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Application News

Natural Product / LCMS-9030 / LabSolutions Insight Explore™

Quick Profiling and Identification of Natural Product Components in Honeysuckle Flower by DDA Method on LCMS-9030 with LabSolutions Insight Explore

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User Benefits

- Effective data-dependent acquisition (DDA) with preferred ions on LCMS-9030 Q-TOF was used for profiling and identification of main components in Honeysuckle Flower extract.
- LabSolutions Insight Explore s/w provides highly-flexible and in-depth data analysis tools for targeted and un-targeted screening, identification and structure elucidation using Analyze, Screen, Precursor, Library Search, Formula and Assign.

■ Introduction

The use of LC-HRMS such as LC-Q-TOF for profiling and identification of natural products and herbal medicines has become an important and effective approach in quality control, research and discovery of new active compounds [1]. The accurate mass (1 ppm accuracy) and rich MS/MS data acquired by data-dependent acquisition (DDA) or data-independent acquisition (DIA) allow researcher to perform targeted and/or untargeted screening as well as identification of unknown components in an extract sample. In this article, we describe DDA analysis approach applying to an extract sample of Honeysuckle flower, ***Lonicera*** *japonicae,* a traditional Chinese medicine (TCM). We demonstrate the in-depth data analysis using a comprehensive software, LabSolutions Insight Explore, for screening and identification using various tools including Analyze, Screen, Precursor, Formula Predictor, Library Search and Assign. ***Lonicera*** has been used as herbal remedies to treat illness since ancient time. It has been documented in China Pharmacopoeia (2005 Edition). It is of great interest to profile and identify the key components present in Honeysuckle flower extracts for quality control and research purpose.

■ Experimental

Reagents and Sample Preparation

LC-MS grade acetonitrile, methanol and formic acid were purchased from commercial suppliers. Dried honeysuckle flower sample was purchased from a local traditional Chinese medicine store. 1 g of dried honeysuckle flower was blend at 250 rpm for 1 min at low temperature (liquid nitrogen). 100 mg of honeysuckle flowers was added into 10 mL of extraction solvent (1:1 Milli-Q water and methanol). Extraction was done at 45°C for 45 min with 150 rpm shaking [2]. The extract was then filtered with 0.22 µm PTFE filter and transferred to HPLC sample vial for analysis.

LC-Q-TOF Analytical Conditions

Shimadzu LCMS-9030, Q-TOF system was used for this sample analysis. Details of the analytical conditions are shown in Table 1.

Table 1 Analytical conditions of Honeysuckle Flower extract on LCMS-9030 Q-TOF

LC Conditions (on Nexera™ UHPLC)	
Column	Shim-Pack™ GIST-HP C18 column (3.0 x 150 mm, 3 µm)
Flow Rate	0.4 mL/min
Mobile Phase	A: 0.1% Formic Acid in Water B: 0.1% Formic Acid in Acetonitrile
LC program	B: 0-2 min, 15% → 20 min, 44% → 23-25 min, 95% → 26 min, 15% → 30 min, stop
Oven Temp.	40°C
Injection Vol.	2 µL
Interface and MS Conditions (on LCMS-9030)	
Interface	Heated ESI
Interface Temp.	300 °C
DL Temp.	250 °C
Heat Block Temp.	400 °C
Nebulizing Gas	3 L/min
Heating Gas Flow	10 L/min
Drying Gas Flow	10 L/min
MS & DDA Mode	MS: *m/z* 250 – 800 (Positive) DDA: *m/z* 100 – 800 with CE 35V (±) 17V Loop time: 0.2 sec

Data-Dependent Acquisition (DDA)

Data-dependent acquisition (DDA) mode was adopted to acquire MS/MS data with a spread CE ranging from 18 V to 52 V. Collection of MS/MS data was triggered based on a pre-set intensity threshold (10,000) and a preferred precursor list (639 ions) which was generated from MS data in a previous run. A delay time of 3 seconds for DDA was applied in order to be able to collect MS/MS data at higher intensity level for spectral quality. The above DDA triggering settings were based on optimization tests. DDA mode may miss significant precursors due to various factors such as triggering intensity, data acquisition speed, interference of noises and LC separation etc. One effective way is to set preferred precursor ions with specific RT ranges, which may improve the DDA coverage significantly (Ref1).

