Poster Reprint

ASMS 2025
Poster number MP 219

Advanced Mass Spectrometry Strategies for Deciphering the Complex Composition of an Unknown Chinese Patent Medicine

Shuna Fu[1],Huakai Wu[2]

[1] Agilent Technologies, Inc., Guangzhou, CHINA

[2] Zhangzhou Pientzehuang Pharmaceutical Co., Ltd., Fujian, CHINA

Introduction

A Chinese patent drug is a specialized form of traditional Chinese medicine (TCM) that adheres to a standardized manufacturing process and has been granted a patent by the relevant authorities in China. These drugs, rooted in ancient herbal formulas and practices, are widely utilized both within China and in regions around the world where TCM is practiced. Despite their widespread use, they possess certain limitations, such as complex active components, unclear mechanisms of action, and challenges in standardizing quality, which necessitate further research and enhancement. Recently, we employed an Agilent 6546 LC/Q-TOF in conjunction with the Agilent PCDL TCM MS/MS Database, Sirius, and Global Natural Product Social Molecular Networking (GNPS) (Figure.1) to analyze an unknown Chinese patent medicine. This analytical approach facilitated a deeper understanding of its botanical composition and active ingredients.

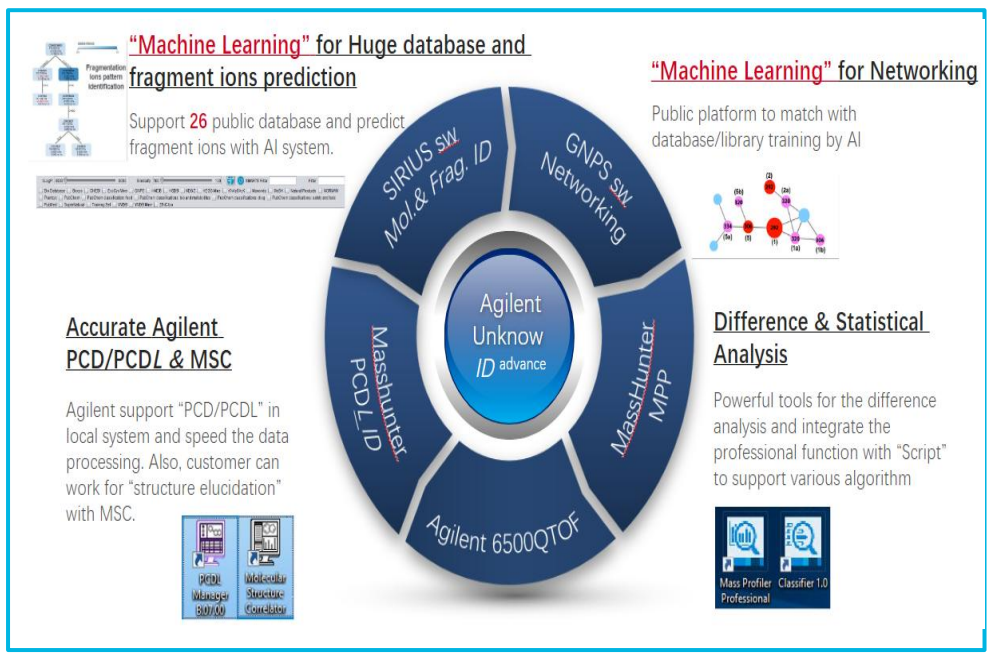

Figure.1 Agilent Innovation "Unknow ID advance "

Experimental

Sample Preparation

1. Weigh out 1.0 g of the powder.
2. Place the powder in a 50 ml centrifuge tube, add 50 ml of 70% ethanol solution, let stand overnight.
3. Ultrasonicate for 30 minutes, then cool to room temperature, shake well and centrifuge at 15000 rmp for 15 min, Immediately collect the supernatant.
4. Inject 1 µL of supernatant onto LC/Q-TOF-MS.

Instrument conditions:

1. Reverse phase chromatography.

LC Conditions		
Column:	Zorbax EC C18,1.8µm, 2.1*100mm	
Column Temperature:	40 ℃	
Injectiong Volume:	1 µL	
Autosampler temp:	15°C	
Flow Rate:	0.4 mL/min	
Mobile Phase A:	Water	
Mobile Phase B:	Acetonitrile	
Gradient:	Time 0.00 15.00 18.00 18.10 22.00	B% 5 100 100 5 5

2. Data independent (All Ions MS/MS) and data dependent iterative MS/MS acquisition were applied to all samples using both positive and negative ion electrospray on Agilent 1290 Infinity II LC coupled to a 6546 LC/Q-TOF(Figure.2)

Figure.2 Agilent 1290 Infinity II LC coupled to a 6546 LC/Q-TOF

1. Targeted Identification using Agilent MassHunter Qualitative Analysis Software (10.1) and Agilent-NatureStandard PCDL.

