Examples of M-RT and MS/MS Search

1. An example of using M-RT to do single mode search.

1). For the M-RT single mode search, the user enters a precursor mass (359.0730) and retention time (425.28 seconds), together with their mass tolerance (5 ppm) and RT tolerance (15 seconds) (see Figure 1). A calibration file with 22 calibration standards also needs to be uploaded. The template of the calibration file can be downloaded under the Introduction on the side bar. The user needs to download it and change the retention time according to the calibration file performed with the metabolite feature. After filling out the retention time, click the "Submit Query" to start the M-RT single mode search.

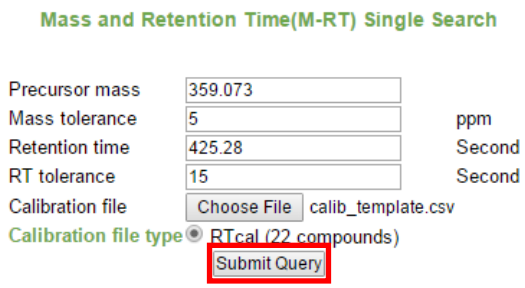

Figure 1. Single mode search parameter

2). The search result is shown in Figure 2.

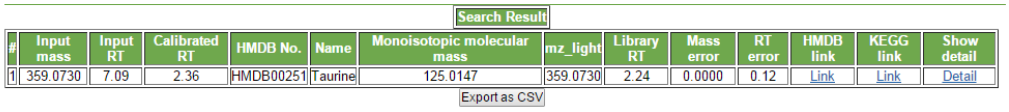

Figure 2. Single search result.

2. An example of using M-RT to do batch mode search.

1). For the M-RT batch mode search, the user enters a mass tolerance (5 ppm) and RT tolerance (15 seconds) (see Figure 1). The user also needs to upload a sample file and a calibration file.

The template of the sample file and calibration file are attached. For the calibration file, the user needs to download it and change the retention time according to the calibration file. After it's all done, click the "Submit Query" to start the M-RT single mode search.

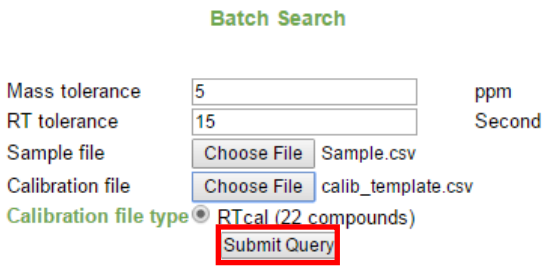

Figure 3. Batch mode search parameters.

2). The search result is shown in Figure 4.

Search Result

#	Input mass	Input RT	Calibrated RT	HMDB No.	Name	Monoisotopic molecular mass	mz_light	Library RT	Mass error	RT error	HMDB link	KEGG link	Show detail
1	375.0785	2.05	1.94	HMDB00224	O-Phosphoethanolamine	141.0191	375.0774	2.02	0.0011	0.08	Link	Link	Detail
2	359.0743	2.31	2.18	HMDB00251	Taurine	125.0147	359.0730	2.24	0.0013	0.06	Link	Link	Detail
3	403.1443	2.32	2.19	HMDB00001	1_Methylhistidine	169.0851	403.1434	2.17	0.0009	0.02	Link	Link	Detail
4	403.1443	2.32	2.19	HMDB00479	3_methyl-histidine	169.0851	403.1434	2.01	0.0009	0.18	Link	Link	Detail
5	408.1708	2.61	2.47	HMDB00517	L-Arginine	174.1117	408.1700	2.44	0.0008	0.03	Link	Link	Detail
6	343.0781	2.64	2.50	HMDB00965	Hypotaurine	109.0197	343.0781	2.47	0.0000	0.03	Link	Link	Detail
7	351.1124	2.81	2.67	HMDB00128	Guanidoacetic acid	117.0538	351.1121	2.74	0.0003	0.07	Link	Link	Detail
8	366.1132	3.09	2.94	HMDB00168	L-Asparagine	132.0535	366.1118	3.0	0.0014	0.06	Link	Link	Detail
9	422.1861	3.21	3.06	HMDB00670	Homo-L-arginine	188.1273	422.1856	3.0	0.0005	0.06	Link	Link	Detail
10	359.1547	3.28	3.14	HMDB01861	3_Methylhistamine	125.0953	359.1536	3.27	0.0011	0.13	Link	Link	Detail
11	436.2014	3.44	3.29	HMDB03334	Symmetric dimethylarginine	202.1430	436.2013	3.05	0.0001	0.24	Link	Link	Detail

Figure 4. Batch mode search result.

