Real-time toxicity prediction of *Aconitum* stewing system using extractive electrospray ionization mass spectrometry

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**Abstract** Due to numerous obstacles such as complex matrices, real-time monitoring of complex reaction systems (e.g., medicinal herb stewing system) has always been a challenge though great values for safe and rational use of drugs. Herein, facilitated by the potential ability on the tolerance of complex matrices of extractive electrospray ionization mass spectrometry, a device was established to realize continuous sampling and real-time quantitative analysis of herb stewing system for the first time. A complete analytical strategy, including data acquisition, data mining, and data evaluation was proposed and implemented with overcoming the usual difficulties in real-time mass spectrometry quantification. The complex Fuzi (the lateral root of *Aconitum*)—meat stewing systems were real-timely monitored in 150 min by qualitative and quantitative analysis of the nine key alkaloids accurately. The results showed that the strategy worked perfectly and the toxicity of the systems were evaluated and predicated accordingly. Stewing with trotters effectively accelerated the detoxification of Fuzi soup and reduced the overall toxicity to 68%, which was recommended to be used practically for treating rheumatic arthritis and enhancing immunity. The established strategy was versatile, simple, and accurate, which would have a wide application prospect in real-time analysis and evaluation of various complex reaction systems.

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1. Introduction

Real-time monitoring of complex reaction systems with complex matrices (e.g., medicinal herb stewing systems) is of great value and significance in many fields, such as environmental chemistry, food safety, drug discovery, and life sciences. However, due to many obstacles like complex matrices, current methods hardly to achieve long-term real-time qualitative and quantitative analysis of complex reaction systems (e.g., medicinal herb stewing system). At present, the commonly used chromatographic–mass spectrometry methods have strong qualitative and quantitative capabilities, but it is hard to realize real-time analysis of complex system as the indispensable complicated pretreatment process of traditional mass spectrometry and time-consuming elution process of chromatography. In recent years, the ambient ionization technologies such as desorption electrospray ionization (EESI), low-temperature plasma probe, and internal extractive electrospray ionization (I-ESI) etc. have been increasingly used for analytes detection in multiple fields (e.g., element analysis, clinical chemistry, metallomics, pharmaceutical and food analysis) due to the unparalleled capability for identification and quantification. Among them, EESI has the characteristics of no sample pretreatment, better stability, and less sample consumption, makes it possible to achieve the task of long-term real-time monitoring of the complex reaction system. Previously, EESI-MS has been focusing on rapid qualitative analysis of various samples such as urine, milk, virgin olive oil, drug, and respiratory gases, etc. have been increasingly used for analytes detection in multiple fields (e.g., element analysis, clinical chemistry, metallomics, pharmaceutical and food analysis) due to the unparalleled capability for identification and quantification. Among them, EESI has the characteristics of no sample pretreatment, better stability, and less sample consumption, makes it possible to achieve the task of long-term real-time monitoring of the complex reaction system. Therefore, EESI-MS has been established, including excellent data acquisition, data processing and data evaluation methods, and applied to complex Fuzi stewing system with strong resistance to matrix interferences successfully. Different meats (i.e., pig’s trotter, chicken, lean pork, and beef) were chosen to stew with Fuzi to evaluate the changes of the detoxification process. The holistic toxicity of Fuzi stewing system was evaluated accurately and the safety time for stewing was calculated respectively. Importantly, the results indicated that stewing with trotters had the best effect on promoting detoxification, which reduced the holistic toxicity to 68% compared with only water. This study used an advanced EESI-MS technology to monitor the complex toxic Fuzi soup in real time without obvious matrix interferences, solved the key problems of mass spectrometry quantification by statistical analysis methods, systematically evaluated the toxicity of different Fuzi–meat soups, and provided an important reference for the safe consumption of Fuzi in clinical practice.

2. Materials and methods

2.1. Reagents, chemicals and materials

Standards of aconitine (C_{34}H_{47}NO_{11}, AT), mesaconine (C_{33}H_{45}NO_{11}, MAT), hyaconitine (C_{31}H_{43}NO_{10}, HAT), benzoylaconine (C_{32}H_{45}NO_{10}, BAC), benzoylmesaconine (C_{31}H_{43}NO_{10}, BMA), benzoylhypaconine (C_{31}H_{43}NO_{9}, BHA), aconine (C_{29}H_{39}NO_{9}, AC), mesaconine (C_{29}H_{39}NO_{9}, MA), and hypaconine (C_{29}H_{39}NO_{9}, HA) were purchased from Beijing Rongcheng Xinde Technology Development Co., Ltd. (Beijing, China, purity > 98%). Berberine (C_{20}H_{21}NO_{6}, BB) was purchased from ANPEL Laboratory Technologies (Shanghai) Inc. (Shanghai, China, purity > 98%) and used as the IS for accurate quantification. The structures of the 10 authentic compounds were shown in Supporting Information Fig. S1. The absolute configurations were provided kindly by the manufacture and confirmed by the literature. De-ionized water (18.2 MΩ·cm) was obtained by Mill-Q water purification system (Billerica, MA, USA). Methanol and dichloromethane (HPLC grade) were purchased from ROE Scientific Inc. (Newark, DE, USA) and used without further purification. Raw Fuzi was collected from Jiangyou, Sichuan province.
2.2. Sample preparation

