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Quantitative analysis of five toxic alkaloids in *Aconitum pendulum* by ultra-performance convergence chromatography (UPC^2) coupled with mass spectrometry

Tang-Juan Zhao[1, 2], Huan-Yang Qi[1], Juan Chen [1, *], Yan-Ping Shi [1, *]

[1] Key Laboratory of Chemistry of Northwestern Plant Resources and Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, People's Republic of China

[2] University of Chinese Academy of Sciences, Beijing 100049, P. R. China

ABSTRACT

A rapid and efficient ultra-performance convergence chromatography (UPC^2) method coupled with electrospray ionization single quadrupole mass spectrometry (ESI-MS) was developed and validated for simultaneous quantification of the five diester diterpenoid alkaloids constituents (3-acetylaconitine, hypaconitine, deoxyaconitine, mesaconitine, aconitine) in *Aconitum pendulum*. Optimum separation was achieved on a BEH 2-EP C18 column (2.1×150 mm i.d., 1.7 μm particle) with a gradient elution of a mixture of A (supercritical CO_2) and B (methanol containing 10 mmol L^{-1} ammonium acetate) at a flow rate of 0.8 mL min^{-1} within 3 minutes, and quantification was performed by mass spectrometry in positive ion ionization mode and selected ion recording (SIR) mode. The influences of column, modifier, additive, column temperature, and back pressure were investigated. The five alkaloids were identified and quantified by the comparison of retention time, ultraviolet spectrum, molecular ion peak (obtained from selective ion recording mode) and peak areas with the reference compounds. The method was validated through linearity, limits of detection, limits of quantification, precision, stability, repeatability, and accuracy. The validated

*Correspondence authors: Juan Chen and Yan-Ping Shi; Tel: 86-931-4968121, Fax: 86-931-4968094, E-mail: chenjuan@licp.cas.cn (J. Chen) and shiyp@licp.cas.cn (Y.-P Shi).

method was applied to analyze *A. pendulum*, which provided a reference for the quality evaluation of *A. pendulum*.

Keywords: ultra-performance convergence chromatography (UPC^2), *Aconitum pendulum*, diester diterpenoid alkaloids (DDAs)

1. Introduction

The Tibetan medicines, which have a history of more than 2500 years, have been attracting wide attention increasingly for their significant curative effects. However, a considerable number of them not only possess significant biological activities but also exhibit considerable toxicity such as *Aconitum*. *Aconitum* is a genus of about 400 diversified species of herbaceous plants belonging to the family of Ranunculaceae, native to temperate regions of the north hemisphere.[1] There are 211 species in China, of which 166 are endemic.[2-4] Although most *Aconitum* species possess excellent analgesic, anti-rheumatic and anti-arrhythmic effects, the extremely high toxicity prove to be the principal obstacle for their extensive medical practice.[1] Natural pharmaceutical chemistry studies have revealed that diterpenoid alkaloids are the main constituents accumulated in many plants of *Aconitum* species responsible for both biological activity and high toxicity.[5]

Aconitum pendulum Busch, known as the name of *Xueshang Yizhihao* in Chinese, is a valuable Tibetan medicine among the *Aconitum* species owing to its analgesic, anti-inflammation, antibacterial activity, and its therapeutic effects of invigorating blood circulation and dispelling rheumatism. *A. pendulum* is widely distributed in the mountain grassy slopes and forest margins of the Qinghai-Tibet plateau, Yunnan Province, Sichuan Province, Gansu Province and Shanxi Province in China, at altitude range of 2300-4500 m.[6] In the previous phytochemical studies, a number of alkaloids, such as aconitine, deoxyaconitine, 3-acetylaconitine, hypaconitine, mesaconitine, 15a-hydroxyneoline, 8-O-acetyl-15ahydroxyneoline, 14-benzoyl-8-O-methylaconine, neoline, benzoylaconine, polyschistine A, polyschistine D, N-deethyl-3-acetylaconitine, N-deethyldeoxyaconitine, secoaconitine,