■ Results and Discussion

Data Analysis Workflow by Insight Explore

The LC-MS TIC and DDA chromatograms of the Honeysuckle flower extract sample are shown in Fig. 1. Data analysis was performed using LabSolutions Insight Explore. The software has multiple functions for in-depth data analysis of screening, identification and structural elucidation. A general workflow (Fig. 2) is described as below:

1) DDA data file is sent to **Analyze** window. Select both MS and MS/MS to import all DDA spectra generated under triggering conditions
2) Upon applying **Analyze**, all precursors (each with a DDA spectrum) are listed
3) Apply **Screen** (contains all targets), Hits are listed
4) All precursors are displayed in **Precursor Pane**. User can view every precursor's XIC (RT), DDA spectrum.
5) Select any interested precursor's DDA spectrum and send it for **Library Search**
6) DDA spectrum also can be sent to **Assign** for database (ChemSpider or PubChem) search and fragment peaks' annotation.

Quick "Screen" for Targeted Compounds

The **Screen** in above Step 3) is for targeted screening. It has been reported in literature that 147 compounds were isolated from various Honeysuckle Flower species [3]. For the mass range of *m/z* 250~800 that the current method has been set up, 85 (including isomers) out of the 147 compounds are compiled into an Excel, which can be searched directly via Screen (Ref2). The criteria of this Screen search is the mass accuracy, which was set to be < 3 ppm based on the measurement conditions.

As shown in Fig. 3a, a total of 1209 precursors, each with a DDA spectrum, were detected in the Honeysuckle flower extract sample. Upon applying Screen for the 85 targets, 250 Hits were found and listed. A selected Hit precursor, chlorogenic acid, *m/z* 355.1028 at 7.98 min, is marked with red square, which DDA spectrum and XIC peak are displayed at the bottom of the Precursor Pane window (Fig. 3b and 3c). The DDA spectrum was sent to NIST library search. The result matched with chlorogenic acid (3-O-caffeoylquinic acid) too. However, if the XIC is displayed in the full elution time (Fig. 3d), two additional peaks appeared at 5.29, and 9.69 min, respectively. Their DDA spectra are almost identical with that at 7.98 min, which indicates that they are isomers. As reported by Lian-Wen Qi et al., [1], three isomers of caffeoylquinic acid (phenolic acid group) are present in **Flos Loonicerae Japonicae** sample with different retentions. In reference to the retention information, we propose that the three peaks corresponding to three precursor isomers are: (a) 5-O-caffeoylquinic acid (neochlorogenic acid, CAS# 906-33-2) at 5.29 min, (b) 3-O-caffeoylquinic acid (or chlorogenic acid, CAS# 327-97-9) at 7.98 min and (c) 4-O-caffeoylquinic acid (CAS# 905-99-7) at 9.69 min (Ref 3).

Quercetin ($C_{15}H_{11}O_7$) is another listed target for Honeysuchkle Flower [1], which belongs to flavonoids. The Screen search revealed at least two main peaks at 13.2 min and 13.9 min for precursor *m/z* 303.0503 (Fig. 4). However, neither of them is quercetin because the actual precursors are found to be *m/z* 611.1617 at 13.2

