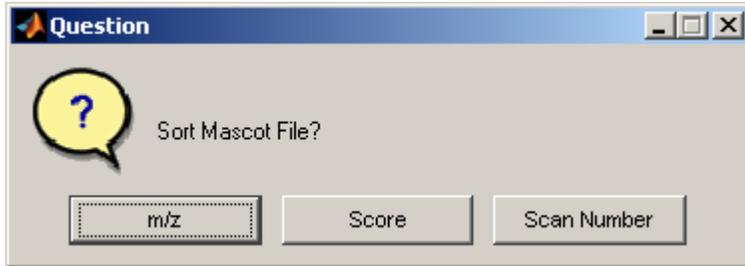
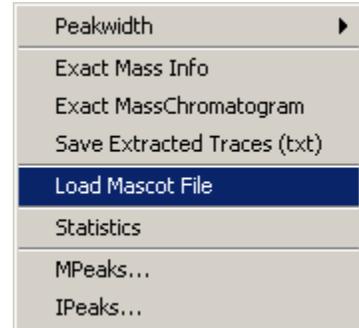


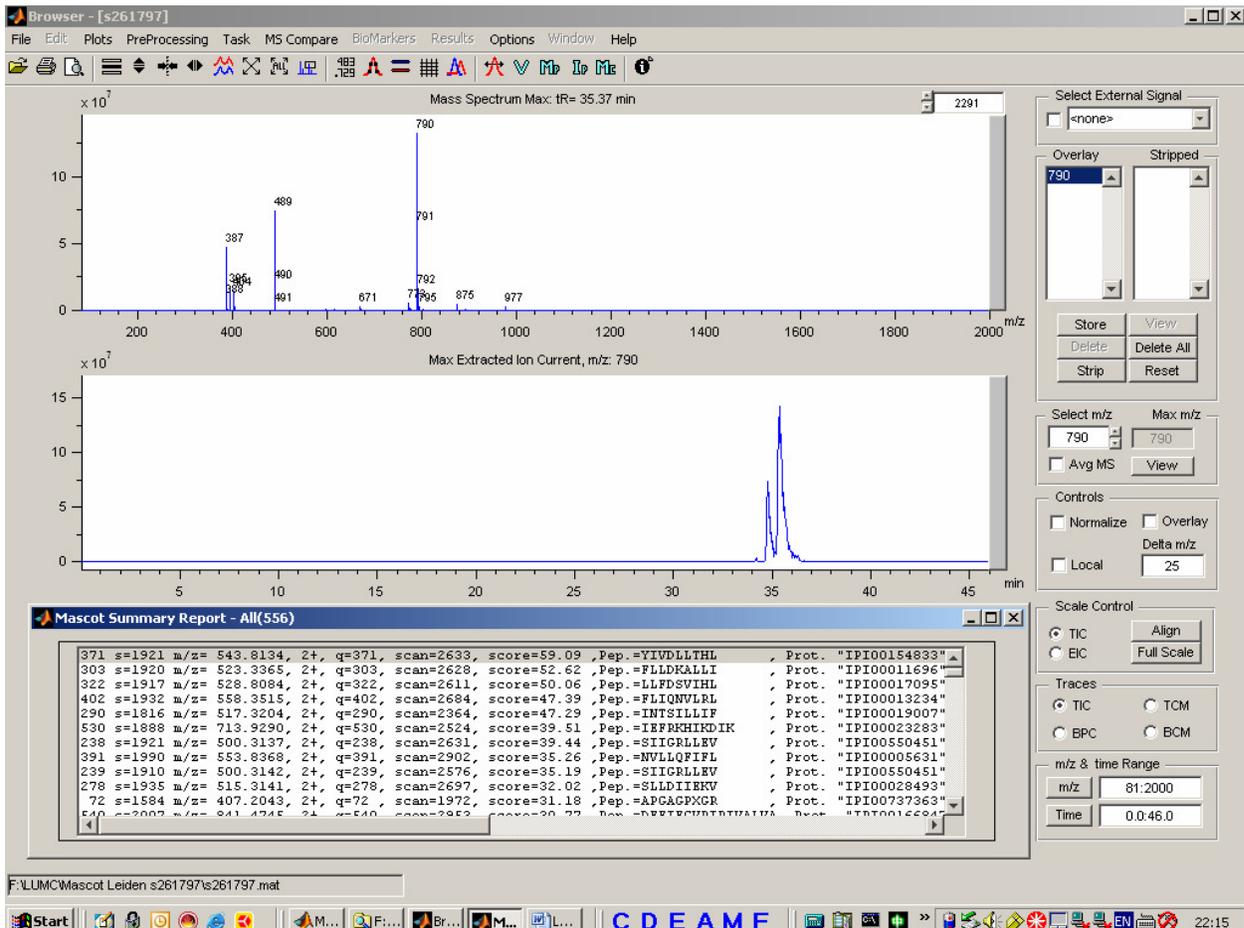
MS-Xelerator: Loading and Viewing Mascot Files:

1. From the Browser Module:

Select **Menu > Task > Load Mascot File**. Locate and select your Mascot file. Be sure that the correct file has been selected – file dependency will not be checked. MS-Xelerator will read in the file and you will be asked on what parameter the file should be sorted (sorting can also be applied afterwards).