Raw Fuzi was cleaned, cut into slices and dried at 30 °C. Standard stock solutions were prepared by dissolving appropriate amounts of each compound in water to obtain final concentrations of 20 μg/mL for AT, MAT, BAC, BMA, AC, MA and 50 μg/mL for HAT, BHA, and HA, respectively. Standard solutions with a series concentration were prepared by diluting the standard stock solution 6, 36, 216, 1296 times, respectively. Stock solution of IS was prepared by dissolving 10.0 mg BB in 10.0 mL de-ionized water.

2.3. Real-time analysis strategy based on EESI-MS

A complete online real-time EESI-MS analysis strategy has been established, including excellent data acquisition, data processing and data evaluation methods (Fig. 1).

2.3.1. Data acquisition

A real-time EESI-MS device for continuously sampling and real-time quantitative analysis of complex reaction systems was developed firstly. The schematic illustration was shown in Fig. 2. A 4.00 g Fuzi powder was soaked in 400.0 mL water with 100.0 g meat (pig’s trotter, lean pork, chicken and beef, respectively) for 30 min, and then heated using a heating mantle at 125 W. The solution was diluted 10 times before analyzed with the flow rate of sample pump and dilution pump were 1.0 and 9.0 μL/min, respectively. The dilution solution was chosen water in this experiment considering the nice water solubility of targeted alkaloids and the solvent compatibility. Nine key alkaloids in Fuzi were dynamic monitored by a linear trap quadrupole mass spectrometer (LTQ-XL, Thermo Scientific, San Jose, CA, USA) coupled with a homemade EESI source. EESI source parameters were manually adjusted according to the literature. The compositions were detected and recorded for 150 min by EESI-MS.

2.3.2. Data mining

Based on the established monitoring device above, concentration profile of each alkaloid could be plotted real-timely and about 900 concentration points obtained in 150 min for each alkaloid. Therefore, the fitting curves on these points were performed to overcome the usual data fluctuations in real-time analysis-based MS caused by the irregular bubbles and instantaneous fluctuations in the MS signal. The fitting software was chosen Matlab (R2016a) owing to its abundant fitting functions (e.g., linear, polynomial, Gaussian, and Fourier fitting) to choose.

2.3.3. Data evaluation

Two evaluation parameters such as the area under the curve (AUC) and the time of the half highest concentration (t₁/₂) were introduced to evaluate specific differences of analytes in different meat stew systems. AUC and t₁/₂ were calculated by Matlab with 3 repetitions. A t-test was performed between each group to evaluate the significance of the differences by Microsoft Excel 2013 and FDR adjusted was performed on the P values using Matlab to eliminate possible false positive results.
2.4. Toxicity prediction of Fuzi—meat soup

The main toxicity and bioactive ingredients in Fuzi are diterpenoid alkaloids which are divided into three types according to the structure (i.e., diester alkaloids, monooester alkaloids, and non-ester alkaloids). Only diester alkaloids (e.g., AT, MAT, and HAT) are the major contributor to the toxicity. The monooester alkaloids (e.g., BAC, BMA, and BHA) are the major contributor to the bioactivity. The toxicological data of the toxic diester alkaloids has been reported before. Hence, combined the toxicological data with the precise concentration curve of each alkaloid obtained by the advanced real-time EESI-MS device, the overall toxicity profile and toxicity change equation of the system could be plotted and obtained. Accordingly, the toxicity of Fuzi—meat soup could be predicted real-timely and the time required for the toxic degradation threshold could be calculated to accurately predict the shortest safe boiling time.

3. Results and discussion

3.1. Development of real-time EESI-MS apparatus

The extraction solution (methanol and dichloromethane, 1:1) was optimized based on the extraction efficiency and used to selectively extract and ionize the target analytes (aconitine-type alkaloids) in the mass spectrometer ion source, charged with +3.5 kV. Ethanoic acid was added with a concentration of 1% to assist in enhancing ionization efficiency. This is the characteristics and advantage of EESI that ionization spray and sample spray separated and interacted in a three dimensional space, which can significantly increase the stability, sensitivity and resistance ability to complex matrix interference. The flow rate of injection was 10.0 μL/min by a syringe (5 mL, Hamilton, GR, Switzerland) with a syringe pump (LSP02-2A, Longer Pump, Baoding, China) to form the ionizing spray. The sample was pumped continuously by a precision peristaltic pump (Longer Pump, Baoding, China) and another one was used to pump water for dilution. The two pipes were connected by a tee branch and lead to a self-made spray nozzle to form the ionizing spray. The sample was pumped independently under optimized experimental conditions. The calibration curves were presented in Fig. 4C. A least-squares linear regression method was used to determine the slop, intercept and correlation coefficient of linear regression equation. From these data we obtained nine equations (R$^2 > 0.99$), e.g., Eqs. (1)–(3) for AT, MAT, and HAT (Supporting Information Table S2).

