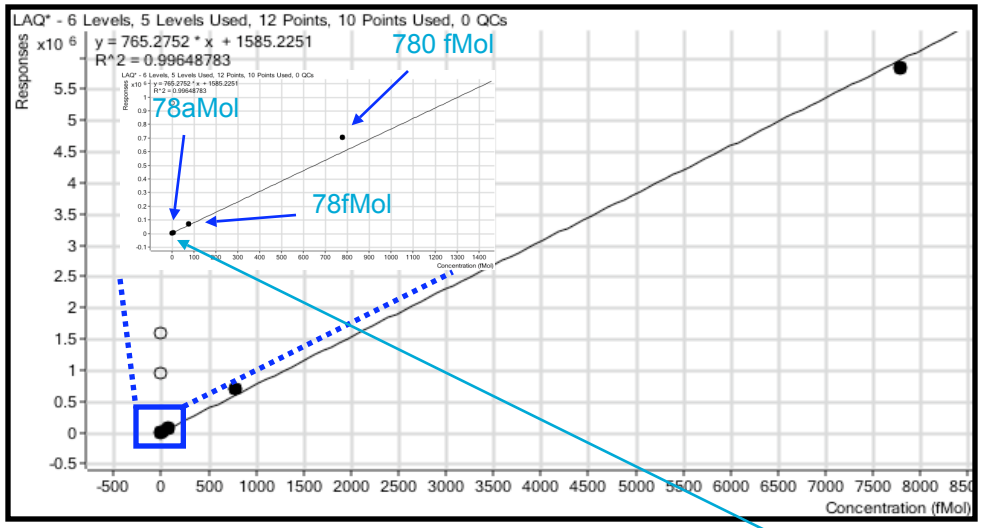
External Calibration on Catalase Peptides Linearity : five order of magnitude



External quantitation curve of catalase peptide L*AQEDPDYGLR from 78 amol to 7800 fmol

RSD < 6%

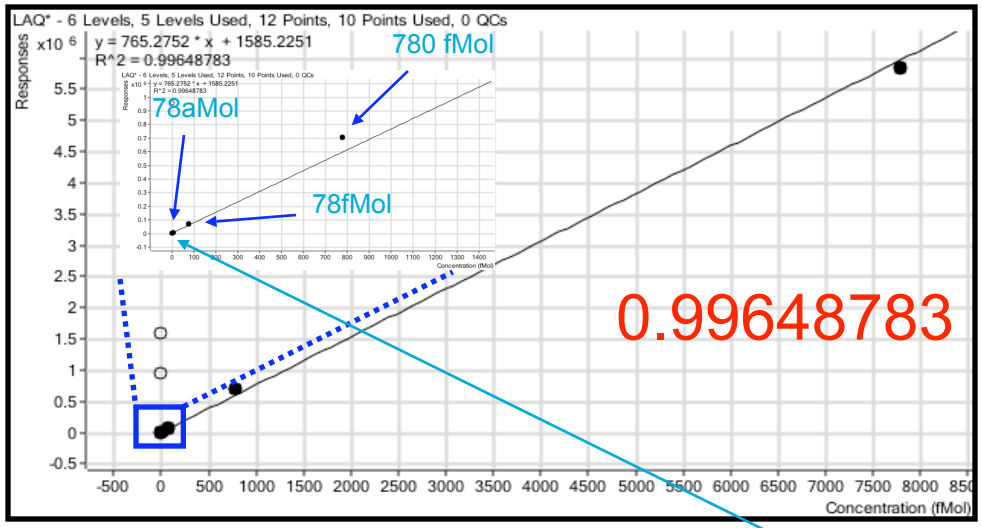
External Calibration on Catalase Peptides Linearity : five order of magnitude



External quantitation curve of catalase peptide L*AQEDPDYGLR from 78 amol to 7800 fmol

