A Strategy for the Discovery and Identification of New Natural Product Metabolites by Orbitrap Mass Spectrometry and Multiple Data-Mining Approaches

Chen Li[1],[+], Ying Liu[2],[+], Jiayu Zhang[2], Siyi Liu[2], Wei Cai[3], Jianqiu Lu[2]
[1]Thermo Fisher Scientific, Shanghai, China
[2]Center of Scientific Experiment, Beijing University of Chinese Medicine, Beijing, China
[3]Hunan University of Medicine, Huaihua, China
[+]: The first two authors contributed equally to this work

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Abstract

Purpose: Demonstrate an effective workflow for natural product metabolites discovery and identification, and its application in profiling and elucidation of asarinin metabolites.
Methods: Biological samples were profiled by using UHPLC coupled with a Thermo Scientific™ quadrupole-Orbitrap™ mass spectrometer. Acquired data were processed using novel node-based Thermo Scientific™ Compound Discoverer™ software.
Results: 38 asarinin metabolites were detected in the biological samples, among which 31 metabolites were discovered for the first time.

Introduction

Traditional Chinese medicine has attracted attention as a natural alternative to treat diseases. However, the obscure transformations of its components *in vivo* hinder the sufficient interpretation of its therapeutic mechanism. Metabolite profiling and identification of its constituent is a crucial step to improve the understanding. But it's always challenging on how to detect trace metabolites in complex biological matrices and maximize the amount of metabolite information without a time-consuming data-mining process.
A novel strategy integrating the Thermo Scientific™ Q Exactive™ Plus high-resolution mass spectrometer and Compound Discoverer software was constructed and then employed to discover and characterize the metabolites of asarinin, a lignan possessing bioactivities such as antihypertensive and hypolipidemic, with only 7 metabolites identified previously.

Materials and Methods

Drug administration and sample preparation
Asarinin was orally administered to rats at a dose of 200 mg/kg body weight. Biological samples were prepared by solid phase extraction method.

Liquid Chromatography
A Thermo Scientific™ UltiMate™ 3000 Binary RSLC system performed separations using 0.1% formic acid and acetonitrile as mobile phase.

Mass Spectrometry
High resolution accurate mass data was acquired in positive ion mode using a Q Exactive Plus Orbitrap mass spectrometer.

MS parameters:
Heat temperature: 350 ˚C; capillary temperature: 300 ˚C; spray voltage: 3 kV (positive mode); sheath gas: 40 arb; auxiliary gas: 10 arb; MS Resolution: 70,000 (FWHM at *m/z* 200); MS^2 Resolution: 17,500 (FWHM at *m/z* 200); HCD fragmentation energy: 40%.

Data Analysis
A node-based processing workflow was custom-built in Compound Discoverer software to search and identify the asarinin metabolites.

Results

Natural product metabolites discovery and characterization workflow using Compound Discoverer software.

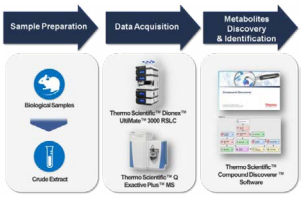

Figure 1. Workflow for natural product metabolites discovery and characterization.

Figure 2. Custom-built workflow for natural product metabolites discovery and characterization in Compound Discoverer software.

The workflow for natural product metabolites discovery and characterization is demonstrated in Figure 1. MS and MS/MS high resolution mass spectrometry data were acquired on the UHPLC-Q-Orbitrap platform using a data dependent scan. The data were then processed with a customized workflow in a drag-and-drop user interface in Compound Discoverer software (Fig. 2). The workflow assembled various data-processing techniques such as mass defect filter and fragment ion search (FISh). The mass defect filter could filter out the vast majority of background ions, while the FISh function could further search out the parent drug structure-related compounds. The structures of the discovered metabolites were further confirmed by fragment ion matching between experimental data and theoretical predictions from Thermo Scientific™ Mass Frontier™ fragmentation libraries.

Discovery of asarinin metabolites.
Common phase I and phase II bio-transformations were preset in Compound Discoverer software, while uncommon ones could be defined through the Transformation Editor (Fig.3). The transformations were automatically combined in the software on the basis of the number of maximum occurrences for each transformation and the maximum number of combinatorial steps, leading to the revelation of metabolites which occurred several transformation steps.
As illustrated in Figure 4, the calculated metabolites were listed in tabular form. Isotopes and adducts were automatically matched and grouped for each compound, reducing false positives from compound hits.

