



Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

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LETTER TO THE EDITOR

Feature-based molecular networking (FBMN): An efficient booster for discovering novel natural products or metabolites



KEY WORDS

Feature-based molecular networking;
Guided separation technology;
Natural products;
Metabolites;
Micro and trace compounds

To the editor:

The comprehensive exploration of natural products and metabolites is essential for unraveling the complexities of natural drug therapies. Inadequate tracking and separation methods result in increased research costs and reduced scientific output, significantly hindering the healthy advancement of related research fields. Emerging molecular networking (MN) technologies help minimize the redundant discovery of known natural products or metabolites. However, they often overlook chromatographic parameters, which are crucial for effectively distinguishing isomers and guiding subsequent separation processes. Feature-based molecular networking (FBMN) is an interactive, online-centric approach to data management and analysis that integrates both structural mass spectrometry (MS) data and the chromatographic behavior of natural products and metabolites¹. FBMN can differentiate between the spectra of positional and stereoisomers in MN that exhibit similar MS but have different retention times. FBMN offers an effective strategy for discovering novel natural products and metabolites, even in trace amounts. It utilizes the freely accessible Global Natural Products Social Molecular Network (GNPS) platform, which provides more diverse and accessible applications compared to expensive commercial mass spectrometry databases, thereby broadening opportunities for the research community. Therefore, to

enhance the identification of FBMN chemical components, more robust mass spectrometry databases are needed. Currently, the FBMN open-source database is in its early stages. Developing an efficient, versatile, and open-source mass spectrometry data format to support the establishment of FBMN databases presents a collective challenge that the research community must address to enhance the research and development of mass spectrometry analysis techniques in related fields. With the high sensitivity of MS and the powerful annotation capabilities of FBMN, this database offers a novel strategy for the comprehensive exploration of natural products and metabolites. However, the promotion of FBMN remains insufficient. This letter aims to provide references for related fields by reviewing the advantages, operations, and applications of FBMN in the discovery of natural products or metabolites.

1. The operational processes of FBMN

The application of FBMN requires attention to three key points: sample processing, optimization of acquired conditions, and analysis of acquired MS/MS data (Fig. 1). Both sample processing and condition optimization significantly impact the successful acquisition of MS/MS data and the accurate identification of the chemical information of test samples. Key natural products or metabolites are often present in micro or trace amounts, making them extremely susceptible to loss during sample processing. Therefore, ideal sample processing should be as straightforward as possible to minimize alterations to the sample composition due to human intervention. Modern extraction techniques are typically utilized to enhance the extraction rate of the target product through pressurization and other auxiliary means, such as supercritical fluid extraction, pressurized liquid extraction, and microwave-assisted extraction. These methods offer advantages such as reduced solvent usage, shortened extraction times, high

Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

<https://doi.org/10.1016/j.apsb.2025.02.019>

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selectivity, and improved retention of trace compounds. Additionally, the choice of extraction method should be based on the chemical types of the samples (Fig. 1A).

High-performance liquid chromatography (HPLC) is the most versatile tool for analyzing a wide range of compounds across various groups with distinct molecular properties. Different columns, elution modes, and chromatographic parameters—such as gradient settings, choice of mobile phase, column temperature, and flow rate—are suitable for the separation of compounds with varying characteristics (Fig. 1B). With the ongoing demand for higher resolution in separation systems, innovative techniques such as capillary liquid chromatography, two-dimensional liquid chromatography, and ion mobility spectrometry have gradually been adopted. Regarding mass spectrometry detection conditions (Fig. 1B), depending on the types of separated compounds, either gas chromatography or liquid chromatography coupled with electrospray ionization (ESI) in both positive and negative ionization modes can be employed.

FBMN is built on chromatographic feature detection and comparison tools (Fig. 1C). It supports multiple software programs for feature detection and alignment processing (<https://ccms-ucsd.github.io/GNPSDocumentation>). Among these, the auto-optimization module can be utilized to fine-tune parameters, which is particularly valuable when using command-line interface tools. Studies have reported that only three different positional isomers could be observed in FBMN using OpenMS. In contrast, MZmine, a popular open-source, cross-platform software for mass spectrometry data processing, successfully distinguished seven isomers in FBMN for the same sample, suggesting that varying treatments and/or parameters may yield different results in FBMN¹.

2. FBMN-based discovery of novel natural products

FBMN plays a crucial role not only in the targeted separation of novel compounds but also in the identification of isomers.