3). At the end of the search result table, there is an "Export as CSV" button (Figure 5). By clicking this button, the user can export the search results into a CSV table shown in Figure 6

113	311.0703	24.64	24.44	HMDB00152	Gentisic acid - multi-tags	154.0266	311.0716	24.69	0.0013	0.25	Link	Link	Detail
114	311.0703	24.64	24.44	HMDB01856	Protocatechuic acid	154.0266	311.0716	24.51	0.0013	0.07	Link	Link	Detail
115	322.1045	24.88	24.67	HMDB00259	Serotonin	176.0950	322.1058	24.65	0.0013	0.02	Link	Link	Detail
116	318.0783	24.94	24.74	HMDB00130	Homogentisic acid	168.0423	318.0794	24.84	0.0011	0.10	Link	Link	Detail
117	317.6047	25.56	25.46	HMDB00022	3_Methoxytyramine	167.0946	317.6056	25.49	0.0009	0.03	Link	Link	Detail
118	317.6047	25.56	25.46	HMDB02182	Phenylephrine	167.0946	317.6056	25.39	0.0009	0.07	Link	Link	Detail
119	302.5998	25.88	25.80	HMDB00306	Tyramine	137.0841	302.6004	25.83	0.0006	0.03	Link	Link	Detail

Export as CSV

Figure 5. "Export as CSV" button.

8	search result:									
9	#	Input mas	Input RT	Calibratec	HMDB No.	Name	Monoisot	mz_light	Library RT	Mass erro	RT error
10	1	375.0785	2.05	1.94	HMDB002	O-Phosphoethanolamine	141.0191	375.0774	2.02	0.0011	0.08
11	2	359.0743	2.31	2.18	HMDB002	Taurine	125.0147	359.073	2.24	0.0013	0.06
12	3	403.1443	2.32	2.19	HMDB000	1_Methylhistidine	169.0851	403.1434	2.17	0.0009	0.02
13	4	403.1443	2.32	2.19	HMDB004	3_methyl-histidine	169.0851	403.1434	2.01	0.0009	0.18
14	5	408.1708	2.61	2.47	HMDB005	L-Arginine	174.1117	408.17	2.44	0.0008	0.03
15	6	343.0781	2.64	2.5	HMDB009	Hypotaurine	109.0197	343.0781	2.47	0	0.03
16	7	351.1124	2.81	2.67	HMDB001	Guanidoacetic acid	117.0538	351.1121	2.74	0.0003	0.07
17	8	366.1132	3.09	2.94	HMDB001	L-Asparagine	132.0535	366.1118	3	0.0014	0.06
18	9	422.1861	3.21	3.06	HMDB006	Homo-L-arginine	188.1273	422.1856	3	0.0005	0.06
19	10	359.1547	3.28	3.14	HMDB018	3_Methylhistamine	125.0953	359.1536	3.27	0.0011	0.13
20	11	436.2014	3.44	3.29	HMDB033	Symmetric dimethylarginine	202.143	436.2013	3.05	0.0001	0.24
21	12	380.1288	3.44	3.3	HMDB006	L-Glutamine	146.0691	380.1275	3.32	0.0013	0.02
22	13	380.1288	3.44	3.3	HMDB034	D-Glutamine	146.0691	380.1275	3.32	0.0013	0.02
23	14	359.1538	3.49	3.34	HMDB018	3_Methylhistamine	125.0953	359.1536	3.27	0.0002	0.07
24	15	409.1551	3.65	3.5	HMDB009	Citrulline	175.0957	409.154	3.74	0.0011	0.24
25	16	399.1049	3.83	3.68	HMDB020	Methionine Sulfoxide	165.046	399.1043	3.72	0.0006	0.04
26	17	307.1223	3.93	3.78	HMDB015	Methylguanidine	73.064	307.1223	3.84	0	0.06

Figure 6. Exported CSV search result.

3. An example of performing M-RT and MS/MS search.

1). For the MS/MS search, the user inputs a precursor mass (581.1214) and select the ion type as [M+H]. Also, a MS/MS list needs to be uploaded. The MS/MS tolerance is defined at a default of 0.005 Da. The match precursor ion and match retention time functions are all turned off, which means the MS/MS search is based on the match of MS/MS fragments with the MS/MS standards. After all the parameters are set, click “Submit Query” to start the MS/MS search.

