Example of Using MyCompoundID

The following describes how to use MyCompoundID using the metabolite 3-hydroxyoctanoylcarnitine as an example.

1. The user has to enter the desired search parameters described in the MyCompoundID Tutorial. In this particular case, one reaction was selected, the ion type selected was $[M+H]^+$, the *m/z* ratio entered (Query Mass) was 304.210568 and finally, the mass tolerance was set to 5 ppm. Please refer to Figure 1.

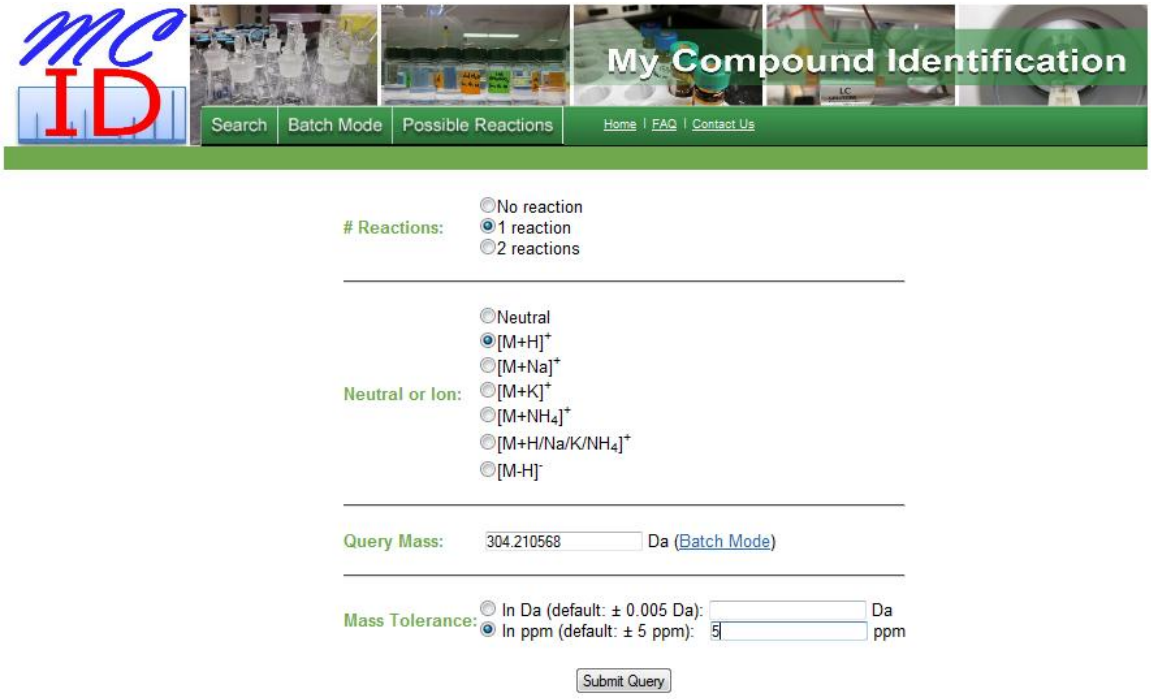

Figure 1. Software interface showing the entered parameters.

2. Once the query is submitted the following page is displayed (Figure 2). For this particular search, there were eight possible hits or matches. The user can then open the structure of any of the possible hits on ChemDraw and use the Fragmentation Tools in ChemDraw to compare the predicted fragments with those observed in an acquired experimental MS/MS spectrum of the query ion. The MS/MS spectrum of 3-hydroxyoctanoylcarnitine acquired on a QTRAP instrument is shown in Figure 3.

