Manual updated on July 16, 2020

Compatible with version 1.0.1

1. About

MS2Compound is a user friendly Graphical User Interface (GUI) for the identification of the compounds from MS/MS metabolomics data. The tool features use of custom database for the identification of compounds. Current version of MS2Compound (v1.0.1) is compatible with the customized database prepared using the fragment prediction using CFM-id tool.

The current version of the tool is compatible with Mascot Generic Format (.mgf) files as input.

The tool has been developed and maintained by Center for Systems Biology and Molecular Medicine (http://csbmm.yenepoya.res.in/), Yenepoya Research Centre, Yenepoya (Deemed to be University).

2. Installing MS2Compound

2.1 System requirements

2.1.1 Operating system

Windows 7, 8 and 10

2.1.2 Minimum system configuration

2GB or more RAM

Intel i3 64-bit CPU

2.2 Installation

2.2.1 Dependencies

The tool is independent from all the pre-requisite dependencies.

2.2.2 MS2Compound installation

- Download the application "MS2Compound_v1.0.1.exe" from https://github.com/beherasan/MS2Compound or https://sourceforge.net/projects/ms2compound/
- Click on "MS2Compound_v1.0.1.exe" to install the program. The default installation directory will be "C:\MS2Compound", user can select the installation directory of choice, however avoid spaces (" ") in the directory path name.

- Please select to create a shortcut of the MS2Compound in desktop for easy access.
- A successful installation will create a directory C:\MS2Compound. This directory will contain the executable "MS2Compound.exe".

3. Start MS2Compound

Click on the shortcut "MS2Compound_v1.0.1", created in desktop after successful completion of installation. If the user has not opted for desktop shortcut during installation, the executable can be start by clicking the "MS2Compound.exe" in "C:\MS2Compound" directory.

4. Availability of test dataset

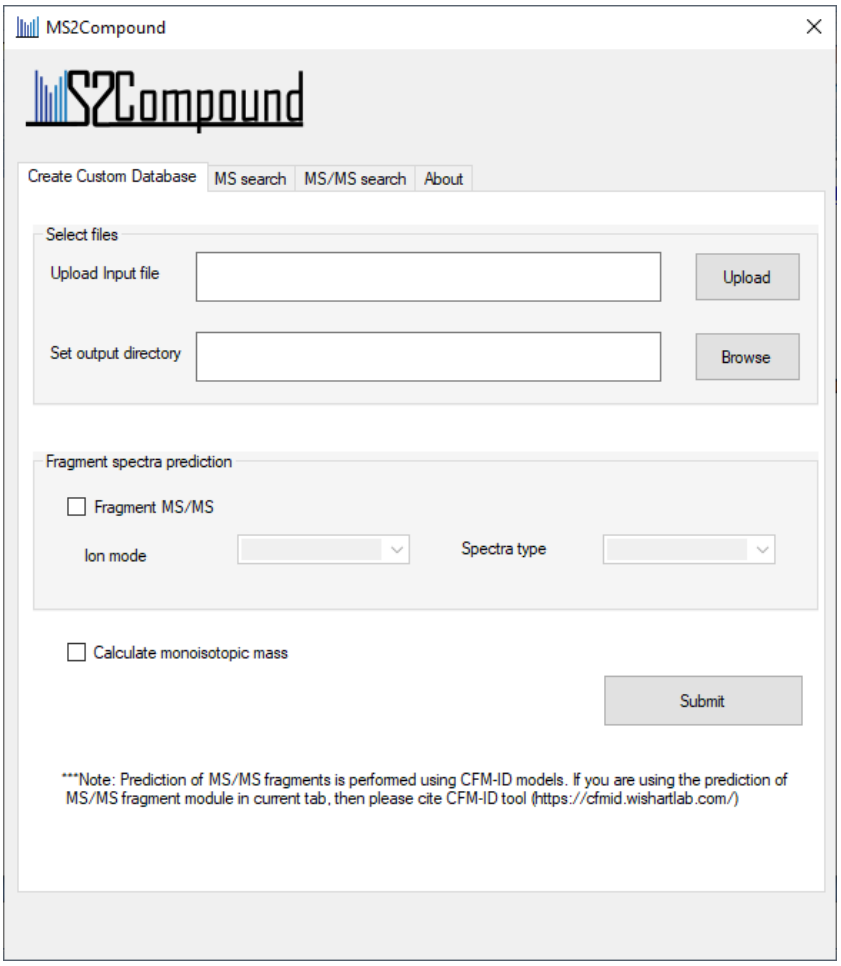
The test dataset for MS2Compound are available at https://github.com/beherasan/MS2Compound/tree/master/TestData

The current version of MS2Compound performs the following five tasks (Link for the test data for each function are provided in parentheses).