RSD < 6%

External Calibration on Catalase Peptides Linearity : five order of magnitude

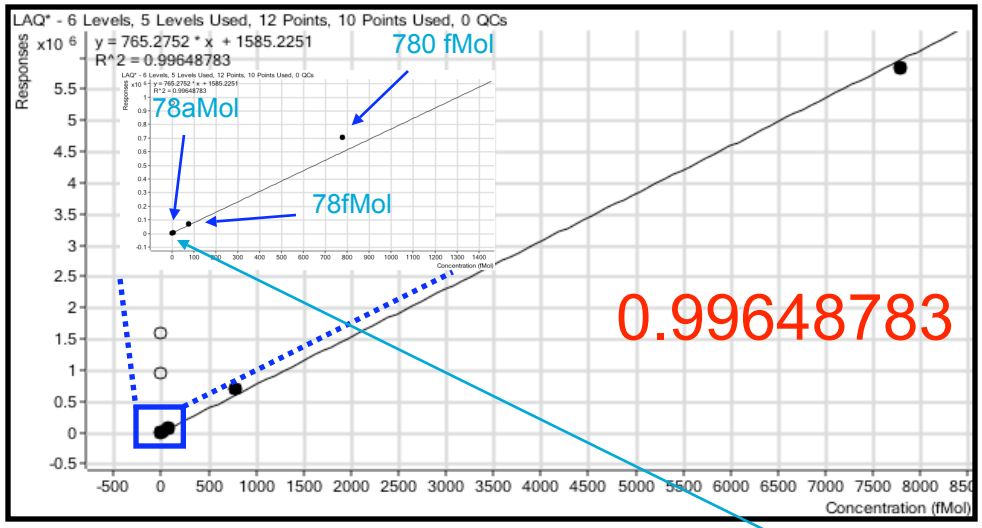


0.99648783

External quantitation curve of catalase peptide L*AQEDPDYGLR from 78 amol to 7800 fmol

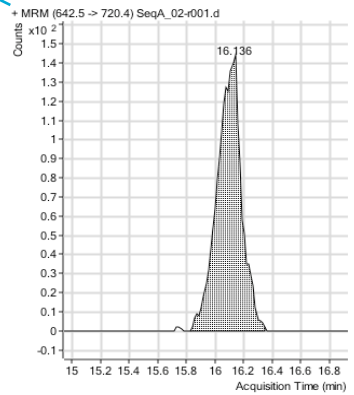
RSD < 6%

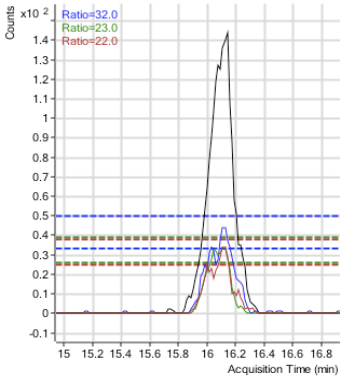
External Calibration on Catalase Peptides Linearity : five order of magnitude



External quantitation curve of catalase peptide L*AQEDPDYGLR from 78 amol to 7800 fmol

