Supplementary Materials

Table S1. Multiple reaction monitoring (MRM) parameters and retention time for target compound.

Analyte	Monoisotopic mass (g/mol)	Precursor ion (*m*/*z*)	Ionization state	Product ion (*m*/*z*)	Collision energy (eV)	Retention time (min)
Ptaquiloside	398.1	399	$[M + H]^+$	<u>181</u>*	16	3.21
				381	10	
				277	10	

*Underlined ion is quantitative ion, and other ions are qualitative ions.

Table S2. Detailed LC–MS parameters.

Parameter	Conditions
Liquid chromatograph Mass spectrometer	Vanquish UHPLC (Thermo Fisher Scientific, California, USA) TSQ Altis (Thermo Fisher Scientific, California, USA)
Column	Kinetex 2.6-μm C18 100 Å (100 × 21 mm^2)
Mobile Phase	A: Water with 0.1% of formic acid (v/v) B: Acetonitrile with 0.1% of formic acid (v/v)
Flow rate	0.5 mL/min
Injection volume	10 μL
Column temperature	35 °C
Ion mode	ESI positive
The gradient elution condition	5% B (0–0.5 min), 5%–95% B (0.5–3 min), 95% B (3–4 min), 95%–5% B (4–4.1 min), and 5% B (4.1–5 min)
Ion Source condition	Ion spray voltage: 3500 V, sheath gas: 50 Arb, aux gas: 10 Arb, sweep gas: 1 Arb, ion transfer–tube temperature: 325 °C, vaporization temperature: 350 °C, and dwell time: 48 ms
MRM transition	Quantitative ion pair: *m*/*z* 399 > 181 (CE: 16 eV) Confirmatory ions pairs: *m*/*z* 399 > 381 (CE: 10 eV) and *m*/*z* 399 > 277 (CE: 10 eV)

Table S3. Ptaquiloside (PTA) calibration curve equation, linearity, the limit of detection (LOD), and limit of quantification (LOQ).

Range (μg/kg)	Equation (y = ax + b)	Linearity (r^2)	LOD (μg/kg)	LOQ (μg/kg)
0.1–50	y = 37732x + 1079.4	0.9979	0.03	0.09

