Comprehensive screening of *Aconitum* alkaloids in Kampo herbal medicine, in human serum and urine by high resolution LC/TOF Mass Spectrometry

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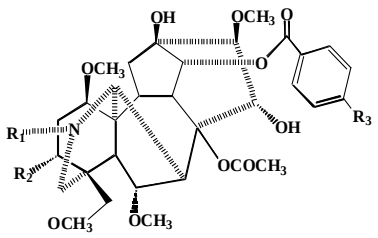
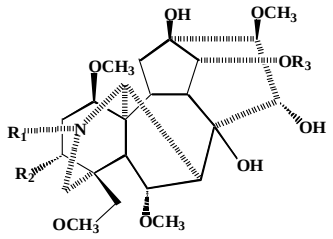
Introduction

The root of *Aconitium carmichaeli* (Fig.1) is called Bushi or Uzu and widely used as traditional herbal medicine in both China and Japan. However, it is a highly toxic herbal medicine because of the presence of diterpene alkaloids such as aconitine, mesaconitine, hypaconitine and jeasaconitine. These alkaloids share a common C19 norditerpenoid skelton and divided into four types, named as non ester diterpeine alkaloids, monoester diterpene alkaloids, diester diterpene alkaloids(Fig.2) and lipoalkaloids. Kampo herbal medicine contained Uzu is hydrolyzed to the non ester and mono ester diterpene alkaloids and detoxified by decocting in boiling water. However, there are several reports of patients who had taken Kampo herbal medicine and developed symptoms of aconite intoxication. The adverse reactions caused by Kampo herbal medicine may originate from adulteration, misidentification, variability in the amount of active ingredients, and improper processing and preparation. Therefore, to guarantee the safety and efficacy of use of aconitum type of Kampo herbal medicine the first prerequisite is to apply comprehensive analytical methods to monitor a large set of active ingredients, i.e. mainly alkaloids. This work describes a novel comprehensive screening method for 11 known *aconitum* alkaloids and other components in Kampo herbal medicine, human serum and urine using high resolution LC/TOFMS and exact mass and retention time(option) database.

Diestre diterpene



Non and mono ester diterpene



Compounds	R_1	R_2	R_3	MW
Aconitine	C_2H_5	OH	H	645
Mesaconitine	CH_3	OH	H	631
Hypaconitine	CH_3	H	H	615
Jesaconitine	C_2H_5	OH	OCH_3	675
Benzoylaconine	C_2H_5	OH	COC_6H_5	603
Benzoylmesaconine	CH_3	OH	COC_6H_5	589
Benzoylhypaconine	CH_3	H	COC_6H_5	573
1,4-Anisoylaconine	C_2H_5	OH	$COC_6H_5OCH_3$	633
Aconine	C_2H_5	OH	H	499
Mesaconine	CH_3	OH	H	485
Hypaconine	CH_3	H	H	469

Fig.2 *Aconitium* alkaloids *and metabolites of Aconitium* alkaloids

Experimental

Table 1. Experimental conditions for LC/TOF-MS

HPLC	: Agilent 1200
Column	: ZORBAX Eclipse Plus C18(100mm,2.1mm,1.8um)
Oven temp	: 40 C
Mobile phase	: A=ACN , B=0.1%HCOOH+10mMHCOONH4
	5%A---(20min)---45%B---(5min)---100%B
Flow rate	: 0.2 mL/min
Injection	: 5 µL
MS	: Agilent 6520 Q-TOF LC-MS
Ionization	: ESI (positive ion mode)
Nebulizer gas	: 345 kPa
Dry gas	: 10 L/min at 350C
Mass range	: 100-1000Da
Reference	: *m/z*=112.050873, *m/z*=922.009798
Resolution	: >10000 at *m/z*=121

Sample preparation

Detoxified Buhi, Uzu, urine, serum and gastric contents (GS) of patient samples were prepared using Solid Phase Extraction (SPE). The scheme is shown in Fig.3

Sample (0.5 ml or 0.5 g)
↓ add 10 mL of ethanol, shaking for 1h
centrifuge at 3,000 rpm for 20 min
Supernatant
↓ evaporate by N_2 gas
Residue
↓ dissolve in 0.4 ml of 10% ethanol
apply
FOCUS (conditioning with 1 mL of methanol and 1 mL of water)
↓ wash with water(1mL)
wash with 10% CH_3CN/water(1mL)
elute with 0.1% TFA/CH_3CN(0.75mL)
+0.2% ammonia/CH_3CN(0.75mL)
Eluate
↓ evaporate by N_2 gas
LC/TOFMS