a. Create custom MS/MS database using CFM-id prediction models. Please cite CFM-id (https://cfmid.wishartlab.com/) if the user has used this module in MS2Compound. (https://github.com/beherasan/MS2Compound/tree/master/TestData/FragmentPrediction)
b. Identification of compounds from LC-MS data using in-built database. (https://github.com/beherasan/MS2Compound/tree/master/TestData/MS_InBuiltDB)
c. Identification of compounds from LC-MS data using custom (user-defined) database. (https://github.com/beherasan/MS2Compound/tree/master/TestData/MS_CustomDB)
d. Identification of compounds from LC-MS/MS data using in-built database. (https://github.com/beherasan/MS2Compound/tree/master/TestData/MSMS_InBuiltDB)
e. Identification of compounds from LC-MS/MS data using custom (user-defined) database. (https://github.com/beherasan/MS2Compound/tree/master/TestData/MSMS_CustomDB)

4. Run MS2Compound

Clicking the shortcut "MS2Compound_v1.0.1" in Desktop or "MS2Compound.exe" in C:\MS2Compound will start the GUI as shown below.



Create Custom Database

This module will help the user to create their custom database from a list of compounds (given the SMILES id of the listed compounds)

The page for creating custom database is the default start page for MS2Compound.

The input file as given the test dataset "inputSMILES.txt" is a tab separated file containing two columns; first column is compound id or any unique identifier, and second column containing the SMILES id as shown below.

```
challenge-001    c1cc(ccc1C1COc2cc(cc(c2C1=O)O)O)O
challenge-002    C=C(C)C(C)c1cc(/C=C/C(=O)c2ccc(cc2)O)c(cc1O)OC
challenge-003    COc1ccc(CCN=C(/C=C/c2ccccc2)O)cc1OC
challenge-004    c1cc(ccc1c1c(c(=O)c2ccc(cc2o1)O)O)O
challenge-005    c1cc(c(cc1/C=C\1/C(=O)c2ccc(cc2O1)O)O)O
```

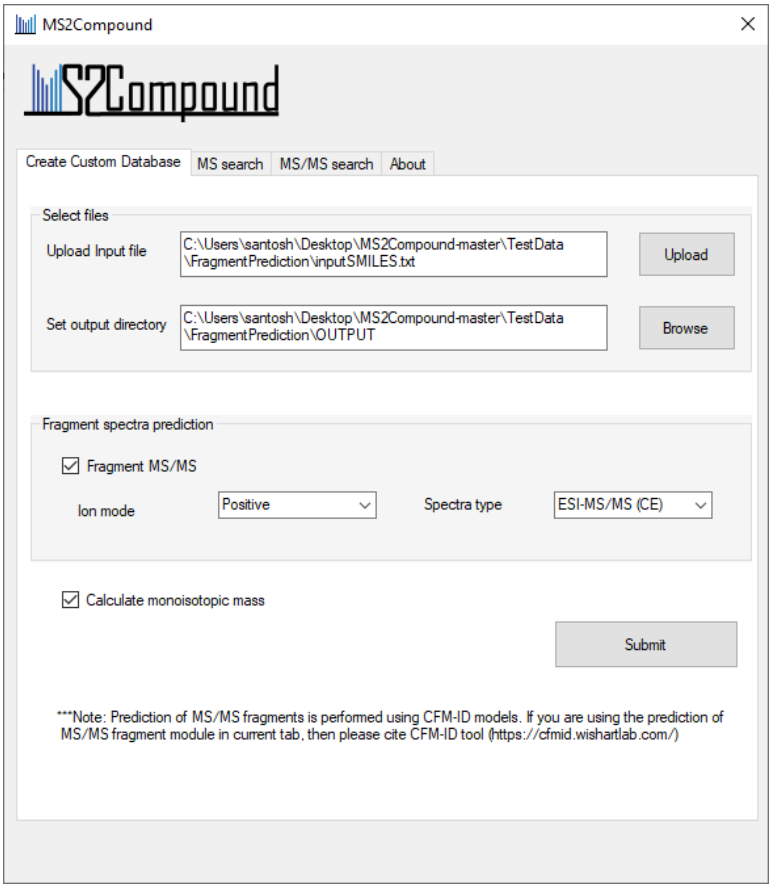
Select the Output directory: Please avoid spaces in name of the directory or the directory path

User can select both the option “Fragment MS/MS” and “Calculate monoisotopic mass” or any one according to the need.

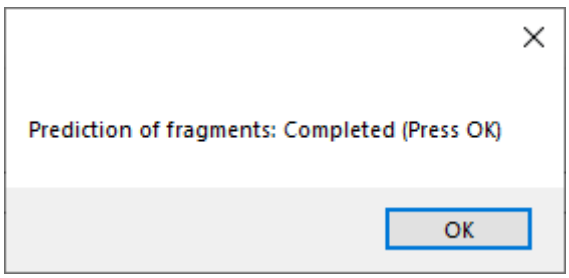
After selecting the “Fragment MS/MS”, please select the desired “Ion mode” and “Spectra type” for the prediction.

Click “Submit after selecting the desired options”. After submit it will start a cmd.exe and the main program cannot be assessed until the current work completed.