benzoyldeoxyaconitine, aconine, dehydrolucidusculline and dehydronapelline, have been isolated from *A. pendulum*.[7-10] Among these alkaloids, the diester diterpenoid alkaloids (DDAs) have captured great attention for its high toxicity and wide range of bioactivities.[11-18] For example, aconitine, an extremely toxic ingredient of *A. pendulum*, possessing a narrow therapeutic index, has striking pharmacological effects such as anti-inflammatory and antinociceptive.[19-20] The poisoning dose of aconitine for human is estimated to be 0.2 mg, and the lethal dose is 1-2 mg. Several fatal accidents have been reported for the administration of the raw material. In general, the raw material of *A. pendulum* is required for a series of processing steps, such as boiling, to reduce its toxicity prior to being used in clinical practice. Therefore, the quality control of this plant is needed to evaluate its toxicological risk and to guarantee its safe use. The related research, however, is still rather limited. Considering the vital role in quality control system played by DDAs, it is a requisite to develop a sensitive and reliable analytical method to quantify DDAs in *A. pendulum*.

For the analysis of DDAs, advanced chromatographic techniques, including gas chromatography (GC), high performance liquid chromatography (HPLC), ultra performance liquid chromatography (UPLC), capillary electrophoresis (CE) and certain hyphenated instrumental techniques have been utilized.[11, 21-25] In light of the characteristics of high molecular weight and low volatility for DDAs, GC is undesirable. Thanks to the technology integration of supercritical fluid chromatography (SFC) and UPLC, ultra-performance convergence chromatography (UPC^2) has provided a new choice for the analysis of DDAs.[26] In contrast with GC and liquid chromatography (LC), the separation performance of UPC^2 depends not only on the interaction between mobile phase and stationary phase, but also on the density of carbon dioxide (CO_2) which relies on its temperature and pressure.[27] Moreover, coupled with mass spectrometry, UPC^2 could provide higher sensitivity and selectivity for the detection of targets. Nowadays, UPC^2 has been applied in the areas of foods[26, 28-29] and drug safety[30], but its application in Tibetan medicines is still limited.

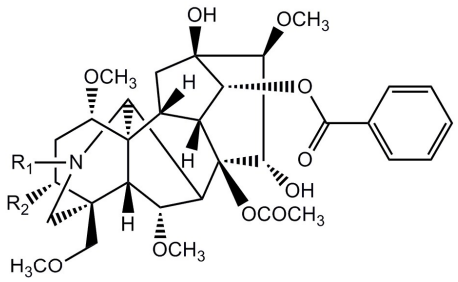
In this work, a sensitive and reliable UPC^2 coupled with electrospray ionization mass spectrometry method was established for the simultaneous quantification of five DDAs, including 3-acetylaconitine, hypaconitine, deoxyaconitine, mesaconitine and aconitine in the roots of *A. pendulum* by a single run. The proposed method was validated and applied to determine five batches of *A. pendulum* collected from different regions. In addition, the proposed method was compared with the reported HPLC-UV, UPLC-UV and UPLC-MS methods in the literature, since these methods were popular in the field of medicinal analysis.

2. Materials and methods

2.1. Chemicals and reagents

Aconitine was purchased from Beijing H&Q Chemical Institute and Beijing Aoke Biological technology Co., LTD (Beijing, China). 3-Acetylaconitine was purchased from Beijing Beina Chuanglian Biotechnology Research Institute (Beijing, China). Mesaconitine, hypaconitine and deoxyaconitine were purchased from Chengdu Herb purify Co., LTD (Chengdu, China). The purities of the above five standards were all above 98% and their chemical structures are shown in Fig. 1. Five batches of *A. pendulum* were obtained from Gansu province (batches S1, S2, S3) and Qinghai province (batches S4, S5).