Fig.3 Sample preparation scheme

Screening target compounds by exact mass database

Extracts were analyzed by LC/TOF-MS and 11 target *aconitum* alkaloids were screened using an exact mass and retention time database with the parameters shown in Tables 2

Table.2 Target screening parameter

Target mass	: $(M+H)^+$
Charge	: Single charge ion
Mass window	: 0.01Da
Retention time window	: 1 min
Relative mass error	: 5 ppm

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Results and Discussion

Analysis of 11 aconitum alkaloid standards

11 *aconitum* alkaloids and metabolite standards shown in Fig.2 were analyzed to construct exact mass and retention time database for target screening. Extract ion chromatograms (EICs) and mass spectra of these compounds are shown in Fig.4.

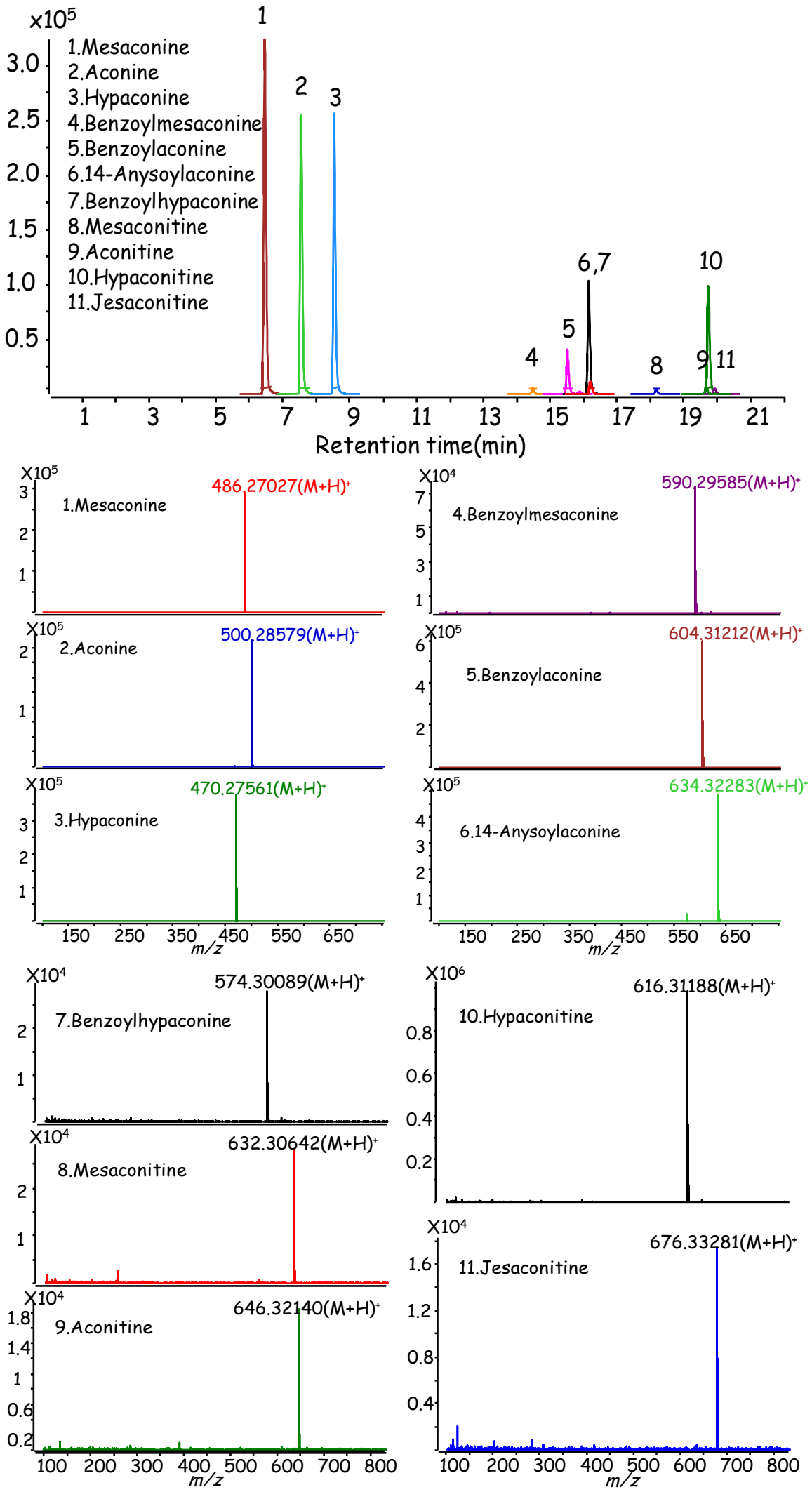

Fig.4 EICs and mass spectra of 11 *aconitum* alkaloids

Target screening of 11 *aconitum* alkloids

Extracts of the detoxified Bushi, Uzu, urine, serum and GS of the patient were analyzed. 11 *aconitum* alkaloids (Fig.4) were searched by using exact mass and retention time database. Mass chromatograms of 11 target alkaloids are shown in Fig.5. Abundance and relative mass error of each aconitum alkaloid are shown in Table 3.