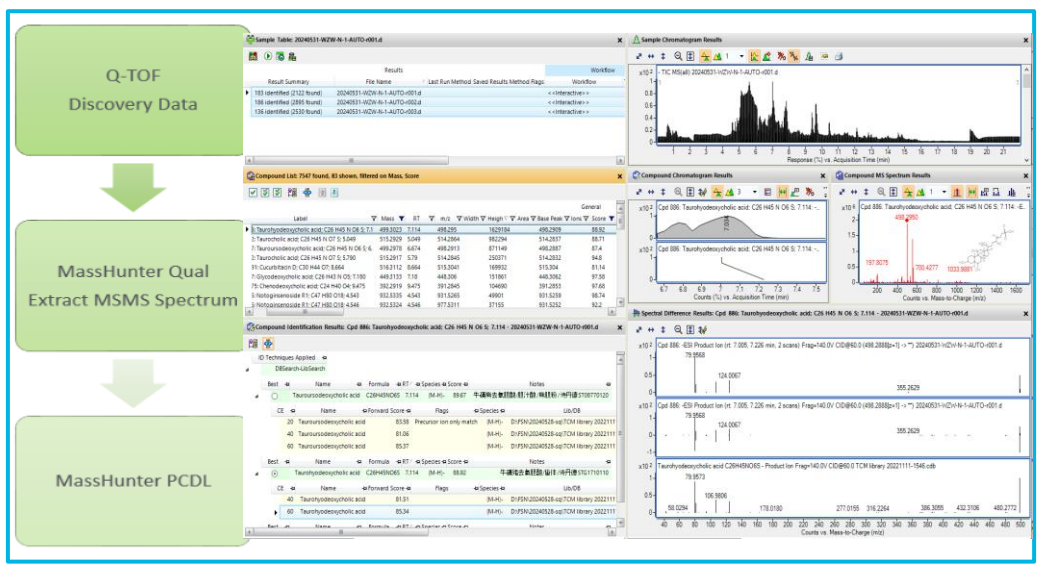

Figure.3 Agilent MH Workflow

- Leveraging the built-in automated workflows(Figure.3) and Agilent-NatureStandard PCDL whichcontains over 1500 natural products with accurate mass, compound name in Chinese, plant source, structural type and structural formula, CAS number and MSMS spectrometry, etc. Covering the standard products included in the Chinese Pharmacopoeia (Figure.4) , we swiftly found 7547 compounds for target compounds screening in data files and display the results in an easy-to-evaluate window, showing only the required content.

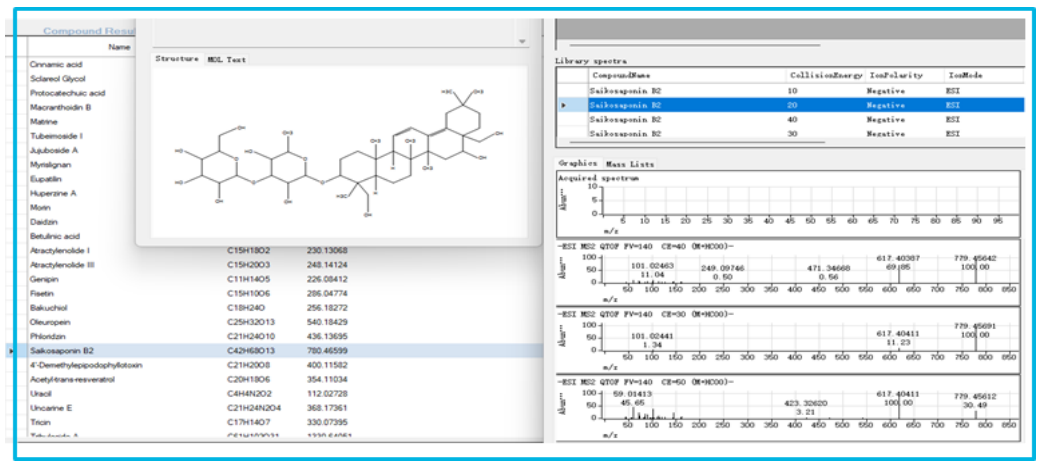

Figure.4 Agilent PCDL Database

- By setting filters and using MSMS matched tag, we identified 83 compounds with high confidence, revealing that saponins and bile acids are the primary bioactive components of the unknown Chinese patent medicine.

2. GNPS Network for Identifying Similar Bioactive but Unknown Compounds:

- We employed GNPS to calculate the similarity of characteristic fragment ions produced by mass spectrometry fragmentation, synthesizing a visual network map where structurally similar compounds clustered together.
- GNPS also facilitated the identification of known compounds, analogs, and automated analysis of compounds within the molecular network. It offers functionalities for copying, linking, and managing secondary mass spectrometry data from multiple sources. For instance, we rapidly identified Ginsenoside Rg1, Ginsenoside Re, Notoginsenoside Ft1 and found several unknown analogs（Figure.5）.