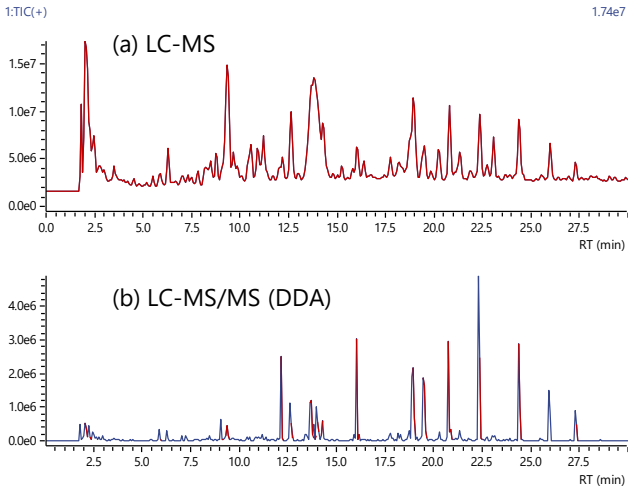


Fig. 1 LC-MS chromatograms (MS and DDA) of Honeysuckle Flower extract on LCMS-9030.

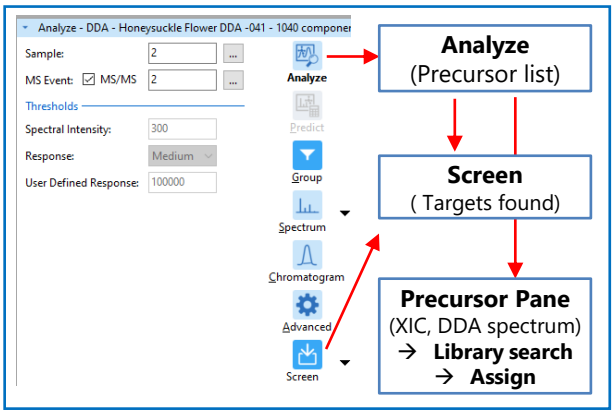


Fig. 2 Illustration of general workflow for DDA data analysis by LabSolutions Insight Explore software.

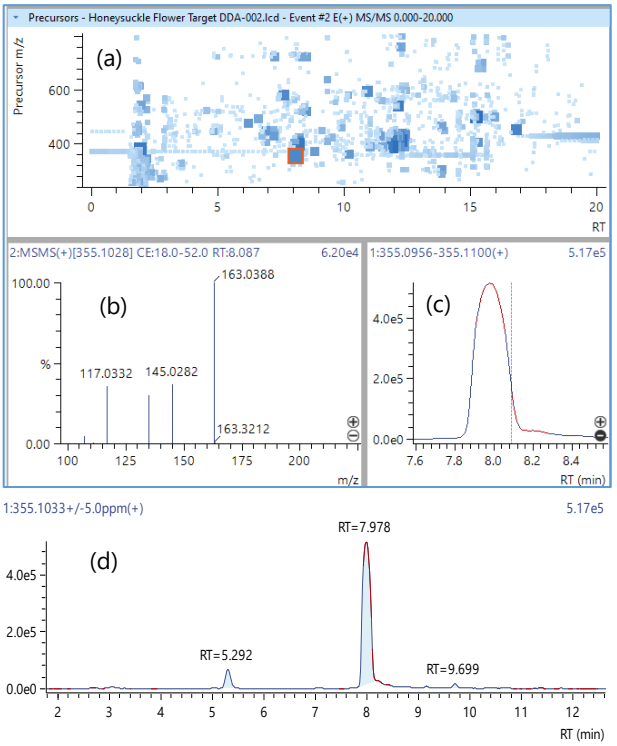


Fig. 3 Precursor Pane displaying all 1209 precursors (a) with DDA spectrum of *m/z* 355.1028 (b) and XIC peak at 7.96 min (c) The full range XIC show three peaks at different RTs (d), which represents three structural isomers.