Search Result

Input Parameter Name	Parameter Value(s)
# Reactions	1
Ion Type	[M+H]+
Query Mass	304.210568 Da
Neutral Mass	303.203292 Da
Mass Tolerance	±5 ppm

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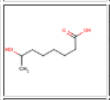
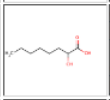
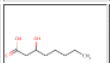
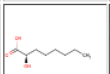
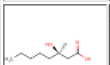
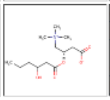
#	HMDB ID	Common Name	Mass (Da)	Formula	Chemical Structure	Explore (for Firefox)	Possible Reactions	Reaction Offset (Da)	Mass Error (ppm)	To Del	Attachments
1	HMDB13324	2-Octenoylcarnitine	285.194	$C_{15}H_{27}NO_4$	Structure	ChemDraw Pro ChemDraw Plugin	[+H_2O]	18.0105650	-4.199987		Add
2	HMDB00486	7-Hydroxyoctanoic acid	160.10994	$C_8H_{16}O_3$		ChemDraw Pro ChemDraw Plugin	[+$C_7H_{13}NO_2$]	143.094629	-4.213180		Add
3	HMDB00711	Hydroxyoctanoic acid	160.10994	$C_8H_{16}O_3$		ChemDraw Pro ChemDraw Plugin	[+$C_7H_{13}NO_2$]	143.094629	-4.213180		Add
4	HMDB01954	3-Hydroxyoctanoic acid	160.10994	$C_8H_{16}O_3$		ChemDraw Pro ChemDraw Plugin	[+$C_7H_{13}NO_2$]	143.094629	-4.213180		Add
5	HMDB02264	(R)-2-Hydroxycaprylic acid	160.10994	$C_8H_{16}O_3$		ChemDraw Pro ChemDraw Plugin	[+$C_7H_{13}NO_2$]	143.094629	-4.213180		Add
6	HMDB10722	(R)-3-Hydroxyoctanoic acid	160.10994	$C_8H_{16}O_3$		ChemDraw Pro ChemDraw Plugin	[+$C_7H_{13}NO_2$]	143.094629	-4.213180		Add
7	HMDB00791	L-Octanoylcarnitine	287.209656	$C_{15}H_{29}NO_4$		ChemDraw Pro ChemDraw Plugin	[+O]	15.9949150	-4.219776		Add
8	HMDB13131	Hydroxyhexanoycarnitine	275.173279	$C_{13}H_{25}NO_5$		ChemDraw Pro ChemDraw Plugin	[+C_2H_4]	28.0313000	-4.246161		Add

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- "Save Attachments" is not available for IE

Figure 2. Parameters and results table displaying a total of eight possible hits.

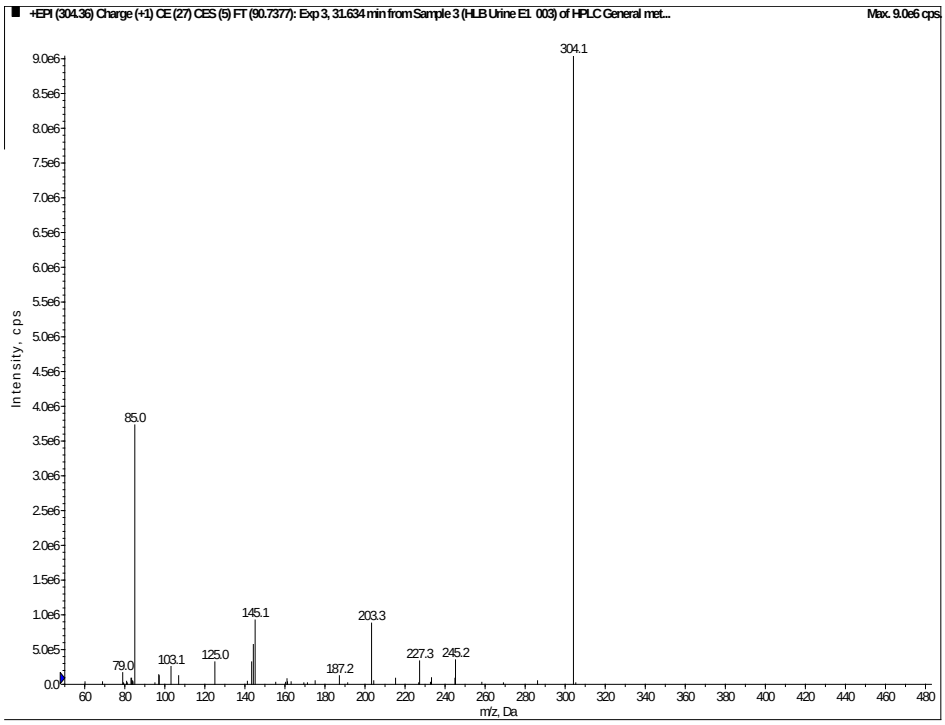

Figure 3. Experimental MS/MS spectrum of 3-hydroxyoctanoylcarnitine found in urine.