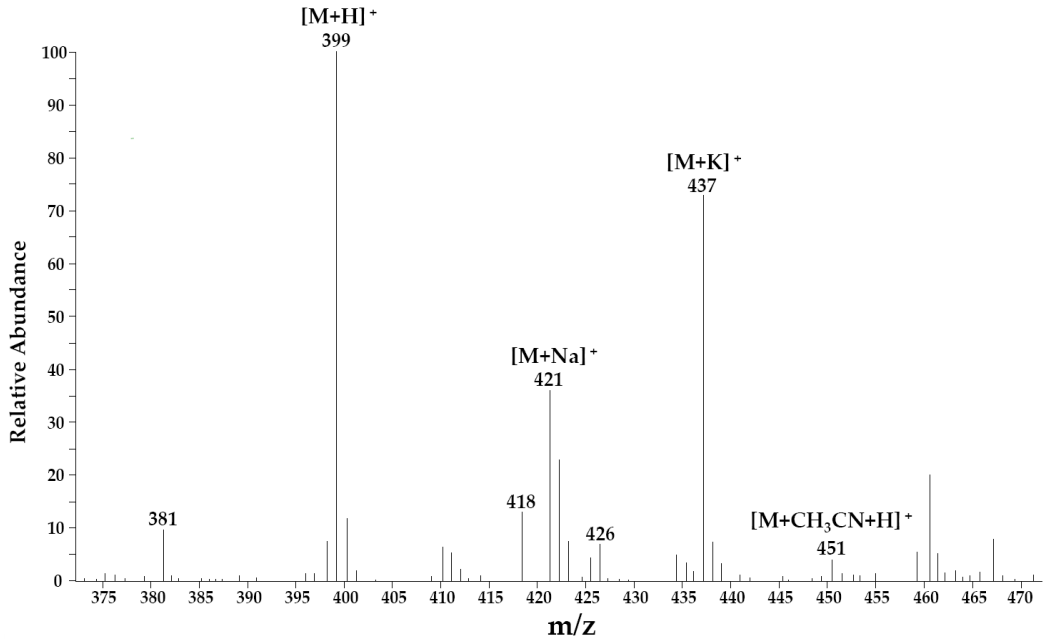
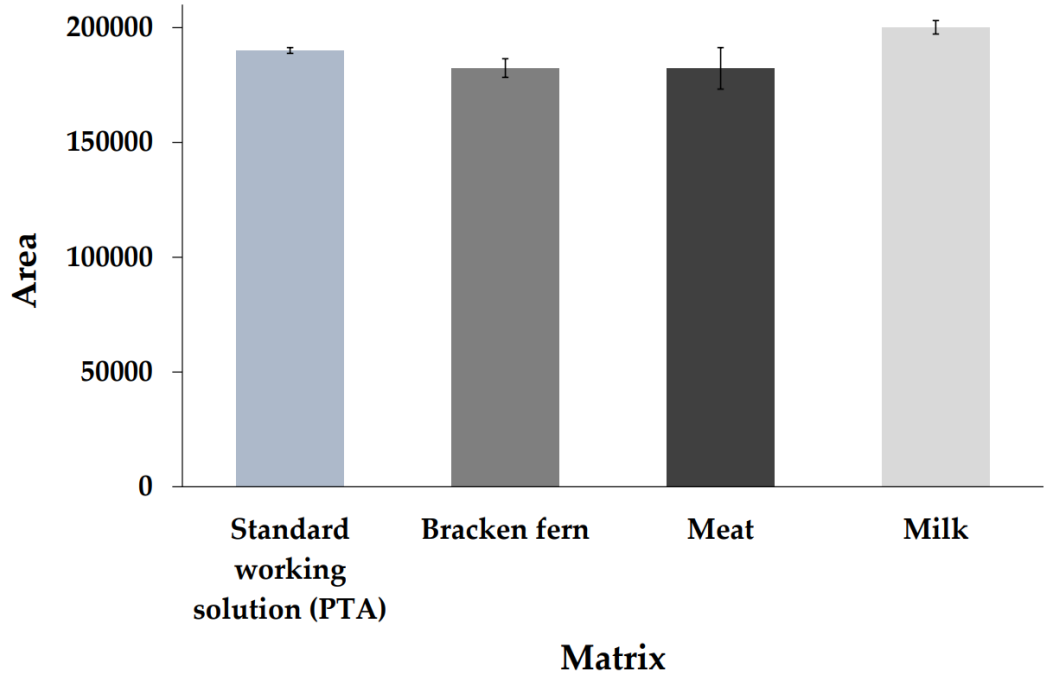


Figure S1. Q3 full scan of ptaquiloside (PTA) standard working solution.



Matrix	The area value difference (%) with the PTA standard working solution [a]
Bracken fern	− 4.0
Meat	− 4.1
Milk	+ 5.3

a: The area value difference (%) with the PTA standard working solution = [(the area value of PTA standard working solution – the area value PTA spiked into each matrix) / the area value of PTA standard working solution] × 100 (n = 3, and error bars represent standard deviation)

Figure S2. Comparison of the relative differences in the peak areas for the same concentration of (20 μg/kg) analyte (ptaquiloside; PTA) in different matrices (bracken fern, meat, and milk) and the standard working solution for PTA.