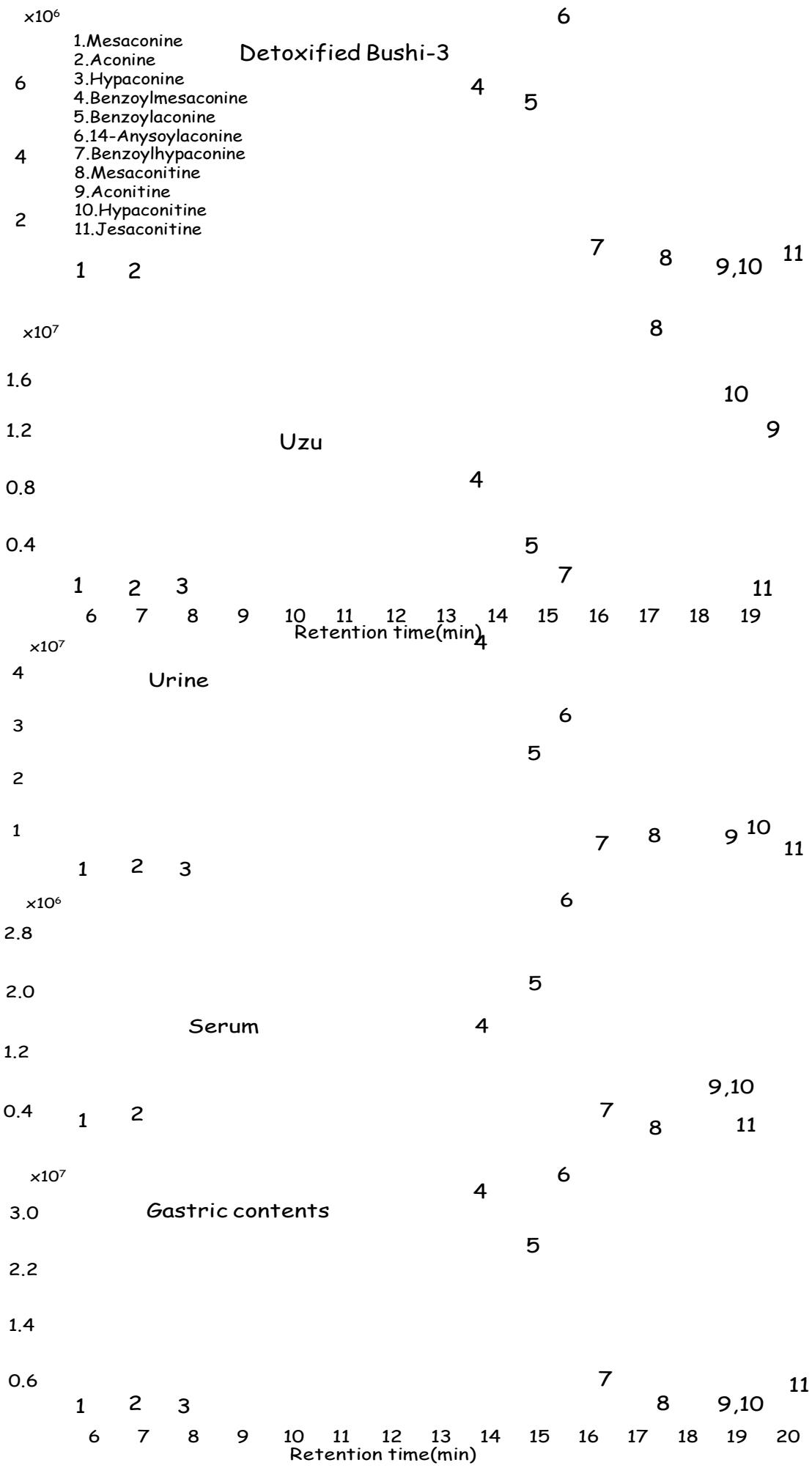

Fig.5 EICs of a*conitum* alkaloids in each extract

Comprehensive screening of *Aconitum* alkaloids in Kampo herbal medicine, in human serum and urine by high resolution LC/TOF Mass Spectrometry

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Table 3. Abundance and relative mass error of each *aconitum* alkaloid.