Chromatographic-grade methanol and acetonitrile were purchased from Merck Co. (Darmstadt, Germany). Chromatographic-grade isopropanol and other chemicals of analytical grade were purchased from Tianjin Chemical Reagent Co. (Tianjin, China). Carbon dioxide (99.999% purity) was purchased from Zhongke Kaite Industry and Trade Co., Ltd. (Lanzhou, China). Ultra-pure water was prepared using an OKPVRE water ultrapure system (Shanghai, China).



	Name	R_1	R_2	$[M+H]^+$	Retention time (min)
1	3-Acetylaconitine	C_2H_5	$C_2H_3O_2$	688	1.02
2	Hypaconitine	CH_3	H	616	1.88
3	Deoxyaconitine	C_2H_5	H	630	2.10
4	Mesacinitine	CH_3	OH	632	2.48
5	Aconitine	C_2H_5	OH	646	2.71

Fig. 1. Chemical structures of the five aconitum alkaloids.

2.2. Apparatus and UPC^2-MS conditions

UPC^2-MS analysis was performed on a Waters ACQUITY ultra-performance convergence chromatography (UPC^2) system (Milford, MA, USA) with a SQ Detector 2 tandem mass spectrometer (Waters, USA). The UPC^2 system was equipped with a binary solvent manager, fixed loop sample manager, column manager and auxiliary manager, convergence manager which controls backpressure and photodiode array detector. The UPC^2 analysis was conducted on a Waters Acquity UPC^{2TM} BEH 2-EP C18 column (2.1×150 mm i.d., 1.7 μm particle), using a linear gradient elution of (A) supercritical CO_2 and (B) methanol with 10 mmol L^{-1} ammonium acetate at a flow rate of 0.8 mL min^{-1}. The gradient elution program was as follows: 0-3 min, A 93-87%; 3-4 min, A 87-93%. The system was re-equilibrated with 93% A for 2 min before the next sample run. The back pressure was set at 2100 psi. The temperatures of column and sample manager room were maintained 55^{o}C and 18^{o}C, respectively. The injection volume was 1 μL, and partial loop with needle overfill was applied for sample injection. Methanol and methanol/islpropanol (1/1, v/v) were used as strong and weak needle wash, respectively. The absorption spectra of the compounds were recorded in the range of 200-400 nm, and the detection wavelength was set at 225 nm with compensation from 350 to 400 nm. The mass spectrometer was equipped with

electrospray ionization (ESI) source, and the MS analysis was performed in a positive ion ionization mode of selected ion recording (SIR). Quantification of the analytical compounds was performed by employing the SIR mode. The effluent from the PDA cell outlet was split to MS and convergence manager by a splitter so that the pressure of CO_2 and modifier could be maintained. The MS analysis conditions were optimized as follows. The source temperature and the desolvation temperature were maintained at 150^{o}C and 350^{o}C, respectively. The capillary voltage and cone voltage were fixed at 2.3 kV and 70 V, respectively. The flow rates of desolvation gas and cone gas (nitrogen was used) were 600 L/h and 50 L/h, respectively. Instrument control, data acquisition and processing were performed by a Masslynx 4.1 workstation (waters, USA).

2.3. Preparation of standard solutions

Stock standard solutions were prepared in methanol at a concentration of 0.5 mg mL^{-1}. Mixed standard solution was prepared by mixing the five stock standard solutions at a concentration of 60 μg mL^{-1}. Calibration standard working solutions were freshly prepared by serially diluting the mixed standard solution to obtain final concentrations of 0.1, 1, 10, 50, 100 and 150 ng mL^{-1}. All the standard solutions prepared above were stored at 0-4^{o}C prior to analysis

