Obtaining Maximum Information from a Peptide Map using Alternating MS and Multiplexed MS/MS Data Acquisition

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Overview

- The peptide map is a basic tool for the identification and characterization of proteins and their modifications.
- Developing an effective peptide map requires analytical technologies for characterizing all the components within a sample, and tracking them as the method is developed.
- The combination of accurate mass and MS/MS information can uniquely identify digested peptides, even from closely related peptides containing deamidations, oxidations, and other modifications.
- Although data directed MS/MS analysis (DDA) on a TOF based MS detector can provide effective qualitative information, multiple runs may be required, and peptide quantitative information is lost.
- We have developed an alternative approach (termed “Protein Expression”) where alternating cycles of MS and multiplexed MS/MS analysis provide quantitative and qualitative information from a single run.
- This approach is illustrated using a yeast enolase peptide map.

Instrumentation

A prototype for the Waters Protein Expression System was produced by combining a CapLC™ separations platform with a Micromass™ Q-Tof Ultima mass detector. Accurate mass was obtained by using a lockspray enabled ESI source.

Sample: 300 ng (~6 pmol) Yeast Enolase tryptic digest in 4 µl Solvent A

Chemistry: 300 µ x 150 mm NanoEase™ Atlantis™ dC18 5µ RP (Waters)

HPLC Parameters:

Solvent A: 0.1% Formic acid in 1% Acetonitrile
Solvent B: 0.1% Formic acid in 80% Acetonitrile
Solvent C: (Lockspray solution) 200 fmol/µl Glu Fibrinogen b peptide and 0.1% Formic Acid in 25% Acetonitrile

Gradient: 0 -100 min 6 %B to 40 %B
20 min 99 %B column wash
30 min column regeneration at 6 %B
Flow rate: 5 µl/min

MS parameters:

Operated in the positive ion V-mode.
Capillary voltage: 3.5 kV
Source Temperature: 80 °C
Cone Voltage: 50 V
Lockspray channel was sampled at 30 sec intervals

MS Scan: Collision cell maintained at 10 V
MS^E Scan: Collision cell stepped between 27 V and 32 V during scan

DDA vs. Protein Expression Analysis

Data Directed Analysis (DDA)

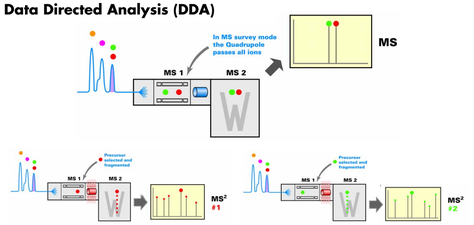


Figure 2: Data Directed LC/MS/(MS) Analysis (DDA). (DDA) employs an MS survey scan to identify abundant components, and series of individual MS/MS scans on several most abundant components. The survey/analysis cycle is repeated throughout the run.

Protein Expression Analysis

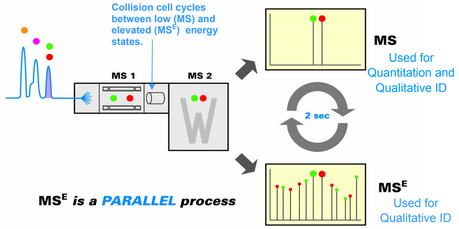


Figure 3: Protein Expression Analysis. Protein Expression analysis utilizes a cycle where there is no bias or selection of precursor ions for MS or MS/MS analysis. During MS acquisition the collision cell is a low potential, and in the MS^E cycle the collision cell is maintained at elevated potential. Data from the MS cycle are analyzed to determine accurate mass (for qualitative component identification) and signal intensities (for quantitative comparisons between analyses). The MS^E spectra are complex, and represent the MS/MS of all components identified during the MS cycle.

