Rapid Identification of Complex Constituents in Cordyceps Cicadae using High Resolution Mass Spectrometry with Targeted and Non-Targeted Processing Workflows

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ABSTRACT

In traditional Chinese medicine (TCM) research, one of the critical tasks is to study active compounds. . Therefore, it is crucial to develop an effective, comprehensive and reliable analytical method for identification of active constituents of TCM. This poster we described:

- Targeted and non-targeted workflows for identifying complex components of Cordyceps Cicadae using TripleTOF ® 5600+ system.
- One hundred and Eighty-nine compounds including 29 previously reported compounds and 35 newly identified / tentatively characterized compounds and 125 Lipid compounds represent the largest dataset of compounds identified in Cordyceps Cicadae extracts.
- The combination of high scan rate speed, highly sensitive accurate mass, and rapid chromatographic separations, and powerful software based workflows enabled the identification of many novel compounds.
- Accurate mass technology has been proved to be a powerful tool in structure characterization

MATERIALS AND METHODS

Sample and instruments:
The extract of the Cordyceps Cicadae was analyzed using TripleTOF® 5600+ scan system (AB SCIEX) coupled with a Shimazhu FPLC system (Figure 1.). Methanol was used as blank sample.

HPLC Conditions:
A 42 minutes gradient on a Phenomena Kinetic C18 column (2.1 × 100mm, 2.7um) was useed to ensure good separation, the eluent were water+0.05% formic acid+5mM ammonium formate (A) and Methanol+0.05% formic acid+5mM ammonium formate (B) at flow rate of 0.35mL/min.

MS/MS Conditions:
TOF MS and MS/MS data in both positive and negative modes were acquired using Information Dependent Acquisition (IDA) workflow with dynamic background subtraction (DBS) enabled. The mass range for MS and MS/MS were M/Z:100~1500, M/Z:50~1500 respectively, and totally 8 MS/MS were employed to follow a survey scan(Figure 2.)

Figure 1. TripleTOF® 5600+ system

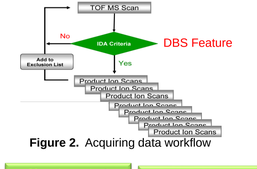

Figure 2. Acquiring data workflow

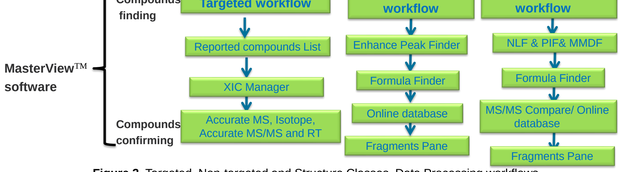

Figure 3. Targeted, Non-targeted and Structure Classes Data Processing workflows

Data processing:
Targeted and non-targeted workflows were implemented on MasterView® software (AB SCIEX) with the XIC Manager and Enhance Peak Finder plugins to search for previously reported compounds and unreported compounds respectively. In addition, as three functions provided by MasterView® software, neutral loss filtering (NLF), product ions filtering (PIF) and Multiplex Mass Defect Filter (MMDF) were also employed to search and identify compounds belonging to certain classes. Figure 3. illustrates the proposed workflows.

RESULTS

A total of One hundred and Eighty-nine compounds were identified or tentatively characterized based on their retention times, accurate mass measurement of molecular and fragment ions. The identified compounds includes 16 adenosine metabolites and nucleosides, 13 bassiatin and beauvericin metabolites, 3 sterol metabolites, 32 amino acids and vitamins, 125 lipid compounds including 23 ceramides. .

In the targeted screening workflow, 29 previously reported compounds were quickly identified by library search, and the structures were confirmed based on accurate mass, isotopic pattern matching and MS/MS fragments, these information directly showed on XIC Manager. Figure 4 illustrates how does the targeted screen workflow.