2.4. Preparation of sample solutions

After being air-dried and crushed into powder, 0.1 g of the roots of *A. pendulum* was accurately weighed and introduced into a 50 mL erlenmeyer flask with a stopper, and then 2.0 mL ammonia solution and 30 mL diethyl ether were successively added. The mixture was sonicated for 30 min (80 kHz), followed by staying for 8 hours at room temperature. The supernatant was collected and the residues were sonicated for another 30 min with 20 mL of diethyl ether. The supernatant was collected and the residue was washed with 10 mL diethyl ether for three times. The extracts were combined and then concentrated to dry. The solid residue was redissolved with 60 mL of methanol, then the insoluble substances were removed by filtration and filtrate was concentrated to a final volume of 50 mL. All the sample solutions were diluted 10 times and the dilution solutions were filtered through 0.2 μm membrane before

injecting into the UPC^2-MS system. All the solutions were stored at 0-4^{o}C prior to analysis.

2.5. Evaluation of the method

The method was validated for linearity, limits of detection (LOD), limits of quantification (LOQ), precision, stability, repeatability, and accuracy according to the guidance for method validation for traditional Chinese medicines in Chinese Pharmacopoeia. [31]

3. Results and discussion

3.1. Optimization of UPC^2 conditions

In order to obtain a good resolution within a reasonable analysis time, optimization of chromatographic parameters was performed through investigating the influence of column, mobile phase, flow rate, column temperature, back pressure, and injection volume.

In this study, three columns were examined to perform the experiments including Waters Acquity UPC^2 BEH 2-EP C18 (2.1 mm × 150 mm, 1.7 μm), BEH C18 (3.0 mm × 100 mm, 1.7 μm), and CSH Fluoro-Phenyl (2.1 mm × 150 mm, 1.7 μm) column. The BEH 2-EP C18 column resulted in better resolution and peak shape within a short analysis time (within 3 minutes). On BEH C18 column, the compounds of aconitine and hypaconitine cannot be baseline separated, and the analysis time is longer than the others. Although five compounds are eluted with a good baseline resolution and peak shape within a short analysis time (within 5 minutes) on CSH Fluoro-Phenyl column, the system pressure is much too high (close to 6000 psi). There is no doubt that the system carries on a considerable burden. So, the BEH 2-EP column was selected in the subsequent experiments.

In order to improve the separation and the shape of peaks, different modifiers (including methanol, methanol:acetonitrile, and methanol:isopropanol) and additives (including ammonium formate, ammonium acetate, and formic acid) were investigated. The results revealed that when the mixed modifiers methanol:acetonitrile and methanol:isopropanol were used, compounds deoxyaconitine and mesaconitine,

and hypaconitine and deoxyaconitine could not be baseline separated. The five compounds, however, could be baseline separated by applying methanol as a modifier.

Furthermore, the influence of additive on peak shape was compared. When 10 mmol L^{-1} ammonium formate was added into methanol as an additive, the compounds of mesaconitine and hypaconitine could not be separated. And when 0.1% formic acid was used as an additive, no chromatographic peaks were found clearly. It seems that the chemical structures of aconitum alkaloids are unstable under acidic conditions in methanol. In other words, the chromatographic separation depends on the pH of solution. The 10 mmol L^{-1} ammonium acetate additive showed the best results in terms of retention time and resolution and thus was selected as the additive. So, the mixed solution of CO_2/methanol with 10 mmol L^{-1} ammonium acetate was used as the mobile phase for UPC^2 analysis. The most suitable injection volume and flow rate were set as 1 μL and 0.8 mL min^{-1}, respectively.

Increasing column temperature has a certain influence on the separation selectivity by decreasing the viscosity of the methanol.[32] In our work, column temperature was examined from 35 to 70^{o}C. As is shown in Fig.2a, with the increase of column temperature, the five aconitum alkaloids had longer retention times and broadened peaks. In the study, an optimal temperature of 55^{o}C was selected for the supercritical fluid chromatography analysis.

Back pressure is a very vital factor which could influence the interaction between the analytes and mobile phase by changing the density of supercritical carbon dioxide in UPC^2. In our work, back pressure was examined from 1500 to 2300 psi. As is shown in Fig.2b, with increasing the back pressure, the retention times of five aconitum alkaloids decreased and the peaks become sharper. Meanwhile, the system pressure will increase. Combined with the effect of column temperature, the optimum back pressure was chosen as 2100 psi.

