Highlights

- An original case of a death after roots powder mislabeled
- Alkaloids (ajmaline, yohimbine and reserpine) were quantified by an original LC-HR-MS method in blood and bile samples
- Consumption of counterfeit products available over the internet represent a worldwide danger

Death related to consumption of *Rauvolfia sp*. powder mislabeled as *Tabernanthe iboga*

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ABSTRACT

Powdered roots of iboga (*Tabernanthe iboga*) contain ibogaine, an alkaloid that has been used to treat addictions. We report the case of a 30-year-old woman who died after ingesting a powder labeled as *Tabernanthe iboga* she had bought online. Analysis of the powder revealed the absence of ibogaine but the presence of toxic alkaloids (ajmaline, yohimbine and reserpine) found in *Rauvolfia sp.* plant species. An original and specific LC-MS/MS method developed to quantify ajmaline, yohimbine and reserpine showed respective concentrations of 109.1 ng/mL, 98.2 ng/mL and 30.8 ng/mL in blood, and 1528.2 ng/mL, 914.2 ng/mL and 561.2 ng/mL in bile. Moreover, systematic toxicological analyses of biological samples showed the presence of oxazepam at therapeutic concentration and cannabinoids. Death could be attributed to ingestion of a substantial quantity of crushed roots of *Rauvolfia* in association with concomitant drug withdrawal.

Keywords: poisoning, *iboga*, *Rauvolfia*, ajmaline, yohimbine, reserpine

1. Introduction

Powdered roots of iboga (*Tabernanthe iboga*) contain ibogaine, an alkaloid considered by some authors, notably on the basis of experiments conducted in rats [1-4], to be a treatment for addiction [5-6]. This hypothesis was put forward as early as 1985 but it has not been scientifically established [7]. Nevertheless, use of iboga has increased due to its online availability. We report the case of a heroin addict who died after oral ingestion of a powder labeled *Tabernanthe iboga* bought online. We set about identifying this powder, and developed a specific method for quantification in blood and bile samples of alkaloids identified in the powder. The relationship between exposure to the powder and death is discussed in relation to the other results of forensic investigations.

2. Case report

2.1. Case history

A 30-year-old white woman, with a history of drug abuse and receiving methadone treatment, was found dead in the caravan where she was living by a friend. Foam was present at her mouth. Cannabis, methadone (one empty bottle with no indication of concentration), oxazepam and a small bag of powder labeled "Top quality Tabernanthe iboga 50g Gabon, Africa" were found in her caravan. This powder would have been consumed orally, mixed with juice or as an infusion, to treat opiate withdrawal symptoms. A friend of the victim said that she had already used iboga in the past as an indication for opiate withdrawal and that she had stopped taking opiates two days before her death, describing loss of appetite, diarrhea and severe fatigue. Her medical history was unremarkable except for anorexia.

2.2. Autopsy findings

An autopsy was performed four days post-mortem. External examination found no lesions other than non-specific injuries on the legs. The autopsy showed congestion of internal organs and pulmonary edema. No organs were injured, and although the cause and manner of death were not established, trauma was definitely ruled out. No previous pathological state was found. Samples of femoral blood, bile and hair were taken for toxicological analysis. Urine was not available (empty bladder). Histopathological analysis of the heart showed no macroscopic or microscopic abnormalities.

3. Analytical methods

Chemicals: *Tabernanthe iboga* was generously donated to us by Dr Alexandre Maciuk (Paris Sud University, France). *Rauvolfia vomitoria* powder and the alkaloids of *Rauvolfia serpentina* (ajmaline and reserpine) were available in the Pharmacognosy's laboratory of Faculty of Pharmacy, Rennes, France. Ajmaline (Gilurytmal®) was purchased from Carinopharm GmbH (Elze, Germany). Formic acid, ammonium acetate, yohimbine, reserpine and flurazepam, as the internal standard (IS), were purchased from Sigma Aldrich (St Louis, MO, USA). Methanol, acetonitrile and water were purchased from Fisher Scientific UK (Loughborough, Leicestershire, UK). All chemicals, reagents and solvents were of LC–MS grade quality. Stock solutions of ajmaline, reserpine, yohimbine and IS were prepared in methanol (1.0 g/L) and then stored at 4°C.

3.1. Analysis of powders

Powders of *Rauvolfia vomitoria*, *Tabernanthe iboga* and the unidentified powder (found in the caravan) were examined under a microscope (Olympus CX41) equipped with a camera (Olympus DP20) in bidistilled water.

Two extractions were carried out, one by maceration in methanol under ultrasonic shaking and a second one based on the specific protocol for extracting alkaloids [8]. Briefly free lipophilic alkaloids were extracted by diethylether in alkaline solution. Then, liquid-liquid extraction was performed after series of acidification and alkalinisation to remove fat soluble impurities and to purify the alkaloids.