$$Y_{AT} = 0.1281X_{AT} + 0.0001$$  \hspace{1cm} (1)

$$Y_{MAT} = 0.2194X_{MAT} + 0.0001$$  \hspace{1cm} (2)
Where $Y_{AT}$, $Y_{MAT}$, and $Y_{HAT}$ represent the peak ratio of AT, MAT, and HAT with IS, and $X_{AT}$, $X_{MAT}$ and $X_{HAT}$ represent the corresponding concentration of AT, MAT, and HAT ($\mu$g/mL), respectively. Notably, the slopes of these equations among the three types (e.g., 0.1281 for AT, 0.1484 for BAT, and 0.1076 for AC) were more similar than classical LC–MS using gradient elution programs previously (e.g., 0.409 for AT, 0.986 for MAT, and 0.156 for HAT)\textsuperscript{46}, indicating that EESI-MS was a better normalized quantification method in complex systems. The developed EESI-MS provided the same solvent background throughout the analysis to significantly eliminate the common variances caused by the mobile phase compositions\textsuperscript{47}. The limit of detection (LOD) and limit of quantitation (LOQ) were obtained when signal (SI) to noise was 3 and 10, respectively. The LODs were 0.251–1.741 ng/mL and LOQs were 0.837–5.803 ng/mL for nine alkaloids (detailed in Table S2). The wide linearity ranges were indicated that the working curves had good quantitative ability at high, medium, and low concentrations (Table S2).

The system precision was given as percent relative standard deviation (RSD, %) of each compound. Six measurements were carried out in parallel to evaluate the precision of the calibration. As the results, the RSDs were 2.91%–6.51% (Supporting Information Table S3) for all the data points employed to make the calibration curves. Accuracy was evaluated using the mixed standard solution and calculated by the relative errors using Eq. (4):

$$\text{RE} \, (\%) = \frac{\text{Measure value} - \text{true value}}{\text{true value}} \times 100 \quad (4)$$

The results showed that for all the 9 alkaloids, RE were 2.49%–11.45% (Table S3). All finding indicated that the developed real-time EESI-MS approach was of high precision and accuracy for analyzing the samples. It is clear that in the stewing process the matrix effect would be increasing and the matrix effect is maximized at the last moment (at 150 min in this study). Therefore, the matrix interferences of the herb extraction and meats soup at 150 min were evaluated and determined by standard addition approach and calculated by Eq. (5)\textsuperscript{48}:

$$M \, (\%) = \frac{\text{Observed amount} - \text{original amount}}{\text{spiked amount}} \times 100 \quad (5)$$

The mixed standard solution was added to the Fuzi–meat soup after being stewed for 150 min. The results were 89.01%–96.90% for matrix effect which were satisfied for quantification (Table S3) and indicated that the established analytical method could tolerate the increasing matrix in the whole process. The precision would not affected by the increasing matrix and calibration curves could work accurately throughout the process. Since there was no pre-treatment process, the results of matrix effect can reflect the satisfactory recovery of the method. All these findings indicated that our developed EESI-MS method could be used to dynamically monitor the whole stewing process of Fuzi and meat real-time with satisfactory resistance to matrix interference which is not possible with traditional ESI-MS.

### 3.3. Curve fitting on the real-timely detected data of the nine key alkaloids in Fuzi–meat soups

Nine key alkaloids in the soup were dynamically and consecutively monitored within 150 min using the established long-term real-time monitoring approach. Compared with the traditional offline method (HPLC and LC–MS) with the drawbacks that based on a limited number (<50) of data for assessing the overall

![Figure 4](image_url)

**Figure 4** Thermo stability of internal standard (IS), calibration effect and calibration curves of nine alkaloids. (A) The concentrations of berberine (BB) heated for 150 min. (B) MS chromatogram of IS, analyte, and analyte after calibration. (C) Calibration curves of nine alkaloids ($n = 6$). C represents for concentration. Data are means±SD.
characteristics, we respectively achieved about 900 measurements for each compound in 150 min, and about 9000 times of data acquisition in the whole process, greatly improved the response accuracy to the actual changes of the components in system. The full-scanned EESI mass spectra of the five stewing system at different times were shown in Supporting Information Fig. S2. It was clear that the chemical composition of each stew system have changed a lot before and after stewing, the diester alkaloids (m/z 616, 632 and 646) disappeared, and the monoester (m/z 574, 590 and 604) and non-ester alkaloids (m/z 470, 486 and 500) appeared. Besides, after optimization of the EESI-MS conditions, the spectra were pure. Most peaks were alkaloids in herbs (m/z 408, 438, 464, 574, 616, etc.) with no apparent matrix interference peaks (Fig. S2). Accordingly, the concentration scatter plots of the nine key alkaloids in the five stewing systems were shown in Supporting Information Fig. S3. The weak changes of the compound in the soup were sensitively captured as in Fig. S3A–C, that the points were continuous though the concentration changes drastically in 20–50 min and different conditions could be clearly distinguished in the whole process.