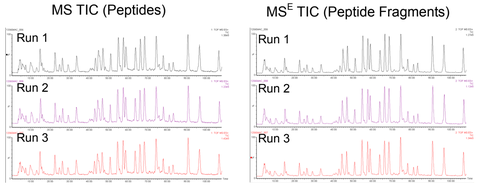
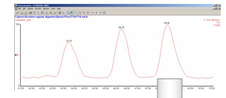
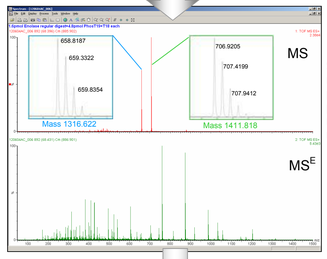


Figure 4: LC/MS/MS^E Enolase peptide maps. As all components are analyzed in both the MS and MS^E modes, the TIC patterns for the intact peptides (LEFT), and MS^E fragments (RIGHT) are comparable. The analysis of three sequential runs demonstrates that the same pattern and intensities of peptides and their fragment ions are observed.

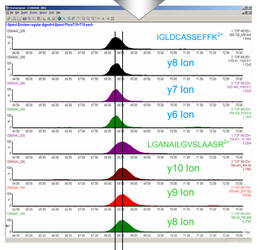
MS^E Spectral “Cleaning”



The TIC segment from 60.5 to 70.5 min reveals three peaks.



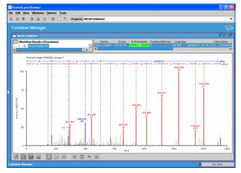
Summing the spectra under the third TIC peak reveals two peptides in the MS mode (TOP), and the resulting complex MS^E spectrum from both peptides (BOTTOM).



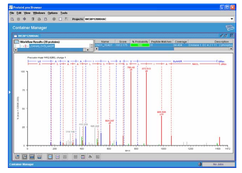
The extracted ion chromatograms (XIC) for fragments of a peptide in the MS^E mode align with that of the originating intact peptide in the MS mode.

In this example, the two peptides produce not only differing peak apex times, but also a characteristic difference in peak width and shape.

(K)IGLDCASSEFFK(D) RT= 68.36 min



(K)LGANAILGVSLAASR(A) RT= 68.57 min



Peak information for every peptide and fragment ion is used to produce reconstructed MS/MS spectra for the individual components that can be searched against a protein sequence database.

Figure 5: Producing individual MS/MS spectra for coeluting components. The example case shown above demonstrates how fragment ions in MS^E spectra are associated with the precursor data from MS scans.

Peptide Map Coverage of Enolase

PEPTIDE SEQUENCE	Mass	START AA	END AA	#AA
K	146.11	199	199	1
K	146.11	337	337	1
R	174.11	200	200	1
R	174.11	288	288	1
R	174.11	329	329	1
SK	233.14	54	55	2
SK	233.14	103	104	2
SK	233.14	139	140	2
SK	233.14	270	271	2
VK	245.17	241	242	2
DGK	318.15	255	257	3
LAK	330.23	406	408	3
DQK	389.19	85	87	3
SER	390.19	403	405	3
AVSK	403.24	1	4	4
DGDK	433.18	50	53	4
SLTK	447.27	195	198	4
GVFR	477.27	28	31	4
VNAR	507.28	5	8	4
WMGK	520.25	56	59	4
TGQIK	545.32	392	396	5
AAAAEK	559.3	120	125	6
TGAPAR	571.31	397	402	6
AAGHDGK	654.31	234	240	7
ANIDVK	658.36	79	84	6
NPNSDK	673.3	264	269	6
GVLHAVK	722.44	60	66	7
SVYDSR	725.33	9	14	6
NVPLYK	732.42	126	131	6
IATAIEK	744.44	330	336	7
LNQLLR	755.47	409	414	6
HLADLSK	782.43	132	138	7
YDLDFK	799.38	258	263	6
TFAEALR	806.43	178	184	7
AADALLLK	813.5	338	345	8
IGSEVYHNLK	1158.6	185	194	10
NVNDVIAPAFVK	1285.7	67	78	12
VNQIGTLSESIK	1287.7	346	357	12
IGLDCASSEFFK	1315.61	243	254	12
LGANAILGVSLAASR	1411.81	105	119	15
GNPTVEVELTTEK	1415.71	15	27	13
AVDDFLISLDGTANK	1577.79	88	102	15
TAGIQIVADDLTVTNPK	1754.94	312	328	17
AAQDSFAAGWGVMVSHR	1788.84	358	374	17
SGETEDTFIADLVVGLR	1820.92	375	391	17
SIVPSGASTGVHEALEMR	1839.92	32	49	18
WLTGPQLADLYHSLMK	1871.96	272	287	16
IEEELGDNAVFAGENFHGDK	2327.05	415	435	21
YPIVSIEDPFAEDDWEAWSHFFK	2827.28	289	311	23
YGASAGNVGDEGGVAPNIQTAEEALDLIVDAIK	3256.61	201	233	33
TSPYVLPVPFLNVLNGGSHAGGALALQEFMIAPTGAK	3736.96	141	177	37
				1
Peptides Identified with 1 Missed Cleavage				1
IEEELGDNAVFAGENFHGDKL	2440.13	415	436	22
RYGASAGNVGDEGGVAPNIQTAEEALDLIVDAIK	3412.71	200	233	34