The *Tabernanthe iboga* and *Rauvolfia vomitoria* extracts and the standards of yohimbine, ajmaline, reserpine, and alkaloids from *Rauvolfia serpentina* were used to identify compounds in the unknown powder. An automated sample applicator, CAMAG ATS-4, was used for spotting High Performance Thin Layer Chromatography (HPTLC) plates and a densitometric UV scanner, CAMAG Scanner type 3, was used for plate analysis (CAMAG, Muttenz, Switzerland). Both instruments were controlled by Wincats software version 1.4.3. The HPTLC analysis for all extracts was carried out on silica gel plates (Merck silica gel $60F_{254}$) with a mobile phase consisting of diethylether/acetone/diethylamine (85/8/7). Ten microliters of extracts (1 $mg.mL^{-1}$) were spotted on TLC plates. After development, plates were scanned using the scanner at 6 wavelengths: 220, 260, 290, 310, 340 and 370 nm. Spot identification

relied on the Rf value, on maximum absorbance wavelength and on visual evaluation after spraying Dragendorff reagent.

3.2. Mass spectrometry analysis

3.2.1. Screening analyses

Powders and biological samples screening analyses were performed using two chromatography systems: GC–MS (Gas Chromatography Mass Spectrometry) and LC-HR-MS (Liquid Chromatography High Resolution Mass Spectrometry) on a Thermo Scientific Finnigan Trace GC Ultra DSQ II (Thermo Scientific, San Jose, USA) and on a Thermo Scientific Q Exactive (San Jose, USA) mass spectrometer, respectively.

3.2.2. Quantitative analyses of biological samples

After decontamination with dichloromethane, hair (16 cm) was segmented into 2 cm segments, extracted and analyzed as per Society of Hair Testing guidelines [9]. Blood was extracted according to specific methods to detect various type of analytes (i.e. psychotropic, narcotic or opioids withdrawal medication). Briefly samples were extracted by liquid-liquid extraction (LLE) or solid phase extraction (SPE) on Bond Elut Agilent C18 (200 mg, 3 mL) columns before analysis under different chromatographic conditions with a LC–MS-MS system (Thermo Scientific, San Jose, USA) including an Accela pump and a tandem triple quadrupole mass spectrometer (TSQ Quantum Ultra) equipped with an electrospray ionization (ESI) source. Data acquisition, peak integration and calibration were performed using Xcalibur 2.1 software.

3.2.3. Quantification of alkaloids

Blood (100 µL) or bile (10 µL) samples were supplemented with 20 µL of 1 µg/mL flurazepam (IS) solution and extracted by basic liquid-liquid extraction (LLE) after adding 2.3 mL of a solvent mix (heptane : isopropanol : 1,2-dichloroethane : dichloromethane (33:17:25:25) (v/v)), 1.8 g of a powders mix (sodium chloride : sodium bicarbonate : sodium carbonate (84:8:8) (w/w)) and 4.5 mL of deionized water (pH=8.9) then mixing by gentle inversion for 10 min. After 10 min centrifugation at 3000 g, supernatants were evaporated to dryness at 50°C under a stream of nitrogen. Residues were dissolved in 500 µL of mobile phase at initial condition (90:10 A:B (v/v)) with solvent A corresponding to 10 mM ammonium acetate buffer containing 0.1% (v/v) formic acid and solvent B consisting of acetonitrile with 0.1% (v/v) formic acid. The analysis was performed by a LC-HR-MS

system equipped with a Thermo Scientific Q Exactive™ (San Jose, USA) mass spectrometer system.

LC separation was performed by an Accela pump (Thermo Scientific, San Jose, USA) on a C18 Accucore column (100 mm x 2.1, 2.6 µm) at a flow rate of 500 µL/min using the following gradient elution : initial conditions of 90:10 (A:B) maintained for 1 min, increasing to 40:60 (A:B) for 4 minutes, and then 1 min for 100% B and return to initial conditions 90:10 (A:B) for equilibration. This corresponded to a total chromatographic run of 8 minutes. All samples were kept at 15°C in the autosampler until injection of 5 µL into the LC-HR-MS system (partial loop) in a thermostated column at 25°C. The MS conditions were as follows: HESI in positive mode, capillary temperature: 300°C; spray voltage: 4500 V; sheath and auxiliary gas (nitrogen) flow-rate: 35 and 15 (arbitrary units), respectively. Data were acquired in targeted MS^2 (t-MS^2) mode, where MRM transitions for quantitative determination were 327.2060 → 144.0805 m/z for ajmaline, 355.2050 → 144.0805 m/z for yohimbine and 609.2796 → 195.0650 m/z for reserpine whereas second transitions were used for confirmation purposes (Table 1). Seven-point calibration curves were obtained by fortifying drug-free human blood with working solution of ajmaline, reserpine, yohimbine at final concentrations of 0, 10, 50, 100, 500, 1000 and 5000 ng/mL. Standard curves corresponded to peak area ratios of each analyte to IS using weighted linear least-squares regression ($1/x^2$) and were linear in the range of 10 to 5000 ng/mL (r^2 >0.99 for ajmaline, reserpine, yohimbine).