Table 2 Summary of compounds found in Honeysuckle Flower Extract via Screen and NIST Library search on LCMS-9030

S/N	Target (Screen)		Found Compound			
	Name of Target	Formula	RT (min), (observed)	Precursor *m/z* (+) (observed)	Fragments of precursor *m/z* (+) - DDA	Error (ppm)
1	Adenosine	C10H13N5O4	2.65	268.1044	136.06, 119.03, 182.08	1.53
2	Guanosine	C10H13N5O5	2.86	284.0991	152.06, 135.03, 110.03	0.63
3	Luteolin	C15H10O6	14.60	287.0551	153.02, 229.05, 121.03	0.14
4	Spiraeoside	C21H20O12	7.90	303.0504	257.05, 229.05, 153.02	0.33
5	Quercetin	C15H10O7	14.92	303.0501	229.05, 153.02, 137.02	-0.53
6	5-O-caffeoylquinic acid (Isochlorogenic acid or Cis-Chlorogenic acid)	C16H18O9	5.28	355.1024	163.04, 135.04, 117.24	0.17
7	3-O-caffeoylquinic acid (Chlorogenic acid)	C16H18O9	7.96	355.1028	163.04, 145.03, 117.03	1.13
8	4-O-caffeoylquinic acid (Neochlorogenic acid)	C16H18O9	9.72	355.1025	163.04, 135.04, 117.03	0.34
9	Sweroside	C16H22O9	10.15	359.1341	197.08, 179.07, 127.04,	1.31
10	3-Ferulicoylquinic	C17H20O9	11.61	369.1181	177.05, 145.03, 117.03	0.33
11	Syringin	C17H24O9	11.05	373.1494	141.05, 211.09, 179.07	0.24
12	Secologanin*	C17H24O10	10.96	389.1446	195.06, 177.05, 151.04	0.85
13	7-Epi vogeloside (Epi-vogeloside)*	C17H24O10	11.66	389.1447	195.06, 177.05, 151.04	1.21
14	Ketologanin*	C17H24O10	11.94	389.1450	195.06, 177.05, 151.04	1.95
15	Vogeloside*	C17H24O10	12.26	389.1448	195.06, 177.05, 151.04	1.54
16	Loganin	C17H26O10	11.11	391.1592	179.07, 193.09, 167.07	-1.79
17	Kingiside	C17H24O11	6.86	405.1394	151.04, 211.06, 125.03	0.62
18	Secoxyloganin	C17H24O11	8.59	405.1391	211.06, 193.04, 125.03	-0.07
19	Astragalin	C21H20O11	13.85	449.1086	287.06, 153.02	1.65
20	Kaempferol 3-O-β-d-glucopyranoside	C21H20O11	15.38	449.1084	287.06, 153.02	1.16
21	Chrysoeriol 7-O-β-d-glucopyranoside	C22H22O11	16.15	463.1242	301.07, 286.05, 258.06	1.51
22	Quercetin 3-O-β-d-glucopyranoside	C21H20O12	7.89	465.1030	303.05, 257.05, 229.05	0.45
23	Hyperoside (Hyperin)	C21H20O12	13.22	465.1026	303.05, 229.05, 153.02	-0.32
24	Isoquercitrin	C21H20O12	13.95	465.1033	303.05, 229.05	1.23
25	Isorhamnetin 3-O-β-d-glucopyranoside	C22H22O12	15.68	479.1188	317.07, 302.04, 153.02	0.88
26	3,5-O-dicaffeoylquinic acid (Isochlorogenic acid A)*	C25H24O12	14.55	517.1348	319.08, 163.03, 145.03	1.35
27	4,5-O-dicaffeoylquinic acid (Isochlorogenic acid C)*	C25H24O12	15.19	517.1348	319.08, 163.03, 145.03	1.53
28	3,4-O-dicaffeoylquinic acid (Isochlorogenic acid B)*	C25H24O12	15.53	517.1347	319.08, 163.03, 145.03	1.31
29	1,3-O-dicaffeoylquinic acid*	C25H24O12	15.88	517.1345	163.03, 145.03, 135.04	0.93
30	Rhoifolin	C27H30O14	15.28	579.1720	271.06	1.97
31	Lonicerin, Kaempferol 3-O-β-d-rutinoside	C27H30O15	13.27	595.1666	449.11, 287.06	1.34
32	Chrysoeirol-7-O-neohesperidoside	C28H32O15	15.71	609.1821	301.07, 286.05	1.21
33	Rutin	C27H30O16	13.22	611.1617	303.05	1.64