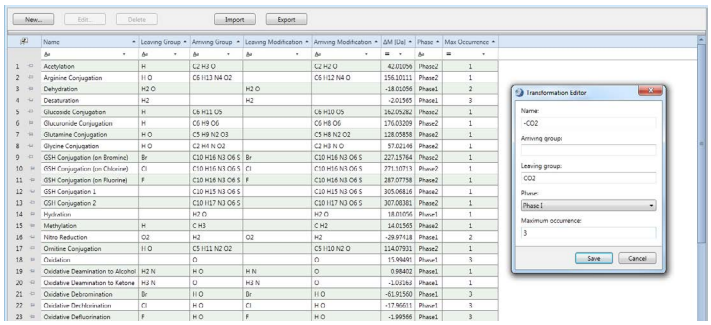

Fig. 3. Transformation Editor in Compound Discoverer software.

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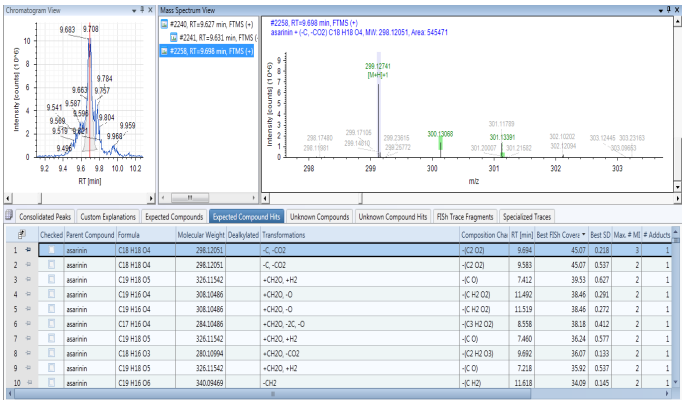


Fig.4. Calculation results of asrainin metabolites in Compound Discoverer software.

Identification of asarinin metabolites.
By employing Result Filters, metabolites of interest could be filtered out by selecting multiple criteria and options (Fig.5). Metabolites with Best FISh Coverage greater than 10 were chosen for identification.

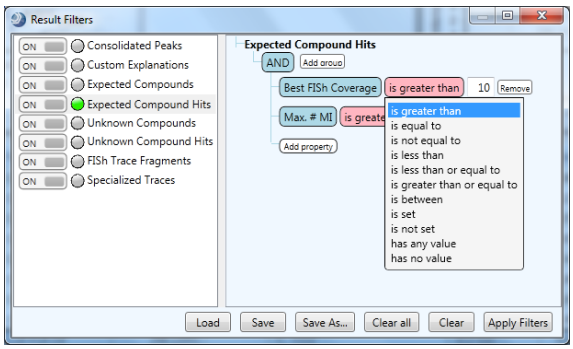


Fig.5. Result Filters in Compound Discoverer software.

FISh Scoring searched the fragmentation spectra, and annotated matching fragment structures directly on the spectra. As seen in the Figure 6, 8 fragment structures matching with those of the parent compound (color-coded in green) and 24 biotransformation shifted fragments (color-coded in blue) were labeled in the MS/MS spectrum, providing a quick visual indication of the modification location.

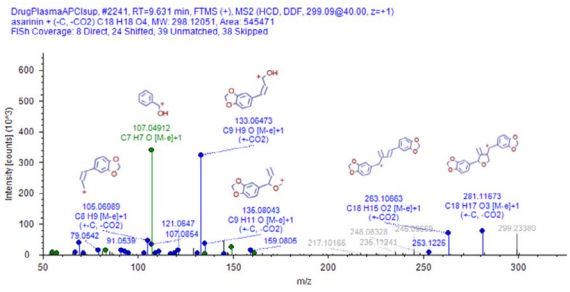


Fig.6. Asarinin metabolite with FISh fragment annotations.

In the custom explanation table, the MS/MS spectrum was automatically interpreted according to the proposed putative structure, giving greater confidence for the metabolite structure assignment. 23 exact match fragments were found in the FISh annotation based on the proposed structure of M18 (Fig.7).

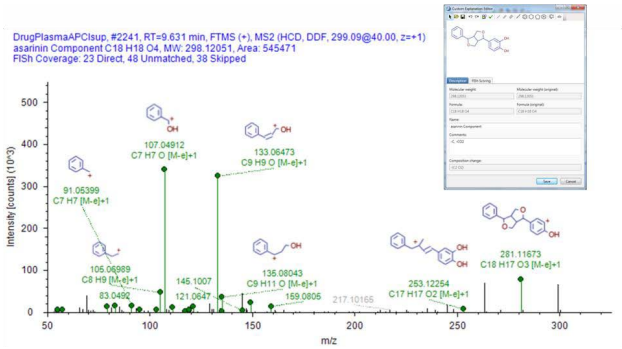
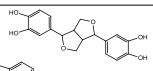
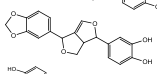
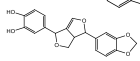
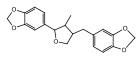
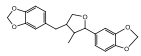
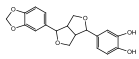
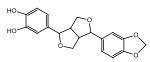
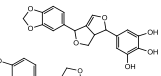
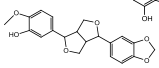
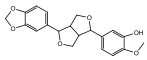
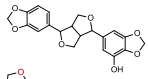
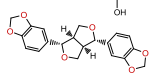


Fig.7. FISh re-annotated MS/MS spectrum based on proposed putative structure for M18.