4. Results

Powders of *Tabernanthe iboga*, *Rauvolfia vomitoria* and the unknown powder were yellow-brownish. Microscopically, all of them showed lignified cork on surface view, starch grains with distinct hilum, isodiametrical and elongated sclereids. Tracheids and xylem vessels were clearly found only in *R. vomitoria*. The unknown powder contained isolated sclereids — not grouped as in iboga — but also simple or grouped starch grains like in *R. vomitoria*, therefore ruling out iboga. Moreover, many prismatic crystals of calcium oxalate were found in the unidentified powder as in *R. vomitoria*. In conclusion, this powder, purportedly derived from roots or barks of *Tabernanthe iboga*, was macroscopically similar to those of the *Apocynaceae* family and had microscopic findings closer to *Rauvolfia* species than to *Tabernanthe iboga*.

Preliminary studies using chromatographic HPTLC analyses show that the methanolic extract and the purified fractions of the unknown powder were different from that of iboga powder (data not shown). Ajmaline and reserpine were only found in *R. vomitoria* and *R. serpentina* as major alkaloids. Screening of the powders by LC-HR-MS and GC-MS showed the presence of ajmaline, reserpine and yohimbine in *R. serpentina*, *R. vomitoria* and the unknown powder. Ibogaine was not found in these powders whereas analysis of authentic *Tabernanthe iboga* powder using the same screening methods confirmed the presence of ibogaine and ibogamine, two indole alkaloids usually contained in iboga roots.

Toxicological analyses of blood samples reported the absence of alcohol and the presence of oxazepam (100.6 ng/mL), delta-9-tetrahydrocannabinol (THC) (3.01 ng/mL) and carboxy-tetrahydrocannabinol (THC-COOH) (105.2 ng/mL). Liquid Chromatography High Resolution Mass Spectrometry (LC-HR-MS) screening revealed the presence of ajmaline, yohimbine and reserpine in blood and bile samples, and the absence of ibogaine, as in the unknown powder (Figure 1). A specific LC-MS/MS method developed to quantify ajmaline, yohimbine and reserpine showed respective concentrations of 109.1 ng/mL, 98.2 ng/mL and 30.8 ng/mL in blood, and 1528.2 ng/mL, 914.2 ng/mL and 561.2 ng/mL in bile.

Hair analysis revealed the presence of morphine, 6-monoacetylmorphine, amphetamine, MDMA (3,4-methylenedioxy-methamphetamine), cocaine, benzoylecgonine, methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), buprenorphine, norbuprenorphine, acetaminophen and ketamine in all hair segments (Table 2). Alkaloids present in the powder were not detected in hair samples when using the specific analytical method.

5. Discussion

Microscopic and toxicological analyses of the powder labeled *Tabernanthe iboga* demonstrated the absence of ibogaine and the presence of ajmaline, yohimbine and reserpine. Allowing that the powder was not *Tabernanthe iboga*, as the labelled on the package. The powder was rather obtained from *Rauvolfia sp*., a shrub of the more common and less expensive *Apocynaceae* family [10]. *Rauvolfia sp*. can easily be confused with *Tabernanthe*

iboga, since powders derived from the crushed roots of these plants were macroscopically similar. *Rauvolfia* genus contains about sixty plant species which are found in all tropical and subtropical regions. Their roots contain a high number of alkaloids including ajmaline, reserpine and yohimbine [11-12].

Alkaloids identified in the powder have also been identified in blood of the deceased, so it can be concluded that she had recently consumed this powder. They were also identified in the bile, at higher concentrations than in post-mortem blood, which is consistent with descriptions in the literature [13]. Furthermore, cannabis and oxazepam were also found in the blood at very low concentrations, such that they can be excluded as contributors to death. Toxicological analysis of the hair confirmed the addiction to narcotics.
Data on the toxic or lethal concentrations of these alkaloids are virtually non-existent. According to literature data, the measured concentrations corresponded, at least for ajmaline and yohimbine, to concentrations observed after consumption of therapeutic doses (200 to 1000 ng/mL for ajmaline; 50 to 300 ng/mL for yohimbine; 1400 to 1800 ng/mL for reserpine), without symptoms of toxicity, and were far below lethal concentrations (above 5500 ng/mL for ajmaline; unknown for yohimbine and reserpine) [14, 15].