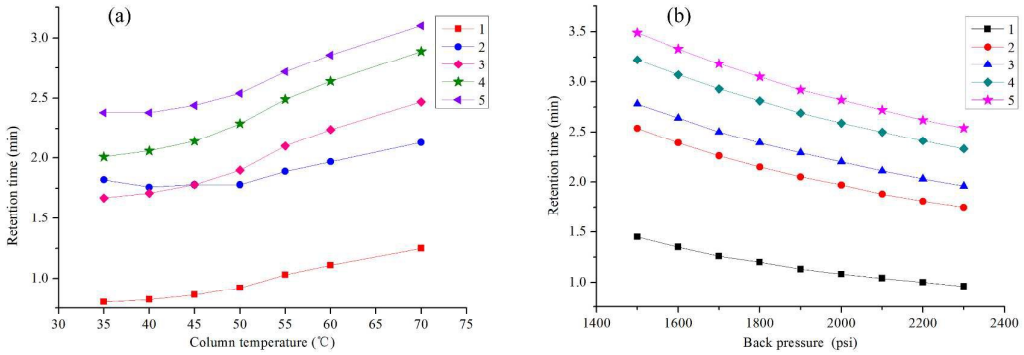


Fig.2. Effects of (a) column temperature and (b) back pressure on the retention time of the analytes. Symbol marking: 1, 3-acetylaconitine; 2, hypaconitine; 3, deoxyaconitine; 4, mesaconitine; 5, aconitine.

3.2. MS analysis of the five aconitum alkaloids

The reference compounds of five aconitum alkaloids were used to optimize the MS parameters and to identify the corresponding compounds presented in this medicinal plant. The effects of desolvation temperature, source temperature, desolvation gas flow, capillary voltage, cone voltage, cone gas flow and ionization mode were separately examined. The source temperature and cone gas (nitrogen) flow were fixed at 150°C and 50 L/h, respectively. And the other parameters were varied as follows: desolvation temperature (300, 350, 400, and 450°C), desolvation gas (nitrogen) flow (600, 700, 800, 900, and 1000 L/h), capillary voltage (2.3, 2.5, 2.8, and 3.0 kV), cone voltage (30, 40, 50, 60, 65, and 70 V). The trials showed that positive ion mode fit the detection for five aconitum alkaloids better than negative ion mode, and cone voltage influenced the ionization significantly. The optimum MS conditions were obtained after several trials.

In a positive ion mode, $[M+H]^+$ ions were observed as the most abundant ions, and molecular weight were determined based on the information of $[M+H]^+$ ions. Herein, five compounds were identified and quantized by comparing the retention time, ultraviolet spectrum and mass data with those of the reference compounds. The UPC^2 chromatogram and total ion chromatogram of a mixed standard solution are shown in Fig.3a and 3b, respectively.