Although the internal standard calibration and the multiple average processing had been performed, the increasing variation in the later period in Fig. S3 was caused by the tiny bubbles generated by intense boiling which is unavoidable in stewing medicinal herbs (Fig. S3D–I). To show the actual concentration curve changes, facilitate comparison of differences of different meats, and effectively decrease the inevitably frequent data fluctuations to eliminate possible errors, fitting curves (Fig. 5) were performed on these points. The complex decoction system involves the slow extraction and dynamic conversion between compounds. Some curves had a bending changes (Fig. 5D–F, 70–120 min) caused by the complex release patterns in stewing of Fuzi with meat. Unlike the concentration point of the diester alkaloids (HAT, MAT, and AT) were approximately normal distribution (Fig. S3A–C), the trends of monoester and non-ester types were more complicated, resulting in a complex waveform, so different fitting models were needed to simulate this complex data change. After optimization, Gaussian functions were used for fitting the three diester alkaloids ($R^2 > 0.97$). Fourier functions were used for the other six alkaloids after comparison with Gaussian functions and polynomial functions as the better fitting result, such as for BHA with only water, the $R^2$ was 0.9862, 0.9840, and 0.9780 for Fourier, Gaussian and polynomial functions, respectively (Supporting Information Fig. S4). The $R^2$ of all fitting equations were greater than 0.90 and mostly located in 0.95–0.99, indicating an excellent fitting effect.

### 3.4. Accurately distinguish and evaluate the effects of different meats on the target ingredients by AUC and $t_{1/2}$

Fig. 5 displayed that the fitted concentration curves were staggered which was difficult to distinguish and evaluate the effect of different meats accurately, such as the comparisons between different meats and water, or the meats compared with each other.
respectively. Hence, in order to clearly indicate the overall characteristics in the dynamic system and intuitively reflect the elimination rate of the analytes, the parameters, AUC and \( t_{1/2} \), were calculated for quantifying the difference, respectively (Fig. 6). The results indicated obviously that the values of AUC for diester alkaloids (AT, MAT and HAT) of pig’s trotters (6.37, 21.37 and 41.12) were the smallest and for monoester (75.02, 131.84, and 269.22) and nonester alkaloids (25.57, 71.42 and 115.11) were the biggest compared with only water or other meats system. The effects of other meats (i.e., chicken, lean pork, and beef) were not much different, most of the values of AUC between the trotters and the water. To assess whether these differences were statistically significant, \( t \)-tests were performed between each other (Supporting Information Table S4) and FDR adjusted was done by Matlab to eliminate possible false results (Supporting Information Table S5). The results indicated that compared with the water system, the effects of trotters on the three key toxic alkaloids in Fuzi were all significant (\( P < 0.05 \)), and of which the effect on MAT and HAT were extremely significant (\( P < 0.01 \)) (Fig. 6A). Other meats had no significant effects on AUC based on the FDR-adjusted \( P \) values (Table S5). Between the trotter system and the only water system, the changes of monoester alkaloids were much bigger than nonester alkaloids (e.g., for BAC and AC, the deviation was 22.77 and 4.00, respectively), indicating the directional transformation of diester alkaloids to monoester alkaloids which is essential for preserving the effect while detoxification.

Interestingly, decocting with pig’s trotter could always faster reach the \( t_{1/2} \) (e.g., 45.52, 46.05, and 45.64 min for AT, MAT, and HAT) than other meats and only water. The \( t \)-test results (Supporting Information Table S6) and FDR-adjusted results (Supporting Information Table S7) were consistent with the AUC’s, that the effect of trotters on each compound (extremely significant on AT, MAT, HAT, BMA, HA) is most pronounced and stronger than that of other meats (Fig. 6B). This probably because of the huge difference in the composition of the trotters from other meats, which contains more fatty components, indicating that the differences may be related to fatty acid\(^{36} \). The results demonstrated that our developed data processing method-based the Gaussian and Fourier fitting coupled with the AUC and \( t_{1/2} \) provided the solving to accurately calculate the differences of the total content and the tiny time simultaneously, which can significantly improve the quantitative accuracy of long-term real-time MS analysis on overall characteristics meanwhile providing efficiency differences between groups.