3. The user should examine all the possible hits and decide which one is the most likely match based on the fragmentation pattern observed in the experimental MS/MS spectrum. In each row, the structure of the core compound from the HMDB is shown along with the added or subtracted group information from the possible biotransformation reaction of the core compound. When the user clicks on the ChemDraw link in the row, the structure of the core compound will be displayed in ChemDraw. Using ChemDraw, the user can add or remove the group from the core compound structure to generate a postulated structure with its mass matched with the Query Mass within the mass tolerance threshold. In this particular case, hit #4 is 3-hydroxyoctanoic acid with the addition of $C_7H_{13}NO_2$ or carnitine. It is known that carnitine conjugation occurs *via* an ester linkage between the carboxylic acid group of the acid and the OH group of carnitine, followed by the loss of water. Thus, a postulated structure from the addition of carnitine to the core structure, 3-hydroxyoctanoic acid, can be drawn in ChemDraw.

4. Using ChemDraw, the user can fragment the compound of interest (e.g., the postulated structure in this example) using the Fragmentation Tools and compare the predicted fragments to those found in the experimental MS/MS spectrum. The MyCompoundID Tutorial contains information on how to use the Fragmentation Tools available in ChemDraw. Figure 4 shows the fragments that were assigned, including the peak at *m/z* 145, which is characteristic of 3-hydroxycarnitines.