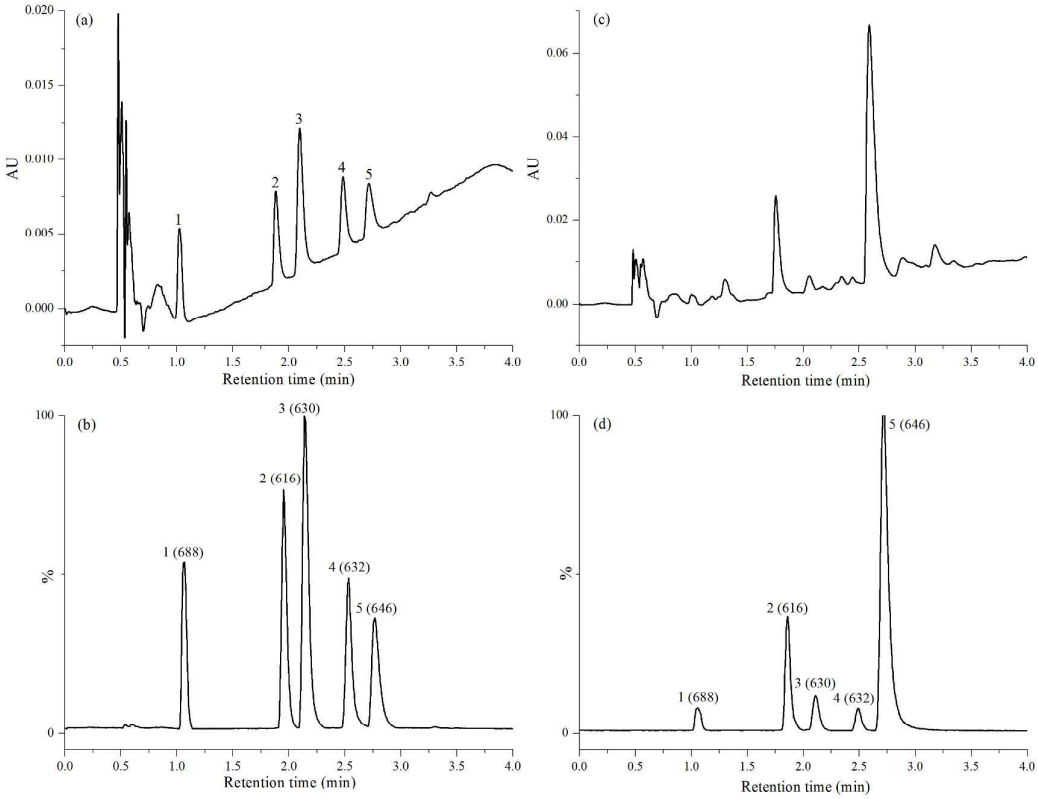


Fig.3. UPC^2 chromatograms at 225 nm and total ion chromatograms obtained from (a, b) a mixed standard solution and (c, d) a real sample solution. Peak numbering is the same as for Fig. 2.

3.3. Method validation

3.3.1. Calibration plots, LODs and LOQs

Under the optimal conditions, calibration standard working solutions at six concentrations of 0.1, 1, 10, 50, 100 and 150 ng mL^{-1} were analyzed by UPC^2-MS method in SIR mode, and three duplicate analyses were performed for each concentration level. The calibration curves were established by plotting the peak areas against the concentrations of analytes with linear regression analysis, and the regression equations were expressed as y=ax+b, where y is peak area and x is concentration (ng mL^{-1}) of analytes. LOD and LOQ were determined as the concentration at the signal to noise ratios of 3 and 10 times, respectively. The regression equations, correlation coefficients (r), linear ranges, LODs, and LOQs are listed in Table 1.

Table 1 Calibration plots, LODs and LOQs

Analyte	Regression equation	r	Linear range (ng mL^{-1})	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
3-acetylaconitine	$y = 52314x + 13174$	0.9994	0.1-150	0.013	0.027
hypaconitine	$y = 60021x + 57111$	0.9991	0.1-150	0.016	0.044
deoxyaconitine	$y = 83512x + 14836$	0.9995	0.1-150	0.034	0.051
mesaconitine	$y = 57768x + 59934$	0.9997	0.1-150	0.011	0.042
aconitine	$y = 60341x + 10002$	0.9997	0.1-150	0.029	0.077

3.3.2. Precision, stability and repeatability

A real sample solution (S2), in which the concentrations of 3-acetylaconitine, hypaconitine, deoxyaconitine, mesaconitine and aconitine were 2.0, 0.6, 0.9, 4.6 and 32.1 ng mL^{-1}, respectively, was used for precision and stability studies. The precision was evaluated by performing intra-day and inter-day variation with consecutive injection of the sample solution. Intra-day variation was estimated by five successive injections within a day, and inter-day variation was measured on five consecutive days. For the stability test, the sample solutions were analyzed at 2 h intervals during storage for 12 h at room temperature. The sample solution can be regarded as stable within 12 h because the RSD% values of both retention times and peak areas were <2%. The specific data are listed in Table 2.

