Journal of Pharmaceutical Analysis 14 (2024) 101016

Contents lists available at ScienceDirect

Journal of Pharmaceutical Analysis

journal homepage: www.elsevier.com/locate/jpa

Short communication

# Rapid toxicity characterization of *Aconitum* herbal medicines using perfusion nano-electrospray ionization mass spectrometry

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ARTICLE INFO

*Article history:*
Received 18 January 2024
Received in revised form
4 May 2024
Accepted 30 May 2024
Available online 5 June 2024

Accurate analysis of the key substances in traditional Chinese medicines (TCMs) is the prerequisite and foundation for the quality evaluation and pharmacological substance research of TCMs [1]. However, the extremely complex characteristics of medicinal origins, preparation processes and chemical compositions make it difficult to accurately analyze TCMs in complex multi-scenarios [2]. Independent innovation of special novel equipment, technologies and methods in line with the complex characteristics of TCMs is an important way to break through the bottlenecks of traditional research [3]. Therefore, this study focuses on the problem of rapid and accurate evaluation of the toxicity of *Aconitum* herbal medicines (AHMs), and independently builds a constant perfusion nano-electrospray ionization mass spectrometry (PnESI-MS) device to achieve direct and rapid analysis of AHMs. Further, a complete rapid evaluation strategy for the toxicity of AHMs was established, including the construction and optimization of proprietary equipment, the quantitative characterization of core index components, and the overall evaluation of holistic weighted toxicity (HWT) and safety limits (SL), in order to promote the quality improvement and standardization development of TCMs.

First of all, a PnESI-MS device with the characteristics of simple, easy operation, high sensitivity, resistance to matrix interference, and strong stability was established. Fig. 1A depicts a schematic illustration of the device, which consists of three key components: the nano-electrospray ionization system, the microfluidic perfusion system, and the versatile three-axis positioning device. Fig. 1B illustrates the nano-electrospray ionization system, which is closed to the MS ion transfer tube and connected to the microfluidic perfusion system through a fused quartz capillary (i.d. 0.1 and o.d. 0.15 mm). The high-voltage field device stands adjacent to the solution delivery pipeline, connected at one end to a high-voltage power supply, and to a conductive copper wire (0.02 mm) at the other end to form an electrode. The copper wire is carefully inserted into the fused quartz capillary to establish a connection with the liquid pipeline. During the experiment, the capillary tip generates a dynamic electromagnetic field when exposed to high-voltage power. This field causes droplets to explode, forming an electrospray that is used for MS analysis, without the need of nitrogen as an auxiliary gas. Compared to classical ionization techniques such as ESI and atmospheric pressure chemical ionization (APCI), PnESI requires little or no sample preparation and has strong resistance against interference from complex matrices, making it ideal for rapid on-site analysis and other special scenarios [4]. Meanwhile, the precision syringe pump enables continuous and stable sample perfusion, which solves the problems of short signal duration, high fluctuation and capillary clogging of traditional nanoESI-MS. Furthermore, PnESI integrates a hands-free, versatile three-axis positioning device that provides precise control over the distance and angle between the capillary tip and MS inlet, thus aiding in accurate quantification. Higher analytical sensitivity is one of the key features of PnESI. Fig. 1C compares the MS fingerprints of Shengfupian (SFP) using PnESI and commercial ESI, showing similar overall profile of the spectra but PnESI with three times greater sensitivity (e.g., the intensity of *m/z* 438 was $5.48\times10^5$ for ESI and $1.35\times10^6$ for PnESI). In summary, PnESI, with its simple and easy-to-use structure, reduced matrix effect and significantly improved analytical sensitivity, is a cost-effective and efficient tool for rapid analysis of herbal components (only 10 s and 1–3 μL of a sample are needed).