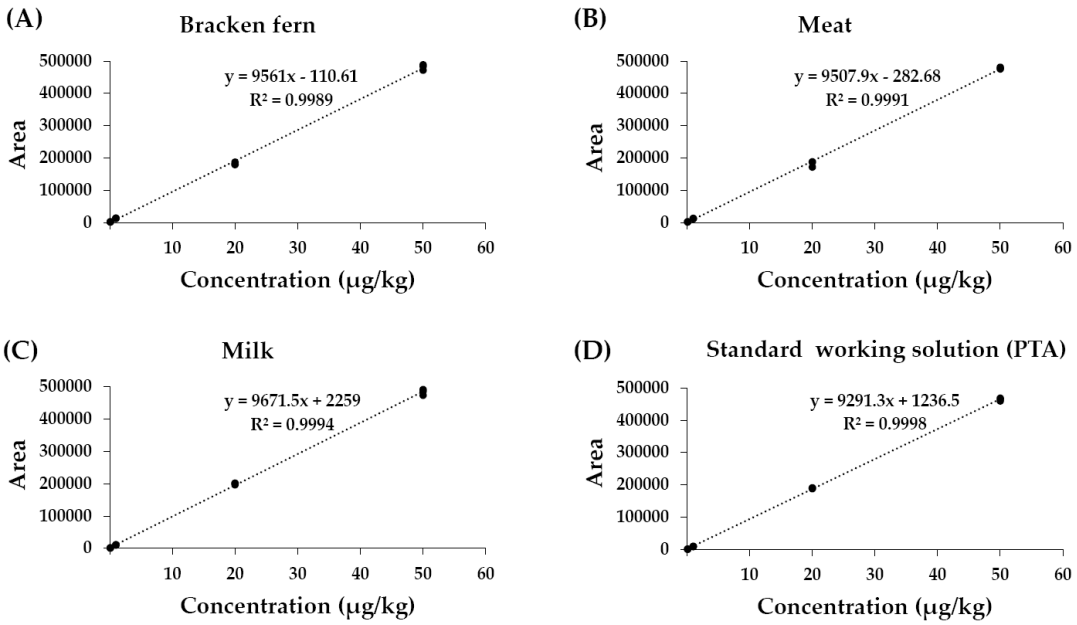


Figure S3. A procedure for verifying the relative matrix effect in different matrices: bracken fern (A), meat (B) milk (C), and PTA standard working solution (D).

Table S4. Comparison of the slope of each matrix-matched standard calibration curve with the slope of the PTA standard calibration curve.

Matrix	Equation (y = ax + b)	Linearity (r^2)	Slope difference (%)[a]	Slope CV (%)[b]
Bracken fern	y = 9561x − 110.61	0.9989	2.9	
Meat	y = 9507.9x − 282.68	0.9991	2.3	
Milk	y = 9671.5x + 2259	0.9994	4.1	1.7
Standard working solution (PTA)	y = 9291.3x + 1236.5	0.9998	-	

a: [(Slope value of standard working solution (PTA) calibration curve − the slope values of each matrix-matched standard calibration) / slope value of standard working solution (PTA)] × 100.
b: Precision value (coefficient of variation; CV, %) = [Standard deviation for the slope values of each matrix-matched calibration curve/Mean for the slope values of each matrix-matched calibration curve] × 100.

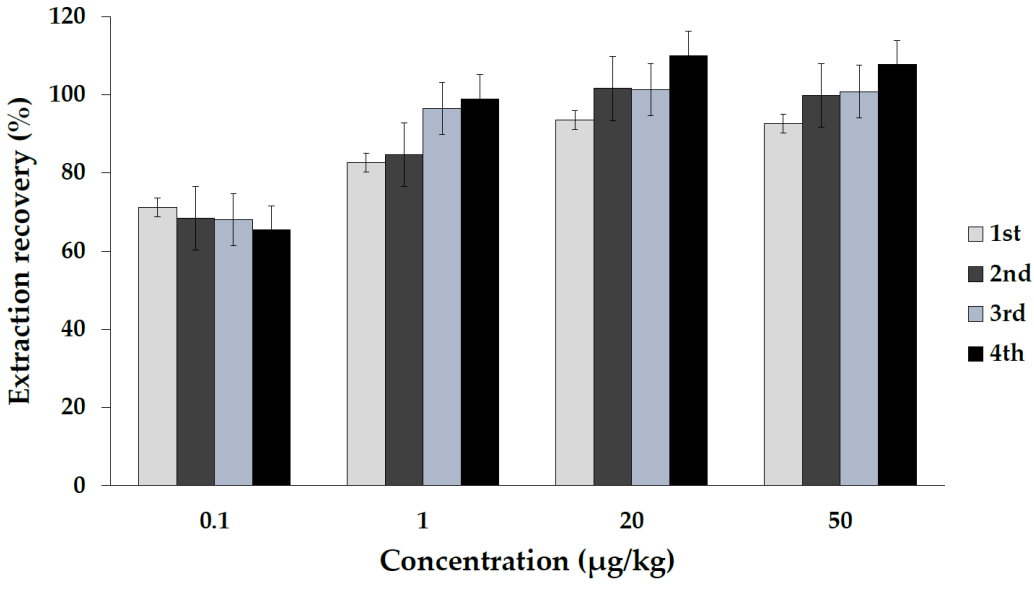


Figure S4. Extraction recovery (%) at 0.1, 1, 20, and 50 (μg/kg) levels of PTA. Measurements were 4 replicates for each concentration ($n = 3$, and *the error bars indicate the standard deviation of three samples per assay batch for each concentration*). In total, there were 4 ×4 × 3 = 96 samples analyzed. Extraction recovery (%) = (matrix spiked before extraction / matrix spiked after extraction) × 100.