Repeatability was performed by the injections of six different sample solutions which were prepared in parallel with the same batch sample (S2) according to the procedure described above, and obtained from the RSD% of the component content. The specific data are listed in Table 2.

Table 2 The RSD% values for precision, repeatability and stability

Analyte	Intra-day precision		Inter-day precision		Stability		Repeatability
	RT[a]	PA[b]	RT	PA	RT	PA	content
3-acetylaconitine	0.0	1.2	0.4	3.2	0.0	1.9	0.6
hypaconitine	0.2	1.5	0.2	3.2	0.2	1.7	1.9
deoxyaconitine	0.2	2.6	0.3	1.7	0.4	0.9	1.4
mesaconitine	0.2	3.9	0.2	4.7	0.0	1.9	0.7
aconitine	0.1	4.3	0.1	4.9	0.2	1.4	0.1

[a] RT: Retention time

[b] PA: Peak area

3.3.3. Accuracy

The accuracy of the method was investigated by spike recovery test. Different amounts of the standards at three levels (low, medium and high level) were separately spiked to an originally analyzed real sample (S2), for which the contents of the compounds of interest were already known. Then three sets of spiked samples were treated according to the procedure of "preparation of sample solution" and analyzed by UPC^2-MS in SIR mode. The accuracy was expressed by the recovery, which was calculated by the following formula: recovery (%) = (found amount-original amount) ×100%/added amount. The results of the recovery test in Table 3 indicate that the proposed method enables highly accurate simultaneous analysis of the five analytes in the roots of *A. pendulum.*

Table 3 Recovery studies for determination of the five alkaloids

Alkaloids	Original amount (μg)	Added amount (μg)	Found amount (μg)	Recovery (%)	Average recovery (%)	RSD (%)
3-acetylaconitine	10.1200	7.83	17.6838	96.6	98.2	1.4
	10.0110	9.78	19.6639	98.7		
	10.0230	12.30	22.2246	99.2		
hypaconitine	2.8336	2.32	5.0956	97.5	96.9	1.9
	2.8031	2.81	5.5681	98.4		
	2.8064	3.36	5.9917	94.8		
deoxyaconitine	4.5540	3.66	8.1042	97.0	95.3	2.6
	4.5050	4.54	8.8816	96.4		
	4.5104	5.42	9.5239	92.5		
mesaconitine	21.9604	17.40	39.4300	100.4	97.6	4.0
	21.7239	21.82	43.3912	99.3		
	21.7499	26.12	46.0676	93.1		
aconitine	156.2528	123.86	278.2549	98.5	97.3	4.7
	154.5698	154.38	310.8024	101.2		
	154.7551	186.44	326.8392	92.3		

3.4. Comparison of analytical methods

In order to evaluate the analytical performance of the proposed UPC^2-MS method, the reported HPLC and UPLC methods in the literature were brought as references for a comparison, the results are summarized in the supporting information (Table S1). Furthermore, to compare the difference between UPC^2 and UPLC more objectively,