The PnESI-MS demonstrates exceptional qualitative and quantitative capabilities for the analysis of complex TCMs. The full-scan

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https://doi.org/10.1016/j.jpha.2024.101016

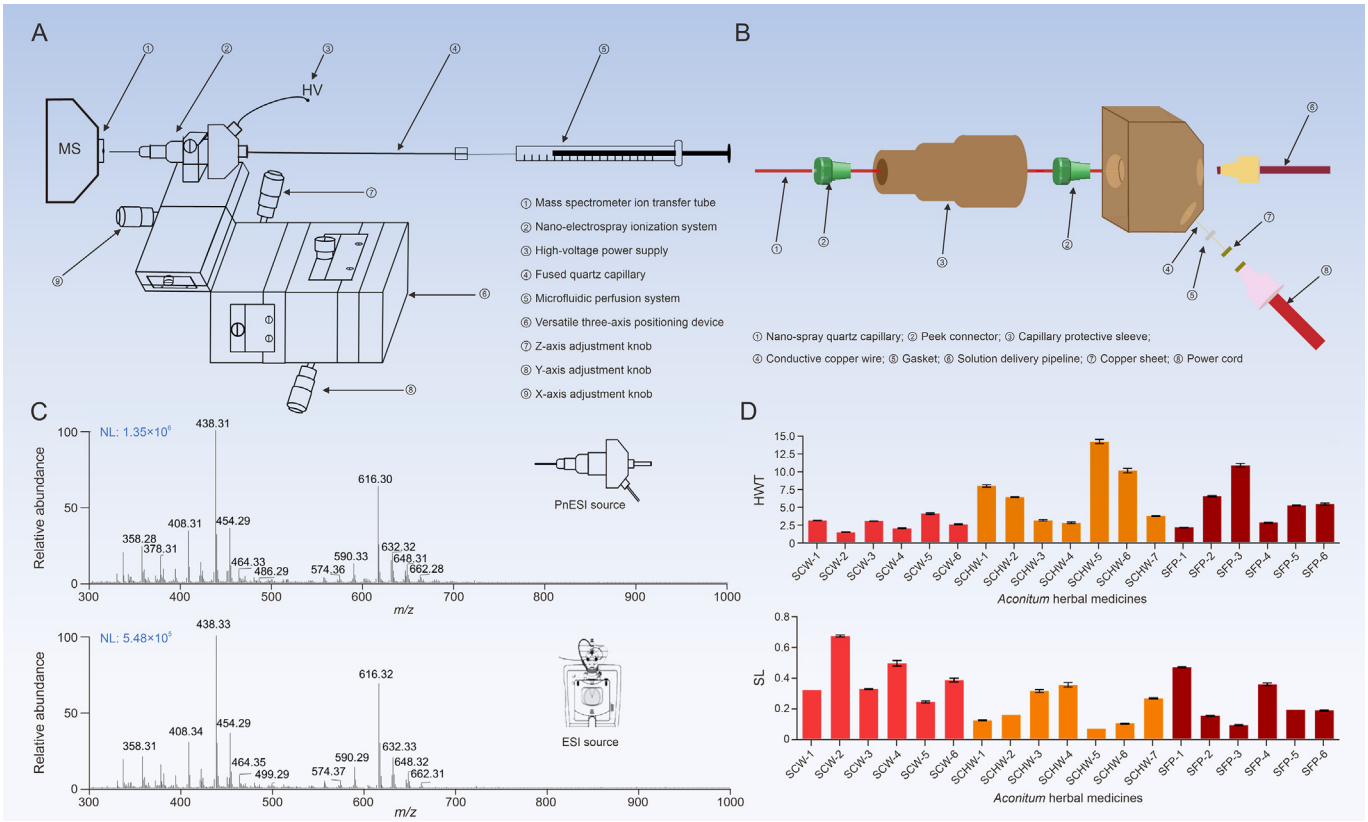


**Fig. 1.** Rapid toxicity characterization of *Aconitum* herbal medicines using perfusion nano-electrospray ionization mass spectrometry (PnESI-MS). (A) Schematic illustration of the overall structure of the device. (B) Schematic illustration of the internal structure of nano-electrospray ionization system. (C) Comparison mass spectrum between the PnESI (upper) and the commercial ESI (lower) ionization source. NL: Normalized Level (D) Toxicity evaluation of 19 batches of samples based on holistic weighted toxicity (HWT) and safety limits (SL). SCW: Shengcaowu; SCHW: Shengchuanwu; SFP: Shengfupian; NL: normalized level.