Recently, with the introduction and application of FBMN, an increasing number of researchers have discovered various natural products featuring new backbones and significant biological activities, providing innovative approaches for the guided separation of natural products. For instance, Padilla-González et al.² utilized FBMN to characterize the structural diversity of caffeic acid esters and to selectively separate novel trace caffeic acid esters from different organs of *Smilax sonchifolius* extracts. In FBMN, the sizes of the nodes indicate semi-quantitative differences in concentrations, while the colors of the nodes correspond to the presence of each metabolite in various organs. This work identified three new compounds, one (I, Supporting Information Fig. S1) of which exhibited a very low isolation yield. Its HMBC correlations were only observed in mixtures. Kakumu et al.³ discovered anti-inflammatory chromene dimers in *Melicope pteleifolia* using FBMN. In comparison to previous studies, in addition to geranylacetylphloroglucinol and acylphloroglucinol C-glucosides, a rare family of trace chromene dimers (II–VI, Fig. S1) was identified through FBMN, demonstrating anti-inflammatory effects with an IC_{50} of up to 5.1 $\mu\text{mol/L}$. Zhang et al.⁴ employed FBMN to systematically analyze compounds in the fruit of *Rosa roxburghii* Tratt. Based on the annotated compounds, four functional and differential compounds were analyzed, and three ascorbyl hexosides were quantified. Further data mining revealed 17 novel and trace ascorbic acid (AA) derivatives, consisting of ascorbic acid units coupled with organic acids, flavonoids, or glucuronides, indicating that AA derivatives extend beyond ascorbyl glycosides, including the AA trace hypothetical derivatives (VII and VIII, Fig. S1). Park et al.⁵ used FBMN to isolate two new ecdysteroids, specasterone A (IX, Fig. S1) and spectasterone B (X, Fig. S1), from *Ajuga spectabilis*, which influence the expression of 11β -hydroxysteroid dehydrogenase type 1 and glucocorticoid receptors, and have not been previously identified in this plant. The results provide a strong rationale for the continued development of ecdysteroids as potential therapeutic candidates for novel anti-aging agents.

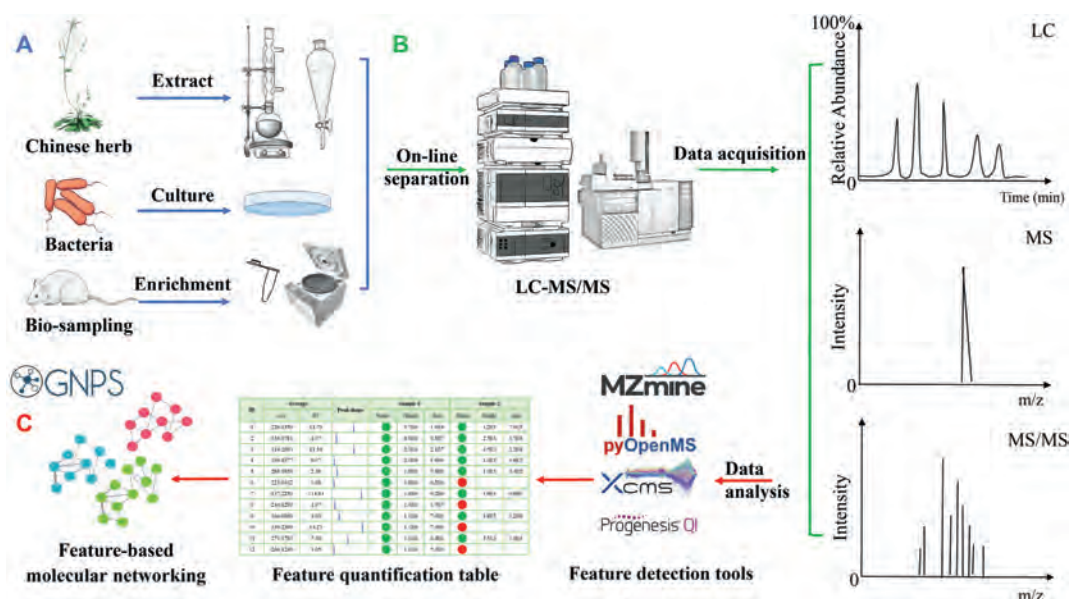


Figure 1 Flow of FBMN study (A) Sample processing. (B) Acquired condition optimization. (C) Acquired MS/MS data analysis.

3. FBMN-based discovery of metabolites

FBMN is rarely utilized to identify metabolites in the health or disease states of organisms. However, it is a powerful tool for annotating micro or even trace amounts of metabolites in both physiological and pathological conditions, as well as for searching for disease biomarkers. Du et al.⁶ employed FBMN to comprehensively identify differential metabolites in rats with type 2 diabetes mellitus (T2DM) and diabetic cognitive dysfunction (DCD). They identified 71 differential metabolites in hippocampal tissue and 179 differential metabolites in urine. Clustered heat maps of the peak areas of these differential metabolites were generated using the relative quantitative function of FBMN, visually representing the metabolic changes between DCD and T2DM rats. Notably, seven trace components in urine were highlighted as key identified metabolites. This study demonstrated that FBMN significantly contributes to the comprehensive identification of differential metabolites in DCD rats, particularly micro or trace *in vivo* metabolites.