an additional experiment was carried out, in which UPLC coupled with MS/MS method in a positive ion ionization mode of multiple reaction monitoring (MRM) was developed and applied to the determination of the same batches samples. The UPLC-MS/MS conditions, along with the UPLC chromatogram, total ion chromatogram and method validation parameters are listed in the supporting information (SF1, Fig. S1 and Table S1). Regarding the HPLC-UV and UPLC-UV methods, as the specific data listed, LOD and LOQ are much higher than that of UPLC-MS and UPC^2-MS methods. Thus, a highly concentrated sample is required for the quantitative analysis of DDAs by UV detection method. However, such kind of sample can cause chromatographic column overload and pollution. So, UV detection is not suitable for analyzing DDAs at such a low concentration in complex sample matrices. Both UPLC and UPC^2 exhibit major advantages over conventional HPLC, such as increased peak capacity, shorter retention time and less solvent consumption, owing to the utilization of sub-2 μm particles as stationary phase. It is worth mentioning that UPC^2 integrates supercritical fluid chromatography (SFC) and UPLC technologies, and thus shows some superiorities compared with UPLC. Supercritical CO_2, the main mobile phase in UPC^2, offers superior solubility for the analytes and induces strong non-polar interactions between the analytes and the mobile phase, also allows a large flow rate, thereby reducing the retention time remarkably. As shown in Table S1, the five DDAs were separated within a very short time using the presented method. In addition, UPC^2 can deliver a reduction in waste generation and disposal compared with UPLC. Furthermore, UPC^2, based on the principles of normal-phase LC, with the ease-of-use of reversed-phase LC, is suitable for separating compounds in a wider range of polarities. Despite these superiorities described above, however, UPC^2 has not been a preferential and popular technique in the field of analysis at present, which might be attributed to expensive instrument, limited instrument manufacture and limited recognition on this technology itself.

4. Sample analysis

The developed method was applied to analyze the five DDAs in five batches of *A.*

pendulum roots samples collected from Qinghai and Gansu Province of China. The UPC^2 chromatogram and total ion chromatogram obtained from a real sample solution are shown in Fig. 3c and 3d. Their contents are presented in Table 4. Total contents of the DDAs in the five batches of samples ranged from 71.5 to 798.2 μg/g. The content of aconitine was higher than the other alkaloids except the batch of sample S4. The results also revealed that the contents of five alkaloids fluctuated largely for different batches. This might be accounted for the variation in different growing environment, growing years, collected regions, harvest seasons, and storage condition for crude herbs. The obtained results further demonstrated the importance and necessity for monitoring the DDAs in *A. pendulum*. In addition, the RSD values of the contents of five alkaloids in the same batches of samples determined by UPC^2 and UPLC methods are less than 15%, which could further prove the applicability of the developed method in real samples.

Table 4 Contents of the five alkaloids in five different batches of *A. pendulum* roots sample

Content (μg/g)	Sample batches				
	S1	S2	S3	S4	S5
3-acetylaconitine	8.8	10.0	11.8	10.1	0.6
hypaconitine	3.8	2.8	6.0	230.0	3.7
deoxyaconitine	7.7	4.5	4.6	5.1	17.9
mesaconitine	6.6	21.7	3.0	434.7	ND
aconitine	133.9	154.4	147.3	113.3	48.3
Total	162.8	196.4	176.7	798.2	71.5

ND: undetected.

5. Conclusion

Monitoring the contents of DDAs in *A. pendulum* is needed to evaluate its toxicological risk and to guarantee its safe use. In this work, an UPC^2 method coupled with mass spectrometry in positive ionization mode possessing high linearity, precision, stability, repeatability, and accuracy was developed for the simultaneous determination of the five aconitum alkaloids in the roots of *A. pendulum*. The developed method involved the use of $[M+H]^+$ ions in the positive ion mode with selective ion recording (SIR). The five aconitum alkaloids constituents were authenticated based on the comparison of their retention times, ultraviolet spectrums

and molecular weights with the reference substance. The established method was successfully applied to the five batches of *A. pendulum* and the results exhibited a substantial fluctuation in the contents of these specific components. This work provided a promise for evaluating the quality of *A. pendulum*.

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Quantitative analysis of five toxic alkaloids in *Aconitum pendulum* by ultra-performance convergence chromatography (UPC^2) coupled with mass spectrometry

Tang-Juan Zhao, Huan-Yang Qi, Juan Chen, Yan-Ping Shi

An UPC^2-MS method for simultaneous quantification of the five toxic alkaloids was developed for the quality evaluation of *A. pendulum*.