MS spectra (Fig. S1A) and MS/MS spectra (Figs. S1B–D) demonstrate its proficiency in capturing targeted molecular ion peaks and provide detailed fragmentation patterns for AHMs. The device enables accurate qualitative characterization, with diagnostic ion signals revealing structural information. For quantitative analysis, employing characteristic fragment ions, favorable precision (relative standard deviation (RSD) 4.78%–9.17%, Table S1) and linearity ($R^2 > 0.99$, Fig. S2) within a concentration range of 0.005–10 ng/mL were yielded. Method repeatability (RSD 3.42%–4.50%, Table S1) and recovery (89.53%–114.69%, Table S1) demonstrate reliability and satisfied matrix interference. The remarkable sensitivity (limit of detection (LOD) = 0.0001–0.001 ng/mL, limit of quantification (LOQ) = 0.001–0.005 ng/mL, Table S2) addresses trace-level quantification needs. In conclusion, PnESI-MS offers a rapid, sensitive, and reliable approach for the quantitative analysis of AHMs components, demonstrating significant value in various applications.

The processing and detoxification mechanisms of *Aconitum* were first characterized using the excellent qualitative and quantitative ability of PnESI-MS. The MS fingerprints of the samples before processing detoxification (SFP) and after processing detoxification (Heishunpian (HSP)) are shown in Fig. S3. Obviously, there are a large number of highly toxic diester alkaloids (e.g., *m/z* 616 was hypaconitine, $8.40\times10^5$) in SFP, while in the processed HSP, these diester alkaloids are converted into the corresponding monoester alkaloids (e.g., *m/z* 574 was benzoylhypaconine, $9.03\times10^4$). Monoester alkaloids have very weak toxicity and good efficacy, which are the main material basis for the efficacy of AHMs in clinical practice. Therefore, the processing process of AHMs can effectively reduce toxicity and increase efficiency. Table S3 displays the results of MS identification for 35 primary substances in SFP and HSP. Moreover, the key three diester-type alkaloids in 19 diverse raw *Aconitum* herbs were quantitatively analyzed utilizing PnESI-MS (Table S4, and Fig. S4). The average total content of three diester alkaloids was in the order of SFP > Shengchuanwu (SCHW) > Shengcaowu (SCW), emphasizing the variability of toxicity among *Aconitum* herbs. More importantly, in order to solve the problem of inaccurate toxicity assessment of different compounds by using the total content, we have systematically calculated the HWT and SL of different *Aconitum* herbs (Fig. 1D) by formula (1):

$$SL = \frac{1}{HWT} = \frac{1}{(C_H/0.0162 + C_M/0.0035 + C_A/0.0051)/60/1000} \quad (1)$$

where HWT stands for the holistic weighted toxicity of ingesting 1.0 g of the herb; SL represents the toxicity threshold, indicating the maximum safe dose; $C_H$ (μg), $C_M$ (μg) and $C_A$ (μg) are the contents of hypaconitine, mesaconitine and aconitine, respectively; 0.0162, 0.0035 and 0.0051 are the minimum toxic dose (mg/kg) of hypaconitine, mesaconitine and aconitine, respectively [5]; 60 is the regular adult weight (kg).

In conclusion, this study developed a PnESI-MS device with significant quantitative advantages for complex TCMs, and developed a systematic strategy for the assessment of HWT and SL of

toxic TCMs, which is expected to contribute to the enhancement of the quality control of TCMs and to guarantee the safe use of medicines in clinics.

## CRediT author statement

**Chaofa Wei** and **Liping Kang**: Conceptualization, Methodology, Investigation, Writing - Reviewing and Editing; **Yaqiu Zhao** and **Li Zhou**: Data curation; **Xiang Li** and **Shuanglong Wang**: Software, Writing - Original draft preparation; **Luqi Huang**: Conceptualization, Funding acquisition; **Zidong Qiu**: Conceptualization, Funding acquisition, Methodology, Writing - Reviewing and Editing.

## Declaration of competing interest

The authors declare that there are no conflicts of interest.

## Acknowledgments

This work was supported by the CACMS Innovation Fund (Grant Nos.: CI2021A05206, and CI2021A04504), the National Natural Science Foundation of China (Grant No.: 82104380), the Fundamental Research Funds for the Central Public Welfare Research Institutes (Grant No.: ZZ14-YQ-047) and the Scientific and Technological Innovation Project of China Academy of Chinese Medical Sciences (Grant Nos.: CI2023E002, and CI2023C039YGL).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpha.2024.101016.

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