After conversion the Mascot file will be opened in a separate window in the lower half of the MS-Xelerator screen



Browser Screen and Mascot Summary Report window:

The Mascot Summary Report lists the total number of queries and has the following fields. All data except the MS-Xelerator Full Scan number is read from the Mascot file:

1. 371, ID number = query number.
2. s=1921, Full Scan number in the MS-Xelerator file closest to the Xcalibur MS2 scan. MS-Xelerator will reorder the full scan numbers during import. The scan number used by MS-Xelerator will therefore be different from the original Finnigan scan number. Example. s=1921 relates to scan 1921 in the MS-Xelerator file. This full scan relates to MS2 scan 2633 in original scan numbers.
3. m/z=543.8134, m/z value of precursor ion, qexp from the Mascot file.
4. 2+, charge state.
5. q=371, query number.
6. scan=2633, Xcalibur's MS2 scan number.
7. score=59.09, Mascot Score.
8. Pep.=YIVDLLTHL, peptide from P1 search. Highest score on MS2.
9. Prot.= "IPI00154833"=42227.41,"Tax_Id=9606 Glucose-fructose oxidoreductase domain containing 2", first protein description found from P1 line.

The screenshot shows a window titled "Mascot Summary Report - All(556)". The table contains the following data:

Query	s	m/z	Charge	q	scan	score	Pep.	Prot.
371	1921	543.8134	2+	371	2633	59.09	YIVDLLTHL	"IPI00154833"
303	1920	523.3365	2+	303	2628	52.62	FLLDKALLI	"IPI00011696"
322	1917	528.8084	2+	322	2611	50.06	LLFDSVIHL	"IPI00017095"
402	1932	558.3515	2+	402	2684	47.39	FLIQNVLR	"IPI00013234"
290	1816	517.3204	2+	290	2364	47.29	INTSILLIF	"IPI00019007"
530	1888	713.9290	2+	530	2524	39.51	IEFRKHIDIK	"IPI00023283"
238	1921	500.3137	2+	238	2631	39.44	SIIGRLLEV	"IPI000550451"
391	1990	553.8368	2+	391	2902	35.26	NVLLQFIFL	"IPI00005631"
239	1910	500.3142	2+	239	2576	35.19	SIIGRLLEV	"IPI000550451"
278	1935	515.3141	2+	278	2697	32.02	SLLDIEKV	"IPI00028493"
72	1584	407.2043	2+	72	1972	31.18	APG&PXGR	"IPI00737363"

Mascot Summary List

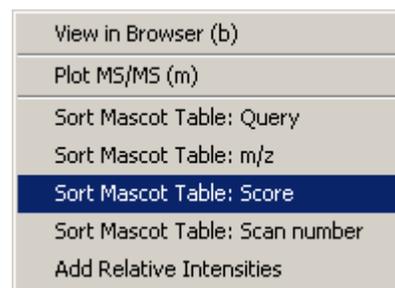
Activating the Mascot Window: the Mascot Summary Report will appear in a separate window. Any operation in MS-Xelerator will minimize this window. To bring it up again press 't' (table).

Viewing the accompanying mass chromatogram in the Browser: select any row from the Mascot list and press 'b' (browser). The mass chromatogram will be plotted in the middle window and the full scan mass spectrum belonging to the selected query will be plotted in the mass spectrum window (+/- 2 Dalton). The scan number will be displayed in the scan number edit box (top right) and the retention time belonging to this scan is marked in the mass chromatogram window using a green vertical line. By using the keyboard (arrow up/down in combination with 'b' and 'm') you may quickly view different mass chromatograms and MS2 spectra.

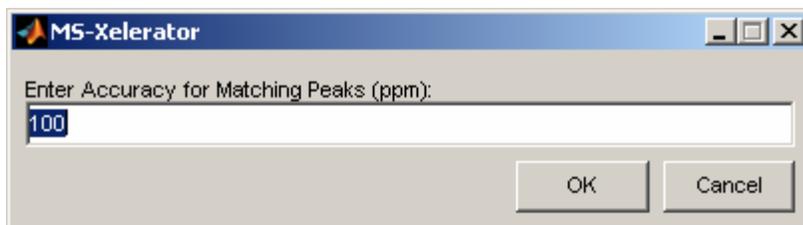
Plotting the MS2 spectrum: to plot the MS2 spectrum press 'm'. The spectrum will be plotted in the top window. The title will indicate the spectrum mode, e.g. MSMS query 343.

