Tutorial for

FlavonoidSearch FsTool GUI

16 May 2017

Contents

Introduction

FlavonoidSearch[1] is a system for annotating flavonoid aglycones using mass spectra obtained from multiple-stage mass spectrometry (MS^n) analyses. FlavonoidSearch consists of two parts: FsDatabase, a manually curated database of predicted mass fragments for approximately 7000 known flavonoids; and FsTool, a Java-based tool to search FsDatabase. FsTool can be used both as an easy-to-use graphical user interface (GUI) tool and a command line tool for high-throughput calculations on servers. This tutorial shows practical operations of the GUI of FsTool (FsTool GUI) using a MS^n data obtained by liquid chromatography (LC)–mass spectrometry (MS) analysis of parsley.

Computer requirements

A PC (64 bit, >2 GB RAM is recommended) with Java Runtime Environment (64 bit, version 1.7 or later) is required to do the operations in this tutorial. An access to the Internet is required for the database search and structure analysis in Step 7.

Preparation

MassChroViewer

In this tutorial, MassChroViewer is used to obtain a MS^n spectrum from the raw mass chromatogram data of parsley. The MassChroViewer tool newer than the version 1.3.2 contains FsTool. Download the program from the following URL and set it up according to the manual.

http://www.kazusa.or.jp/komics/software/MassChroViewer/

FsTool

You don't have to install FsTool, when you use MassChroViewer 1.3.2 or later. If you want to install FsTool as a standalone tool, download it at the URL below and setup it up following the instructions given in the manual.

http://www.kazusa.or.jp/komics/software/FlavonoidSearch

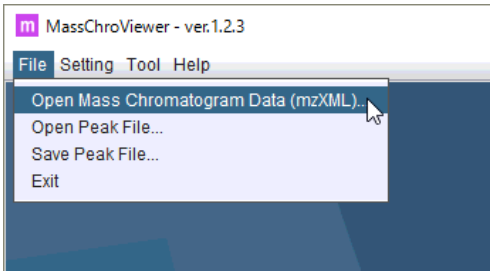
The Mass Chromatogram Data of Parsley

Raw mass chromatogram data of parsley is available at the abovementioned MassChroViewer website. Download the file named 'S2_Cont_LRes_DX_ms2-3.zip' and decompress it using a decompression software such as 7zip. A file named 'S2_Cont_LRes_DX_ms2-3.mzXML' is generated.

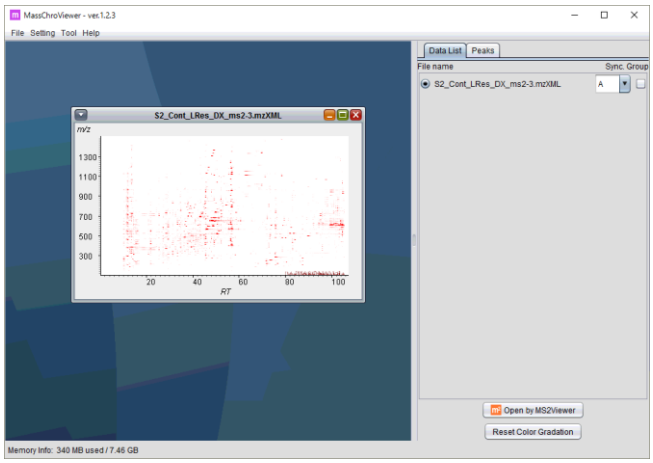
Analysis procedures

Step 1) Opening the mass chromatogram data using MassChroViewer

Run MassChroViewer and select 'Open Mass Chromatogram Data (mzXML)' from the 'File' menu. Select the file 'S2_Cont_LRes_DX_ms2-3.mzXML' from the dialog window.

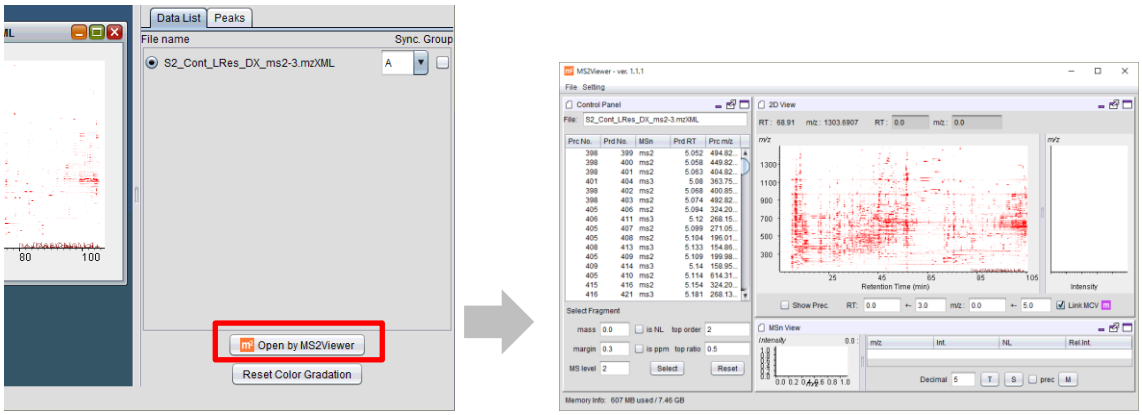


A 2D mass chromatogram will be presented in the window.

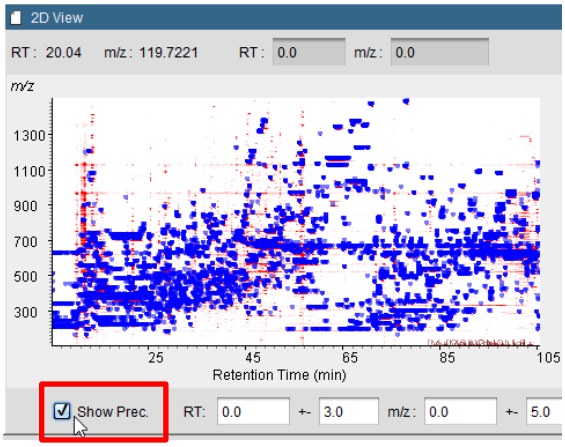


Step 2) Execution of MS2Viewer

Click a button 'Open by MS2Viewer' at the bottom of the 'Data List' panel. The MS2Viewer window will open.