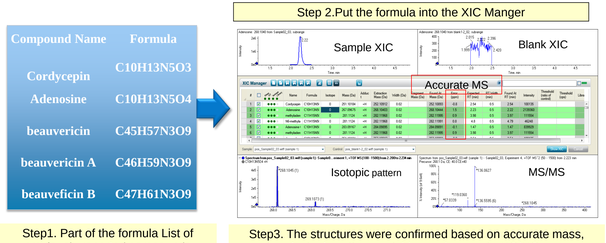

Figure 4. How does the targeted screen workflow work

For the Non-targeted workflow, firstly, we used the Enhance Peak Finder built-in MasterView® software (AB SCIEX) to search for unknown compounds. Figure 5 shows all the perspective constituents found by Enhance Peak Finder and their formula were predicted by software. Not surprisingly, the constituents of Cordyceps Cicadae were very complex, there were more than 3500 molecular features found in a single injection.

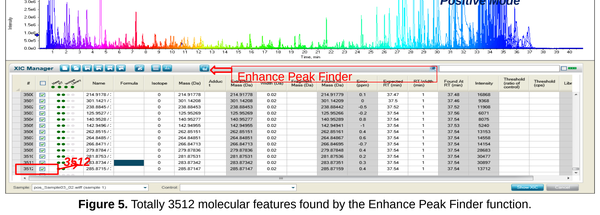

Figure 5. Totally 3512 molecular features found by the Enhance Peak Finder function.

Besides targeted workflow and Non-targeted workflow, we also used NLF, PIF and MMDF to search and identify compounds belonging to certain classes. Table 1.shows the Bassiatin and beauvericin metabolites list, which was achieved using PIF of m/z134.0966, which is a characteristic fragment ion of the Bassiatin and beauvericin metabolites

No.	Compound Name	Formular	Polarity Type	Experiment m/z	Error(ppm)	RT	Intensity	Fragment
1	beauvericin	C45H57N3O9	+NH4	801.4446	1.6	28.74	4459215	m/z134.0966, m/z244.1326
2	beauvericin A	C46H59N3O9	+NH4	815.4609	2.4	29.21	144317	m/z134.0966, m/z244.1326
3	beauvericin B	C47H61N3O9	+NH4	829.4762	1.9	28.74	95859	m/z134.0966, m/z244.1326
4	beauvericin C	C48H63N3O9	+NH4	843.4913	1.3	29.21	5281	m/z134.0966, m/z244.1326
5	allobeauvericin A	C46H59N3O9	+NH4	815.4605	1.9	28.62	68666	m/z134.0966, m/z244.1326
6	allobeauvericin C	C48H63N3O9	+NH4	843.4908	0.6	28.62	5160	m/z134.0966, m/z244.1326
7	beauvericin D	C44H55N3O9	+NH4	787.4297	2.6	28.62	1134329	m/z134.0966, m/z244.1326
8	beauvericin H	C43H53N3O9	+NH4	773.4137	2.2	28.47	107085	m/z134.0966, m/z244.1327
9	bassiatin	C15H19NO3	+H	262.1440	0.8	19.90	187923	m/z134.0966, m/z180.1010,m/z119.0724
10	epibassiatin	C15H19NO3	+H	262.1440	0.9	25.70	67195	m/z134.0966, m/z180.1010,m/z119.0724
11	Dioxdation-bassiatin	C15H21NO5	+H	296.1497	1.5	14.57	144659	m/z134.0966, m/z180.1010,m/z119.0724
12	bassiatin hydrolysate	C15H21NO4	+H	280.1550	2.3	19.85	234182	m/z134.0966, m/z180.1010,m/z119.0724
13	bassiatin hydrolysate isomer	C15H21NO4	+H	280.1549	2.1	19.47	36247	m/z134.0966, m/z180.1010,m/z119.0724

Table 1. The Bassiatin and beauvericin metabolites list was achieved using PIF of m/z134.0966

With Dynamic background subtraction (DBS) enabled and IDA criteria, TripleTOF™ 5600+ system is capable to acquire both MS and MS/MS data in a single run. With only one injection, we were able to acquire enough data for the structural elucidation. In addition, the TOF MS and MS/MS spectra are of high resolution and high mass accuracy, which eliminate many false-positives and improve the confidence for identification. Figure 6 illustrates how DBS effectively acquired the TOF MS/MS.