Sorting the Mascot Summary Table: the Mascot table can be sorted on: Query Number, m/z Value, Mascot Score and Scan Number. Press the right mouse button and select the type of sorting from the context menu.

To add Relative Intensities: to calculate a relative intensity for each precursor m/z value, select **Add Relative Intensities**. You will be asked to what accuracy (ppm) the mass peaks should match.



Example: suppose the precursor ion has a m/z value of 543.8134. Selecting a value of 100 ppm will extract the highest intensity (peak height) in the range m/z 543.8134 +/- 0.054. After all intensities have been determined, the relative intensities are calculated and added to the Mascot table, **Field: IRel%..**



Specify the accuracy for calculating the relative intensity

Irel%	s	m/z	q	scan	score	Pep.	Prot.
4.96	371	543.8134	2+	q=371, scan=2633	59.09	YIVDLLTHL	Prot.
2.33	303	523.3365	2+	q=303, scan=2628	52.62	FLLDKALLI	Prot.
6.09	322	528.8084	2+	q=322, scan=2611	50.06	LLFDSVIHL	Prot.
0.72	402	558.3515	2+	q=402, scan=2684	47.39	FLIQNVLRRL	Prot.
0.96	290	517.3204	2+	q=290, scan=2364	47.29	INTSILLIF	Prot.
0.84	530	713.9290	2+	q=530, scan=2524	39.51	IEFRKHIDIK	Prot.
13.07	238	500.3137	2+	q=238, scan=2631	39.44	SIIGRLLEV	Prot.
1.35	391	553.8368	2+	q=391, scan=2902	35.26	NVLLQFIFL	Prot.
10.08	239	500.3142	2+	q=239, scan=2576	35.19	SIIGRLLEV	Prot.
9.19	278	515.3141	2+	q=278, scan=2697	32.02	SLLDIIEKV	Prot.
0.20	72	407.2043	2+	q=72, scan=1972	31.18	APGAGPXGR	Prot.

Relative intensities added to Mascot Summary Table

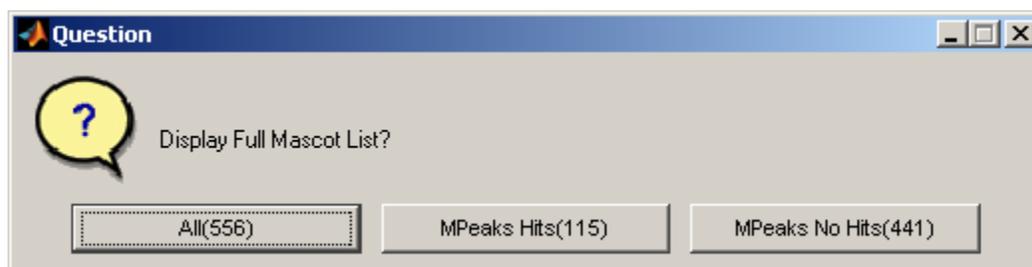
2. Linking a Mascot file from the MPeaks Module:

After the MPeaks algorithm has been run you may link the MPeaks output to a Mascot file. For every peak in the MPeaks table the procedure will determine if a MS2 spectrum has been recorded. In this way it will be possible to check on peaks lacking MS2 spectra and further investigation would be necessary. The Linking process will also determine the number of queries for which MPeaks did not find a peak. Perhaps you should increase the sensitivity of peak detection. However, often MS2 spectra will be recorded where no peak elutes.

To perform Mascot labeling select from the Menu: **Tasks > Link to Mascot output File**. Select the Mascot file. In the case that the MPeaks table has been converted to accurate m/z values, you will be asked to enter the accuracy for peak matching. A default value of 100 ppm will be used. Accept the default value and press OK. If no accurate conversion was performed a default nominal m/z matching will be used. You might also want to check and compare charge states between MPeak's method and the output from Xcalibur (perform the charge state calculation after conversion to accurate m/z values using, Menu: **Accurate Mass > Calculate Charge State**). Deviations between both methods are possible and most often seen for the smaller peaks or in situations where mixtures of peaks are present.

The next window will give an overview on the number of Mascot queries that could be linked to peaks in the Mpeaks table. For the example shown, the Mascot output file contains 556 queries. A total of 115 MS2 spectra could be linked to detected peaks using MPeaks. 441 queries did not meet the search criteria and could not be linked.

The Mascot output file may be viewed as described on the previous pages. You may select to view all results, only those queries for which real peaks were found or you might be interested in queries that could not be linked to existing peaks from the MPeaks table.



Probably you will select All. The Mascot output will be shown as below. The second field will have the value zero or one. The value zero means that no peak could be found for this query, a value of one corresponds to a peak for which a query matched.