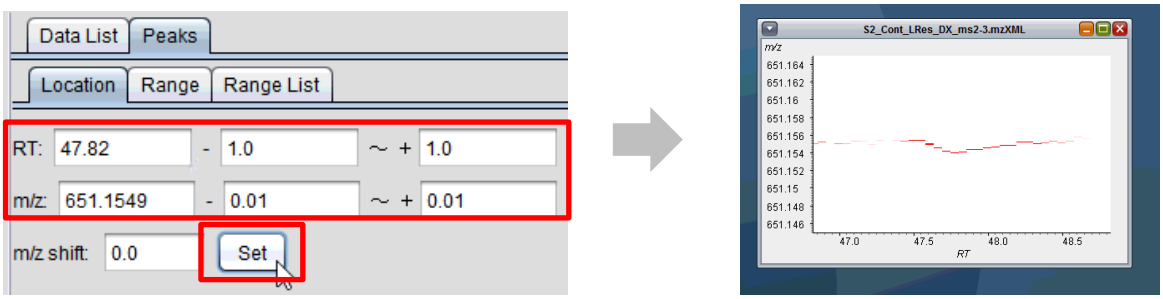
Check the box next to 'Show Prec.' at the bottom of the '2D View' panel in MS2Viewer. The positions of the precursor ions of the MS^n analyses will be represented as blue markers.



Step 3) Selection of the target peak

Using MassChroViewer, select the most intense peak in parsley data which appears around retention time (RT) 47.82 min and at *m/z* 651.1549.

Enter the following values in the 'Location' subpanel in the 'Peaks' tab. Click 'Set' button to display the RT and *m/z* region in the 2D window.



You can select this peak directly on the 2D window by operations similar to those of Google Map:

Modifications	Mouse operations	Note
Changing the colour strength.	CTRL + SHIFT + wheel rotation	
Zooming in the selected area	Right button click and drag	
Zooming full out	Right button double click	*1
Zooming in/out	Wheel rotation	*1

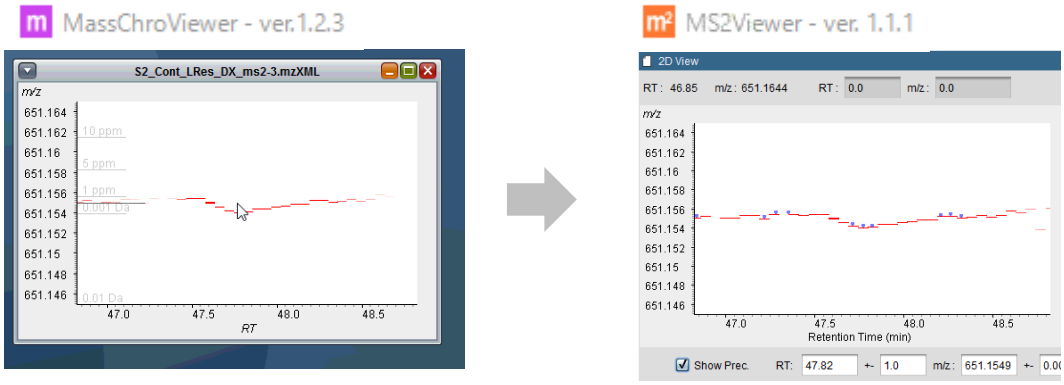
Moving	Left button click and drag	*1
Picking up values	Left button double click	The RT and *m/z* at the position will be used as the base of Mass Ruler and other link functions

*1 The direction of zooming in/out and moving can be fixed using CTRL and SHIFT keys.

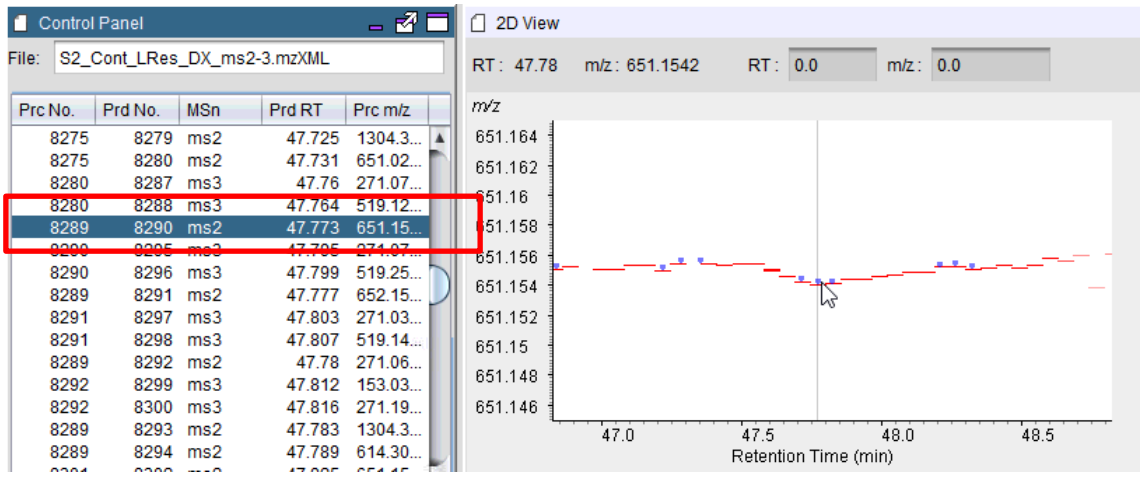
- Operations with the CTRL key restricts the modifications only to y-direction.
- Operations with the SHIFT key restricts the modifications only to x-direction.

