Supporting information

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

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The supporting information includes UPLC-MS conditions for the determination of the five DDAs in five batches of *A. pendulum* roots samples (SF1), UPLC chromatogram and total ion chromatogram of a mixed standard solution and a real sample solution (Fig. S1) and comparison of the proposed method with the reported methods in the literature (Table S1).

SF1 UPLC-MS conditions for the determination of the five DDAs in five batches of *A. pendulum* roots samples

The UPLC-MS/MS analysis was performed on a Waters ACQUITY UPLC™ system (Waters, Milford, MA, USA) equipped with a Waters ACQUITY tandem triple quadrupole mass spectrometer (TQD-MS). The identifications and quantifications were performed on TQD-MS equipped with a Z-Spray ion interface. The UPLC analysis was conducted on a Waters Acquity UPLC BEH C18 column (50 × 2.1 mm i.d., 1.7 μm particle), using a linear gradient elution program of (A)

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acetonitrile and (B) 10 mmol L^{-1} ammonium acetate at a flow rate of 0.3 mL min-1. The gradient elution program was applied as follows: 0-4.5 min, 30-33% A; 4.5-5.0 min, 33-70% A; 5.0-6.0 min, 70% A; 6.0-7.0 min, 70-30% A; 7.0-9.0 min, 30% A. The temperatures of column and sample manager room were maintained at 30°C and 18°C, respectively. The injection volume was 2 μL, and the partial loop with needle overfill was applied for sample injection. The absorption spectra of the compounds were recorded in the range of 190-400 nm (3 D-plots were recorded), and the detection wavelength was set at 225 nm. The mass spectrometer was equipped with ESI source, and the MS analysis was performed in a positive ion ionization mode of multiple reaction monitoring (MRM) and operated under the following optimal conditions: The source temperature and the desolvation temperature were maintained at 140°C and 350°C, respectively. The electrospray capillary voltage, cone voltage and collision voltage were maintained at 2.8 kV, 50 V and 50 V, respectively. The desolvation gas and cone gas (high-purity nitrogen was used) flows were 800 L h^{-1} and 50 L h^{-1}, respectively. The collision gas (high-purity argon was used) flow was set at 0.15 ml min^{-1}. The protonated molecular ions ($[M+H]^+$) of aconitine, mesaconitine, hypaconitine, 3-acetylaconitine and deoxyaconitine, and the fragmentations of their corresponding daughter ions for quantification were selected as follows: aconitine (*m/z* 646 → 586), mesaconitine (*m/z* 632 → 572), hypaconitine (*m/z* 616 → 556), 3-acetylaconitine (*m/z* 688 → 628) and deoxyaconitine (*m/z* 630 → 570). The UPLC chromatogram and total ion chromatogram of a mixed standard solution and a real sample solution are shown in Fig.S1.