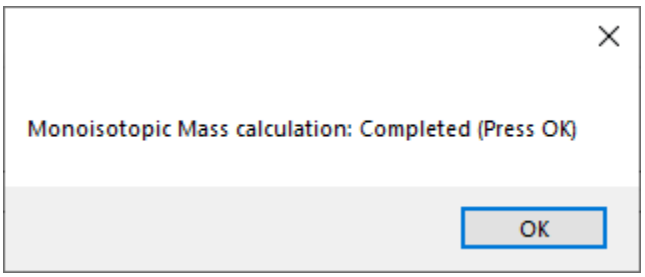
A filled form is shown below:



After completion of prediction program it will create a message box as follows:

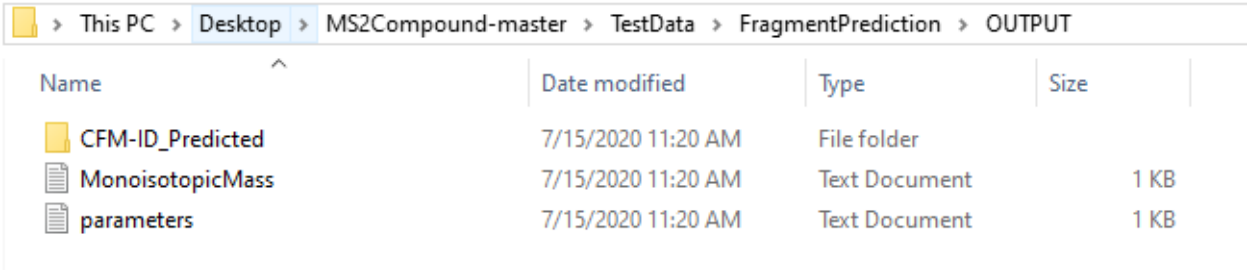


Click “OK” for the monoisotopic mass calculation program to execute. After the completion of mass calculation it will generate a message box as follows:



Press “OK” to finish the task.

This program will generate a directory “CFM-ID_Predicted” inside the selected output directory for the MS/MS fragments. If the directory “CFM-ID_Predicted” already exists in selected output directory, program will be halted with an error. Please rename the existing file or delete it before running the command. After successful completion of the prediction it will create separate log files for each compounds in “CFM-ID_Predicted” directory, this directory can be used as the custom database in the MS/MS search using custom Database module in the tool. The output directory will be generated as follows:



The parameters used for prediction of MS/MS fragments were saved in “parameters.log” file

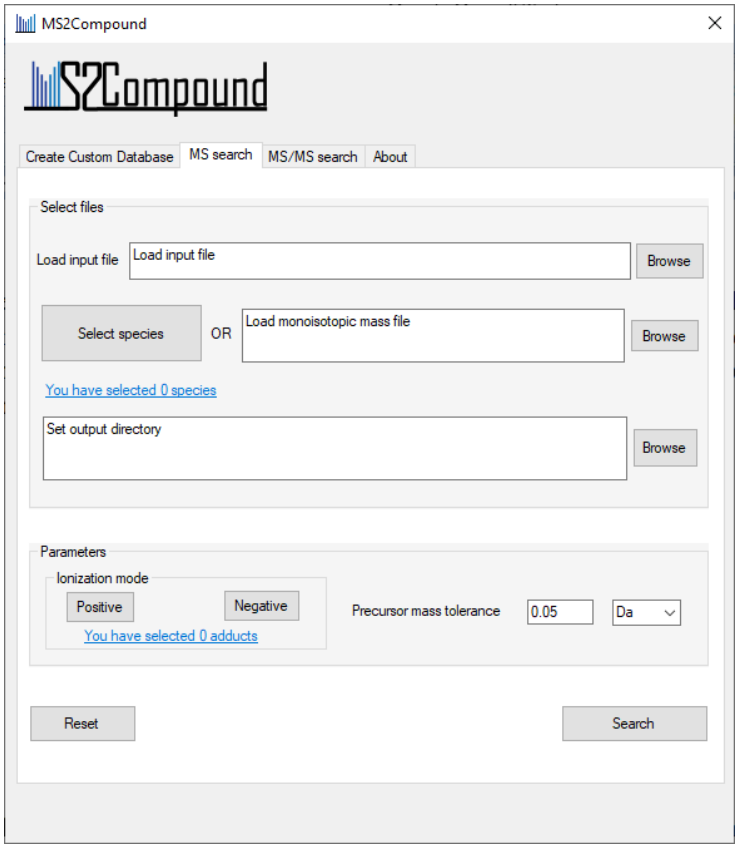
Alongside the program will create an output file named “MonoisotopicMass.txt” for the calculated monoisotopic masses.

The sample outputs from this module using the given test data, and “Positive” ionization mode and “ESI-MS/MS (CE)” spectra type is provided in https://github.com/beherasan/MS2Compound/tree/master/TestData/FragmentPrediction/OUTPUT .