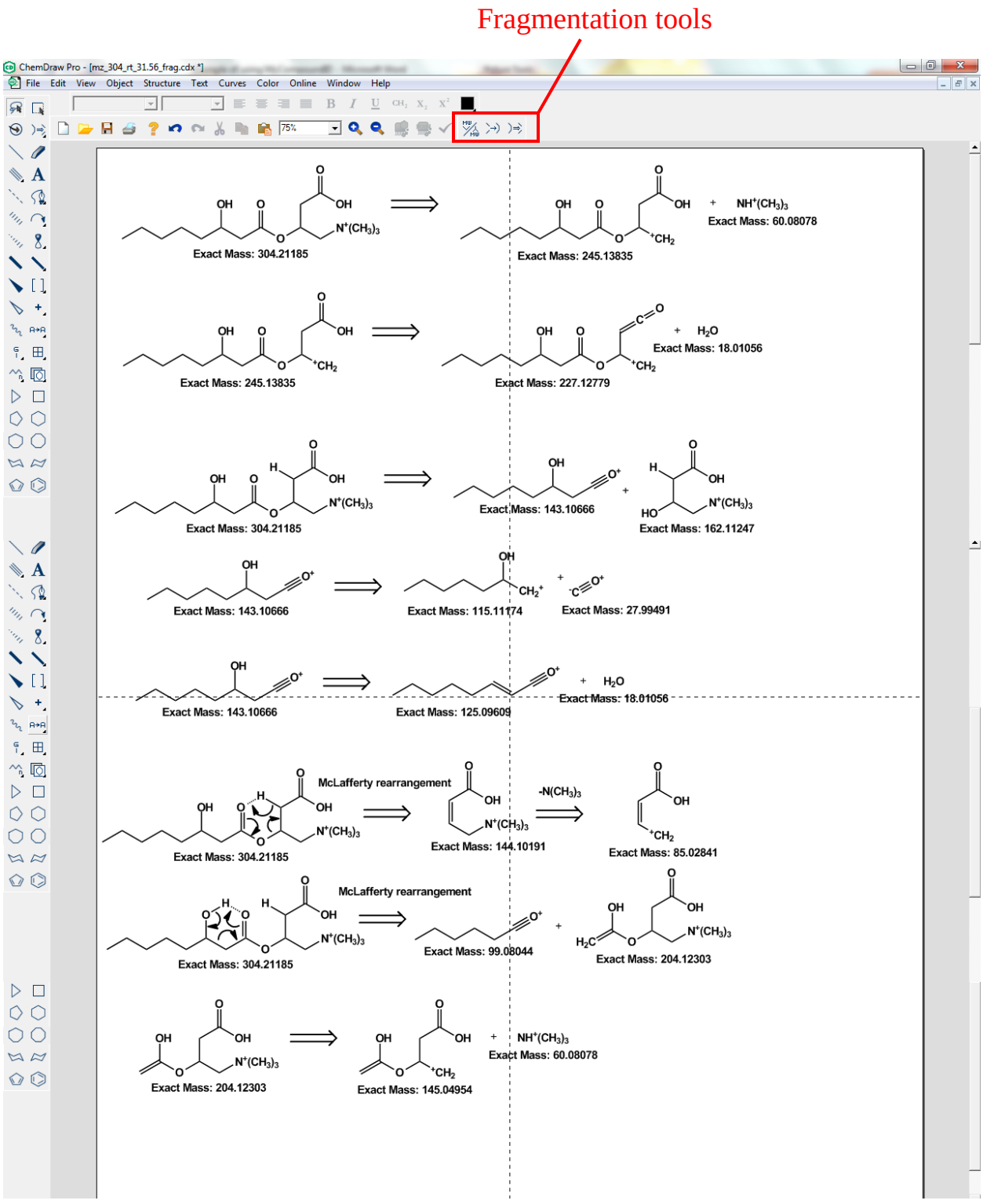

Figure 4. Fragmentation of 3-hydroxyoctanoylcarnitine. The retro synthesis tool was used in order to display both fragments that originate from bond cleavages.

5. Based on the fragmentation pattern, the user is able to select hit # 4 as the most likely match, since the peak at *m/z* 145 is characteristic of hydroxyacylcarnitines that contain the OH group in the 3 position. Hit #4 is highlighted in Figure 5 displaying a mass error of 4.2 ppm, which is lower than the mass tolerance selected. The structure of 3-hydroxyoctanoylcarnitine is shown in Figure 6.

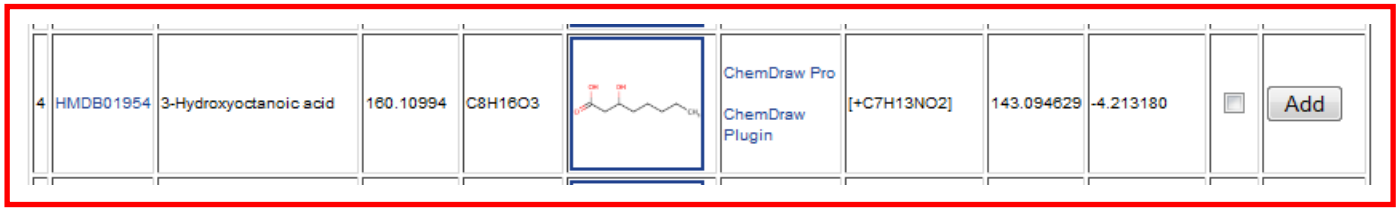

Figure 5. The row corresponding to hit #4 is highlighted.

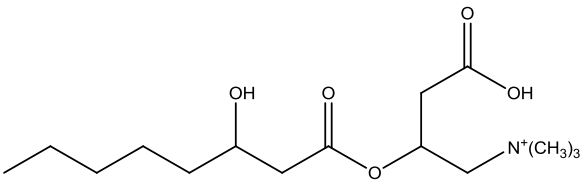

Figure 6. Structure of the putative identification of a feature with *m/z* 304.210568 and retention time of 31.56 min.

6. Finally the user can save all the files in the same folder using the save attachment option on the search results table. This way the user can compile all the evidence supporting the putative identification(s). **Note: The save attachment function does not work with Internet Explorer; please use Firefox.**