Step 4) Obtaining MS^n spectrum of the target peak

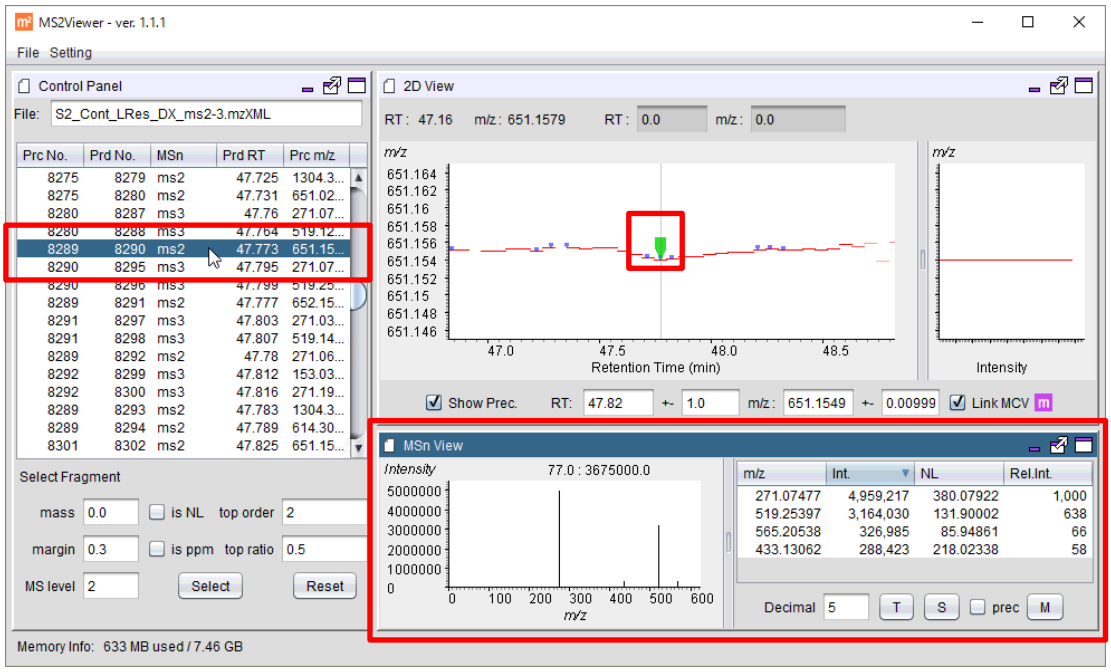
Double click at the top of the target peak. The same RT and *m/z* ranges are displayed in MS2Viewer.



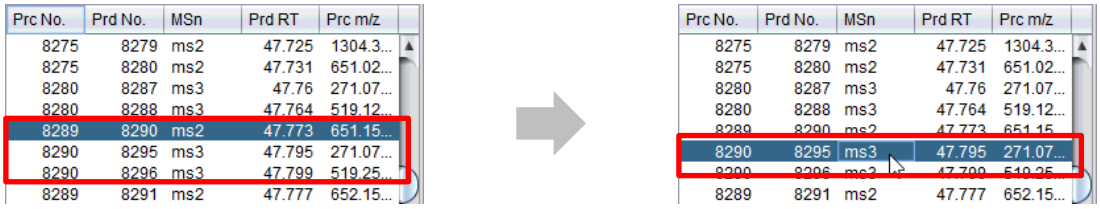
Click on a blue marker in MS2Viewer. The information of the clicked precursor will be highlighted in the table of the control panel (hereafter referred to as 'Precursor table').



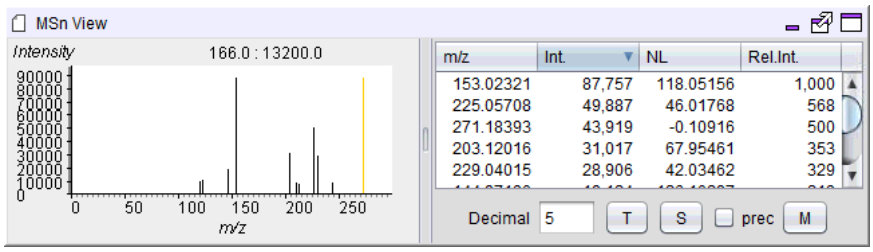
Click on the highlighted row; the accurate position of the precursor will be represented by a green marker. The MS^n spectrum will be displayed in the 'MSn View' panel.



As shown in the precursor table, the selected precursor ion (*m/z* 651.15) is used for MS^2 analysis. For the current parsley data, data dependent MS^3 analysis is also performed. Therefore, the MS^3 scans are displayed in the subsequent rows. Select the next MS^3 row where the MS^2 product ion (*m/z* 271.07) is analysed (the product scan No. [Prd. No.] 8295).



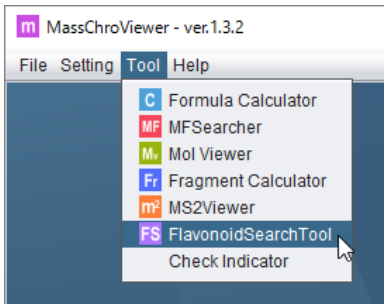
The MS^3 spectrum will be displayed.