*** *The CFM-id prediction will take some time based on the number of the compounds in input file. The current monoisotopic mass calculation cannot be used for the isotopes of compounds.*

MS-Search using in-built Database

Click on MS search tab. This will open a window as following:

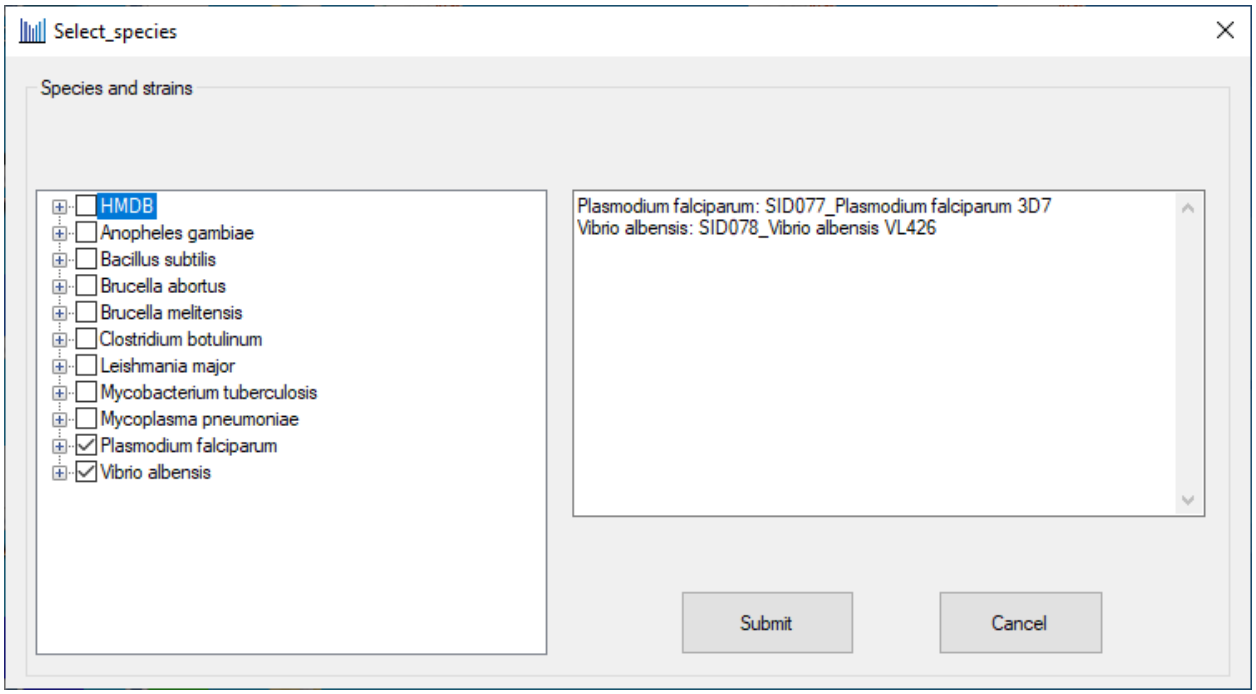


The input file should be a tab separated file with three columns; first column containing unique identifiers, second containing the precursor m/z value, and the third column with charge information. The format of the charge should be "a numeric number followed by + or - sign", for example "1+". A sample file has been shown below:

1	145.54	1+
2	530.3015	1+
3	758.2237	1+
4	336.2166	2+
5	747.3701	1+
6	345.2206	2+
7	445.2429	2+
8	536.1644	
9	610.1874	
10	182.9848	1+

If the charge information is provided the match will consider particular adducts for the given charge, else it will search for all adducts selected.

After uploading the input file, select the “Select species” button. User can select any species of interest, or the search can be strain specific by clicking the “+” sign of species, and then select the desired strain. The species selection is shown as following:

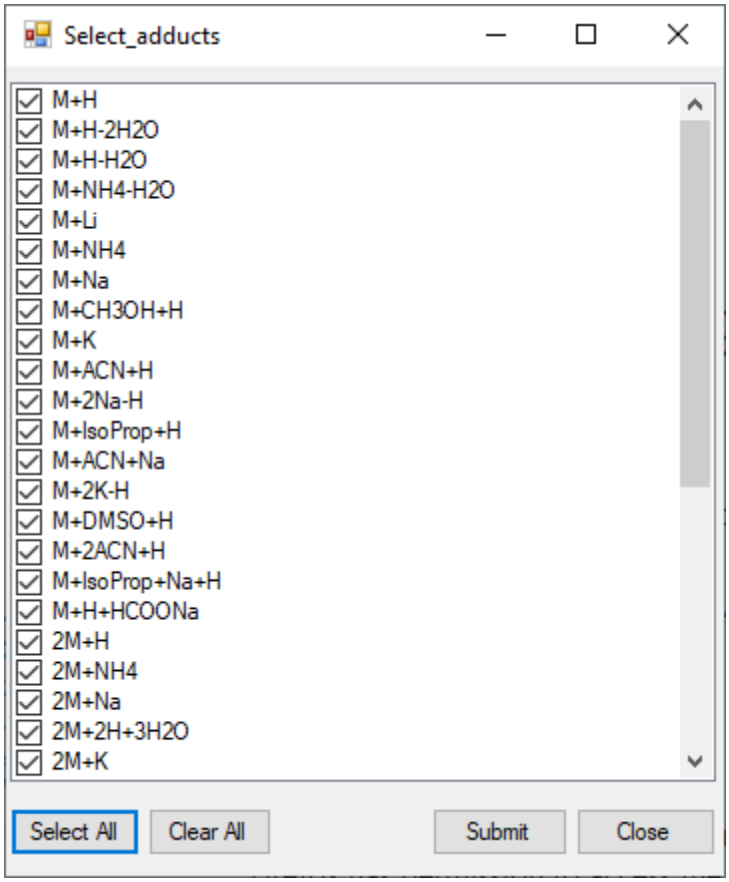


Please ignore the “SID…” as these are the in-built ids provided by MS2Compound backend database

Click “Submit” after selecting the species.