[redacted]

Non-target screening of *aconitum* alkloids

15 alkaloids and 12 lipo-alkaloids were searched using exact mass database. Mass chromatograms are shown in Fig.6. Abundance and relative mass error of each compound are shown in table 4.

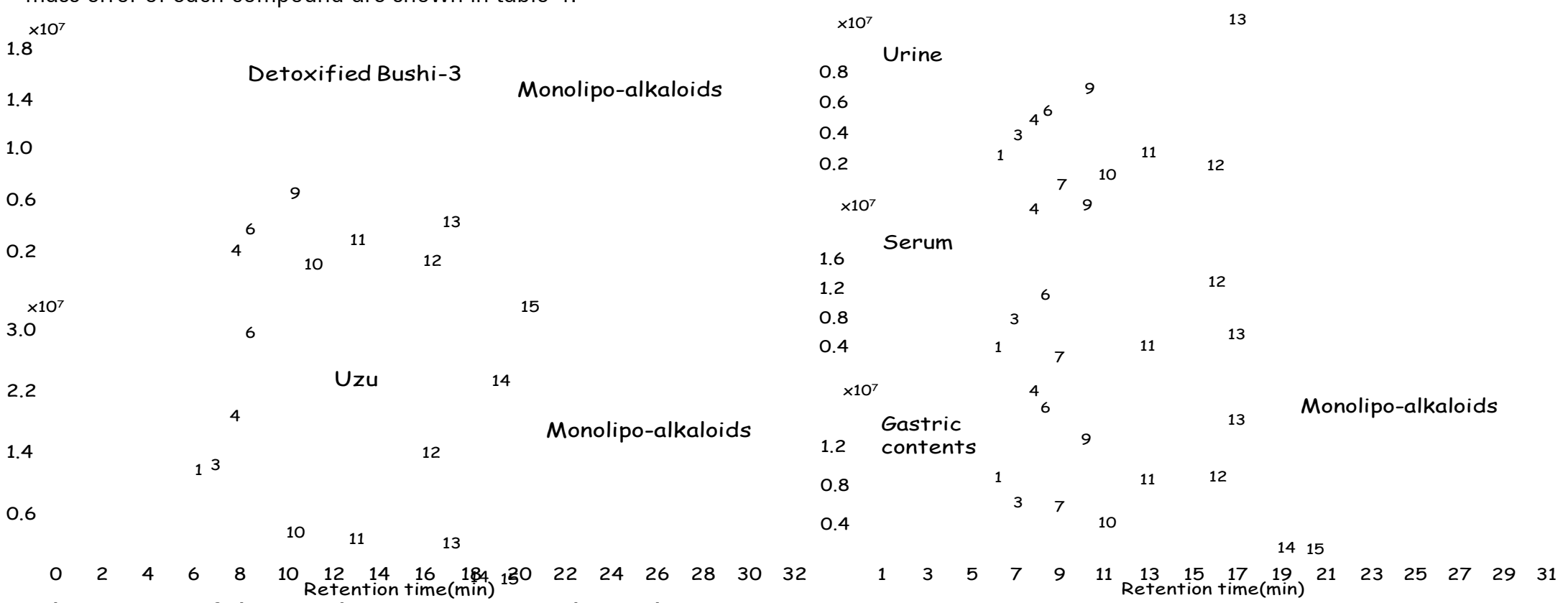

Fig.6 EICs of detected 19 components in each extract

Table 4. Abundance and relative mass error of each components.

[redacted]

Conclusions

1. 11 target aconitum alkaloids gave protonated molecules as the base ion in the mass spectrum with relative mass errors of less than 3 ppm.
2. All target alkaloids were detected in detoxified Bushi and Uzu. However, amounts of diester alkaloids in detoxified Bushi were less than 10% of the levels found in Uzu.
3. All diester alkaloids were detected in urine, serum and gastric contents of the patient.
3. 15 alkaloids and 4 lipo-alkaloids were identified in detoxified Bushi and Uzu using an exact mass database with less than 5 ppm error.
4. 4 lipo-alkaloids were detected in only gastric contents of the patient.
5. We have demonstrated that the use of LC-QToF together with an accurate mass database can be used to screen for targeted alkaloid compounds in herbal samples.