### 3.5. Holistic weighted toxicity evaluation and safety stewing time calculation of Fuzi—meat soups

To confirm that all the alkaloids would dissolve in the water, the dregs of Aconitum and meat were collected and ultrasonically extracted by methanol after stewing, and then analyzed by ESI-LTQ-MS. Based on the MS spectra of the extraction (Supporting Information Fig. S5), almost no residual toxic alkaloids (i.e., AT \( m/z \) 646, MAT \( m/z \) 632, HAT \( m/z \) 616) was found in the extraction indicating that all the target toxic alkaloids were water soluble and not absorbed in meats. Hence, the toxicity of the soup was the holistic toxicity of the system. As the monoester alkaloids are the main active ingredients and nonester alkaloids are ineffective, excessive decocting may completely lose its effectiveness. So, on the premise of ensuring safety, the shortest stewing time was important for better preservation of efficacy. Therefore, based on the real-time monitoring approach to capture the whole process of the reaction precisely, the shortest safety stewing time could be calculated to ensure the best efficacy without toxicity, which was difficult to achieve by conventional analysis (HPLC/LC–MS)\(^{49,50} \). The complex multi-component

![Figure 6](image)

**Figure 6** AUC (A) and \( t_{1/2} \) (B) values and FDR adjusted \( t \)-test results of the nine alkaloids in five stewing system. *\( 0.01 < P < 0.05 \), **\( P < 0.01 \) (\( n = 3 \)). Data are mean±SD.
characteristics of medicinal herbs determined the complexity of its toxicity evaluation and usually, the overall toxicity is contributed by multiple different toxicity components. Therefore, it is necessary to comprehensively consider the toxicity of different components and comprehensively evaluate the holistic toxicity of the system. The toxicity evaluation of *Aconitum* is based on the content of the three key diester alkaloids (AT, MAT, and HAT). A large number of detailed pharmacological and toxicological data for these three components have been reported in previous study, such as for HAT, MAT, and AT, the lowest dose with relevant signs of toxicity (LDT) for mice were 0.1470, 0.0316, and 0.0464 mg/kg, respectively. Hence, the equivalent safety doses for human could be calculated as 0.0162 (HAT), 0.0035 (MAT) and 0.0051 (AT) mg/kg, because of the 9.1-fold conversion relationship between humans and mice in pharmacology. Then, the holistic weighted toxicity (HWT) for Fuzi soup could be calculated by the following Eq. (6):

\[
\text{HWT} = \frac{C_1}{0.0051} + \frac{C_2}{0.0162} + \frac{C_3}{0.0035}/3/W \times V
\]

Where \( C_1, C_2, \) and \( C_3 \) were respectively represent the concentrations of the three toxic alkaloids (AT, HAT, and MAT) (mg/mL). \( W \) was the weight of the consumer, taking the conventional 60 kg. \( V \) was the amount of soup that was drunk (mL), the default was 500 mL. Then we could get the HWT real-time by combining the real-time concentration equations with HWT formula. Further we can calculate the safety stewing time of different meats systems when HWT = 1.0, which were 59.01 min for trotters, 59.99 min for chicken, 61.08 min for lean pork, 63.39 min for beef, and 62.44 min for water only, indicating that stewing with trotters took the shortest time and had the best bioactivity.

To systematically evaluate the overall toxicity of the stewing system throughout the process (150 min), the HWT curves were drawn and the AUCs were integrated, respectively (Fig. 7). This took into account the strength contribution of the toxicity of different compounds, and intuitively shown the toxicity of the system within 150 min, avoided the deviation that may be caused by the single time point evaluation. Finally, the AUCs were calculated as 404.58 for only water, 275.14 for trotters, 324.42 for chicken, 333.81 for lean pork, and 377.38 for beef, indicated that the overall toxicity stew with trotters almost reduced to 68% compared with only water, which was the best meat to accelerate the detoxification of Fuzi.

4. Conclusions

A complete analytical strategy, including data acquisition, data mining, data evaluation and practical applications were proposed for real time analysis of medicinal herb stewing system based on the real-time EESI-MS. The usual obstacles in real-time analysis of complex reaction system were clear and nine key alkaloids in Fuzi stewing system were real-time monitored for 150 min. The established method realized the precise quantification of the chemical substances in the complex stewing system, and accurately evaluated the variations of different meat systems through data processing. The holistic toxicity and safety stewing time of Fuzi soup with different meats were calculated and predicted, respectively. It was found that pig’s trotter could directionally transform the toxic diester alkaloids to monoester alkaloids in 59.01 min and was the best material to stew with hazardous Fuzi for detoxification and preservation of activity compared with other three meats (chicken, lean pork and beef), which would provide an effective reference for the safe and rational consumption of Fuzi.

Real-time qualitative and quantitative analysis of complex reaction systems can continuously and accurately obtain the transformation law of substances, capture the reaction characteristics of each moment, and discover the best reaction conditions and results. All of these are extremely important in drug development and application, such as for evaluation and prediction of the efficacy and toxicity. The method is easy to operate and has good universality for real-time online analysis, which is expected to play a key role in many fields such as environmental protection, food and drug safety, and industrial production.

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Author contributions

Zi-Dong Qiu performed the experiments of this work and wrote the manuscript. Jin-Long Chen and Wen Zeng analyzed the results and processed the data. Ying Ma, Tong Chen, and Jin-Fu Tang contributed to writing. Chang-Jiang-Sheng Lai and Lu-Qi Huang designed the research.