Select the “Output directory”

Select the “Ionization mode” by clicking either “Positive” or “Negative” button. This will open a new window for adducts.

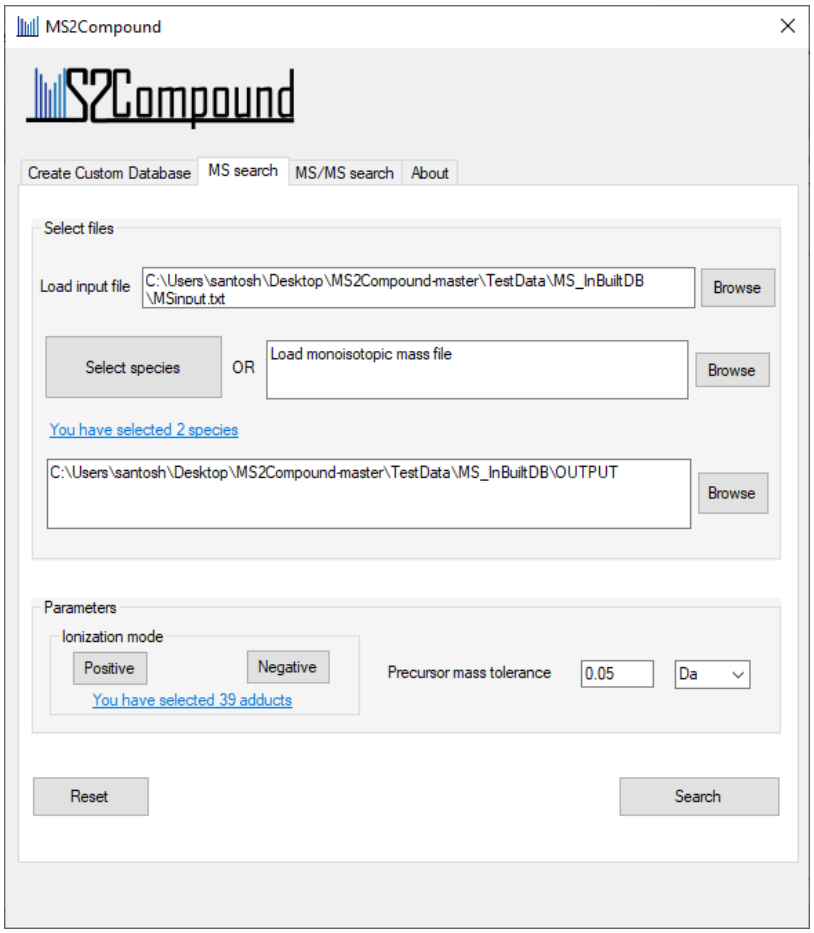


If adducts are not known please select all adducts.

Select the “Precursor mass tolerance”, and the unit.

Click “Search”

A filled form is shown below:



This generates three output files in selected output directory.

1. Assigned.txt

2. Assigned.html

3. Unassigned.txt

4. parameters_MS_search.log

Assigned.html can be opened with any browser. A screenshot for the same is shown below:

Please Cite MS2Compound if you are using this as a part of your analysis

ID	mz	Rank	Adduct	Charge	Compound ID	Da error	BioCyc UniqId	Common Name	Molecular Weight	Monoisotopic Mass	HMDB	PubChem
2	530.3015	1	M+CH3OH+H	1+	CID000394	0.000521749100016677	CPD66-21	leukotriene-D4	495.653	497.2685327491		52940265
2	530.3015	2	2M+ACN+Na	1+	CID001564	0.0291208333999862	N2-SUCCINYLORNITHINE	N^2-succinyl-L-ornithine	231.228	233.1137466666	HMDB01199	25244550
2	530.3015	2	2M+ACN+Na	1+	CID002786	0.0291208333999862	CPD-9000	4-(L-γ-glutamylamino)butanoate	231.228	233.1137466666	HMDB12161	25245457
2	530.3015	3	2M+ACN+H	1+	CID001736	0.045675419700018	BIOTIN	biotin	243.3	244.0881630803	HMDB00030	6560210
3	758.2237	1	2M+ACN+H	1+	CID001844	0.00141980339998327	PANTETHEINE-P	4'-phosphopantetheine	356.33	358.0963583034	HMDB01416	25245905
3	758.2237	2	2M+ACN+H	1+	CID000385	0.00195101500003148	ALPHA-RIBAZOLE-5-P	α-ribazole 5'-phosphate	356.271	358.092987485	HMDB03882	25244046
3	758.2237	3	2M+ACN+Na	1+	CID001425	0.0408831599000337	CPD-3706	adenosine 3'-monophosphate	345.208	347.0630843401	HMDB03540	15938966
3	758.2237	3	2M+ACN+Na	1+	CID002589	0.0408831599000337	AMP	AMP	345.208	347.0630843401	HMDB00045	15938965