In addition, the study of *in vivo* metabolites and metabolic pathways of drugs and other active substances through FBMN is receiving renewed attention. Albuquerque Cavalcanti et al.⁷ utilized data mining and FBMN for the non-targeted screening of synthetic cannabinoids (SC) in oral fluids. The FBMN analysis created a unique integrated network for most of the low concentrations (20 ng/mL) of SC assessed in oral fluids. Renai et al. combined FBMN with mass spectrometry libraries to analyze nutrient metabolomics, focusing on postprandial urinary metabolomic analyses within the context of a *Vaccinium* supplementation intervention. The results indicate that this approach is a powerful strategy for longitudinal studies, as it reduces the unknown chemical space by enhancing annotation capabilities to characterize biochemically relevant metabolites in human biological fluids and identify *in vivo* metabolites at trace levels (Supporting Information Table S1). Kwak et al. investigated the phase I metabolites of SR9009, a peroxisome proliferator-activated receptor δ (PPAR δ) agonist, in horse liver microsomes *in vitro* using FBMN, identifying a total of 15 metabolites, including a new major metabolite, the *N*-dealkylated metabolite, which indicated various metabolic modifications (Table S1). Wu et al.⁸ elucidated the *in vitro* metabolism of seongsanamide A in human liver microsomes using untargeted metabolomics and FBMN, resulting in the identification of four metabolites. The main metabolic pathways associated with seongsanamide A were found to be hydroxylation and oxidative hydrolysis. Wang et al.⁹ analyzed the metabolites of ofloxacin in mice based on FBMN, identifying five metabolites, probable metabolic pathways, and the primary metabolic site. FBMN is rapid, accurate, does not require standards, and has a wide range of applications in the analysis of various metabolites.

4. FBMN combined with network pharmacology helps to explain the mechanism of action of traditional Chinese medicine

In recent years, emerging network pharmacology has been widely utilized in the field of traditional Chinese medicine (TCM). This approach plays a crucial role in exploring the pharmacological mechanisms of medicinal plants, elucidating the principles of TCM, and facilitating the development of new drugs. However, the lack of integration of disparate and quantitative data has

resulted in findings being limited to the predictive stage, primarily due to a high dependence on aggregated data. Integrating FBMN into network pharmacology can address this limitation of genomic technology. Since FBMN provides relative quantitative information for each feature, it allows for the construction of the strongest correlated features and biological parameters derived from MS/MS quantification. Consequently, the chemical structure spectra resolved by MS/MS may serve as an effective tool for screening active or toxic chemicals. Zhao et al.¹⁰ investigated the hepatotoxic components and mechanisms of intrinsic hepatotoxicity of *Epimedium Folium* using an integrated strategy that combines network toxicology and FBMN. The results indicated that the combination of FBMN and network pharmacology could enhance the understanding of the mechanisms of action of medicinal plants and aid in the discovery of bioactive components.

5. Conclusions and perspectives

As an emerging mass spectrometry technique, FBMN leverages the exceptional separation capabilities of the liquid phase alongside the powerful characterization abilities of mass spectrometry. It offers significant advantages in distinguishing isomers and stereoisomers, providing a robust methodology for the discovery, comprehensive characterization, and isolation of novel natural products and metabolites. The growing adoption of FBMN is poised to accelerate the evolution of mass spectrometry databases and analytical methodologies, providing a wealth of valuable strategies for related research initiatives. Additionally, FBMN can effectively extract mass spectral response intensities of molecules with varying retention times, enabling more accurate relative quantification compared to traditional mass spectrometry analysis techniques.

In contrast to expensive commercial mass spectrometry databases, FBMN utilizes the freely accessible GNPS platform, broadening opportunities for a wider research community with more accessible and diverse applications. Consequently, the significance of FBMN in the discovery of novel trace or micro-natural products and metabolites, along with the identification of other bioactive compounds, has garnered increasing recognition among researchers in recent years (Table S1 and Fig. S2–S4). However, the application scenarios and scope of FBMN still require further exploration.

Natural medicines often possess a complex material foundation. With the rapid advancement of network pharmacology and bioinformatics, the comprehensive research strategy for natural medicines has reached a new level. However, systematic research approaches regarding their material basis remain underdeveloped, frequently failing to provide robust data on active ingredients. As a result, the characterization of pharmacodynamic substances is often one-dimensional, and various ethnic drugs or different preparations of the same ethnic drug tend to rely on the same material basis in their studies. Online mass spectrometry, utilizing strategies such as FBMN, can offer more precise information about the material composition of samples, and the integration of diverse bioinformatics approaches holds significant application value.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (82430116 and 82204607), The special fund of Central committee high level Chinese medicine

hospital (CZ015-DZMG-LJRC-0014, DZMG-LJRC0013, China), and Young Elite Scientists Sponsorship Program by CACM (2023-QNRC2-B18, China).

Author contributions

Meiru Han: Writing-original draft, Investigation. Huan Xia: Investigation. Guiyang Xia: Review & editing. Xiaohong Wei: Review & editing. Jinyu Li: Review & editing, Project administration, Funding acquisition, Supervision. Yuzhuo Wu: Writing-review & editing, Project administration, Funding acquisition, Supervision. Sheng Lin: Review & editing, Project administration, Funding acquisition, Supervision. All of the authors have read and approved the final manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

Appendix A. Supporting information

Supporting information to this article can be found online at <https://doi.org/10.1016/j.apsb.2025.02.019>.

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Received 31 December 2024

Received in revised form 17 February 2025

Accepted 18 February 2025