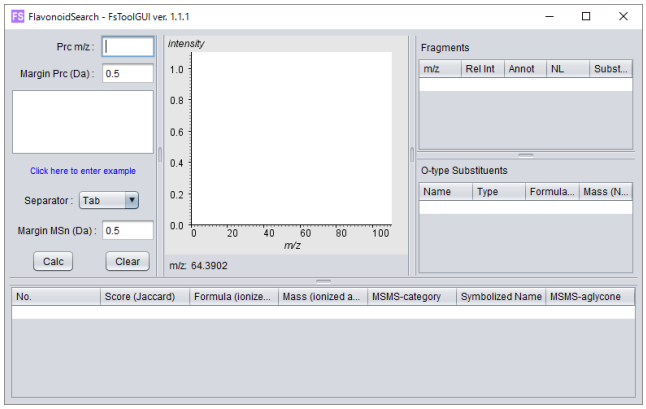


Step 5) Prediction of flavonoid aglycones using FsTool GUI

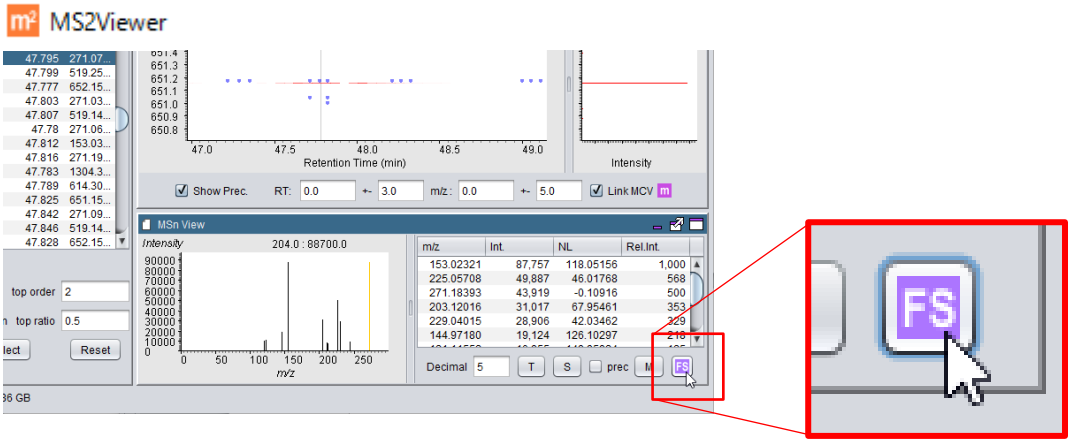
MassChroViewer 1.3.2 or newer

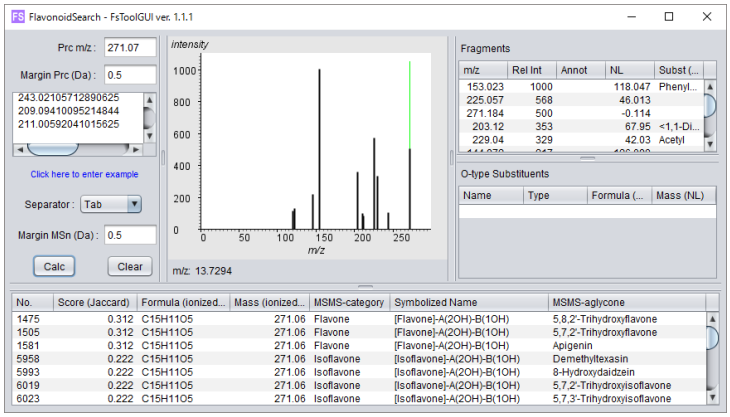
Run FsTool by selecting 'FlavonoidSearch Tool' from the 'Tool' menu of MassChroViewer.





Click the 'FS' button at the bottom-right of the MSn Viewer panel of MS2Viewer. The mass spectrum data are loaded in the FsTool, and the results are displayed.



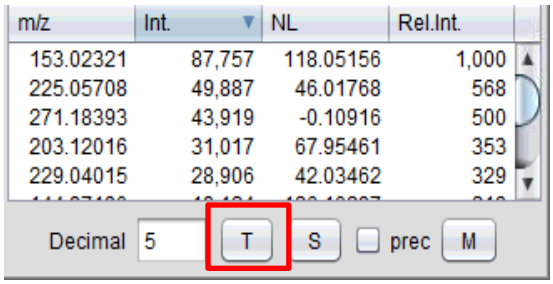


MassChroViewer older than 1.3.2

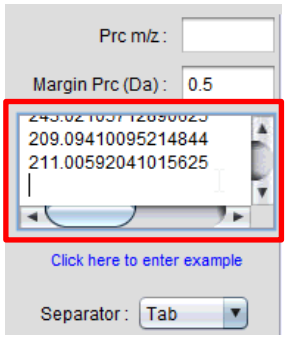
FsTool is not contained in MassChroViewer older than 1.3.2. In this case, run FsTool as

a standalone tool following the manual of FsTool. Then, copy and paste the spactrum data manually, and execute a calculation by clicking the 'Calc' button as below.

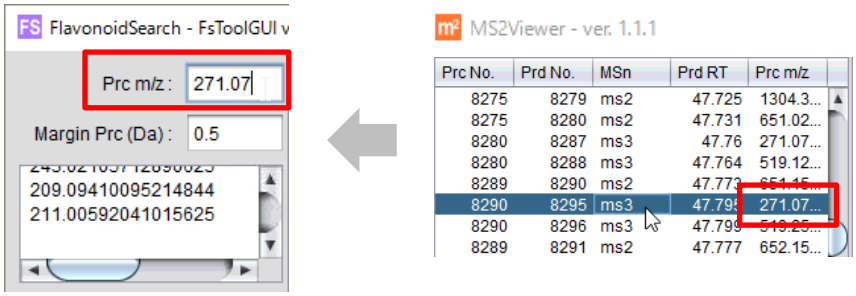
Click the 'T' button in the MSn View panel. The values for the fragment ions will be copied to the clipboard as tab delimited text data.



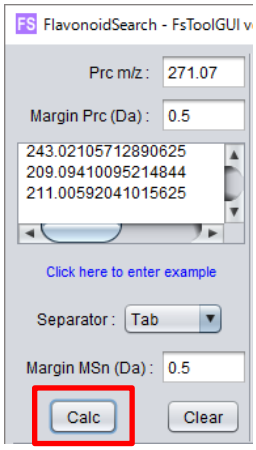
Use CTRL + V to paste the fragment ion data copied to the clipboard in Step 4 into the text area of FsTool.



Enter the *m/z* value in the 'Prc. m/z' field. In this tutorial, enter the *m/z* value '271.07' displayed in the precursor table.