The sample output files can be found at "https://github.com/beherasan/MS2Compound/tree/master/TestData/MS_InBuiltDB/OUTPUT"

MS-Search using Custom Database

This module allows the user to search MS data against a user defined monoisotopic mass file for a selected list of compounds.

The input file will be similar as that of the input for "**MS-Search using in-built Database**".

The monoisotopic mass file should be a tab separated file with two columns; one for unique identifier, and the second one for corresponding monoisotopic mass.

Remaining parameters and the output will be similar as that of "**MS-Search using in-built Database**".

MS/MS-Search using in-built Database

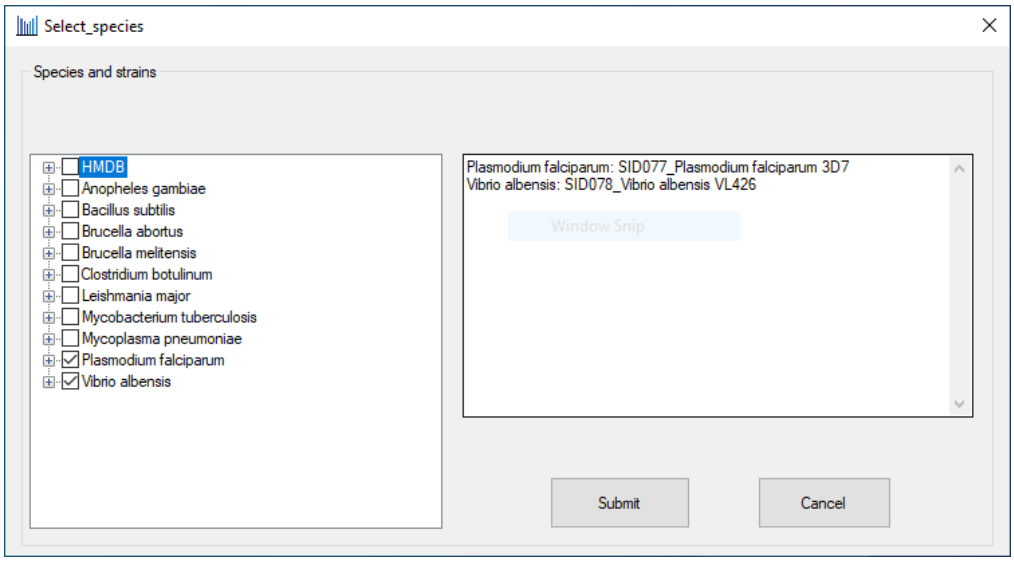
This module allows the user to search MS/MS data against in-built MS/MS database. The current version (1.0.0) of MS2Compound supports Mascot Generic Format (MGF) file as input.

The screenshot for a sample input is shown below:

```
BEGIN IONS
FEATURE_ID=2
PEPMASS=245.1289
RTINSECONDS=145.54
CHARGE=1+
MSLEVEL=2
75.72264099121094 0.0921952008202265
76.5239486694336 0.105720467448089
77.03893280029297 0.129845590210377
82.06517791748047 0.157307460571153
85.88349914550781 0.0928718868766921
87.49651336669922 0.100132314717048
91.05423736572266 0.650206850612891
93.07019805908203 0.282088145881232
96.9111328125 0.109116483030762
98.06049346923828 20.5369436388339
102.48599243164062 0.0955491634067138
103.05403900146484 0.858005857680907
105.0699234008789 0.199647936413473
108.80220031738281 0.0939097949997296
113.07038879394531 0.0998121146098996
120.08045196533203 100
121.7412338256836 0.0913062209685591
125.07101440429688 0.462762398350365
126.07872772216797 0.274709301805809
131.04966735839844 1.98450060608678
137.071044921875 0.153365507697125
148.0762939453125 0.511827029879847
```

The MS/MS search can be performed with following steps:

