

Supplementary Materials: A Simple and High-Throughput Analysis of Amatoxins and Phallotoxins in Human Plasma, Serum and Urine Using UPLC-MS/MS Combined with PRiME HLB μ Elution

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The extraction procedures for WAX, WCX, HLB and PRiME HLB cartridges in detail:

WAX: a WAX 1cc/30 mg cartridge was preconditioned with methanol and water, then 1 mL 10 ng/mL water-dissolved standard mixture was loaded onto the cartridge. After washing the cartridge with 1 mL 2% formic acid in water and 1 mL methanol, the analytes were eluted with 1 mL 5% $\text{NH}_3\cdot\text{H}_2\text{O}$ in methanol. The eluent was evaporated under N_2 and the residues were diluted with initial mobile phase.

WCX: a WCX 1cc/30 mg cartridge was preconditioned with methanol and water, then 1 mL 10 ng/mL water-dissolved standard mixture was loaded onto the cartridge. After washing the cartridge with 1 mL 2% $\text{NH}_3\cdot\text{H}_2\text{O}$ in water and 1 mL methanol, the analytes were eluted with 1 mL 2% formic acid in methanol. The eluent was evaporated under N_2 and the residues were diluted with initial mobile phase.

HLB: a HLB 1cc/30 mg cartridge was preconditioned with methanol and water, then 1 mL 10 ng/mL water-dissolved standard mixture was loaded onto the cartridge. After washing the cartridge with 1 mL 5% methanol in water, the analytes were eluted with 1 mL methanol. The eluent was evaporated under N_2 and the residues were diluted with initial mobile phase.

PRiME HLB: 1 mL 10 ng/mL water-dissolved standard mixture was loaded onto the PRiME HLB cartridge. After washing the cartridge with 1 mL 5% methanol in water, the analytes were eluted with 1 mL methanol. The eluent was evaporated under N_2 and the residues were diluted with initial mobile phase.

The HLB μ Elution and PRiME HLB μ Elution protocols:

HLB μ Elution: after conditioning the HLB μ Elution 96-well plate with 200 μL of MeOH and water, 100 μL of 10 ng/mL plasma QC sample and 100 μL 4% H_3PO_4 -water solution mixture was transferred onto the well. The loaded plate was then washed with 200 μL of 5% methanol in water twice and the sample was eluted with 25 μL of methanol–water (95/5, *v/v*) twice. The eluent was diluted with 450 μL initial mobile phase.

PRiME HLB μ Elution: 100 μL of 10 ng/mL plasma QC sample and 100 μL 4% H_3PO_4 -water solution mixture was transferred onto the PRiME HLB μ Elution 96-well plate. The loaded plate was then washed with 200 μL of 5% methanol in water twice and the sample was eluted with 25 μL of methanol-water (95/5, *v/v*) twice. The eluent was diluted with 450 μL initial mobile phase.