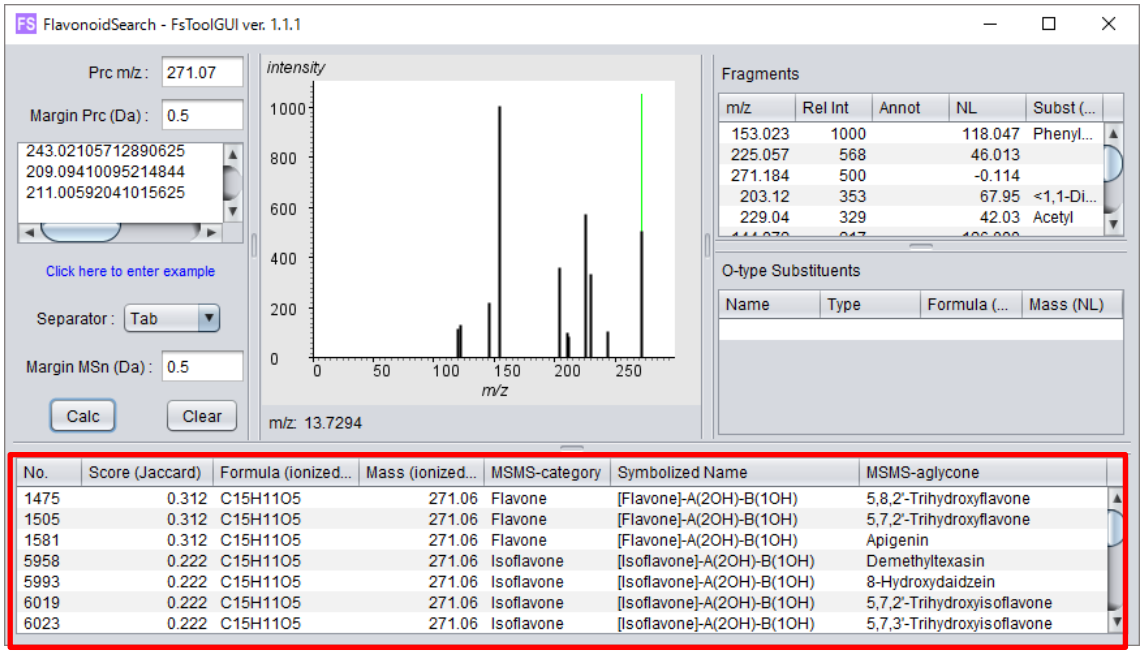


Click the 'Calc' button.



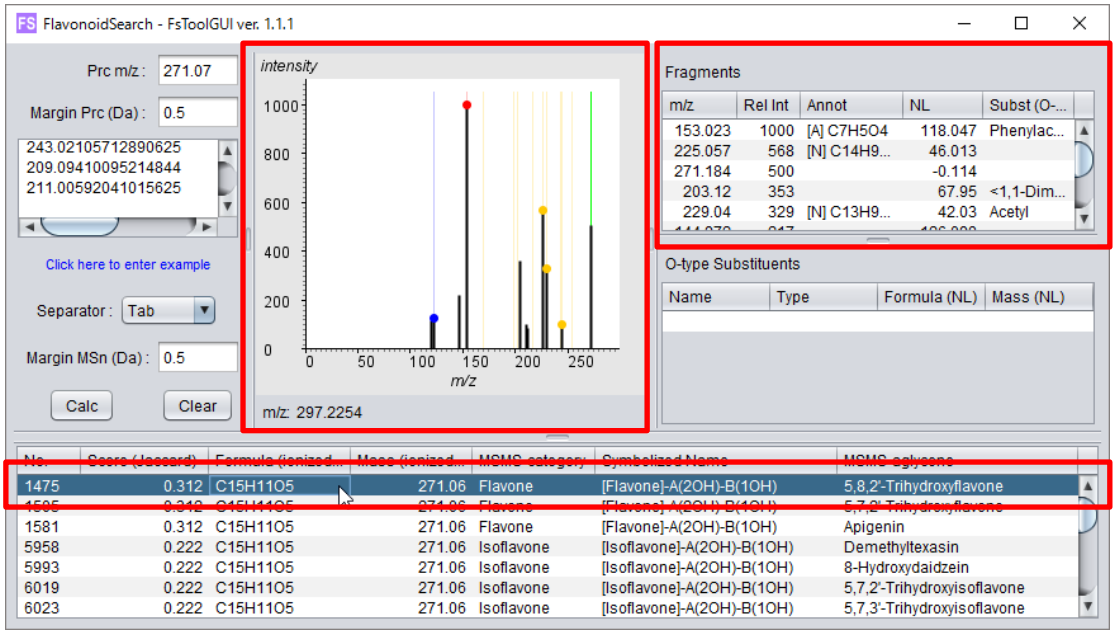
Search Results

The search results will be listed in the table at the bottom of the tool.



The aglycones with the symbolized name [Flavone]-A(2OH)-B(1OH) including apigenin, a typical aglycone in parsley, are shown at the top of the list.

Click on a row of the table. The measured mass spectrum (black lines) and the predicted fragment of the aglycone corresponding to the selected row in FsDatabase (red, blue and yellow lines) will be displayed. The matched measured fragments are represented with circles.

