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Online Analysis of Chinese Medicine Pharmaceutical Processes Using a Miniature Mass Spectrometer: A Case Study on Active Ingredient Extraction

Benjamin Miller[1]*, Chloe Taylor[1], Samuel Garcia[1]

[1]Department of Pharmacy Practice, College of Pharmacy, University of Toronto, Toronto, Canada.

***E-mail** ✉ benjamin.miller@yahoo.com

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ABSTRACT

The automation of traditional Chinese medicine (TCM) manufacturing has advanced process analysis from offline to online modes. However, most existing online analytical technologies rely on spectroscopic methods, which still face difficulties in accurately identifying and quantifying individual compounds. In this study, we established a quality control (QC) system utilizing paper spray ionization miniature mass spectrometry (mini-MS) to monitor TCM production processes. This approach enabled, for the first time, real-time online qualitative and quantitative assessment of specific components in herbal extracts using mini-MS without the need for chromatographic separation. The dynamic variation of alkaloid compounds in Aconiti Lateralis Radix Praeparata (Fuzi) during the decoction process served as a demonstration, alongside an exploration of the underlying principles of Fuzi compatibility. Moreover, the system demonstrated reliable performance during continuous pilot-scale extraction over several hours. This mini-MS–based online analytical platform holds great promise for expanding QC applications across diverse pharmaceutical manufacturing processes.

Keywords: Online analysis, Miniature mass spectrometry, TCM Pharmaceuticals, Process analytical technology

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Introduction

Process Analytical Technology (PAT) refers to methods used to assess the physical and chemical properties of natural or manufactured products during production, allowing real-time monitoring and quality control (QC) of process variations. The U.S. Food and Drug Administration (FDA) officially defined PAT in 2004 as part of its regulatory framework [1]. In recent years, the rapid advancement of PAT has significantly promoted the modernization of the traditional Chinese medicine (TCM) industry [2]. PAT tools have become essential for QC in TCM pharmaceutical manufacturing [3], serving roles in raw material evaluation, monitoring of processing steps, quantification of bioactive compounds, and real-time analysis of extraction processes. The development of QC systems for production is a critical step toward ensuring the safety, efficacy, and consistency of herbal medicines [4]. The TCM industry is thus shifting from a “quality by test” approach to a “quality by design” paradigm [5], in which quality is embedded in every stage of production rather than assessed only after completion. This transformation has encouraged the evolution of TCM process analysis from traditional offline testing toward real-time online monitoring.

A wide range of analytical chemistry techniques has been employed in TCM studies, with spectroscopic methods—such as ultraviolet (UV), infrared (IR), near-infrared (NIR), and Raman spectroscopy—being the predominant choices for online analysis. Among them, NIR spectroscopy is extensively applied for monitoring multiple stages of TCM manufacturing, including extraction, concentration, alcohol precipitation, drying, blending, granulation, and coating [2, 6]. Spectroscopy-based technologies offer benefits such as broad applicability, simple operation, and non-destructive measurements. However, because these methods capture

spectral information primarily based on functional groups or chemical bonds, overlapping signals often occur in complex TCM mixtures. This overlap hinders the ability to perform targeted qualitative and quantitative assessments of individual compounds. Consequently, spectroscopic process analyses generally rely on constructing chemometric models, which require large datasets and multiple algorithmic combinations. These models are often costly to develop and must be frequently updated to accommodate new analytes.

Mass spectrometry (MS) represents a more powerful analytical alternative, capable of both qualitative and quantitative characterization of components in complex matrices, and it has been widely adopted in TCM research [7, 8]. Conventional laboratory MS instruments exhibit outstanding performance in sensitivity, resolution, and mass range but are typically large, stationary systems that demand skilled operators. Moreover, traditional sample preparation—such as extraction, dilution, centrifugation, and filtration—is labor-intensive and time-consuming. As a result, despite their analytical accuracy, benchtop MS systems are less suited for on-site or real-time applications. There is therefore a pressing need for compact, user-friendly MS technologies that can deliver rapid feedback during production for immediate decision-making by field personnel.

Miniature mass spectrometry (mini-MS) provides an ideal solution owing to its small footprint, energy efficiency, and portability [9-13]. The field has advanced rapidly, with some instruments now weighing as little as 4 kg [14]. These systems support advanced detection modes, such as multiple reaction monitoring (MRM), allowing the selective analysis of target analytes [15]. Continuous progress in ion source design—such as membrane inlet or low-gas-intake interfaces—has also enabled the creation of portable gas chromatography (GC)-MS instruments for field analysis of volatile substances [16-18]. Additionally, compact electrospray ionization (ESI)-MS systems have been developed for both small molecules and biomolecules, often integrated with liquid chromatography (LC) or nanoESI setups [19, 20]. Although miniaturized GC-MS and LC-MS systems are powerful, the emergence of ambient ionization methods in 2004 marked a major breakthrough, allowing rapid analysis of samples in their native form without complex pretreatment [21, 22].

Ambient ionization MS now plays an important role in TCM studies, including component identification [23], QC monitoring [24], detection of pesticide residues [25], and herbal authentication [26]. These systems are durable, versatile, and capable of generating ions in non-laboratory settings, greatly expanding the scope of mini-MS applications in field-based analysis. Several ambient ionization techniques—such as paper spray ionization (PSI) [27, 28], desorption electrospray ionization [21, 29], and laser spray ionization [30]—can be efficiently coupled with mini-MS instruments for the direct examination of complex mixtures without chromatographic separation.