ID	scan	s	m/z	charge	q	scan	score	peptide
371	1	1921	543.8134	2+	q=371	scan=2633	score=59.09	Pep.=YIVDL
303	1	1920	523.3365	2+	q=303	scan=2628	score=52.62	Pep.=FLLDK
322	1	1917	528.8084	2+	q=322	scan=2611	score=50.06	Pep.=LLFDS
402	0	1932	558.3515	2+	q=402	scan=2684	score=47.39	Pep.=FLIQN
290	1	1816	517.3204	2+	q=290	scan=2364	score=47.29	Pep.=INTSI
530	1	1888	713.9290	2+	q=530	scan=2524	score=39.51	Pep.=IEFRK
238	1	1921	500.3137	2+	q=238	scan=2631	score=39.44	Pep.=SIIGP
391	1	1990	553.8368	2+	q=391	scan=2902	score=35.26	Pep.=NVLLQ
239	0	1910	500.3142	2+	q=239	scan=2576	score=35.19	Pep.=SIIGP
278	0	1935	515.3141	2+	q=278	scan=2697	score=32.02	Pep.=SLLDI
72	0	1584	407.2043	2+	q=72	scan=1972	score=31.18	Pep.=APGAC
540	1	2007	841.4745	2+	q=540	scan=2953	score=30.77	Pep.=DEEIF
277	1	1919	515.3083	2+	q=277	scan=2621	score=30.37	Pep.=GLLDD
415	0	1967	566.8421	2+	q=415	scan=2815	score=29.84	Pep.=MLLAL

Mascot Summary Report: matched peaks listed in 2nd column (0/1)

Queries that could be linked to peaks in the table will be labeled as shown below. To put all the labeled peaks on top, sort on e.g. Peak height and then on ID using the sort control in the **Output Control Area**.

Exact Mass Spectrum: t= 24.61 min scan=1734

--- Results (1167) ---

Peak	m/z	Charge	tR	PH	%PH	%Area	Mascot ID()
1	446.17065	2	24.61	2.1e+007	14.55	0.77	s=1735 m/z= 446.1698, 2+,
2	395.19934	2	34.79	1.0e+007	7.05	4.90	s=2250 m/z= 395.1678, 2+,
3	477.32410	2	29.39	7.8e+006	5.48	2.10	s=1967 m/z= 477.3201, 2+,
4	524.79987	2	29.67	6.7e+006	4.71	1.42	s=1962 m/z= 524.7873, 2+,
5	535.84216	2	28.86	4.0e+006	2.83	1.14	s=1925 m/z= 535.8439, 2+,
6	487.30914	2	29.36	3.4e+006	2.36	1.10	s=1951 m/z= 487.3112, 2+,
7	841.44843	2	30.84	3.4e+006	2.36	1.52	s=2007 m/z= 841.4745, 2+,
8	964.65259	2	29.39	2.8e+006	1.97	0.46	s=1960 m/z= 964.6238, 2+,
9	515.32104	2	28.57	1.7e+006	1.22	0.38	s=1919 m/z= 515.3083, 2+,
10	464.27124	2	33.86	1.7e+006	1.17	0.73	s=2177 m/z= 464.2709, 2+,
11	456.28662	2	33.84	1.5e+006	1.06	0.62	s=2177 m/z= 456.2845, 2+,
12	528.81580	2	28.48	1.5e+006	1.04	0.23	s=1917 m/z= 528.8084, 2+,
13	497.82898	2	33.73	1.5e+006	1.03	0.29	s=2184 m/z= 497.8296, 2+,
14	497.82898	2	34.02	1.5e+006	1.03	0.62	s=2184 m/z= 497.8296, 2+,

MPeaks Screen showing table and Mascot labeled peaks

Sorting the MPeaks table on Mascot Score:

To sort the MPeaks table on Mascot Score use: Menu: **Options > Sort Mascot Score**.

View MS2 Spectra;

To view the MS2 spectra having a label, press '2'. The MS2 spectrum will be shown in the plot window. Toggle between MS, Full Scan and the Mass Chromatogram by using the keyboard keys '2' and 'm'. The plot title of the selected mass chromatogram will contain the scan number of the peak's maximum. You can compare it with the accompanying MS2 scan number displayed in the label.

Finding peaks having no MS2 spectrum:

To identify peaks in the MPeaks table not matching any queries, sort on peak height and scroll downwards. Peaks having no MS2 spectra or peaks that could not be matched based on the search criteria will have no label. As a prefiltering step you might want to delete all 1+ charge states first (select from the menu: **Accurate Mass > Delete Charge State** and make your selection).

Viewing Full Scan, MS2 spectra and mass chromatograms in the Browser:

From the Mascot List Window press 'b' or 'm' and the data will be copied to the Browser. The same keys and functionality applies as described in the first chapter.