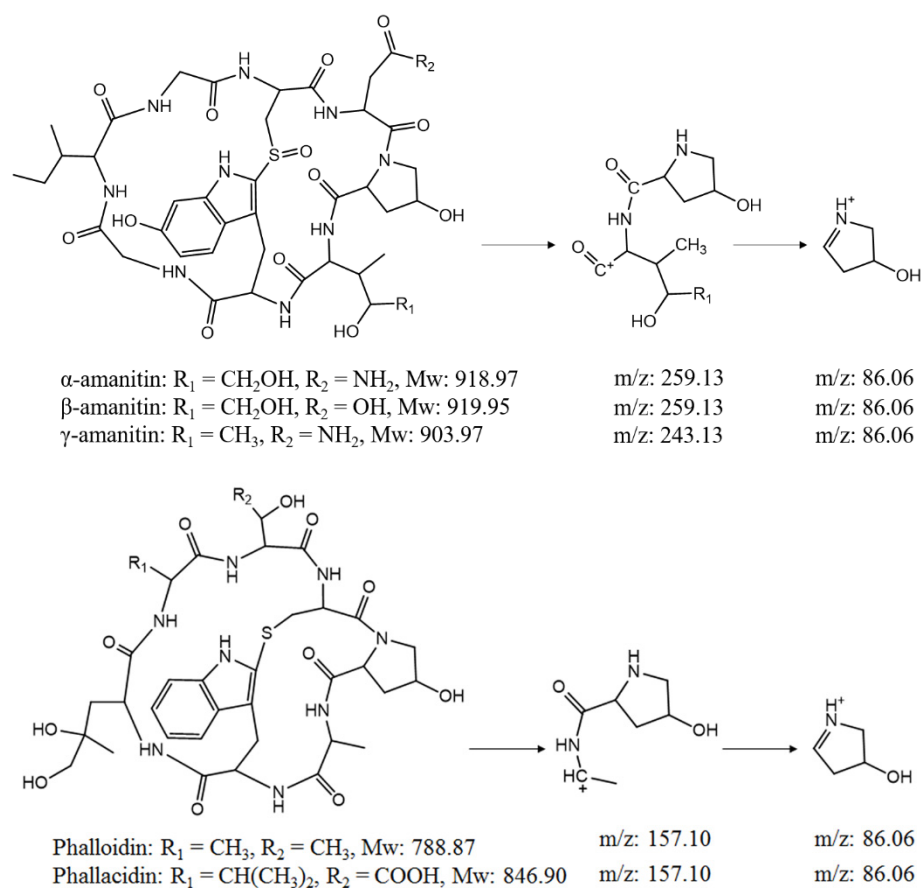


Figure S1. The proposed fragmentation of the analytes by MS/MS mode to their products

Table S1. The information of 24 urine samples with mushroom poisoning

Case	Gender	Age (year)	Time post ingestion (h)	Symptom	Intoxicated mushroom	Toxins analysis	Sample
1	F	38	74.5	Nausea, vomiting, abdominal pain, and watery diarrhea	Unknown	ND	1
2	F	57	72	Nausea, vomiting, diarrhea and palpitation	Unknown	ND	2
3	M	61	80			ND	3
3	M	61	96	Nausea, vomiting and diarrhea	Unknown	ND	4
3	M	61	116			ND	5
4	M	48	48	Abdominal pain, muscular pain	<i>Russula nigricans</i> and <i>Russula griseocarnea</i>	ND	6
4	M	48	72			ND	7
7	M	52	Unknown	Nausea, vomiting and diarrhea	Unknown	ND	8
8	F	51	Unknown	Nausea, vomiting and diarrhea	Unknown	ND	9
9	M	70	96.5	Nausea, vomiting and palpitation	Unknown	ND	10
10	M	55	102	Nausea, vomiting and palpitation	Unknown	ND	11

Table S1. Cont.

11	F	59	144	Nausea, vomiting and skin rash	Unknown	ND	12
12	M	12	96	Vomiting, abdominal pain, and watery diarrhea	Unknown	ND	13
13	M	36	96	Vomiting, abdominal pain, and watery diarrhea	Unknown	ND	14
14	M	35	Unknown	Dizziness, nausea and diarrhea	Unknown	ND	15
15	M	43	72	Vomiting and diarrhea	Unknown	ND	16
16	M	11	74	Vomiting and diarrhea	Unknown	ND	17
17	M	59	77	Vomiting and diarrhea	Unknown	ND	18
18	M	49	74	Nausea, vomiting and diarrhea	<i>Amanita exitialis</i>	+	19
19	M	59	74	Nausea, vomiting and diarrhea	<i>Amanita exitialis</i>	+	20
20	F	44	72	Diarrhea and palpitation	Unknown	ND	21
			80			ND	22
21	M	34	192	Nausea and diarrhea	Unknown	ND	23
22	F	53	72	Nausea and diarrhea	<i>Russula virescens</i>	ND	24

M: male; F: female; ND: not detected (< LOD); +: positive.