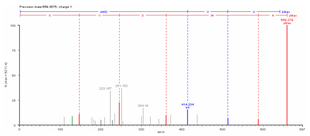
AVSKVYARSVYDSRGNPTVEVELTTEKGVFRSIVPSGASTGVHEALEMRD
GDKSKWMGKGVLHAVKNVNDVIAPAFVKANIDVKDQKAVDDFLISLDGTA
NKSKLGANAILGVSLAASRAAAAEKNVPLYKHLADLSKSKTSPYVLPVPF
LNVLNGGSHAGGALALQEFMIAPTGAKTFAEALRIGSEVYHNLKSLTKKR
YGASAGNVGDEGGVAPNIQTAEEALDLIVDAIKAAGHDGKVKIGLDCASS
EFFKDGKYDLDFKNPNSDKSKWLTGPQLADLYHSLMKRYPIVSIEDPFAE
DDWEAWSHFFKTAGIQIVADDLTVTNPKRIATAIEKKAADALLLKVNQIG
TLSESIKAAQDSFAAGWGVMVSHRSGETEDTFIADLVVGLRTGQIKTGAP
ARSERLAKLNQLLRIEEELGDNAVFAGENFHHGDKL

Figure 6: Yeast enolase peptide map coverage map. This map was produced from a single expression analysis run, and depicts peptides automatically identified by the Protein Expression informatics using accurate mass and MS^E peptide sequence information.

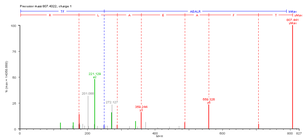
Black= AA from observed from tryptic peptides
Blue= AA from tryptic peptides that were not automatically identified
Green= AA from a tryptic peptide with a single missed cleavage.

Overall RMS (average) mass error of identified peptides was 7.5 (6.2) ppm.
Coverage: Overall (89% of Sequence), Peptides w/ 5+ AA (98.5%)

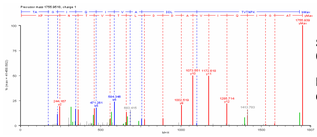
MS/MS Spectra of Selected Enolase Peptides



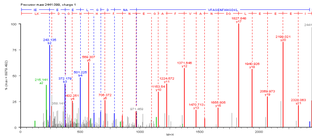
Sequence: (K)ANIDVK(D)
Predicted Mass: 658.371
Observed Mass: 658.365



Sequence: (K)TFAEALR(I)
Predicted Mass: 806.434
Observed Mass: 806.429



Sequence: (K)TAGIQIVADDLTVTNPK(R)
Predicted Mass: 1754.944
Observed Mass: 1754.942



Sequence: C-terminal Peptide (R)IEEELGDNAVFAGENFHHGDKL(-) (1 Missed Cleavage)
Predicted Mass: 2440.092
Observed Mass: 2440.129

Conclusions

- Peptide mapping data, with high MS/MS sequence coverage, was obtained using a single Protein Expression analysis run.
- Processing of peptide map data from Protein Expression analysis can be accomplished using fully automated informatics tools.
- Peptide map data in both the MS and MS^E (multiplexed MS/MS) scans were shown to be reproducible from run to run.
- Both qualitative (components within a run) and quantitative (component intensities between runs) peptide map analysis can be accomplished using this approach.
- It was demonstrated that clear and concise MS/MS data could be obtained from peptides with overlapping elution profiles.
- This approach should prove valuable for developing better peptide mapping methods.