RSD < 6%

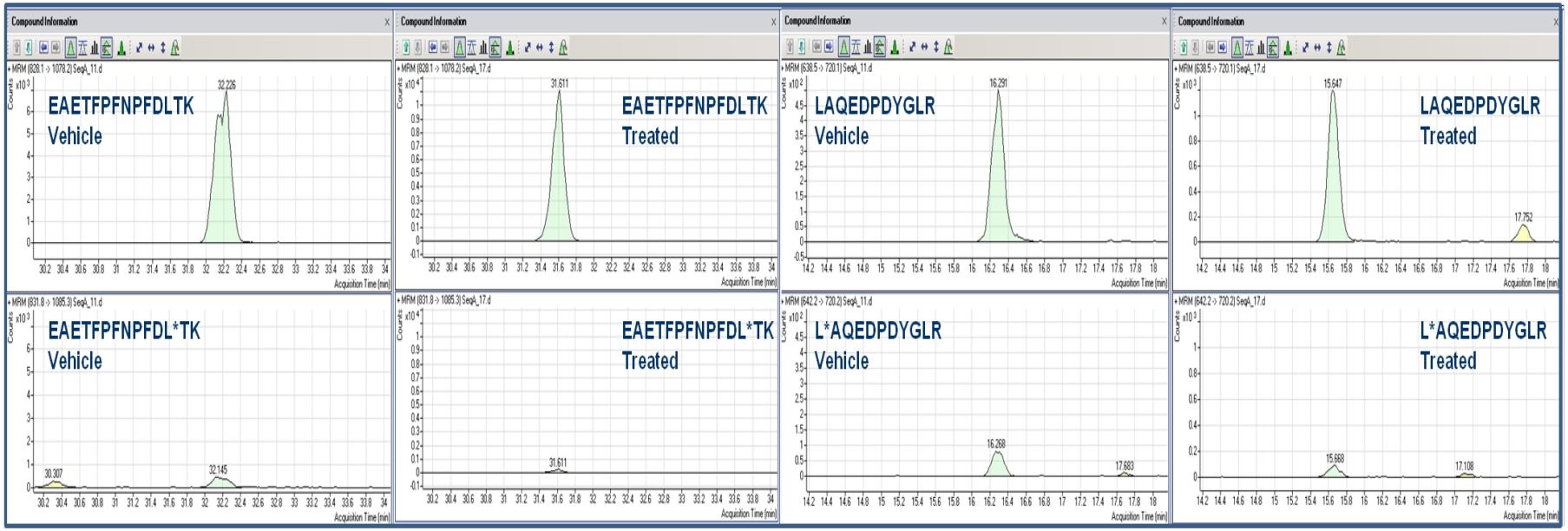




Catalase Quantitation Results

Sample					EAE Met...	EAE Results						Qualifier (828.1 -> 720.1) Results			EAE* (ISTD) Results		Qualifier (831.8 -> 727.3) Results		
		Name	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	S/N	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	S/N	MI	RT	Resp.	Ratio	S/N	MI
▶		RL_Veh_1ppb	Cal	1	2/27/2008 7:49 AM		32.226	85633	169.81		14.6295	14.6295		10.9	44.30		32.145	5853	20.6	2.71	
		RL_HDose_1ppb	Cal	1	2/27/2008 3:09 PM		31.611	104655	298.12		54.3647	54.3647		10.6	71.79		31.611	1925	11.2	1.03	

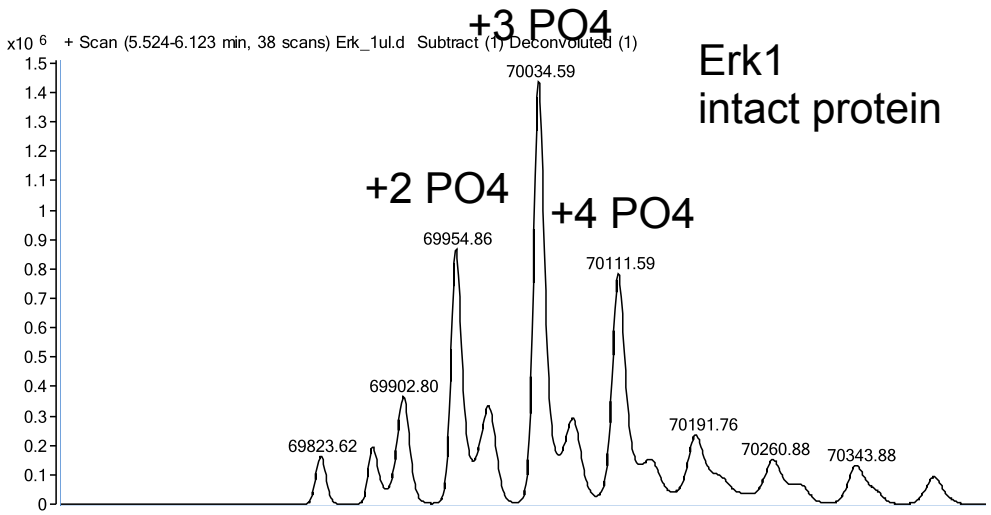
Sample					LAQ Met...	LAQ Results						Qualifier (638.5 -> 964.2) Results			LAQ* (ISTD) Results		Qualifier (642.2 -> 964.2) Results		
		Name	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	S/N	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	S/N	MI	RT	Resp.	Ratio	S/N	MI
▶		RL_Veh_1ppb	Cal	1	2/27/2008 7:49 AM		16.291	4781	71.89		5.8898	5.8898		55.4	35.63		16.268	812	49.9	13.65	
		RL_HDose_1ppb	Cal	1	2/27/2008 3:09 PM		15.647	10560	77.02		12.9958	12.9958		51.2	50.83		15.668	813	44.1	6.34	