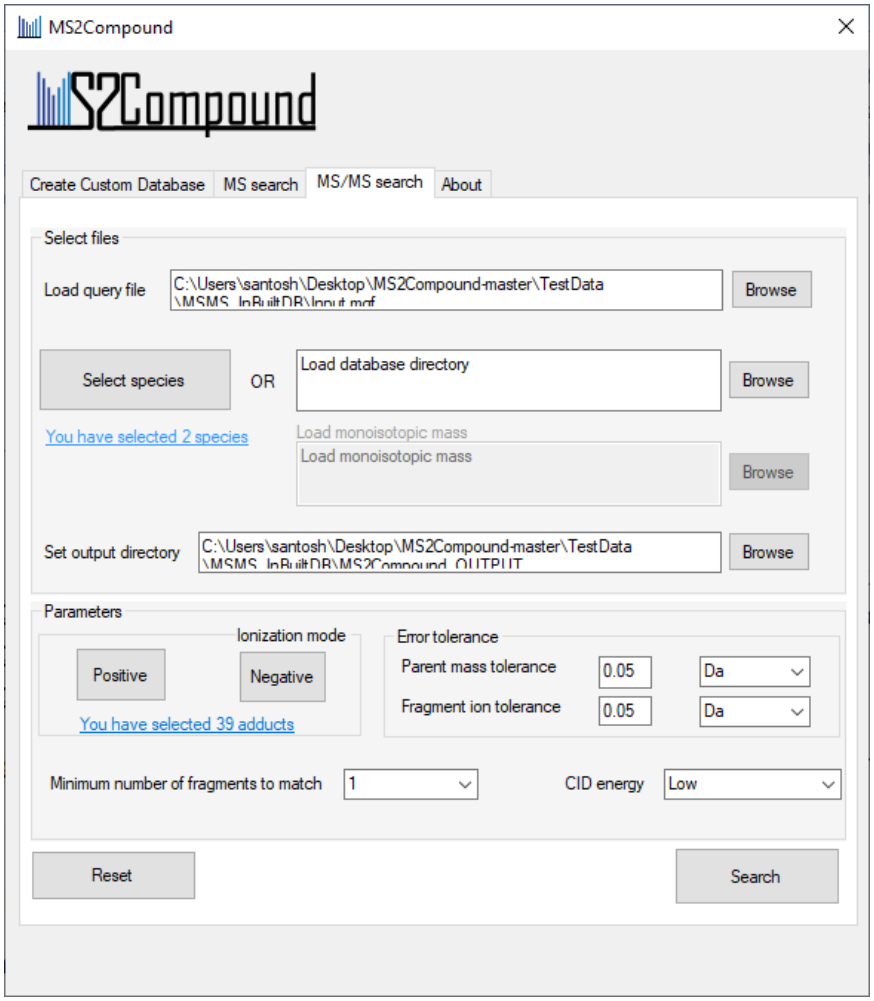
- Load MGF file as Query file. The sample input is provided at https://github.com/beherasan/MS2Compound/tree/master/TestData/MSMS_InBuiltDB
- Select the species of interest by clicking “Select species” button.



- Set the output directory

- Click on ionization mode (either Positive or Negative), and select adducts. If the adducts are not known please select all the adducts
- Select the error tolerance for precursor and fragment match
- Select the “Minimum number of fragments to match” for number of fragment matches.
- Select the CID energy for MS/MS fragmentation
- Select “Search” button

A filled is shown below:



This will create a directory “MS2Compound_OUTPUT” in selected output directory. If the “MS2Compound_OUTPUT” directory already exists in the output directory path, it will create “MS2Compound_OUTPUT_1” for a new search. In same search it will replace the results in “MS2Compound_OUTPUT”. This directory contains the

This generates three output files in “MS2Compound_OUTPUT” directory.

1. Assigned.txt

2. Assigned.html

3. Unassigned.mgf

4. allMapped.txt

5. parameters_MSMS_search.log

The sample output files can be found at https://github.com/beherasan/MS2Compound/tree/master/TestData/MSMS_InBuiltDB/MS2Compound_OUTPUT

"Assigned.html" can be open with any browser (such as firefox). A screenshot of "Assigned.html" is shown below:

Please Cite MS2Compound if you are using this as a part of your analysis

Feature ID	Rank	Adduct	Charge	Compound ID	mS-score	BioCyc UniqId	Common Name	Molecular Weight	Monoisotopic Mass	HMDB	PubChem
2	1	M+DMSO+H	1+	CID002075	2.80010	PHE	L-phenylalanine	165.191	166.0868036346	HMDB00159	6925665
2	2	2M+H	1+	CID000058	2.50043	PHENYLETHYLAMINE	2-phenylethylamine	122.189	122.0969743904	HMDB12275	448751
2	3	2M+H	1+	CID002969	2.11706	CPD-7035	2-phenylethanol	122.166	122.0731649431	HMDB33944	6054
2	4	M+IsoProp+H	1+	CID000272	0.12337	CPD-11497	3-methoxy-4-hydroxyphenylglycol	184.191	184.0735588736	HMDB01490	10805
2	5	M+IsoProp+Na+H	1+	CID002572	0.05672	N-ACETYL-L-24-DIAMINOBUTANOATE	*N*-acetyl-L-2,4-diaminobutanoate	160.172	161.092617294		25245066
2	6	M+NH4	1+	CID003368	-0.91380	DEOXYCYTIDINE	2'-deoxycytidine	227.219	227.0906059213	HMDB00014	13711
2	7	M+IsoProp+H	1+	CID000245	-0.93770	CPD-11875	normetanephrine	184.214	184.0973683209	HMDB00819	1237
2	8	M+IsoProp+H	1+	CID002755	-0.95498	L-EPINEPHRINE	(R)-adrenaline	184.214	184.0973683209	HMDB00068	4102253
2	9	M+IsoProp+Na+H	1+	CID003484	-1.03396	D-ALA-D-ALA	D-alanyl-D-alanine	160.172	161.092617294	HMDB03459	6992112

The assigned compounds to a particular Feature ID are ranked based on the *mS-score* and sorted in order.