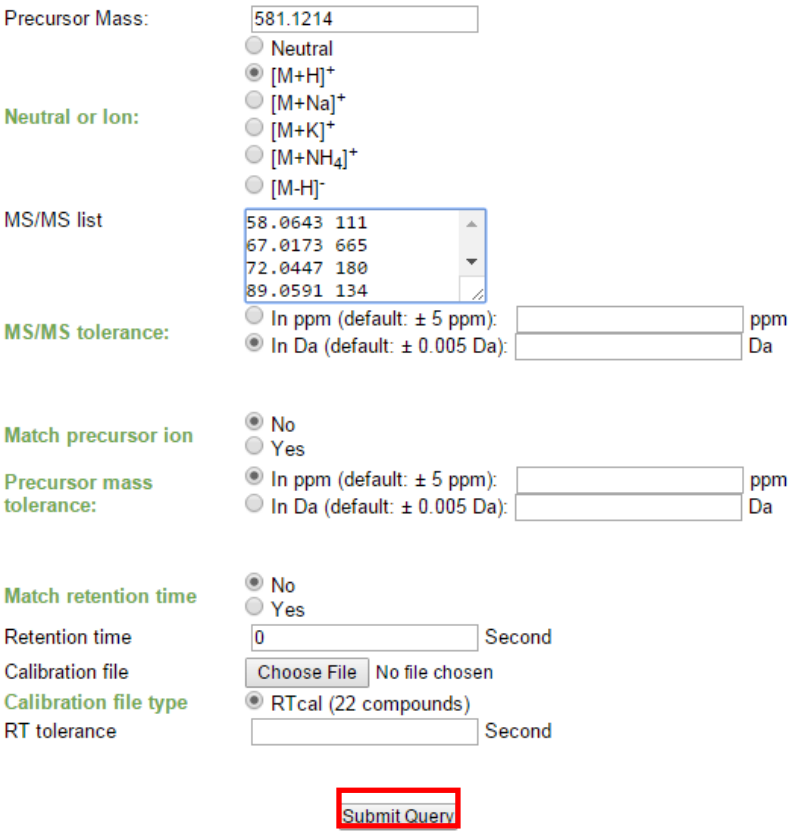

Figure 7. MS/MS search parameters.

2). The MS/MS search result is shown in Figure 8.

Search Result

#	Input mass	Input RT	Calibrated RT	HMDB No.	Name	Monoisotopic molecular mass	mz_light	Library RT	Mass error	RT error	HMDB link	KEGG link	MS/MS score ▲	Show detail
7	581.1214	NA	NA	HMDB00045	Adenosine monophosphate	347.0631	581.1214	1.75	0.0000	NA	Link	Link	1.00	Detail
160	581.1214	NA	NA	HMDB01341	ADP	427.0294	661.0877	1.49	79.9663	NA	Link	Link	0.97	Detail
146	581.1214	NA	NA	HMDB01044	2'-Deoxyguanosine 5'-monophosphate	347.0631	581.1214	5.57	0.0000	NA	Link	Link	0.53	Detail
155	581.1214	NA	NA	HMDB01173	5'-Methylthioadenosine	297.0896	531.1479	6.97	49.9735	NA	Link	Link	0.23	Detail
138	581.1214	NA	NA	HMDB00939	S-Adenosylhomocysteine	384.1216	426.1191	10.52	155.0023	NA	Link	Link	0.22	Detail
300	581.1214	NA	NA	HMDB60003	Isovanillic acid	168.0423	402.1006	15.69	179.0208	NA	Link	Link	0.21	Detail
299	581.1214	NA	NA	HMDB59966	3-5-Dimethoxyphenol	154.0630	388.1213	23.75	193.0001	NA	Link	Link	0.21	Detail
8	581.1214	NA	NA	HMDB00050	Adenosine	267.0968	501.1551	3.94	79.9663	NA	Link	Link	0.20	Detail
280	581.1214	NA	NA	HMDB28937	Leucyl-Proline	228.1474	462.2057	12.99	118.9157	NA	Link	Link	0.18	Detail
269	581.1214	NA	NA	HMDB28691	Alanyl-Leucine	202.1317	436.1901	11.36	144.9313	NA	Link	Link	0.18	Detail
234	581.1214	NA	NA	HMDB02991	Cysteamine	77.0299	311.0882	15.08	270.0332	NA	Link	Link	0.17	Detail

Figure 8. MS/MS search result.