In this study, we established an online QC system integrating PSI with mini-MS for real-time monitoring of TCM pharmaceutical processes. Since extraction is a crucial phase in the preparation of herbal medicines, we used it as a representative case study. Aconiti Lateralis Radix Praeparata (commonly known as “Fuzi”) was chosen as the model material due to its well-documented pharmacological properties [31, 32]. The raw Fuzi contains diester alkaloids—its primary toxic constituents—which can be hydrolyzed into monoester alkaloids during processing and decoction, leading to reduced toxicity and enhanced therapeutic efficacy. Using our mini-MS system, we continuously tracked the transformation of three representative diester alkaloids into their corresponding monoester derivatives during decoction, providing real-time quantitative insight into the chemical conversion process. Furthermore, we investigated the impact of herbal compatibility on Fuzi’s “detoxification and efficacy enhancement” mechanisms through dynamic online monitoring. The system was subsequently validated under pilot-scale extraction conditions, demonstrating stable performance over extended operation.

Compared with conventional spectroscopic methods, this LC-independent MS-based online system enables real-time quantitative detection of target compounds in TCM extracts. Its compact design, low energy demand, and operational simplicity make it easy to integrate into production lines, where it can be operated by technicians with minimal training. Beyond TCM, this approach contributes to the broader advancement of decentralized PAT applications in modern pharmaceutical manufacturing.

Table 1. The amount of three herbs in the six recipes.

Herbs	Recipe 1	Recipe 2	Recipe 3	Recipe 4	Recipe 5	Recipe 6
Fuzi (g)	8	8	8	8	8	8
Ganjiang (g)	0	8	0	8	4	8
Zhigancao (g)	0	0	8	8	8	4

Sample transfer and PSI–mini-MS analysis

Standard solutions were employed to study the fragmentation patterns of analytes in PSI–mini-MS/MS and to establish calibration curves. Consequently, all experiments involving standard injections were performed manually using a micropipette rather than the automated moving platform. A modified version of the general PSI–MS sampling procedure was adopted [28]. For each analysis, two μL of the standard solution was applied directly onto a triangular piece of chromatography paper. The paper tip was positioned approximately 5 mm from the MS inlet, after which 20 μL of elution solvent (methanol containing 0.1% v/v formic acid) was added, and a spray voltage of 3.5 kV was applied. All mass spectrometric analyses were carried out in positive ionization mode. The analytical method was initially optimized in the laboratory and validated with quality control samples before further application.

For online monitoring of the TCM decoction, the liquid sample from the flask was continuously transferred to the PSI–mini-MS system using a peristaltic pump connected via a silicone tube (inner diameter: 0.25 mm, length: 40 cm). Although a shorter tube could have been used in the laboratory to minimize dead volume, a 40 cm length was selected to maintain consistency with the pilot-scale extraction setup. The tube outlet was linked to two polyether ether ketone (PEEK) capillaries through a three-way valve: one (inner diameter 25 μm, length 40 cm) directed the sample toward loading, while the other (inner diameter 0.17 mm) returned the residual decoction to the flask. The injection system had an estimated dead volume of approximately 20 μL. The peristaltic pump was operated at a flow rate of 0.01 mL/min. The automated platform advanced by 1 cm every 10 minutes, ensuring that a fresh section of paper carrying the sample reached the detection point at each interval. The elution solvent (methanol with 0.1% v/v formic acid) was delivered via a quartz capillary (inner diameter: 50 μm) driven by a syringe pump at a rate of 2 μL/min. The primary components of the analytical setup are illustrated in **Figure 1**.