** The isomers cannot be differentiated by library search of DDA MS/MS spectra.*

min and *m/z* 465.1033 at 13.9 min, respectively. The *m/z* 303.0503 is only a neutral loss ion (of the glycoside). Library search (NIST) suggests that they are likely rutin ($C_{27}H_{30}O_{16}$) at 13.22 min and hyperoside (or hyperin, $C_{21}H_{20}O_{12}$) at 13.9 min. A very small peak at 14.9 min is likely attributed by quercetin (*m/z* 303.0501). Due to structural similarity (glycosides), Rutin and hyperoside are easily undergoing neutral loss (NL) at ESI ionization step to form a common ion of *m/z* 303.0503 (Fig. 5).

The above Screen function provides a fast approach for targeted screening. Table 2 summarizes the identification results in the Honeysuckle Flower sample. These results are essentially in accordance with NIST HR library results. However, library search cannot differentiate isomers. For example, for XIC *m/z* 287.0552,

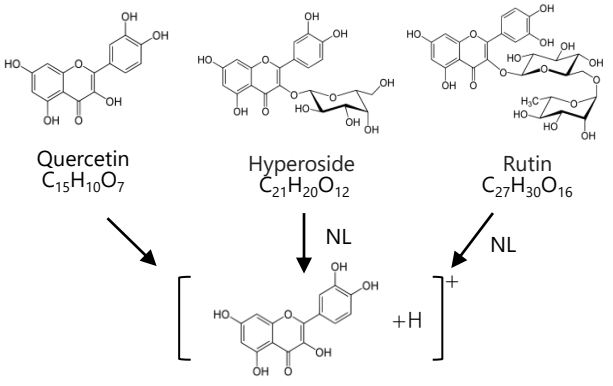


Fig. 5 Structures of flavonoids found in Honeysuckle Flower Extract. The glycoside tends to undergo neutral loss (NL) during ESI to form *m/z* 303.0503 as $[M\text{-}NL+H]^+$.

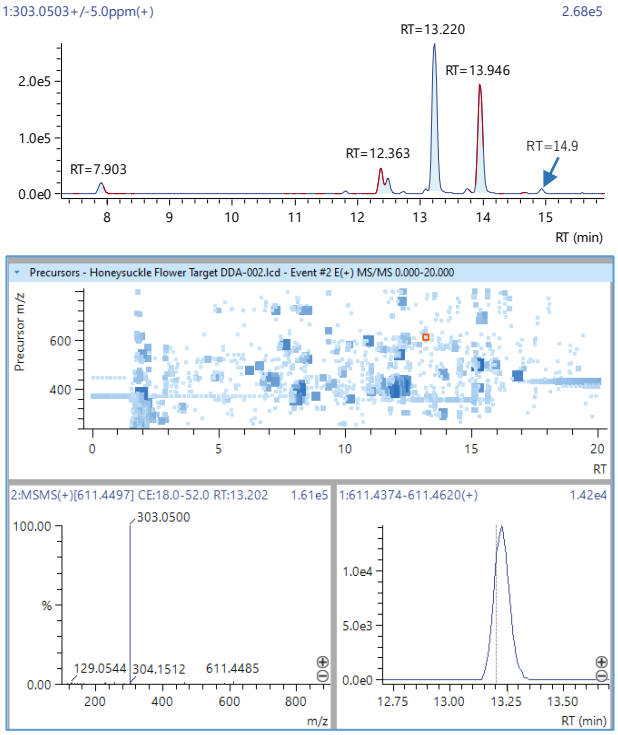


Fig. 4 XIC of *m/z* 303.0503 (top); Precursor display with DDA spectra at 13.2 min (Bottom).