Catalase Quantitation Results

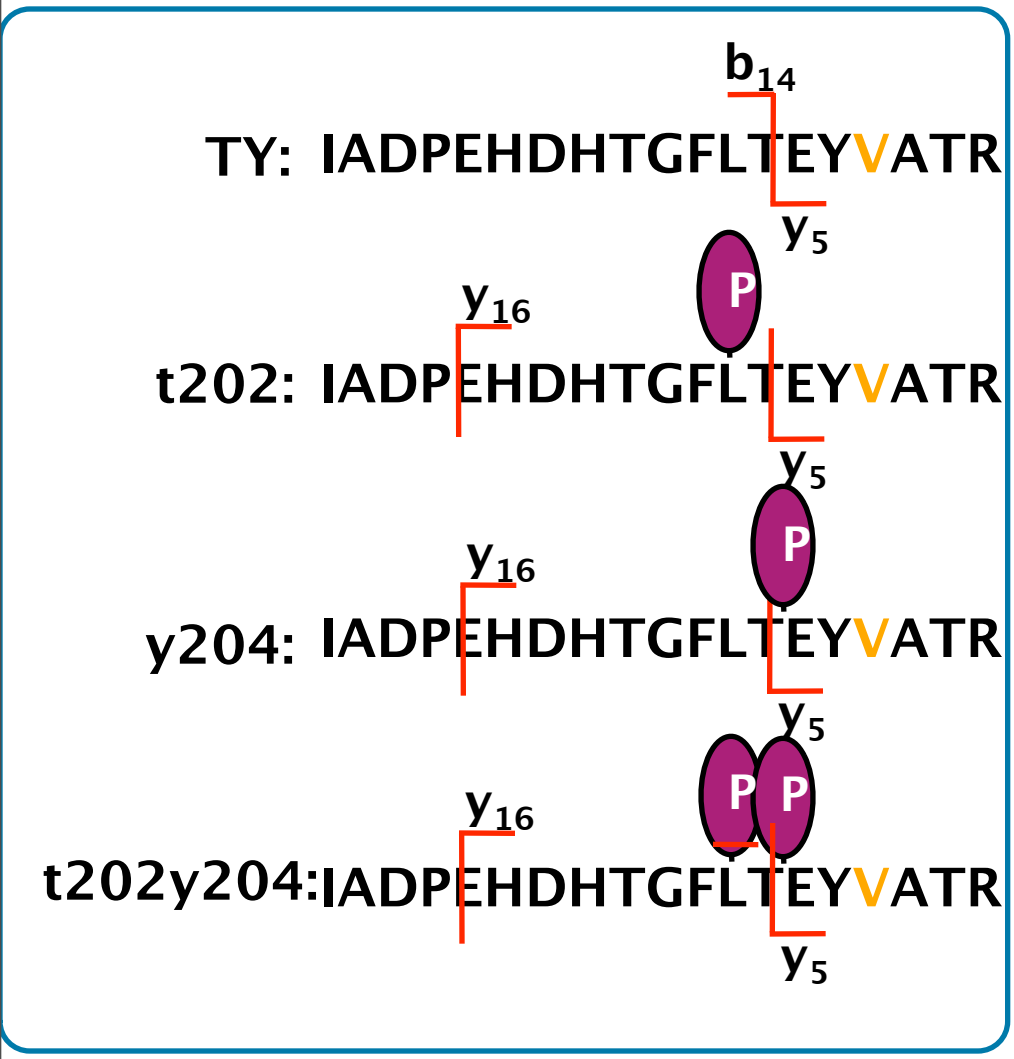
Sample	Peptide	Catalase (fmol/ug protein)	Catalase (pg/ug protein)	Fold Change EAETFPFNPFDLTK	Fold Change LAQEDPDYGLR	Fold Change 2D-DIGE
Vehicle Treated	EAETFPFNPFDLTK	8.84	14.63	1.00	1.00	1.00
	LAQEDPDYGLR	4.61	5.89			
Troglitazone Treated	EAETFPFNPFDLTK	32.69	54.36	3.7	2.2	1.45
	LAQEDPDYGLR	10.13	13.00			