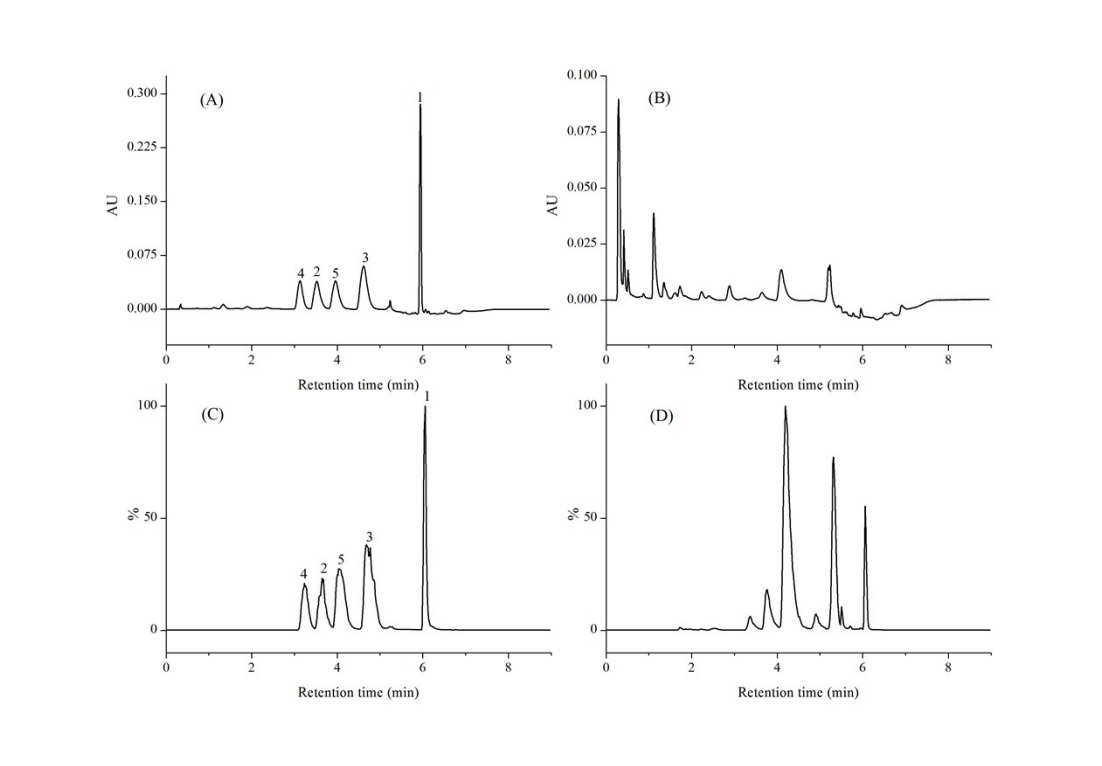

Fig. S1. UPLC chromatograms at 225 nm and total ion chromatograms obtained from (A, B) a mixed standard solution and (C, D) a real sample solution. Peak numbering: 1, 3-acetylaconitine; 2, hypaconitine; 3, deoxyaconitine; 4, mesaconitine; 5, aconitine.

Table S1 Comparison of the proposed method with the reported methods in the literature

Analytical method	Matrices	Analytes[a]	RT[b] (min)	LOD[c]	LOQ[c]	Precision (RSD%)	Repeatability (RSD%)	Stability (RSD%)	Accuracy (RSD%)	Ref.
HPLC-UV	*A. carmichaeli*	4	31.98	20	–	0.14-0.67	–	0-0.98	97.1-101.9	33
	A. pendulum	5	46.97	24						
	A. hemsleyanum	2	49.20	27						
	A. transsectum	3	63.26	30						
UPLC-UV	*Radix Aconiti*	4	20.82	200	630	＜3.3	–	–	103-112	34
		5	27.58	280	800					
		2	31.47	300	1000					
UPLC-MS EIC	*Radix Aconiti*	4	20.82	4	10	＜3.3	–	–	103-112	34
		5	27.58	6	15					
		2	31.47	3	9					
UPLC-MS SIR	*Xiaohuoluo Pill*	4	2.61	0.45	1.41	2.4-2.82	1.79-3.78	1.11-1.76	99.8-101.7	35
		5	3.94	0.39	1.20					
		2	4.07	0.65	1.92					
UPLC-MS SIR	*SanhuangXiexin Tang*	5	7.29	1.00	5.00	0.72-3.99	–	–	98.7-104.8	36
	FuziXiexin Tang	2	7.21	0.38	0.76					
		4	6.37	2.50	5.00					
UPC^2-MS SIR	*A. pendulum*	1	1.02	0.013	0.027	1.2-4.3	0.6-1.4	0.9-1.9	95.3-98.2	This one
		2	1.88	0.016	0.044					
		3	2.10	0.034	0.051					
		4	2.48	0.011	0.042					
		5	2.71	0.029	0.077					
UPLC-MS MRM	*A. pendulum*	4	3.28	3.20	8.00	4.1-5.1	2.2-6.4	3.9-5.1	98.0-100.1	This one
		2	3.71	1.20	2.40					
		5	4.10	0.73	2.18					
		3	4.82	0.28	1.40					
		1	6.05	0.08	0.24					

[a] Analyte numbering: 1, 3-acetylaconitine; 2, hypaconitine; 3, deoxyaconitine; 4, mesaconitine; 5, aconitine

[b] RT: Retention time

[c] ng mL^{-1}