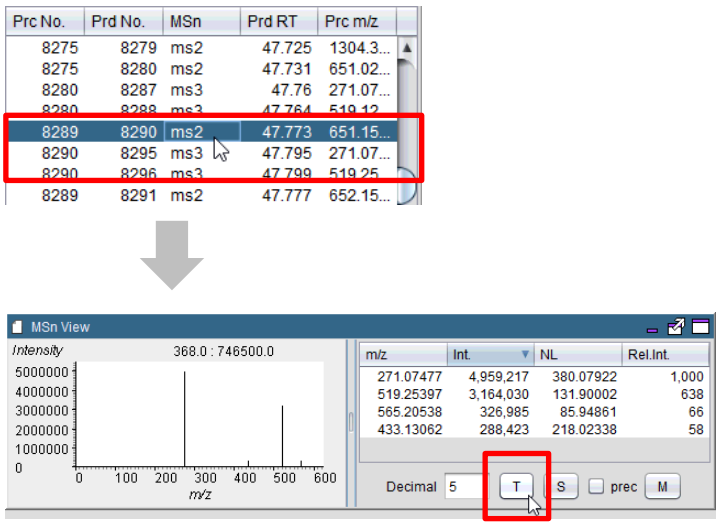


The red, blue and yellow colour of the lines and symbols represent the fragments derived from A-ring, B-ring and neutral loss, respectively. The green line shows the *m/z* of the precursor ion. The frequently observed fragments (Essential fragments[1]) are represented as thick lines and bordered circles.

In the 'Fragments' table at the right-hand side, information of the predicted fragments is displayed in the 'Annot' column. The letters A, B, and N in the parentheses [ ] denote the fragment derived from A-ring, B-ring and neutral loss, respectively. The essential fragments are represented as asterisks '*'.

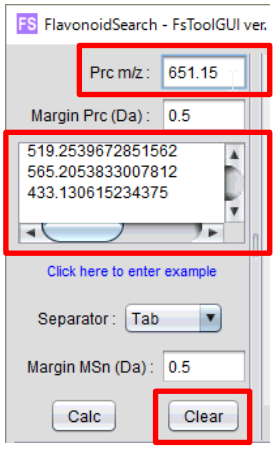
Step 6) Prediction of the substituents

Return to MS2Viewer. Select the precursor scan (MS^2 scan) of this MS^3 spectrum. Click on the row (with the Prd. No. 8290) previous to the current row.

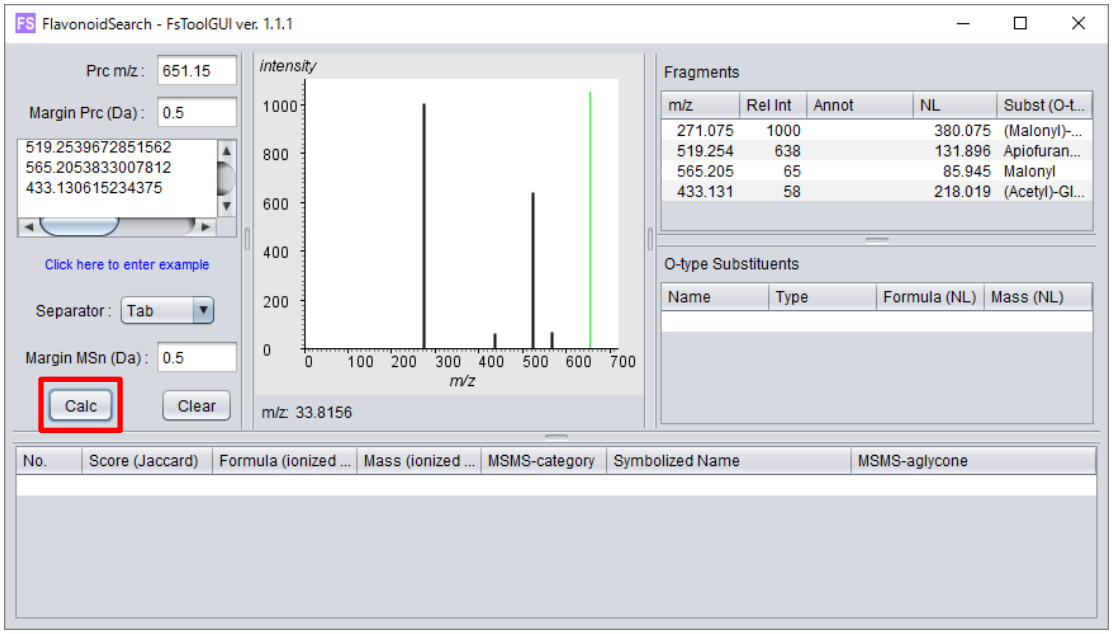


Click the 'T' button to copy the fragment data to the clipboard.

Click the 'Clear' button in FsTool to clear the previous data. Then paste the fragment data with the same procedure as Step 5. Enter the *m/z* value of the precursor, 651.15.

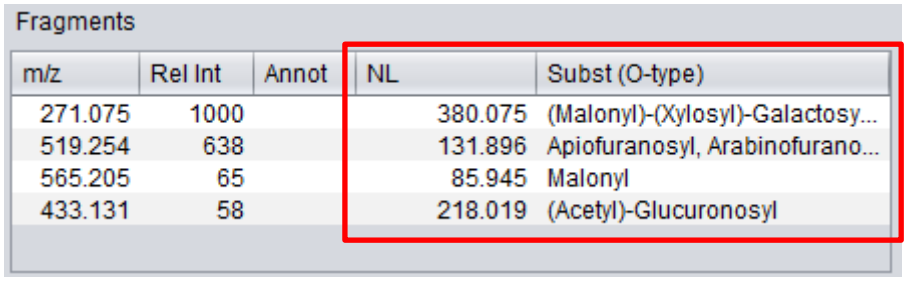


Click the 'Calc' button.

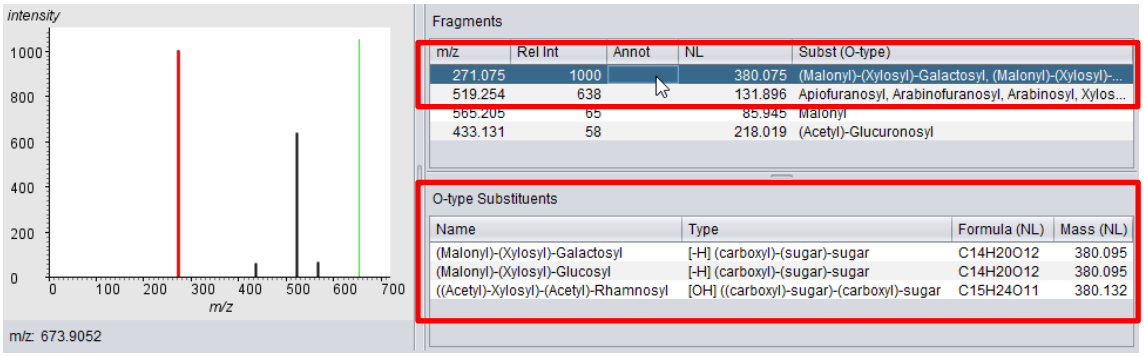


In this case, no aglycones were found.