four peaks appeared at 13.77, 13.90, 14.61 and 15.38 min, respectively and their DDA spectra are almost identified. However, library search could not differentiate these isomers ($C_{15}H_{10}O_6$) among Luteolin (CAS# 491-70-3), Kaempferol (CAS# 520-18-3) and Datiscetin (CAS# 480-15-9) etc. Further identification study is needed.

Identification of Unknown Compounds

Apart from targeted screening using the Screen with Library search, there are still many precursors remained not being identified. The Insight Explore with Assign program provides a tool for identification of unknown precursors through formula prediction, database search and fragment peaks annotation. Here we describe two representative examples to demonstrate this procedure.

Fig. 6 shows an unknown precursor *m/z* 397.1111 at 8.18 min. Library search (NIST) result of the DDA spectrum suggests that it is likely ketologanic acid ($C_{16}H_{22}O_{10}$, CAS#170382-11-3) or swertiamarin ($C_{16}H_{22}O_{10}$, CAS# 7388-39-5). The precursor *m/z* 397.1111 was sent to Formula Predictor and the most-likely candidate is also $C_{16}H_{22}O_{10}$. However, the Assign with ChemSpider database search did not generate reasonable results. Another example is precursor *m/z* 403.1605 at 15.26 min, which is confirmed to be a protonated ion $[M+H]^+$. However, library search of its DDA spectrum did not generate matched results. Formula Predictor generated

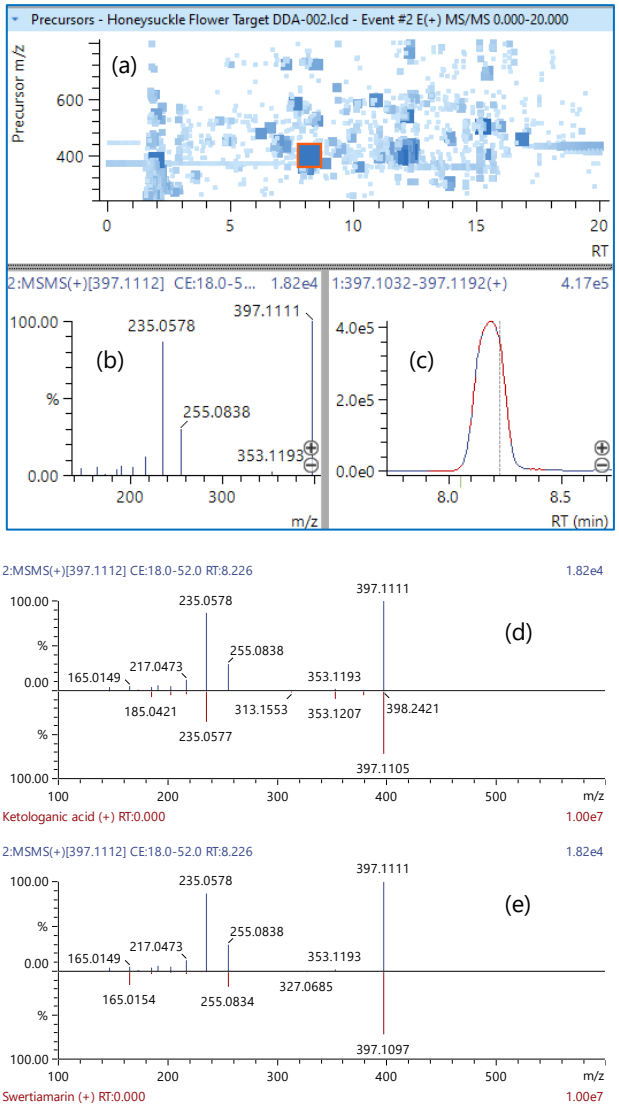
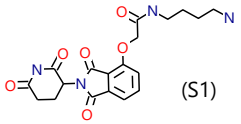
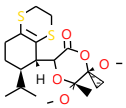


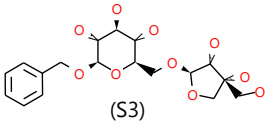
Fig. 6 Display of unknown precursor of *m/z* 397.1111 at 8.18 min (a), XIC (b), DDA spectrum (c) and matched NIST Library search results (d & e).