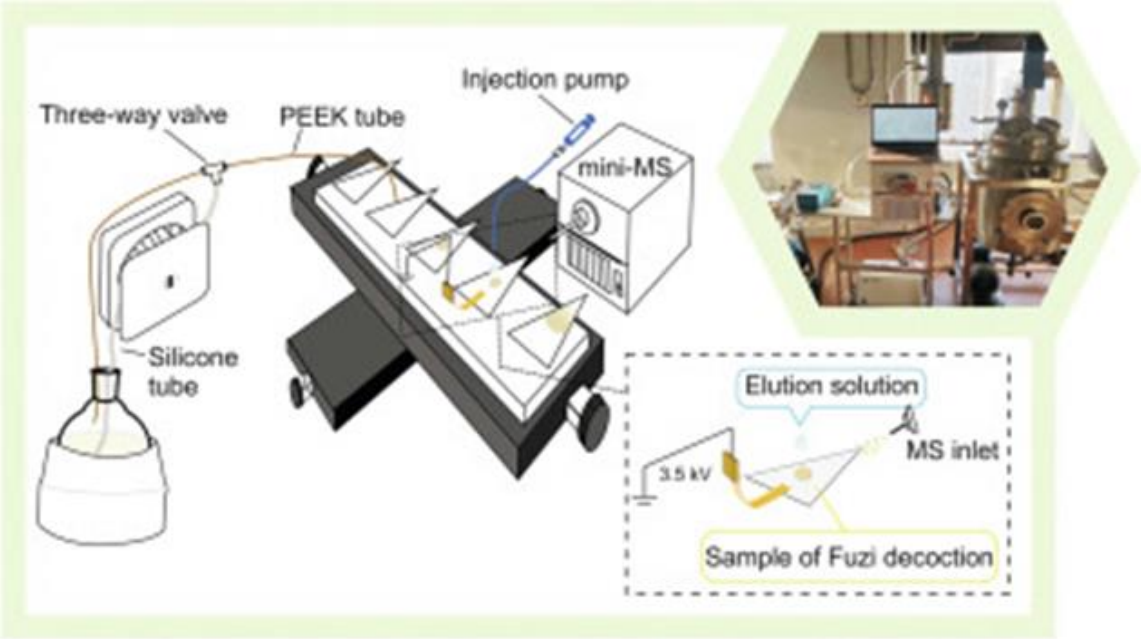


Figure 1. Schematic representation of the paper spray ionization miniature mass spectrometry (PSI–mini-MS) system for real-time monitoring of the traditional Chinese medicine (TCM) extraction process. PEEK: polyether ether ketone.

Pilot-scale extraction studies were performed using a multifunctional extraction unit at Huayi Pharmaceutical Co., Ltd. (Beijing, China), where the mini-MS device was directly integrated with the extraction tank. The same sample transfer setup used in laboratory experiments was maintained to ensure consistency and reliability during on-site testing.

Brick-L mini-MS instrumentation

All analyses were conducted on a portable ion trap mass spectrometer (Brick-L series, Nier Instruments, Kunshan, China) coupled with the PSI interface. A motorized platform—adapted from previously reported systems—was employed to automate sample loading for both laboratory-scale and pilot-scale decoction experiments [33]. The

Brick-L mini-MS device measured 31 × 28 × 31 cm (L × W × H) and weighed approximately 24 kg, including the vacuum pump. It was controlled via a laptop computer connected through a USB interface. Collision-induced dissociation (CID) served as the fragmentation technique, consistent with standard ion trap MS operation. A key feature of the Brick-L instrument was its unique pseudo-multiple reaction monitoring (pseudo-MRM) mode [15, 34], which minimized space charge effects within the ion trap, thereby enhancing signal intensity, detection sensitivity, and quantification precision compared to traditional ion traps. In this study, CID was performed using an alternating current voltage of 1.2 V for 20 ms. The inlet temperature was maintained at 80 °C, with ion funnel voltages set to 0 V for DC_in and 40 V for DC_out, and the skimmer DC set to 20 V. The scanning mass range was configured between m/z 500–700.

Results and Discussion

Fuzi—a processed derivative of the secondary roots of Aconitum carmichaelii Debx.—is extensively applied in TCM formulations for its anti-inflammatory, analgesic, anti-rheumatic, and cardioprotective activities [35]. Despite its therapeutic potential, raw Fuzi contains diester alkaloids that are highly toxic, posing significant risks to cardiac and nervous system function [36, 37], which has historically constrained its clinical use. During the decoction or processing of Fuzi, diester alkaloids undergo hydrolysis, losing an acetyl group and forming monoester alkaloids with markedly lower toxicity—approximately 1/200 that of their parent compounds [38]. These monoester alkaloids are considered the principal bioactive constituents responsible for Fuzi's pharmacological benefits [39]. Hence, the hydrolysis of diester alkaloids is recognized as a key transformation that simultaneously enhances therapeutic efficacy and reduces toxicity.

In this study, three representative diester alkaloids—aconitine (AC), mesaconitine (MA), and hypaconitine (HA)—were selected as model toxic compounds. Their hydrolysis reactions, yielding benzoylmesaconine (BMA), benzoylaconine (BAC), and benzoylhypaconine (BHA), respectively, are illustrated in **Figure 2a**. The concentrations of these six alkaloids (three reactants and their corresponding hydrolysates) were continuously monitored in real time throughout the decoction process based on their peak intensities in the mass spectra. To confirm the accuracy of compound identification, the MS/MS fragmentation profiles of the six alkaloids in the decoction samples were compared against those of their respective standards **(Figure 3)**. The reference MS/MS spectra obtained under identical instrumental conditions, were used to establish the qualitative identification method. Additionally, CID fragmentation data were analyzed to interpret the product ion patterns of these alkaloid analytes.