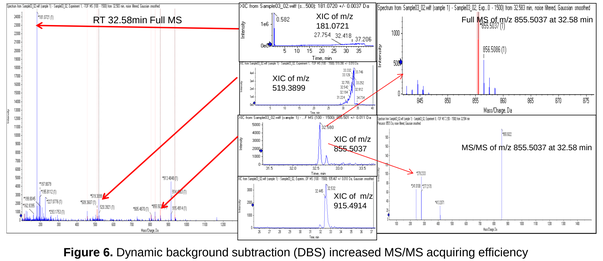

Figure 6. Dynamic background subtraction (DBS) increased MS/MS acquiring efficiency

As another important function built in MasterView® software, Formula Finder function could automatically calculate the most probable formula for each molecular features found by Enhance Peak Finder or NLF & PIF, as showed in Figure 7, the formula were calculated by using the accurate mass of MS and MS/MS and isotopic pattern, and the system ranked them by the matching degree. Then the proposed formula was submitted to the online databases, e.g. ChemSpider, for identification (Figure 8).

After the proposed structure was selected in the database, it would be identified and ratified with the high resolution MS/MS spectrum by the fragments pane (Figure 9).

We found much more lipid compounds in Cordyceps Cicadae Extract. These lipids were identified by LipidView™ software, which features a lipid fragments database composed of over 600 fragment ions across 50 lipid classes and over 25000 individual lipid species (Figure 10).

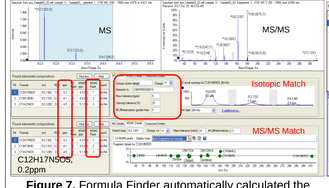

Figure 7. Formula Finder automatically calculated the formula and ranked them by the matching degree

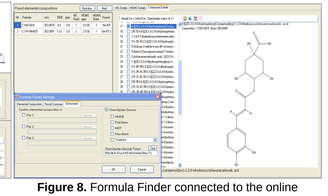

Figure 8. Formula Finder connected to the online database and searched for the possible structure

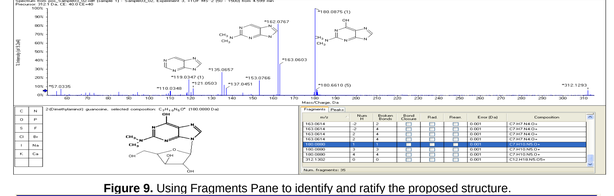

Figure 9. Using Fragments Pane to identify and ratify the proposed structure.

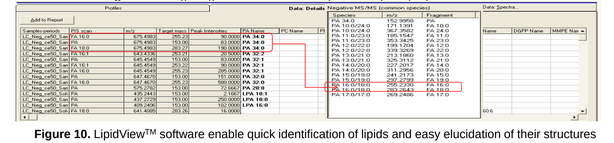

Figure 10. LipidView™ software enable quick identification of lipids and easy elucidation of their structures based on the built-in database of MS and MS/MS fragments with high mass accuracy

CONCLUSIONS

■ Dynamic background subtraction (DBS) is a powerful tool for data acquiring which can significantly increase the MS/MS acquiring efficiency, in combination with information-dependent (IDA) data acquisition, it can provide sufficient MS/MS data for structural elucidation.

■ Targeted, non-targeted and structure classes workflows are very effective for identifying complex components of TCM. With XIC Manager, Formula Finder, Fragments Pane plugins built in MasterView® software, targeted workflow presents a quick method for identification of previously reported compounds, while the non-targeted and structure classes workflow provides a specific and efficient approach for identification of unreported compounds.

TRADEMARKS/LICENSING