Look at the 'Subst (O-type)' column in the 'Fragments' table. If the neutral loss value matches to the masses of the *O*-type substituents of known flavonoids (Supplementary Table S8[1]), the information of the substituents is displayed.



Click the row of the 'Fragments' table. The candidates of the substituents are listed in the 'O-type Substituents' table.

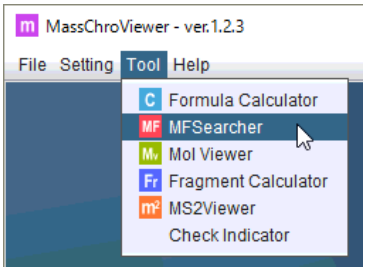


* The selected fragment in the 'Fragments' table is highlighted in red in the spectrum panel.

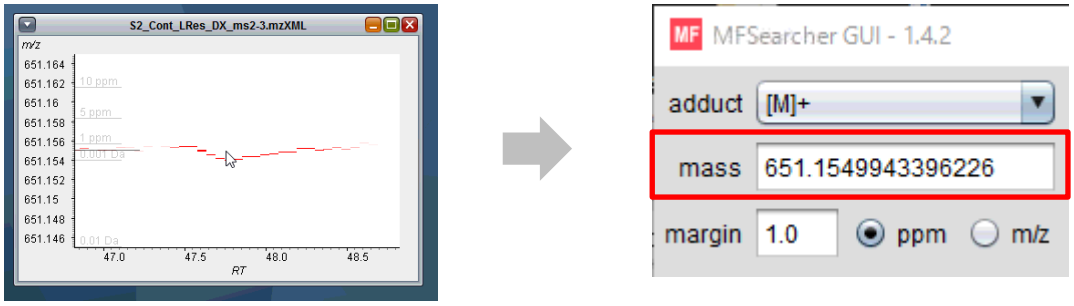
For this tutorial, a neutral loss value of 380.075 for the fragment ion 271.075 is suggested as a dissociation of a substituent ($C_{14}H_{20}O_{12}$) in which malonyl and xylosyl residues are conjugated to a hexose (galactose or glucose).

Step 7) Assessment of the annotation

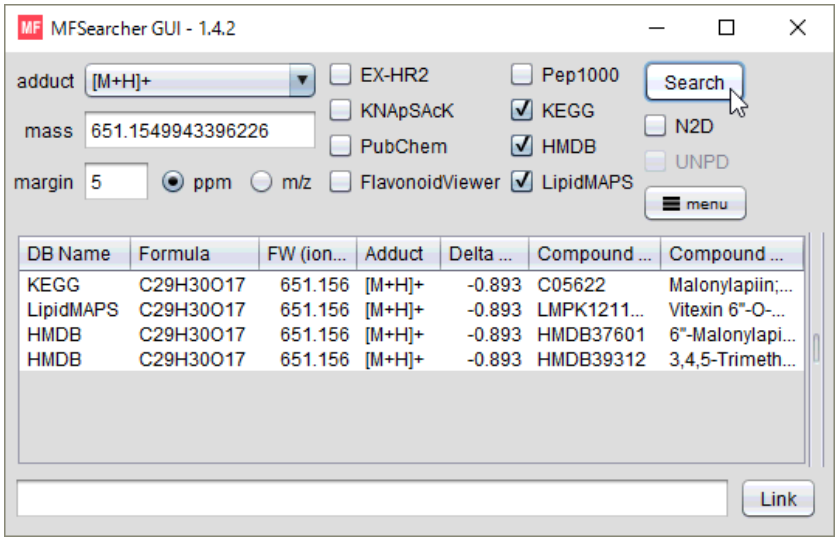
Return to MassChroViewer, and select 'MFSearcher' in the 'Tool' menu. MFSearcher is a tool to query a mass value in major compound databases.



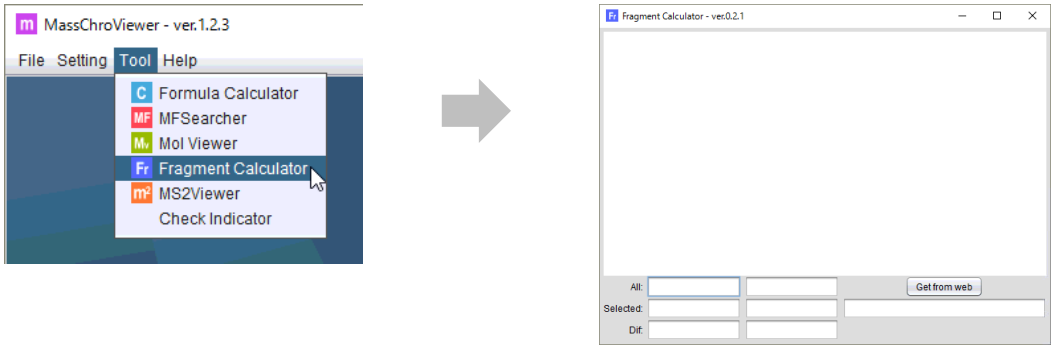
Double click again at the top of the peak drawn in the 2D Window. The accurate *m/z* value at the clicked position is represented in the 'mass' field of MFSearcher.