Quantitation of protein phosphorylation using MRM



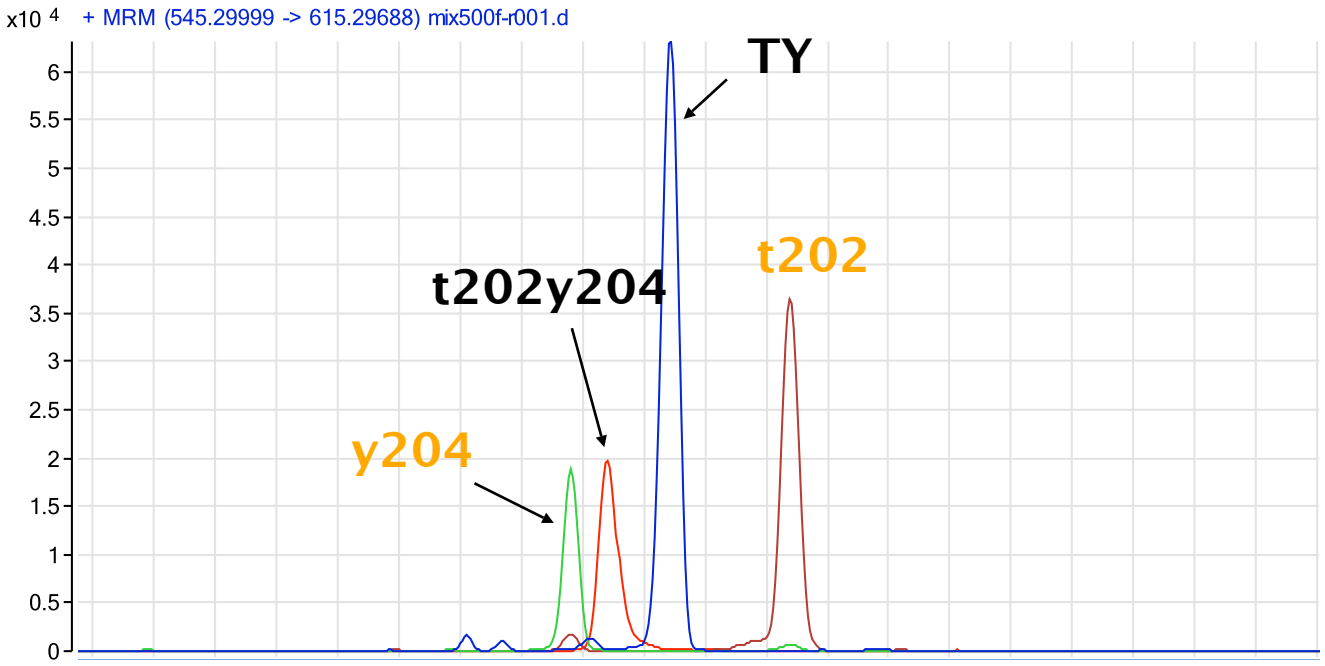
Agilent Technologies

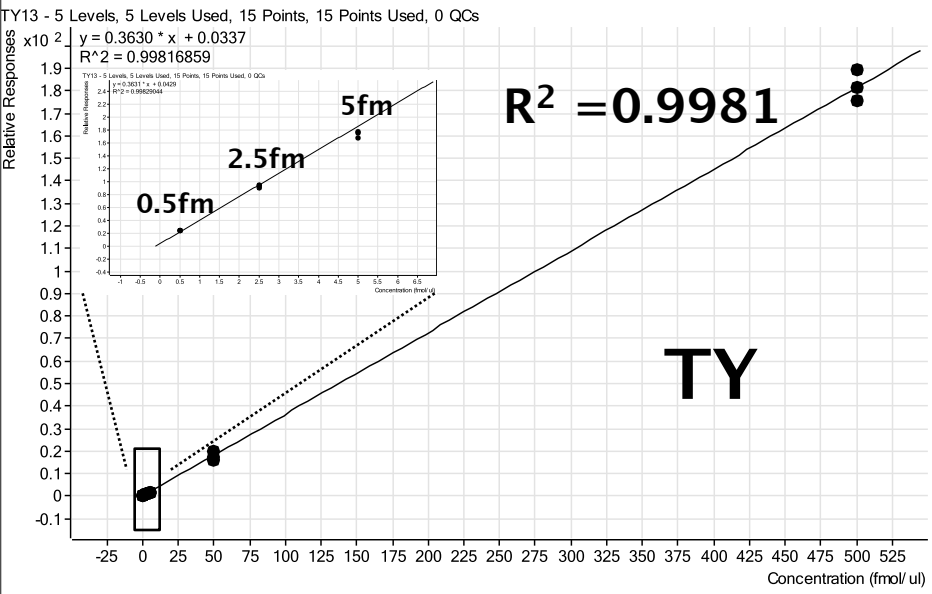
Selection of MRM transitions

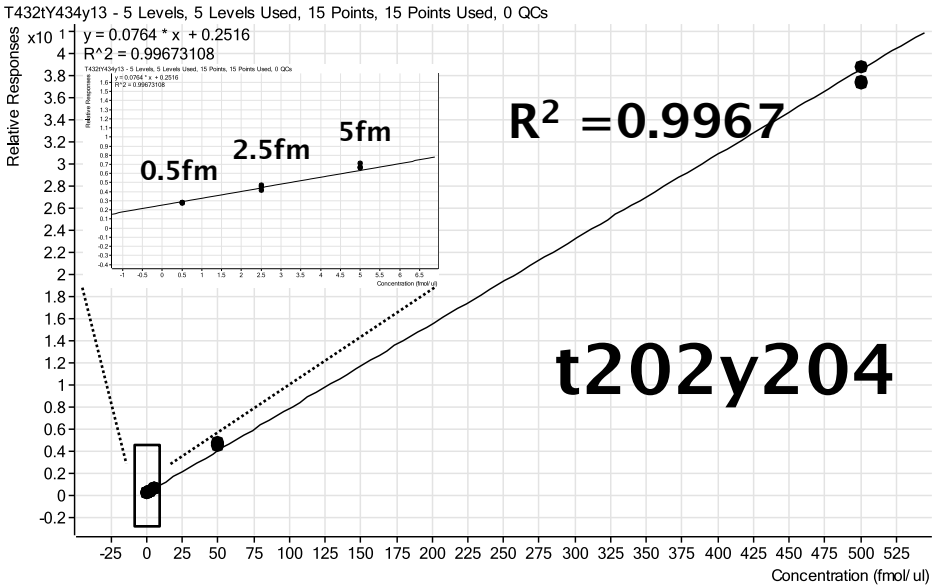


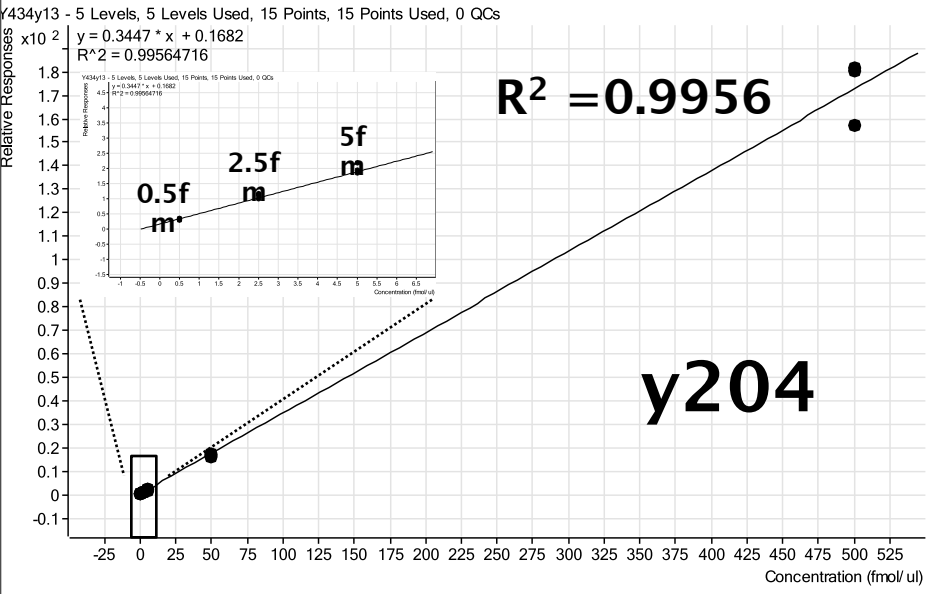
	Precursor ion	Product ions	
TY	545.3	615.3	782.5
	[M+3H] $^{3+}$	y_5	b_{14}^{2+}
t202	753.3	615.3	979.9
	[M+2H] $^{2+}$	y_5	y_{16}^{2+}
y204	753.3	979.9	695.3
	[M+2H] $^{2+}$	y_{16}^{2+}	y_5
t202y204	780.0	647.6	695.3
	[M+2H] $^{2+}$	$y_{16}^{3+}-H_3PO_4$	y_5