Conflicts of interest

All authors have read and approved this version of the article, and due care has been taken to ensure the integrity of the work. We assure here that the manuscript is not under consideration for publication and has not been published elsewhere in any medium including electronic journals and computer databases of a public nature and it is not being submitted to any other journal. The authors declare no conflict of interest.
Appendix A. Supporting information

Supporting data to this article can be found online at https://doi.org/10.1016/j.apbs.2019.08.012.

References

1. Dalton CN, Jaouli M, Kamens RM, Glish GL. Continuous real-time analysis of products from the reaction of some monoterpenes with ozone using atmospheric sampling glow discharge ionization coupled to a quadrupole ion trap mass spectrometer. Anal Chem 2005;77:3156–63.

2. Lei J, Li P, Zhang Q, Wang Y, Zhang Z, Ding X, et al. Anti-idiotypic nanobody-phage based real-time immuno-PCR for detection of hepatocarcinogen aflatoxin in grains and feedstuffs. Anal Chem 2014;86:10841–6.

3. Petucci C, Diffendal J, Kaufman D, Mekonnen B, Terefenko G, Musselman B. Direct analysis in real time for reaction monitoring in drug discovery. Anal Chem 2007;79:5064–70.

4. Chen L, West J, Auroux PA, Manz A, Day PJ. Ultrasensitive PCR and real-time detection from human genomic samples using a bidirectional flow microreactor. Anal Chem 2007;79:9185–90.

5. Zhu Z, Bartmess JE, McNally ME, Hoffman RM, Cook KD, Song L. Quantitative real-time monitoring of chemical reactions by autosampling flow injection analysis coupled with atmospheric pressure chemical ionization mass spectrometry. Anal Chem 2012;84:7547–54.

6. Liu Y, Song Q, Liu W, Li P, Li J, Zhao Y, et al. Authentic compound-free strategy for simultaneous determination of primary coumarins in Peucedani Radix using offline high performance liquid chromatography—nuclear magnetic resonance spectroscopy—tandem mass spectrometry. Acta Pharm Sin 2018;48:645–54.

7. Garran TA, Ji R, Chen JL, Xie D, Guo L, Huang LQ, et al. Elucidation of metabolite isomers of Leonurus japonicus and Leonurus cardiaca using discriminating metabolite isomerism strategy based on ultra-high performance liquid chromatography tandem quadrupole time-of-flight mass spectrometry. J Chromatogr A 2019;1598:141–53.

8. Hu T, Tie C, Wang Z, Zhang JL. Highly sensitive and specific derivatization strategy to profile and quantitate eicosanoids by UPLC–MS/MS. Anal Chim Acta 2017;950:108–18.

9. Qiao X, Lin XH, Ji S, Zhang ZX, Bo T, Guo DA, et al. Global profiling and novel structure discovery using multiple neutral loss precursor ion scanning combined with substructure recognition and statistical analysis (MNPS): characterization of terpene-conjugated curcuminoids in curcuma longa as a case study. Anal Chem 2016;88:703–10.

10. Wang X, Yang B, Sun H, Zhang A. Pattern recognition approaches and computational systems tools for ultra performance liquid chromatography—mass spectrometry-based comprehensive metabolic profiling and pathways analysis of biological data sets. Anal Chem 2012;84:428–39.

11. Stojanovska N, Tahtouh M, Kelly T, Beavis A, Fu S. Qualitative analysis of seized cocaine samples using desorption electrospray ionization-mass spectrometry (DESI-MS). Drug Test Anal 2015;7:393–400.

12. Veach BT, Mudalige TK, Rye P. Rapidfire mass spectrometry with enhanced throughput as an alternative to liquid–liquid salt assisted extraction and LC/MS analysis for sulfonamides in honey. Anal Chem 2017;89:3256–60.

13. Chen Y, Zhou Z, Yang W, Bi N, Xu J, He J, et al. Development of a data-independent targeted metabolomics method for relative quantification using liquid chromatography coupled with tandem mass spectrometry. Anal Chem 2017;89:6954–62.

14. Ewing KJ, Gibson D, Sanghera J, Mikkos F. Desorption electrospray ionization—mass spectrometric analysis of low vapor pressure chemical particulates collected from a surface. Anal Chim Acta 2015;853:368–74.

15. Chingin K, Chen H, Gamez G, Zhu L, Zenobi R. Detection of diethyl phthalate in perfumes by extractive electrospray ionization mass spectrometry. Anal Chem 2009;81:123–9.

16. Xu J, Xu S, Xiao Y, Chingin K, Lu H, Yan R, et al. Quantitative determination of bulk molecular concentrations of β-agonists in pork tissue samples by direct internal extractive electrospray ionization-mass spectrometry. Anal Chem 2017;89:11252–8.