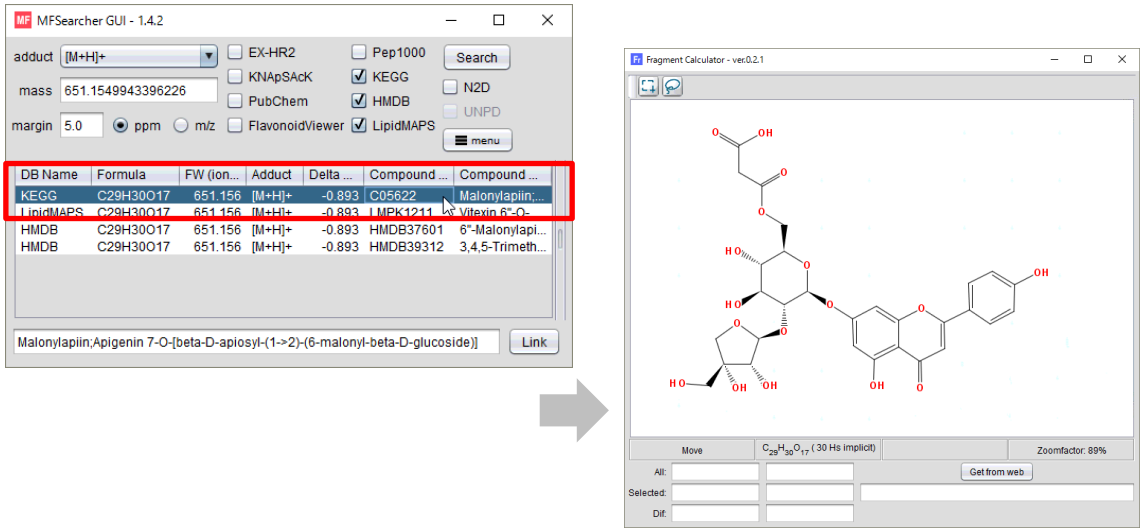
Set the search condition as follows: adduct, [M+H]+; margin, 5 ppm; target databases, KEGG, HMDB, LIPID MAPS. Click the 'Search' button. Several candidates will be listed in the result table.



Return to MassChroViewer, and select 'Fragment Calculator' from the 'Tool' menu.

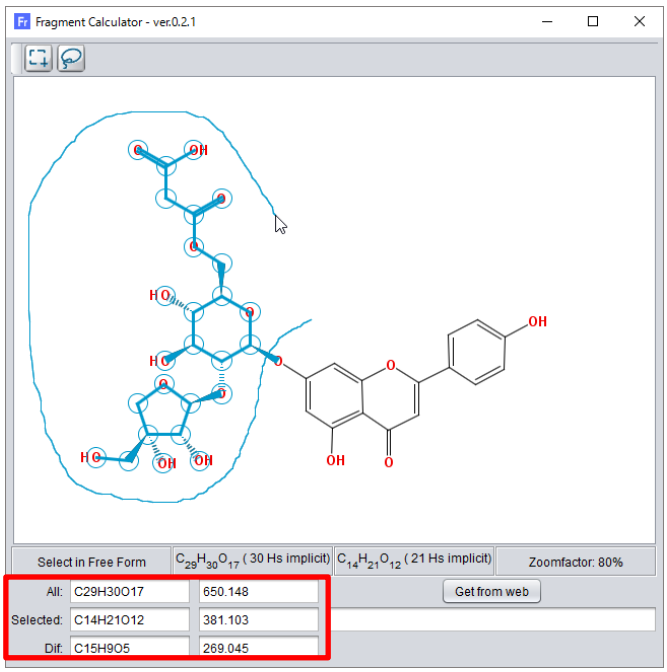


Return to the MFSearcher and select a row of record C05622 in KEGG. The chemical structure of this candidate is displayed in the 'Fragment Calculator' window.



This structure is an apigenin derivative with the predicted *O*-type substituent at the 7-position. This is a potential compound for the targeted peak.

Atoms on the structure can be selected using the square or the lasso selection tools. The mass values and formulae of the selected and unselected atoms are displayed in the panel.

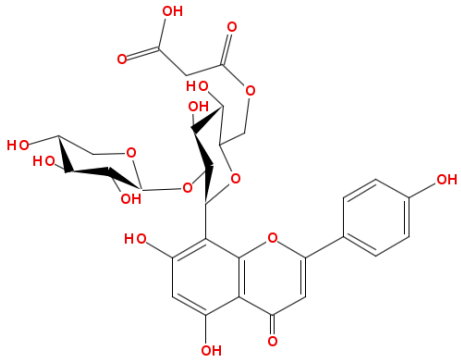


In this example, the formula and mass value of the selected structure ($C_{14}H_{21}O_{12}$, 381.103) match to those of the substituent candidates ($C_{14}H_{20}O_{12}$, 380.095) when a

hydrogen is removed from the selected structure.

Some other candidates, including a vitexin derivative, are listed in MFSearcher results. As vitexin is a *C*-glucoside of apigenin, the mass value of precursor ion for MS^3 analysis and the MS^3 spectral pattern of the vitexin derivative are remarkably different from those of apigenin *O*-glycoside. This candidate is not selected by FsTool.

DB Name	Formula	FW (ioniz...	Adduct	Delta ppm	Compou...	Compou...
KEGG	C29H30...	651.156	[M+H]+	-1.038	C05622	Malonyla...
LipidMAPS	C29H30...	651.156	[M+H]+	-1.038	LMPK121...	Vitexin 6"-...
HMDB	C29H30...	651.156	[M+H]+	-1.038	HMDB37...	6"-Malony...
HMDB	C29H30...	651.156	[M+H]+	-1.038	HMDB39...	3,4,5-Tri...



As shown in this tutorial, users can use FsTool to annotate potential flavonoids among other isomers and isobars.

About us

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References

1 Akimoto, N. *et al.* FlavonoidSearch: A system for comprehensive flavonoid annotation by mass spectrometry. *Sci Rep* **7**, 1243 (2017).