Example of Using PEP Search

The following describes how to use PEP Search using di-/tri peptide standards as an example.

1. The user has to enter the desired search parameters described in the PEP Search Tutorial. In this particular case, MH Tol and Peak Tol was set to 0.05 Da; RT shift from: -600 second; RT shift to: +60 second; a ion intensity threshold: 5 times of base intensity; Please refer to Figure 1.

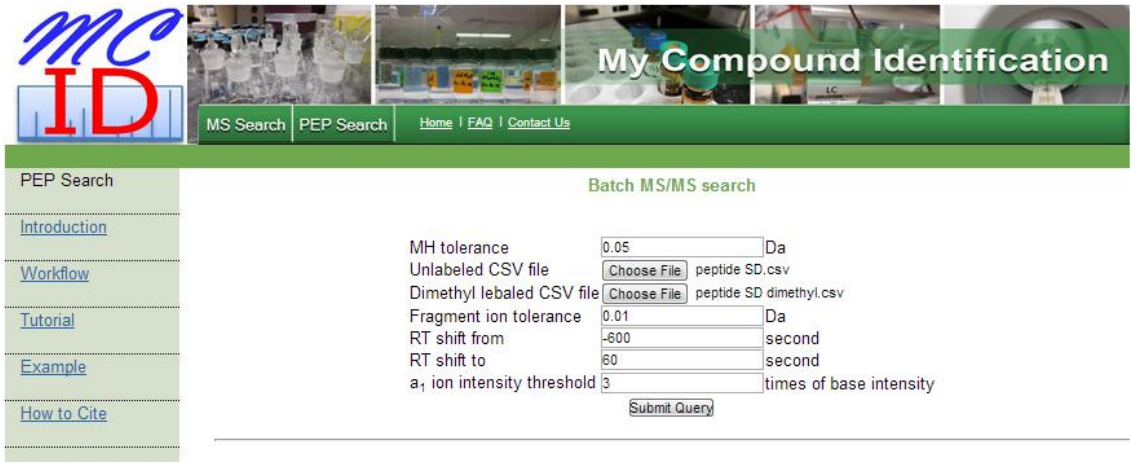


Figure 1. Software interface showing the entered parameters.

2. Once the query is submitted the following page is displayed (Figure 2). For this particular search, there were 81 entries came back from database search. The user can then open the possible identifications in entries, and exam their sequences and scores as shown in Figure 3.

Search parameters

Parameter Name	Parameter Value(s)
MH Tol	0.05
Uploaded File	peptide SD.csv
Di-Methyl File	peptide SD dimethyl.csv
mass list Tol	0.01
r.t. from	-600
r.t. to	60
a Ion Intensity Thres	3
tri Booster	true

Search results

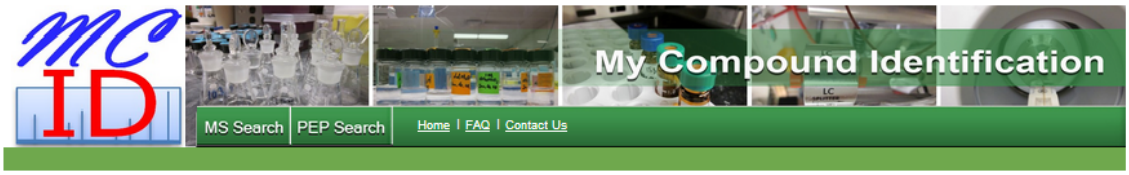
index	$[M+H]^+$ (mass)	retention time (min)	unlabeled peptide matches	ambiguous Id	dimethyl a_1 ions	confirmed Id
1	244.19719	2.107	2	PK	...	
2	205.10223	2.780	1	EG	...	
3	343.17450	3.543	4	NLP	...	
4	459.36008	4.794	1	KRR	...	
5	460.23615	6.355	7	FFF	F...	FFF
6	460.23654	6.455	16	FFF	F...	FFF
7	352.17528	8.919	26	WF	W...	WF
8	279.17821	9.632	3	FL	FY...	FL
9	335.14798	9.682	50	NVC	...	
10	318.19058	10.303	51	LW	WL(I)A...	WL
11	364.17613	10.731	13	HAH	...	
12	352.17561	10.747	46	WF	W...	WF
13	313.16389	11.234	3	FF	F...	FF
14	313.16394	11.366	3	FF	F...	FF
15	279.17868	11.837	3	FL	FY...	FL
16	265.16232	12.466	2	FV	F...	FV

Figure 2. Parameters and results table displaying matched entries.

Sequence	Score
FV	274.8
VF	49.1

Figure 3. Identification table displaying matched sequences and their scores for each entry.

3. For each entry, the top ranked identification is considered correct, when it has corresponding dimethyl labeled a_1 ion match as confirmation. In cases that the second ranked identification also confirmed by dimethyl labeled a_1 ion, its MS/MS spectrum matches should be carefully checked before taking this identification as correct. Users can exam all matched sequence by clicking the sequence in identification table and check their MS/MS fragments and spectrum matches (Figure 4).



Search Results
MS/MS Fragmentation of FV

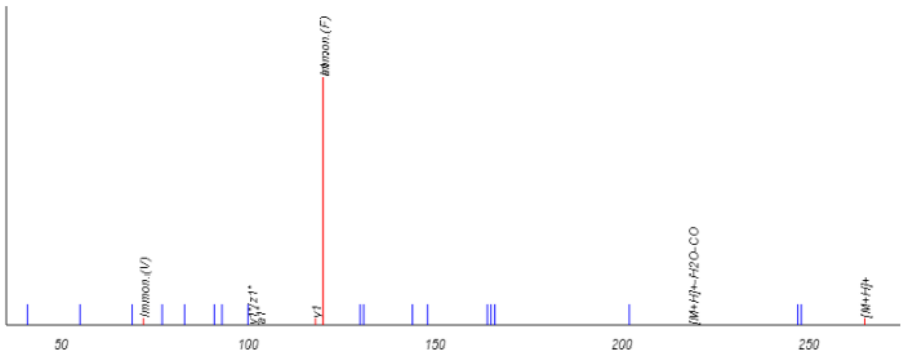
Match to Query: 265.16232

Calc. $[M+H]^+$: 265.15466

Calc. Monoisotopic Mass of Neutral Peptide: 264.14738

Mass Error: 0.00766

Match Score: 274.8



Matches (Bold Red): 8/26

#	Immon.	a	a*	b	b*	b°	c	c + H	c - H	Seq.
1	**120.081**	**120.081**	**103.054**	148.076	131.049	130.065	165.102	166.110	164.094	F
2	**72.081**									V

Seq.	x	y	y*/z	y°	z - H_2O	#
F						2
V	144.066	**118.086**	**101.060**	100.076	83.049	1

$[M+H]^+$	**265.155**
$[M+H]^+ - NH_3$	248.128
$[M+H]^+ - H_2O$	247.144
$[M+H]^+ - H_2O - CO$	**219.149**
$[M+H]^+ - H_2O - CO - NH_3$	202.123
$[M+H]^+ - SCH_3O$	
$[M+H]^+ - H_2O - CO - HSCH_3$	
Immonium ion (V) - NH_3	55.054,
Immonium ion (F) related ions	77.039, 91.054, 93.070,
Immonium ion (V) related ions	41.039, 69.058,

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Figure 4. Webpage displaying MS/MS spectrum matches and fragmentation tables for matched sequence.