"Assigned.txt" has the similar information as that of "Assigned.html", and can be opened in any text editor or excel sheet to view the results.

MS/MS-Search using Custom Database

This module allows the user to search MS/MS data against custom/user generated MS/MS database. The current version (1.0.0) of MS2Compound supports Mascot Generic Format (MGF) file as input as described in "**MS/MS-Search using in-built Database**" section.

Unlike that of "**MS/MS-Search using in-built Database**", in this module user have to upload a Database directory (as "ReferenceDB" in https://github.com/beherasan/MS2Compound/tree/master/TestData/MSMS_CustomDB), and the

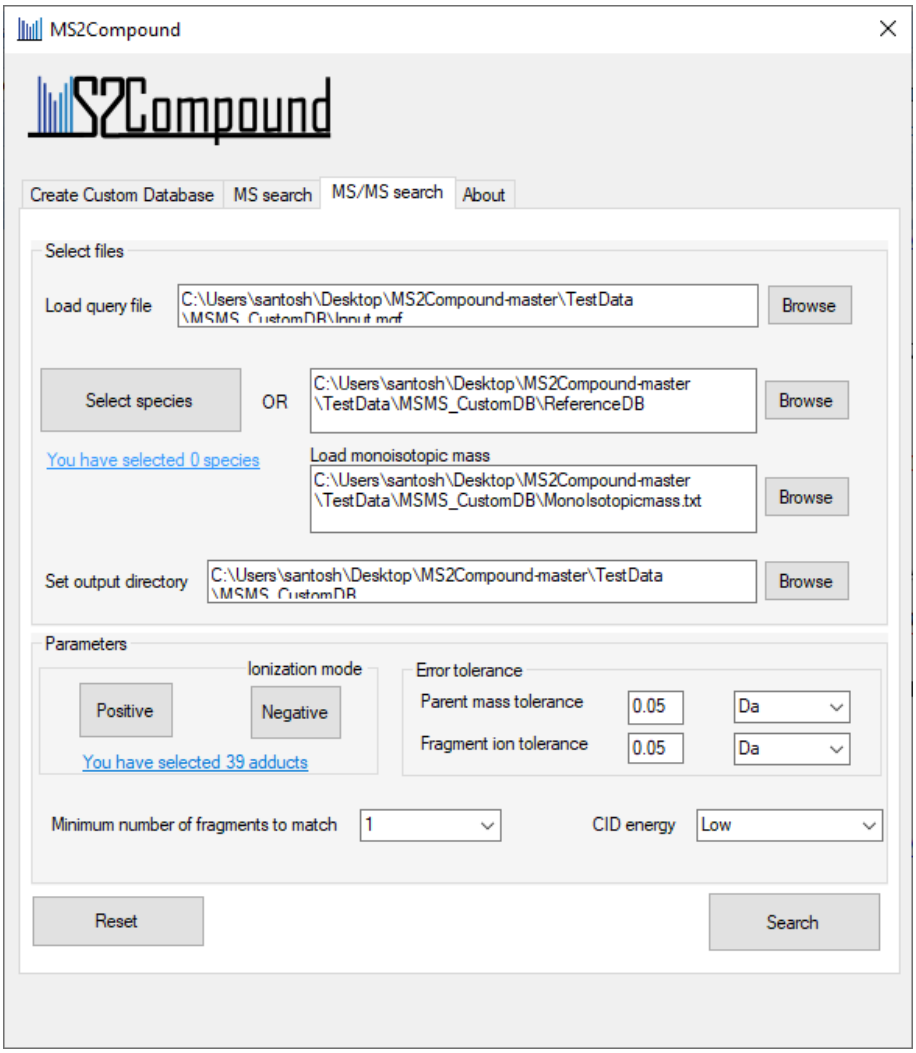
monoisotopic mass file (as "MonoIsotopicmass.txt" in https://github.com/beherasan/MS2Compound/tree/master/TestData/MSMS_CustomDB) instead of selecting species of interest.

"MonoIsotopicmass.txt" is a tab separated file containing compound unique identifier (UID) and the corresponding monoisotopic mass.

The MS/MS fragment file names should be UID.log in "ReferenceDB". Please maintain the naming for proper functioning of the tool.

If you are using our module "**Create Custom Database**" for creating your database, then you can directly load the Database directory and the monoisotopic mass file for the search.

A filled form is shown below:



The result file will be similar as that of the "**MS/MS-Search using in-built Database**", however it will not provide the compound details.

The sample output files are provided at https://github.com/beherasan/MS2Compound/tree/master/TestData/MSMS_CustomDB/MS2Compound_OUTPUT