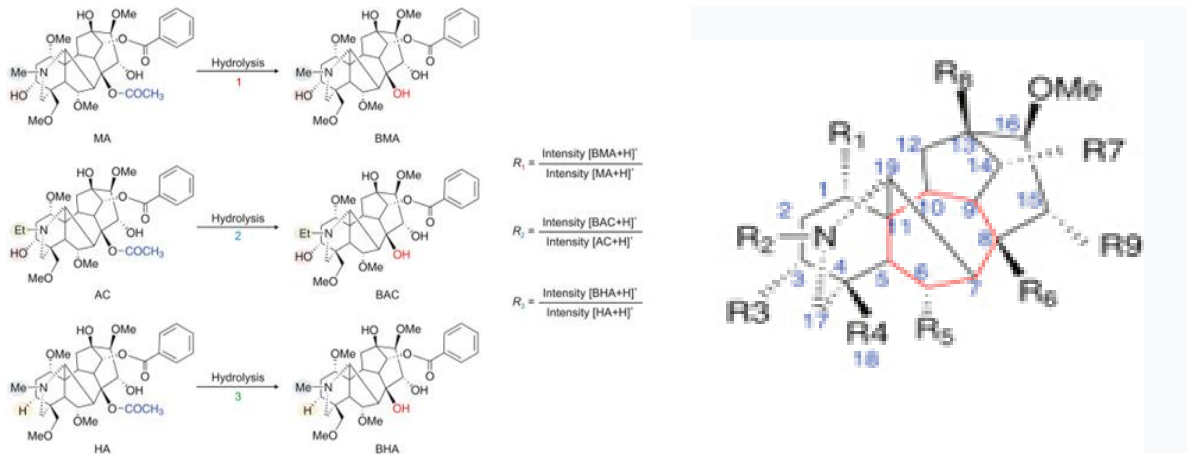


Figure 2. Illustration of the hydrolysis pathways of three representative diester alkaloids and the method used to determine their respective R-values. (a) Depiction of the hydrolysis transformations of mesaconitine (MA), aconitine (AC), and hypaconitine (HA) into their corresponding monoester derivatives—benzoylmesaconine (BMA), benzoylaconine (BAC), and benzoylhypaconine (BHA)—along with the R-value calculation method. The parameters R1, R2, and R3 were calculated as the intensity ratios [BMA+H]+/[MA+H]+, [BAC+H]+/[AC+H]+, and [BHA+H]+/[HA+H]+, respectively. (b) Chemical structures of the C-19 diterpenoid alkaloids present in Fuzi.

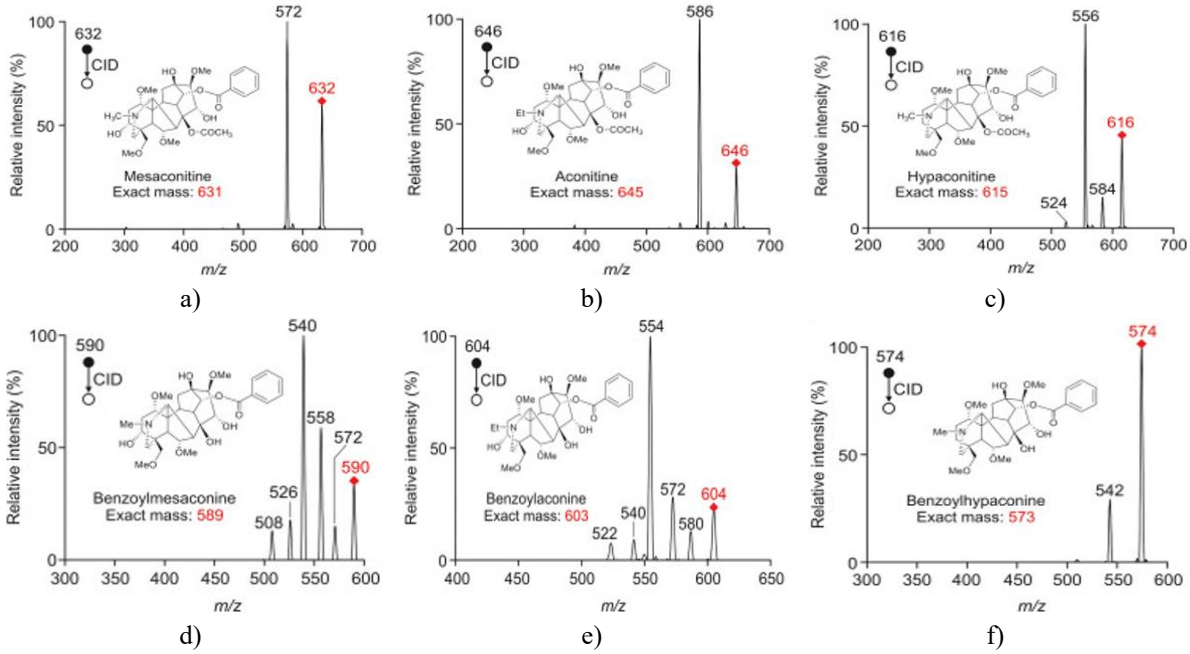


Figure 3. MS/MS spectra of six alkaloids detected in Fuzi decoction by paper spray ionization miniature mass spectrometry (PSI-mini-MS). (a–c) Represent the three precursor diester alkaloids, while (d–f) correspond to the hydrolyzed monoester alkaloids. CID: collision-induced dissociation.

The PSI-mini-MS system enabled real-time, quantitative tracking of the hydrolysis of the three major Fuzi alkaloids—mesaconitine (MA), aconitine (AC), and hypaconitine (HA)—throughout the decoction process. The extent of hydrolysis was assessed using R-values, defined as the ratio between the ion intensities of each hydrolyzed product and its corresponding precursor, thereby eliminating the need for an internal standard. Specifically, R1, R2, and R3 corresponded to the ratios [BMA+H]+/[MA+H]+, [BAC+H]+/[AC+H]+, and [BHA+H]+/[HA+H]+, respectively, as shown in **Figure 2a**.

Throughout the decoction, all three diester alkaloids gradually converted to their monoester counterparts, though to varying extents. MA exhibited the highest hydrolysis rate, whereas HA was the most resistant. As presented in **Figure 4**, R1 increased sharply during heating, while R3 rose at a much slower pace, suggesting that the hydroxyl substituent at the C3-position acts as an electron-donating group that accelerates hydrolysis through an inductive effect. This group strongly affects the structural stability of the C5–C11 seven-membered ring (red ring in **Figure 2b**). The MS/MS comparison of the hydrolysates further supported this finding: both BMA and BAC generated several dehydration-related product ions under CID conditions, while BHA did not.