Table 3 Display of candidates for an unknown precursor of *m/z* 403.1605 at 15.26 min

#	Meas. *m/z*	Formula Predictor				Assign	
		Formula (M)	Ion	Diff. (ppm)	DBE	Candidate	Structure
1	403.1605	C19H22N4O6	[M+H]+	-1.8	11	N-(4-Aminobutyl)-2-{[2-(2,6-dioxo-3-piperidinyl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]oxy}acetamide	S1
2	403.1605	C19H30O5S2	[M+H]+	-0.6	5	(5R,6R)-3-[(5S,6R)-6-Isopropyl-2,3,5,6,7,8-hexahydro-1,4-benzodithiin-5-yl]-5,6-dimethoxy-5,6-dimethyl-1,4-dioxan-2-one	S2
3	403.1605	C18H26O10	[M+H]+	1.5	6	Icariside F2	S3


(S1)


(S2)


(S3)

three most-likely candidates as shown in Table 3. Sending the DDA spectrum to Assign together with each of the formulas, it leads to several matched structures from ChemSpider database. Only one of such structures for every formula is listed (S1, S2 and S3) in Table 3. Fig. 7 shows a snapshot of Assign window for fragment annotation for a plausible structure S3 obtained from ChemSpider database search. It is needed to note that the results are considered as reference of possible structures, rather than a conclusion. Therefore, further structural elucidation study is needed for drawing a conclusion.

■ Conclusion

In this study, DDA method was used in analysis of natural product extract (honeysuckle flower) on LCMS-9030 using LabSolutions Insight Explore software. Optimized DDA trigging parameters were set to obtain DDA spectra of all significant precursors. The Insight Explore software provides highly-flexible and effective tools including Analyze, Screen, Precursor, Library Search, Formula and Assign for targeted and untargeted screening, and unknown identification etc. With this comprehensive software, user can perform targeted screening easily for complex DDA data. The HRMS library search may further confirm or reject the identification. This approach is fast, easy and highly efficient. However, due to presence of isomers and neutral loss (like glycosides), the above identification may be confused or lead to wrong results. Therefore, careful checking of data set for every compound is necessary. The Insight Explore also enables unknown identification using Formula predictor and Assign. The full usage of these tools may facilitate unknown identification and structural elucidation, although a conclusion may not be drawn.

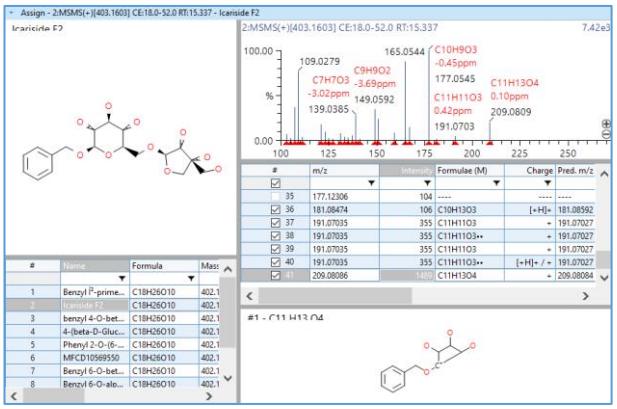


Fig. 7 Assign program for fragment annotation of a plausible structure (Icariside F2) obtained from ChemSpider database search.

■ References

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