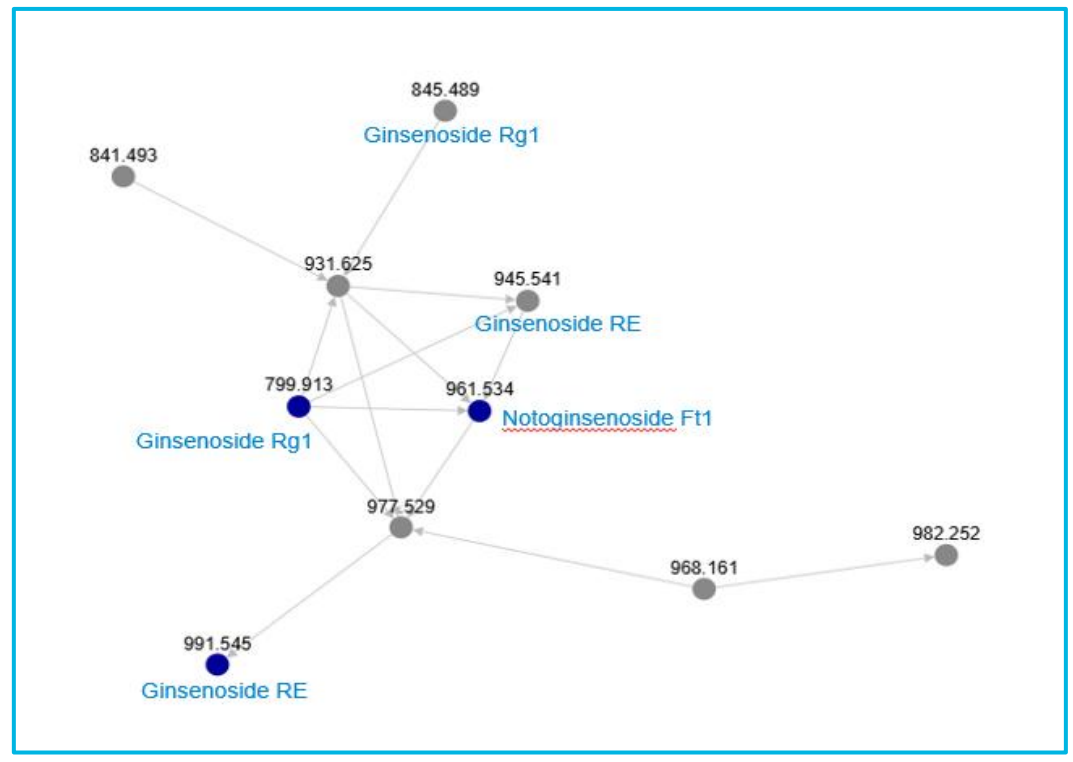

Figure.5 GNPS Network of structurally similar compounds

3. Untargeted Identification using Sirius with Online Databases:

We imported high-resolution tandem mass spectrometry (LC-MS/MS) data into SIRIUS （Figure.6a & Figure.6b ） to identify unknown analogs, SIRIUS provides de novo molecular formula annotation, accurately inferring the molecular formulas of compounds. And it integrates CSI:FingerID which can search molecular structure databases. For example, it aided in the identification of the unknown analog Notoginsenoside R1 （Figure.7）.