However, the comparative hydrolysis behavior of AC and HA suggested that the impact of the hydroxyl group might be modest, likely due to their low natural abundance in Fuzi. Quantitative analysis based on MS spectra and calibration curves revealed approximate concentrations of 0.85 μg/mL for MA, 0.18 μg/mL for AC, and 0.15 μg/mL for HA after 10 minutes of decoction with Zhigancao, consistent with previous reports [40]. Consequently, the low levels of HA and AC contributed to their small R-values and limited hydrolysis rates.

In this study, the temporal evolution of R-values provided a direct measure of the chemical transformations in Fuzi during extraction. Standard curves for each alkaloid were additionally established by spiking known quantities of reference compounds into herbal extract matrices lacking these alkaloids and analyzing them via PSI-mini-MS. To assess whether paper variability affected ionization efficiency, MA and BMA were used as representative analytes at a concentration of 0.5 μg/mL in alkaloid-free herbal matrices. Each was analyzed five times using PSI-mini-MS with different chromatography papers. The variation in ionization efficiency due to paper type was negligible.

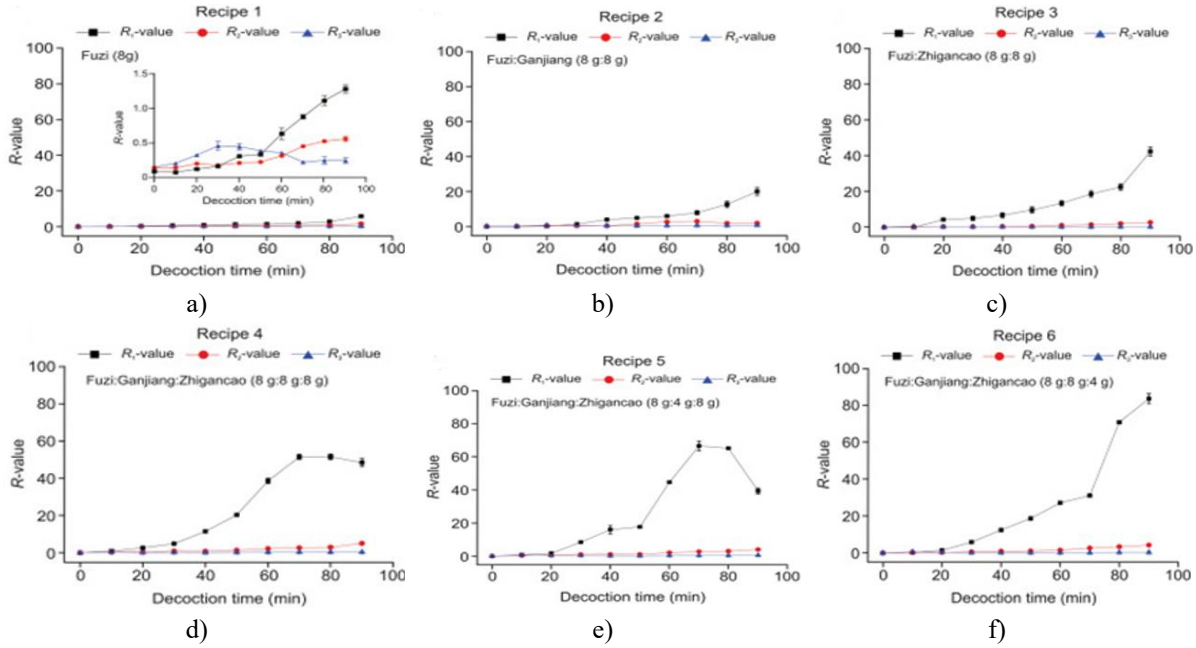


Figure 4. Time-dependent variation of R-values during Fuzi decoction, demonstrating how compatibility influences diester alkaloid hydrolysis: (a) Fuzi alone (inset: zoom-in with 0–1.5 scale), (b) Fuzi: Ganjiang = 1:1, (c) Fuzi: Zhigancao = 1:1, (d–f) Fuzi: Ganjiang: Zhigancao combinations at different dose ratios. R1, R2, and R3 are defined as [BMA+H]+/[MA+H]+, [BAC+H]+/[AC+H]+, and [BHA+H]+/[HA+H]+, respectively.

In clinical Traditional Chinese Medicine (TCM), single-herb treatments are used far less frequently than prescriptions formed through herb compatibility, a concept in which two or more medicinal plants are combined to reinforce therapeutic benefits while mitigating toxicity [41]. Classic formulas, including "Sini Decoction," routinely integrate Fuzi with Ganjiang and/or Zhigancao [38]. Therefore, in addition to testing Fuzi alone, we evaluated its co-decoction with these commonly paired herbs to understand their effect on the hydrolysis of Fuzi diester alkaloids **(Figure 4)**.

A comparison of Fuzi decocted individually versus its 1:1 mixtures with Ganjiang or Zhigancao revealed a clear enhancement in all R-values when compatibility herbs were present **(Figsures 4a–4c)**. This indicates that both Ganjiang and Zhigancao accelerate the transformation of diester alkaloids into less-toxic monoester forms, with Zhigancao exerting the stronger catalytic influence.

