

A Novel Strategy for the Discovery and Characterization of New Natural Products from Traditional Chinese Medicine by Using Benchtop Orbitrap Mass Spectrometer

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Overview

Purpose: To establish a workflow for TCM microconstituents discovery and identification, and demonstrate its efficacy in the analysis of components in blister beetle.

Methods: Blister beetle extract was profiled by using UHPLC coupled with a quadrupole orbitrap mass spectrometer. Acquired data were processed by using Compound Discoverer and Mass Frontier software.

Results: 21 cantharidins, including 16 new cantharidins, were detected in the blister beetle extract.



Introduction

Traditional Chinese medicines (TCMs) have been utilized to treat various diseases for several millennia. They have gained increasing attention worldwide due to limitations of western medicines. However, their components with diverse chemistries and polarities usually exist at very low level within complex matrices, hindering the revelation of unknown microconstituents that were not discovered by phytochemistry techniques.

A new methodology combined with the Thermo Scientific™ Q Exactive™ high resolution mass spectrometer and the Thermo Scientific™ Compound Discoverer software and Thermo Scientific™ Mass Frontier software were established to rapidly screen and identify the unknown constituents of TCMs. Blister beetle, one of the animal TCMs most frequently used in traditional prescriptions, with few components discovered so far, was used as an example to verify the effectiveness of this workflow.

Methods

Sample preparation

The blister beetles were ground and extracted with methanol using ultrasonic extraction. The extract was centrifuged, and the supernatant was then used for LC-MS analysis.

Liquid Chromatography

A Thermo Scientific™ UltiMate 3000 Binary RSLC system performed separations using 0.1% formic acid and acetonitrile as mobile phase and a Thermo Scientific™ Hypersil Gold C18 (150×2.1mm, 3μm) as column. The flow rate was 0.3 mL/min and elution conditions at 35°C applied with a linear gradient as follows: 0–20 min, 5–35% B; 20–24 min, 35–45% B; 24–27 min, 45–70% B; 27–47 min, 70–90% B.

Mass Spectrometry

High resolution accurate mass data was acquired in both positive and negative ion mode using a Q Exactive mass spectrometer. Data dependant tandem mass spectra were acquired by Higher Energy Collision Induced Dissociation (HCD).

MS parameters:

Heat temperature: 350 °C; capillary temperature: 300 °C; spray voltage: 3 kV (positive mode), 2.8 kV (negative mode); sheath gas: 40 arb; auxiliary gas: 10 arb; MS Resolution: 70,000 FWHM (at m/z 200); MS2 Resolution: 17,500 FWHM (at m/z 200); HCD fragmentation energy: 35%.

Data Analysis

Acquired data were processed using a custom-built workflow in Compound Discoverer software. Mass Frontier software was then employed to interpret MS/MS spectrum by assigning structures to the fragment ions automatically.

Results

TCM microconstituents discovery and characterization workflow using Compound Discoverer and Mass Frontier software.

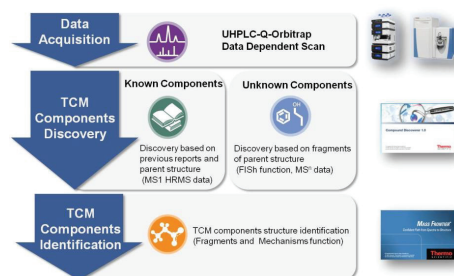


Figure 1. Workflow for TCM microconstituents discovery and characterization.

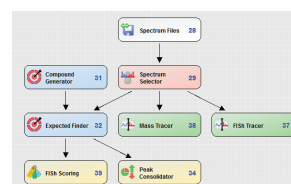


Figure 2. Custom-built workflow for TCM components discovery in Compound Discoverer software.

Figure 1 shows the workflow for TCM microconstituents discovery and characterization. MS and MS/MS HRMS data were acquired by UHPLC Q Exactive MS using data dependent scan. The data were first filtered with a custom-built workflow by Compound Discoverer software for TCM components discovery (Figure 2). Then the spectrum of discovered components were interpreted by the Fragments and Mechanisms function of Mass Frontier software, which based on a fragmentation library containing abundant literature-proposed fragmentation mechanisms.

Discovery and identification of known cantharidins.

For the known cantharidins, a list of transformations was set up with the transformation editor in the Compound Discoverer software based on the structure of cantharidinimide. The known cantharidins were then searched out in the form of EIC, and the [M+H]⁺ related ions such as adducts and isotopes were automatically matched with the theoretical ones (Figure 3). Their structures were then confirmed by Mass Frontier software (Table 1, rows with no shadow).