17. Law WS, Chen H, Ding J, Yang S, Zhu L, Gamez G, et al. Rapid characterization of complex viscous liquids at the molecular level. Angew Chem Int Ed Engl 2009;48:8277–80.

18. Ma X, Zhang S, Lin Z, Liu Y, Xing Z, Yang C, et al. Real-time monitoring of chemical reactions by mass spectrometry utilizing a low-temperature plasma probe. Analyst 2009;134:1863–7.

19. Song L, Xu J, Chingin K, Zhu T, Zhang Y, Tian Y, et al. Rapid identification of meat species by the internal extractive electrospray ionization mass spectrometry of hemoglobin selectively captured on functionalized graphene oxide. J Agric Food Chem 2017;65:7006–11.

20. Zhang H, Chingin K, Zhu L, Chen H. Molecular characterization of ongoing enzymatic reactions in raw garlic cloves using extractive electrospray ionization mass spectrometry. Anal Chem 2015;87:2878–83.

21. Zhang H, Zhu L, Luo L, Wang N, Chingin K, Guo X, et al. Direct assessment of phytochemicals inherent in plant tissues using extractive electrospray ionization mass spectrometry. J Agric Food Chem 2013;61:10691–8.

22. Chen H, Venter A, Cooks RG. Extractive electrospray ionization for direct analysis of undiluted urine, milk and other complex mixtures without sample preparation. Chem Commun (Camb) 2006;2006:2042–4.

23. Law WS, Chen HW, Balabin R, Berchtold C, Meier L, Zenobi R. Rapid fingerprinting and classification of extra virgin olive oil by microjet sampling and extractive electrospray ionization mass spectrometry. Analyst 2010;135:773–8.

24. Gu H, Hu B, Li J, Yang S, Han J, Chen H. Rapid analysis of aerosol drugs using nano extractive electrospray ionization tandem mass spectrometry. Analyst 2010;135:1259–67.

25. Li M, Ding J, Gu H, Zhang Y, Pan S, Xu N, et al. Facilitated diffusion of acetonic acid revealed by quantitative breath analysis using extractive electrospray ionization mass spectrometry. Sci Rep 2013;3:1205.

26. Pan S, Tian Y, Li M, Zhao J, Zhu L, Zhang W, et al. Quantitative detection of nitric oxide in exhaled human breath by extractive electrospray ionization mass spectrometry. Sci Rep 2015;5:8725.

27. Tian Y, Yu M, Chen J, Liu C, Shi J, Chen H, et al. Real time on-line correction of mass shifts and intensity fluctuations in extractive electrospray ionization mass spectrometry. Anal Chem 2015;87:11962–6.

28. Nah T, Chan M, Leone SR, Wilson KR. Real time in situ chemical characterization of submicrometer organic particles using direct analysis in real-time mass-spectrometry. Anal Chem 2013;85:2087–95.

29. Yue Y, Qiu ZD, Qu XY, Deng AP, Yuan Y, Huang LQ, et al. Discoursing on Soxhlet extraction of ginseng using association analysis and scanning electron microscopy. J Pharm Anal 2018;8:312–7.

30. Jiang H, Zeng J, Li W, Bifano M, Gu H, Tisch C, et al. Practical and efficient strategy for evaluating oral absolute bioavailability with an intravenous microdose of a stable isotopically-labeled drug using a selected reaction monitoring mass spectrometry assay. Anal Chem 2012;84:10031–7.

31. Guo Q, Xia H, Meng X, Shi G, Xu C, Zhu C, et al. C19-Diterpenoid alkaloid arabinosides from an aqueous extract of the lateral root of Aconitum carmichaeli and their analgesic activities. Acta Pharm Sin B 2018;8:409–19.

32. Guo L, Peng C, Dai O, Geng Z, Guo YP, Xie XF, et al. Two new pyrazines from the parent roots of Aconitum carmichaeli. Biochem Syst Ecol 2013;48:92–5.

33. Wang X, Wang H, Zhang A, Lu X, Sun H, Dong H, et al. Metabolomics study on the toxicity of aconite root and its processed products using ultra-performance liquid-chromatography/electrospray-ionization synapt high-definition mass spectrometry coupled with pattern recognition approach and ingenuity pathways analysis. J Proteome Res 2012;11:1284–301.
34. Liu S, Li F, Li Y, Li W, Xu J, Du H. A review of traditional and current methods used to potentially reduce toxicity of Aconitum roots in traditional Chinese medicine. *J Ethnopharmacol* 2017;207:237–50.

35. Zhang Q, Wang CH, Ma YM, Zhu EY, Wang ZT. UPLC–ESI/MS quantification of 17 active constituents in two categorized formulas of traditional Chinese medicine, Sanhuang Xiexin Tang and Fuzi Xiexin Tang: application in comparing the differences in decoctions and macerations. *Biomed Chromatogr* 2013;27:1079–88.