Results and Discussion

Figure.6a Identification of compounds in Sirius

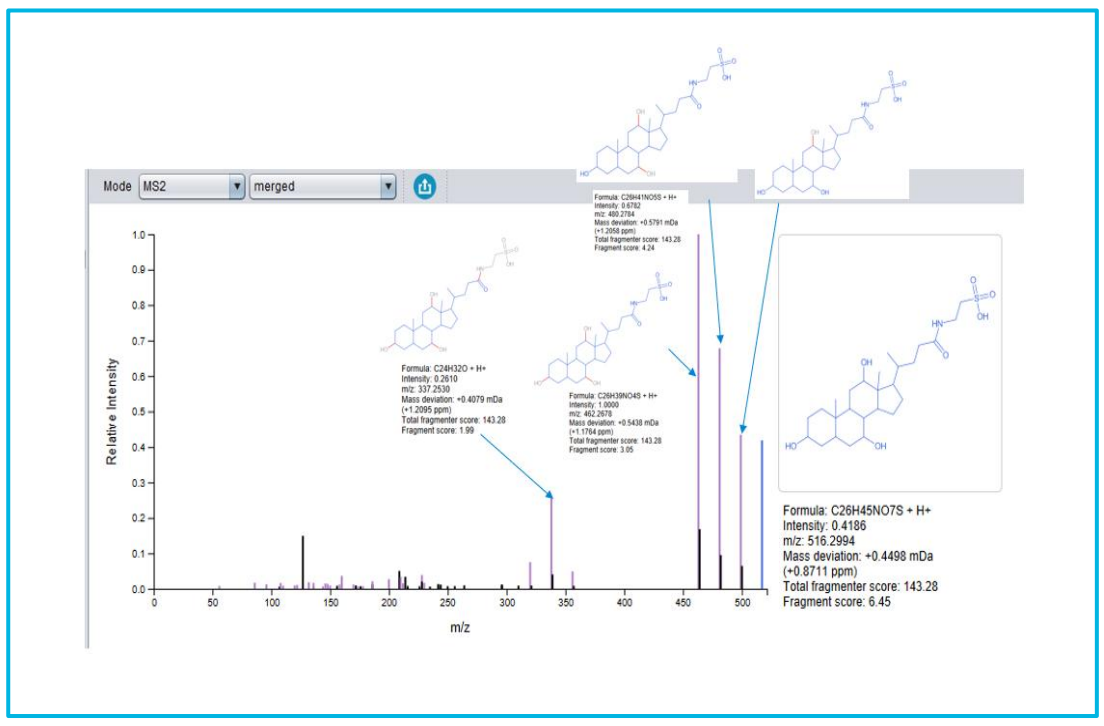

Figure.6b Fragment analysis in Sirius

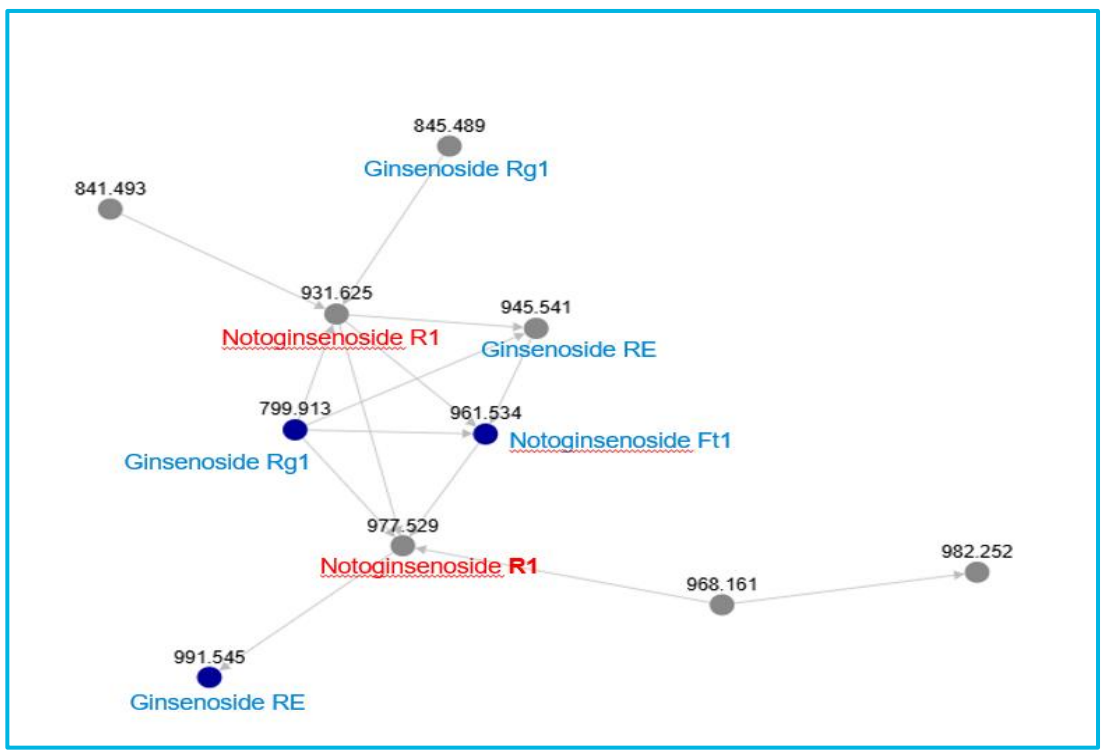

Figure.7 GNPS Network of Notoginsenoside R1

Conclusions

- By integrating the Agilent-NatureStandard PCDL (MS/MS Library), Sirius, and GNPS, we successfully identified more bioactive compounds within the unknown Chinese patent medicine, confirming that its main components are Panax notoginseng, snake gallbladder, and cow bezoar.
- The combination of Agilent-NatureStandard PCDL (MS/MS Library) and Sirius with GNPS represents a innovative approach for identifying a broader spectrum of bioactive compounds and new compounds .

https://www.agilent.com/en/promotions/asms

DE-006632

Published in USA, May 15, 2025

Agilent
Trusted Answers