We then examined three triple-herb decoction ratios—1:1:1 (8 g each, **Figure 4d**), 2:1:2 (8 g, 4 g, 8 g, **Figure 4e**), and 2:2:1 (8 g, 8 g, 4 g, **Figure 4f**)—based on clinically used dose proportions. When Zhigancao was introduced into the Fuzi–Ganjiang combination (compare **Figure 4b** with **Figures 4d and 4f**), the R-values increased further, demonstrating enhanced hydrolysis promotion. Interestingly, a smaller amount of Zhigancao (4 g) produced the most pronounced rise in R-values **(Figure 4f)**, whereas doubling the dose led to a dampened increase **(Figure 4d)**. This pattern likely arises because a higher level of Zhigancao not only drives diester → monoester conversion but also intensifies secondary hydrolysis, removing benzoyl groups from the monoesters and diminishing R-value growth—a process previously documented [31, 42]. A similar inverse relationship between dose and effect was observed when Ganjiang amounts were adjusted **(Figures 4c–4e)**.

Another compatibility-related mechanism involves increased alkaloid dissolution: components such as ginger ether from Ganjiang and glycyrrhetinic acid from Zhigancao facilitate the release of diester alkaloids during extraction [43-45]. Since neither herb contains notable alkaloid levels [46, 47], their impact results from indirect interactions rather than additional alkaloid contributions. Moreover, Zhigancao has been reported to induce precipitation and hydrolysis of Fuzi alkaloids [44].

Collectively, these observations emphasize that compatibility in TCM represents a multidimensional molecular interplay, not merely additive contributions from each herb. Fuzi provides a longstanding example: the famous physician Zhongjing Zhang of the Han Dynasty emphasized that Ganjiang "activates" Fuzi's warming properties—summarized as "Fuzi is not hot without Ganjiang" [48]. This empirical wisdom led to enduring formulas such as "Ganjiang Fuzi Decoction" and "Sini Decoction" [49]. Uncovering the biochemical mechanisms underpinning these traditional principles will require further comprehensive investigation.

The PSI-mini-MS online analysis platform enables high-frequency, real-time quantitative monitoring of target constituents—at millisecond-level capability—though measurements here were slowed to a per-minute basis to match alkaloid hydrolysis kinetics. Analytical performance was rigorously evaluated: limits of detection, limits of quantification, and calibration curves for all six alkaloids are listed in **(Table 2)**, while **(Table 3)** summarizes results for method validation. Low, medium, and high QC samples were prepared by fortifying Zhigancao extract matrices (naturally free of the six target alkaloids) and analyzed on three separate days (five replicates per sample). The outcomes confirmed that PSI-mini-MS delivers reliable precision, accuracy, and recovery for quantitative detection in simulated TCM matrices. Although LC-MS/MS still exhibits slightly superior accuracy [40, 50], the elimination of sample pretreatment represents a significant practical advantage.

Additionally, successful validation was assisted by the pseudo-MRM function of the Brick-L mini-MS [15, 34]. In this mode, only parent ions are retained in the ion trap during injection, and all others are immediately expelled, greatly reducing space-charge effects. A standard CID step then isolates fragment ions for measurement. This focused trapping strategy generates enhanced ion signals and improved sensitivity and quantitation accuracy—making pseudo-MRM a valuable innovation for robust miniature-MS-based online analysis.

Table 2. Standard curves of alkaloids plotted based on paper spray ionization miniature mass spectrometry (PSI-mini-MS) analysis (n = 5).

Analyte	Ion (*m/z*)	Equation (concentration vs. intenstiy)	Linear range (ng/mL)	r^2	Limit of detection (ng/mL, SNR = 3)	Limit of quantitation (ng/mL, SNR = 3)
Mesaconitine (MA)	632	$y = 1750.3x + 11.3090$	5–2000	0.9994	1	5
Aconitine (AC)	646	$y = 1586.4x - 9.4632$	5–2000	0.9991	1	5
Hypaconitine (HA)	616	$y = 1403.1x + 4.7877$	5–2000	0.9993	1	5
Benzoylmesaconine (BMA)	590	$y = 1587.9x + 17.0300$	5–2000	0.9991	1	5
Benzoylaconine (BAC)	604	$y = 1306.2x + 2.8183$	5–2000	0.9993	1	5
Benzoylhypaconine (BHA)	574	$y = 1256.3x - 3.7815$	5–2000	0.9994	1	5

SNR: signal-noise ratio.

Table 3. Precision, accuracy, and recovery rates of six alkaloids in background traditional Chinese medicine (TCM) extracts using paper spray ionization miniature mass spectrometry (PSI-mini-MS) (n = 5, three days).

Analyte	Ion (*m/z*)	Concentration of quality control sample (μg/mL)	Precision (RSD%)		Accuracy (RE%)		Recovery rate (%)
			Intra-day	Inter-day	Intra-day	Inter-day	
MA	632	0.01	4.1	4.8	5.3	5.4	97.4 ± 3.2
		0.10	4.3	3.2	3.1	−0.7	98.5 ± 2.1
		1.00	0.9	1.4	1.2	1.5	99.8 ± 1.1
AC	646	0.01	6.5	6.1	7.8	7.0	100.6 ± 1.2
		0.10	1.1	1.3	1.3	1.9	95.8 ± 3.9
		1.00	0.4	0.9	1.2	0.6	99.8 ± 2.4
HA	616	0.01	5.8	5.1	6.0	6.1	101.6 ± 0.3
		0.10	3.0	2.9	2.8	2.0	96.8 ± 1.9
		1.00	0.8	1.8	0.7	1.0	98.2 ± 1.7
BMA	590	0.01	6.8	6.0	4.9	−1.6	96.8 ± 3.1