36. Wei X, Qiu Z, Chen J, Sun R, Huang L, Lai C. Research advancement in mechanisms of processing and compatibility for detoxication of Aconitums. *China J Chin Mater Med* 2019;44:3695–704.

37. Hao H, Cui N, Wang G, Xiang B, Liang Y, Xu X, et al. Global detection and identification of nontarget components from herbal preparations by liquid chromatography hybrid ion trap time-of-flight mass spectrometry and a strategy. *Anal Chem* 2008;80:8187–94.

38. Tang M, Zhao LC, Xu M, Leng J, Tang N, Hu ZY, et al. Chemical constituents and pharmacological activities of *Aconiti lateralis* Radix Praeparata. *Guilaiia* 2017;37:1614–27.

39. Luo M, Hu B, Zhang X, Peng D, Chen H, Zhang L, et al. Extractive electrospray ionization mass spectrometry for sensitive detection of uranyl species in natural water sample. *Anal Chem* 2010;82:282–9.

40. Link H, Fuhrer T, Gerosa L, Zamboni N, Sauer U. Real-time metabolome profiling of the metabolic switch between starvation and growth. *Nat Methods* 2015;12:1091–7.

41. Bicker W, Monticelli F, Bauer A, Roider G, Keller T. Quantification of aconitine in post-mortem specimens by validated liquid chromatography–tandem mass spectrometry method: three case reports on fatal ‘monkshood’ poisoning. *Drug Test Anal* 2013;5:753–62.

42. Tang L, Gong Y, Lv C, Ye L, Liu L, Liu Z. Pharmacokinetics of aconitine as the targeted marker of *Fuzi* (*Aconitum carmichaelii*) following single and multiple oral administrations of *Fuzi* extracts in rat by UPLC/MS/MS. *J Ethnopharmacol* 2012;141:736–41.

43. Gutser UT, Friese J, Heubach JF, Matthiesen T, Selve N, Wilfert B, et al. Mode of antinociceptive and toxic action of alkaloids of *Aconitum* spec. *Naunyn Schmiedebergs Arch Pharmacol* 1997;357:39–48.

44. Friese J, Gleitz J, Gutser UT, Heubach JF, Matthiesen T, Wilfert B, et al. *Aconitum* sp. alkaloids: the modulation of voltage-dependent Na⁺ channels, toxicity and antinociceptive properties. *Eur J Pharmacol* 1997;337:165–74.

45. Lai CJ, Tan T, Zeng SL, Xu LR, Qi LW, Liu EH, et al. An enzymatic protocol for absolute quantification of analogues: application to specific propanediol-type ginsenosides. *Green Chem* 2015;17:2580–6.

46. Liu M, Cao Y, Lv D, Zhang W, Zhu Z, Zhang H, et al. Effect of processing on the alkaloids in *Aconitum* tubers by HPLC–TOF/MS. *J Pharm Anal* 2017;7:170–5.

47. Lai CJ, Tan T, Zeng SL, Dong X, Liu EH, Li P. Relative quantification of multi-components in *Panax notoginseng* (Sanqi) by high-performance liquid chromatography with mass spectrometry using mobile phase compensation. *J Pharm Biomed Anal* 2015;102:150–6.

48. Liu Y, Shi XW, Liu EH, Sheng LS, Qi LW, Li P. More accurate matrix-matched quantification using standard superposition method for herbal medicines. *J Chromatogr A* 2012;1254:43–50.

49. Zhang XL, Wu XF, Wang K, Wu QN, Yang GS, Sun X. Determination of 9 aconitum alkaloids in Fengshi Gutong capsules by HPLC–QTOF-MS. *Chin J Pharm Anal* 2015;35:414–9.

50. Zhang Y, Tian D, Huang Y, Li L, Mao J, Tian J, et al. Pharmacokinetic evaluation of Shenfu Injection in beagle dogs after intravenous drip administration. *Acta Pharm Sin B* 2016;6:584–92.

51. Kang C, Lai CJ, Zhao D, Zhou T, Liu DH, Lv C, et al. A practical protocol for comprehensive evaluation of sulfur-fumigation of *Gastrodia Rhizoma* using metabolome and health risk assessment analysis. *J Hazard Mater* 2017;340:221–30.

52. Han B, Huang W, Xie X, Peng C. Thought and methodology of toxicity-efficacy network integrated analysis of toxic *Aconitum*. *World Chin Med* 2017;12:2585–97.

53. Lu G, Dong Z, Wang Q, Qian G, Huang W, Jiang Z, et al. Toxicity assessment of nine types of decoction pieces from the daughter root of *Aconitum carmichaeli* (Fuzi) based on the chemical analysis of their diaster diterpenoid alkaloids. *Plant Med* 2010;76:825–30.

54. Jin SR, Zhu BD, Qin XH, Chen HL. Effects of xihuang pill on cell cycles of human hepatoma carcinoma cell strain (SMMC7721) and mouse uterine cervix cancer (U14). *Lishizhen Med Mater Med Res* 2007;18:2782–3.