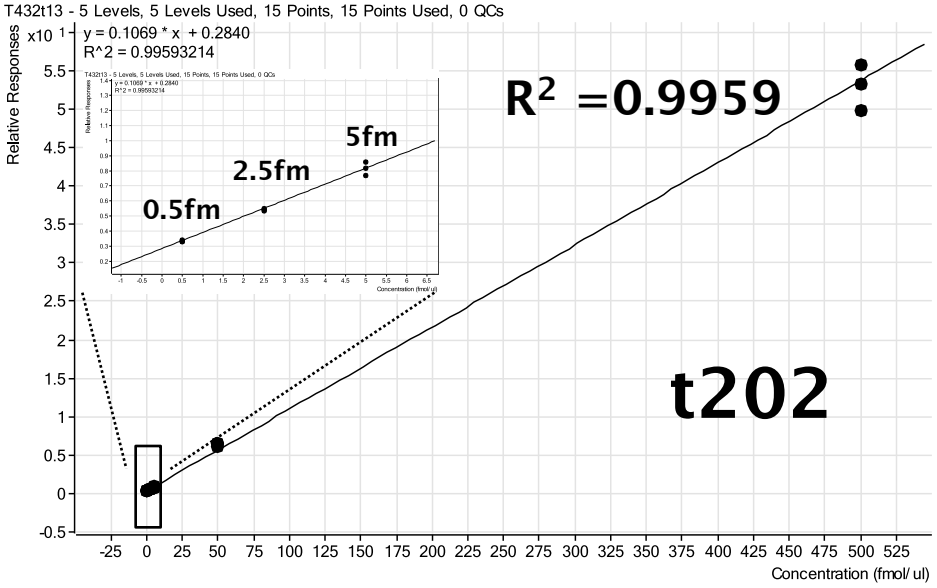
Chromatographic Separation of the Four Peptide Standards allowed the selection of the same Q1 and Q3 transitions for two different peptides




TY13 - 5 Levels, 5 Levels Used, 15 Points, 15 Points Used, 0 QCs
y = 0.3630 * x + 0.0337
R^2 = 0.99816859
R² =0.9981
0.5fm
2.5fm
5fm
TY
Relative Responses
Concentration (fmol/ ul)


T432tY434y13 - 5 Levels, 5 Levels Used, 15 Points, 15 Points Used, 0 QCs
y = 0.0764 * x + 0.2516
R^2 = 0.99673108
R² =0.9967
0.5fm
2.5fm
5fm
t202y204
Relative Responses
Concentration (fmol/ ul)


Y434y13 - 5 Levels, 5 Levels Used, 15 Points, 15 Points Used, 0 QCs
y = 0.3447 * x + 0.1682
R^2 = 0.99564716
R² =0.9956
0.5fm
2.5fm
5fm
y204
Relative Responses
Concentration (fmol/ ul)


T432t13 - 5 Levels, 5 Levels Used, 15 Points, 15 Points Used, 0 QCs
y = 0.1069 * x + 0.2840
R^2 = 0.99593214
R² =0.9959
0.5fm
2.5fm
5fm
t202
Relative Responses
Concentration (fmol/ ul)

Quantitation of the degree of phosphorylation at T202 and Y204 in active Erk1 protein

peptide	% Molar ratio	RSD (n=9)
TY	**20%**	**0.13**
t202	**25%**	**0.15**
y204	**21%**	**0.12**
t202y204	**34%**	**0.08**

In this batch of active Erk1 sample, 59% of T202 and 55% of Y204 were phosphorylated

HPLC-Chip/QQQ System

- Provide high sensitivity and large dynamic range
- Robust and stable nanoflow with HPLC-Chip
- Good retention time and MS detection reproducibility
- Peptide selector helps choosing MRM transitions