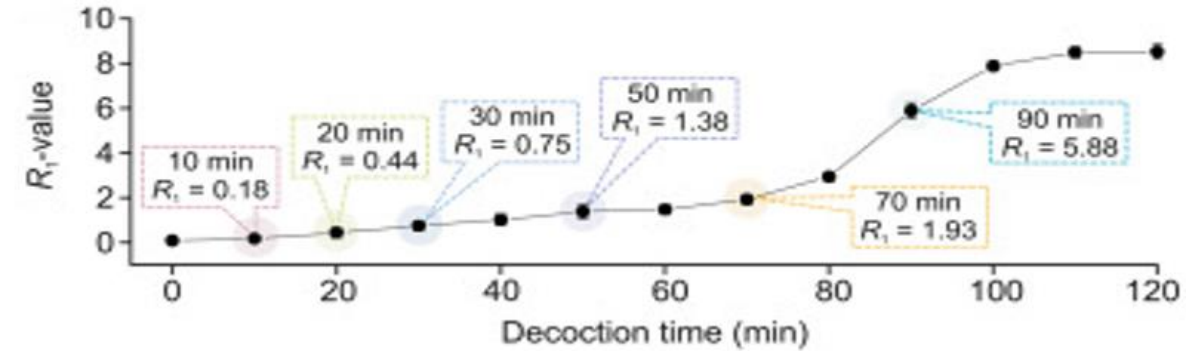
		0.10	4.0	4.9	4.0	4.2	99.8 ± 1.1
		1.00	0.7	1.0	1.1	0.9	100.2 ± 0.9
BAC	604	0.01	7.0	7.7	6.9	7.8	101.7 ± 1.2
		0.10	3.4	2.8	2.7	3.2	95.3 ± 2.8
		1.00	1.2	1.9	1.0	1.2	100.7 ± 2.1
BHA	574	0.01	7.7	7.8	6.8	8.3	100.2 ± 0.9
		0.10	3.4	3.5	3.8	3.1	95.2 ± 2.1
		1.00	1.4	2.0	1.4	1.1	96.3 ± 2.2

RSD: relative standard deviation; RE: relative error.

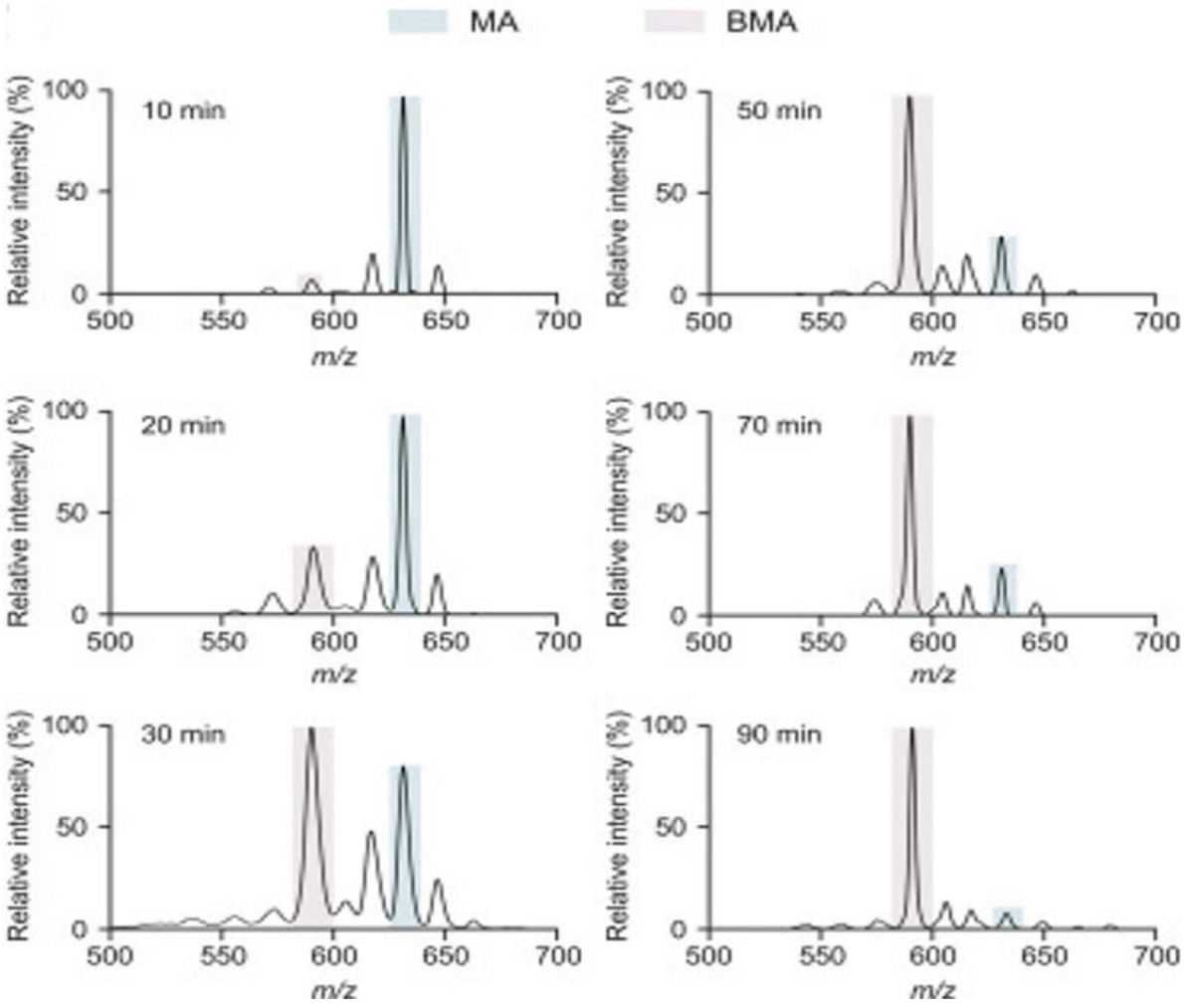
Despite the advantages of the PSI-mini-MS platform in quantifying alkaloids within complex matrices such as Fuzi decoctions, certain limitations remain inherent to this analytical approach. Although the mini-MS system achieves unit mass resolution, it is fundamentally a low-resolution instrument, which restricts its ability to fully discriminate between structurally similar compounds. Even so, its analytical capability is sufficient for specific targeted applications. A major challenge in using low-resolution MS for qualitative and quantitative assessments of TCM extracts lies in isomeric interference, which may compromise measurement accuracy. Typically, to minimize such interference, researchers employ multiple reaction monitoring (MRM) to selectively quantify fragment ions; however, in our system, this method is impractical because the paper spray ionization (PSI) process sustains a stable spray for only about 1–1.5 minutes, a duration insufficient to achieve simultaneous accurate quantification of all six alkaloids.