In total, 38 asarinin metabolites with mass accuracy less than 1 ppm were identified (Table 1), among which 31 metabolites were discovered for the first time.

Table 1. Identification results of asarinin metabolites.

No.	t_R	Theoretical Mass *m/z*	Experimental Mass *m/z*	Precision (ppm)	Formula	Structure
M1	11.646	269.11722	269.11703	-0.71	$C_{17}H_{16}O_3$	
M2	28.915	269.11722	269.11703	-0.71	$C_{17}H_{16}O_3$	
M3	26.722	269.15361	269.1535	-0.41	$C_{18}H_{20}O_2$	
M4	29.014	269.15361	269.15341	-0.74	$C_{18}H_{20}O_2$	
M5	11.264	271.09649	271.09623	-0.96	$C_{16}H_{14}O_4$	
M6	7.420	281.11722	281.11743	0.75	$C_{18}H_{16}O_3$	
M7	8.828	281.11722	281.11719	-0.11	$C_{18}H_{16}O_3$	
M8	9.967	281.11722	281.11731	0.32	$C_{18}H_{16}O_3$	
M9	12.170	281.11722	281.1171	-0.43	$C_{18}H_{16}O_3$	
M10	19.770	281.11722	281.11695	-0.96	$C_{18}H_{16}O_3$	
M11	28.991	281.11722	281.1171	-0.43	$C_{18}H_{16}O_3$	
M12	7.225	283.09649	283.09638	-0.39	$C_{17}H_{14}O_4$	
M13	8.565	285.11214	285.11209	-0.18	$C_{17}H_{16}O_4$	
M14	10.305	285.14852	285.14871	0.67	$C_{18}H_{20}O_3$	
M15	19.852	295.16926	295.16905	-0.71	$C_{20}H_{22}O_2$	
M16	20.820	295.16926	295.16942	0.54	$C_{20}H_{22}O_2$	
M17	8.830	299.12779	299.12784	0.17	$C_{18}H_{18}O_4$	
M18	9.631	299.12779	299.12778	-0.03	$C_{18}H_{18}O_4$	
M19	14.626	309.11214	309.11203	-0.36	$C_{19}H_{16}O_4$	
M20	11.667	309.11214	309.11189	-0.81	$C_{19}H_{16}O_4$	
M21	8.358	315.12270	315.1229	0.63	$C_{19}H_{18}O_5$	
M22	9.810	315.12270	315.1225	-0.63	$C_{19}H_{18}O_5$	
M23	7.218	327.12270	327.12241	-0.89	$C_{19}H_{18}O_5$	
M24	7.412	327.12270	327.12238	-0.98	$C_{19}H_{18}O_5$	
M25	9.992	327.12270	327.12247	-0.70	$C_{19}H_{18}O_5$	
M26	14.593	327.12270	327.12259	-0.34	$C_{19}H_{18}O_5$	

No.	t_R	Theoretical Mass *m/z*	Experimental Mass *m/z*	Precision (ppm)	Formula	Structure
M27	9.095	331.11761	331.1178	0.57	$C_{18}H_{18}O_6$	
M28	12.843	341.10196	341.10187	-0.26	$C_{19}H_{16}O_6$	
M29	17.776	341.10196	341.10172	-0.70	$C_{19}H_{16}O_6$	
M30	11.616	341.13835	341.13812	-0.67	$C_{20}H_{20}O_5$	
M31	11.929	341.13835	341.13818	-0.50	$C_{20}H_{20}O_5$	
M32	9.995	343.11761	343.11743	-0.52	$C_{19}H_{18}O_6$	
M33	12.831	343.11761	343.11749	-0.35	$C_{19}H_{18}O_6$	
M34	11.687	357.09688	357.09664	-0.67	$C_{19}H_{16}O_7$	
M35	11.662	357.13326	357.133	-0.73	$C_{20}H_{20}O_6$	
M36	11.825	357.13326	357.13303	-0.64	$C_{20}H_{20}O_6$	
M37	14.593	371.11253	371.11258	0.13	$C_{20}H_{18}O_7$	
M38	11.672	355.11761	355.11739	-0.62	$C_{20}H_{18}O_6$	

Conclusions

- Effective and confident natural product metabolites discovery and identification were achieved using the Q Exactive Plus high resolution mass spectrometer and Compound Discoverer software.
- In total, 38 asarinin metabolites, with mass accuracy less than 1 ppm, were identified, of which 31 metabolites were discovered for the first time.
- Consolidating various complimentary metabolite detection strategies, Compound Discoverer software provides significant speed and organizational benefits to accelerate the natural product MetID process in one customizable platform.

References

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