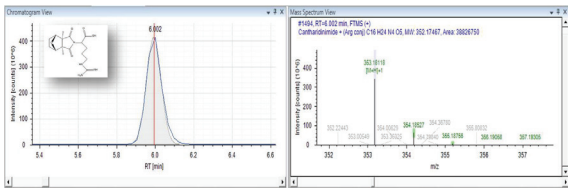


Figure 3. EIC and matched isotope pattern of one known cantharidin.

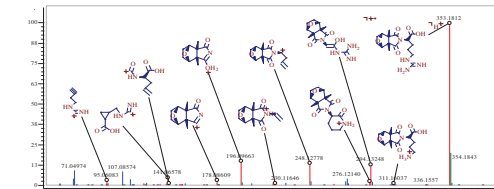


Figure 4. MS/MS spectrum interpretation result of one known cantharidin by Mass Frontier software.

Discovery and identification of unknown cantharidins.

For the unknown cantharidins, fragment ion search (FISH) function was applied using cantharidinimide as the parent structure. The cantharidins sharing the similar parent structure were screened out and then interpreted by Mass Frontier software. 16 new cantharidins were discovered and identified with mass accuracy less than 1 ppm (Table 1, rows shadowed in blue).

Figure 5 illustrates the MS and MS/MS spectrum of one unknown cantharidin. The polarity switching ability and the high mass accuracy in all MS stage level bring more confidence to the characterization result of the unknown constituents.

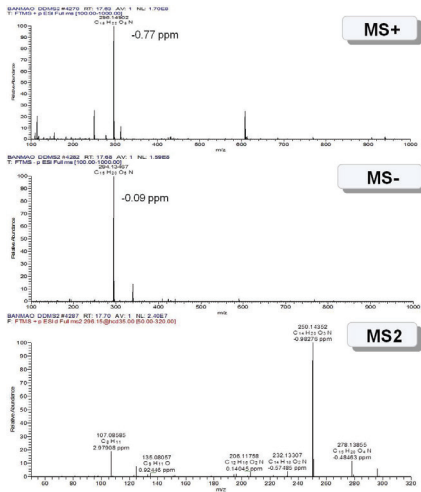


Figure 5. MS and MS/MS spectrum of one unknown cantharidin.

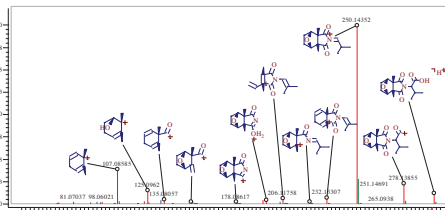








Figure 6. MS/MS spectrum interpretation result of one unknown cantharidin by Mass Frontier software.

Table 1. Identification results of known and unknown cantharidins in blister beetle.

No.	RT (min)	Formula	Theoretical Mass	Measured Mass	Precision (ppm)	Structure
1	4.5	C15H22O5N2	311.16015	311.15994	-0.67	
2	4.56	C10H13O3N	196.09682	196.0968	-0.1	
3	5.4	C16H24O5N2	325.1758	325.17551	-0.89	
4	6	C16H24O5N4	353.18195	353.18179	-0.45	
5	7.21	C13H17NO6	284.11286	284.11282	-0.15	
6	7.43	C14H18N2O6	311.12376	311.12366	-0.33	
7	7.78	C14H17NO7	312.10778	312.1077	-0.25	
8	7.81	C15H20N2O6	325.13941	325.13937	-0.13	
9	8.62	C12H15NO5	254.1023	254.10219	-0.43	
10	9.18	C14H19NO6	298.12851	298.12845	-0.21	
11	9.24	C15H19NO7	326.12343	326.12347	0.13	
12	9.67	C13H17NO5	268.11795	268.11792	-0.11	
13	10.03	C10H12O4	197.08084	197.08084	0.03	
14	14.05	C22H35O6N3	438.25986	438.25983	-0.07	
15	17.23	C15H21NO5S	328.12132	328.12128	-0.12	

16	17.63	C15H21NO5	296.14925	296.14902	-0.77	
17	18.39	C21H30O8N2	439.20749	439.20743	-0.14	
18	19.94	C21H22N2O5	383.16015	383.16003	-0.31	
19	20.66	C16H23NO5	310.1649	310.16467	-0.74	
20	21.34	C16H23NO5	310.1649	310.16479	-0.35	
21	26.2	C22H34O6N2	423.24896	423.24863	-0.78	

Conclusion

The Q Exactive high resolution mass spectrometer combined with Compound Discoverer and Mass Frontier software was successfully applied to rapidly screen and identify the unknown constituents of TCMs.

Totally 21 cantharidins with mass accuracy less than 1 ppm were identified, among which 16 components were discovered in blister beetles for the first time.

The functions of Compound Discoverer and Mass Frontier software including Fragment Ion Search (FiSh), allow for automated localization of components with similar structures, enabling the quick discovery and interpretation of unknown microconstituents in the complex matrices of TCMs.

References

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