Paper spray ionization was deliberately selected for this study because it represents one of the simplest, fastest, and most robust ambient ionization methods, enabling direct sample introduction without pre-separation and thereby facilitating rapid online MS analysis. To compensate for the intrinsic constraints of PSI and low-resolution detection, we implemented specific measures to enhance analytical reliability. We validated that the MS/MS spectra of the six alkaloid precursor ions in Fuzi extracts were identical to those of their corresponding standards, as verified in laboratory-scale decoction experiments **(Figure 3)**. Moreover, rather than relying on absolute quantitative measurements, we described alkaloid transformation using R-values, defined as the ratio of product-to-reactant ion intensities. This approach effectively minimizes isomer-related interference and provides a robust indicator of hydrolytic progression during decoction.

Ultimately, the entire online analytical setup was successfully integrated into a pilot-scale multifunctional extraction system for real-world validation at Huayi Pharmaceutical Co., Ltd.. The integrated platform demonstrated stable operation over extended hourly monitoring periods. As illustrated in **Figure 5a**, the R1-value—representing the hydrolysis of mesaconitine (MA) to benzoylmesaconine (BMA)—increased steadily with decoction time in the pilot-scale run, confirming consistent hydrolytic behavior. Corresponding dynamic MS spectra captured at various time points are displayed in **Figure 5b**. Similar real-time monitoring was also achieved for aconitine (AC) and hypaconitine (HA), with their respective R2- and R3-value profiles. Consistent with laboratory results, R1 and R2 exhibited the greatest increases during decoction, while R3 changed least markedly. The compact, lightweight design and low energy demand of the mini-MS instrument make it exceptionally practical for field applications. The system's ease of use and operational safety allow pharmaceutical technicians to perform online monitoring independently after minimal training. Beyond laboratory use, this portable PSI-mini-MS technology offers broad potential for on-site quality control, including extraction workshops, concentration and purification facilities, hospital decoction centers, and temporary field laboratories—signaling a transition of mass spectrometry from the analytical bench to the production floor.



a)



b)

Figure 5. Real-time tracking of mesaconitine (MA) hydrolysis into benzoylmesaconine (BMA) during the pilot-scale Fuzi extraction process. (a) Time-dependent variation of the R1-value throughout the decoction. (b) Mass spectrometry (MS) spectra obtained from online paper spray ionization miniature MS (PSI-mini-MS) analysis. The R1-value represents the ratio of ion intensities [BMA+H]+/[MA+H]+.

Conclusion

In this study, we established a quality control (QC) platform for traditional Chinese medicine (TCM) manufacturing processes using a PSI-mini-MS-based system. This approach enabled, for the first time, real-time and online qualitative and quantitative detection of specific compounds in herbal extracts without the need for chromatographic separation. The major advancement of this system, compared to previous spectroscopy-based or portable LC-MS analytical methods, lies in its ability to conduct direct, continuous monitoring during production.

Extraction was chosen as a representative pharmaceutical step, given its importance in herbal medicine preparation, with Fuzi selected as the model herb. Using mini-MS, we dynamically and simultaneously observed the hydrolysis process in which toxic diester alkaloids were converted into their less toxic monoester forms during decoction. The quantitative approach underwent strict validation to ensure accuracy. Moreover, the study provided new insights into the compatibility mechanism of Fuzi, experimentally confirming the traditional concept of "enhancing efficacy and reducing toxicity." The fully integrated system was tested on a pilot-scale extractor, demonstrating stable operation over extended time periods. Overall, this PSI-mini-MS-based online analytical method offers a promising and efficient QC tool for broader pharmaceutical applications, with the advantages of real-time data acquisition, improved process reliability, and potential cost savings in production.

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Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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