The low blood concentrations must be interpreted with caution, taking into account certain limitations; although the body was discovered shortly after death, the autopsy was carried out 4 days later. In the absence of data on the stability of these compounds, one cannot rule out post-mortem degradation and hence underestimation of their concentration in the blood at the time of death. Moreover, degradation of ajmaline and yohimbine leads to formation of active metabolites such as ajmaline-N-oxide and hydroxy-yohimbine, which were not identified. The *Rauvolfia sp*. powder also possibly contained other xenobiotics that could not be identified using the laboratory's analytical techniques, and so their possible involvement in the death cannot be discussed.

There are few scientific data about the toxicity of *Rauvolfia*, which varies in accordance with the nature and concentration of alkaloids contained in the plant. The alkaloids are primarily concentrated in the roots and especially in the bark [11]. The concentration of alkaloids is affected by the *Rauvolfia* species, the geographical area where it grows, the age of the plant, the season and the harvesting technique [11, 16]. The root extract is used, in south Asia, as the

main constituent of certain medications notably used to treat high blood pressure and certain mental illnesses [17].

Reserpine is described as the most potent alkaloid of the plant, responsible for the hypotensive activity of *Rauvolfia* [16]. It acts on receptors of the central and peripheral nervous system, lowering the level of endogenous bioamines (norepinephrine, serotonin and dopamine), by specifically inhibiting the uptake of these amines by storage vesicles [18]. This mechanism induces depression of the central nervous system and a reduction in blood pressure [17-18]. Reported cases of reserpine intoxication are rare. The observed toxic effects were gastrointestinal (hypersalivation, gastric hypersecretion, nausea, vomiting, diarrhea), skin flushes, nasal congestion, sedation or even coma, hypotension and bradycardia [19]. As with any substance acting on a tissue receptor, the blood concentration of the xenobiotic is not representative of its clinical effects, and if intoxication is suspected, the blood concentration is a priori uninformative for assessing clinical gravity. Moreover, a characteristic of the molecule is that a low proportion of administered reserpine binds specifically and irreversibly to its site of action [20-21] leading to long-lasting clinical signs. After the end of consumption of reserpine, these clinical signs only subside when storage of endogenous bioamines is restored, a process which requires several days or even weeks. A study was carried out to evaluate the efficacy of *Rauvolfia serpentina* in people with essential hypertension: the hypotensive effect induced by ingestion of *R. serpentina* persisted 2 to 4 weeks following discontinuation of the "treatment" [22]. The fraction bound to tissue receptors, responsible for clinical signs, is not detectable in blood, while the free form (not bound to the receptor, quantifiable in the blood), representing the inactive portion, is metabolized and/or excreted [23]. A clinical-biological discordance is therefore possible, in which clinical or even toxicity symptoms are present when reserpine is no longer detectable or is detected in small amounts in the blood [24].

The potentially toxic nature of the other alkaloids identified in the powder must not be under-estimated. Ajmaline is an inhibitor of β-adrenergic receptors used for its antihypertensive and antiarrhythmic properties [25]. Its toxicity is primarily cardiac, by induction of rhythm and conduction disorders [26-31]. One of the metabolites of ajmaline, ajmaline-N-oxide, is pharmacologically active in animals [31]. Yohimbine is a selective antagonist of α2-adrenergic receptors that increases the release of norepinephrine and dopamine [32]. One of its metabolites, 1-hydroxy-yohimbine, is pharmacologically active [33]. Among the rare reported cases of yohimbine intoxication, the adverse events included: increase in blood pressure, tachycardia, paresthesias, loss of coordination, headaches, gastrointestinal disorders,

agitation and even coma [25, 34]. A case of atrial fibrillation after ingestion of 350 mg yohimbine has been reported [35].

The toxicity of each of these alkaloids, considered separately, is partially known, but there are very few scientific articles concerning the toxicity of these alkaloids consumed concomitantly. There is one report in which medicinal plants were adulterated by alkaloids derived from *R. serpentina* (containing ajmaline and reserpine), in a dietary supplement of Italian origin: the people who ingested the powder experienced hypotensive problems [36]. To our knowledge, there are no cases of death in the literature related to simultaneous consumption of these three alkaloids. The symptoms reported by witnesses, nausea and diarrhea in combination with fatigue, are compatible with opiate withdrawal syndrome. However, muscle pain, rhinorrhea, hyperhidrosis, increased gastrointestinal motility with diarrhea and vomiting have also been described in association with reserpine or yohimbine intoxication. It is therefore not possible, on the basis of symptomatology, to distinguish between the withdrawal syndrome and possible intoxication by *Rauvolfia*.

6. Conclusion

Post-mortem investigations did not find any active pathological processes at the time of death or a previous pathological state. Exposure to a powder containing three toxic alkaloids could be demonstrated. These data suggest that the death, which occurred violently after initiation of the “treatment”, was due to the cardiovascular effects of the powder. The present case is therefore the first reported case of death following consumption of *Rauvolfia sp*. powder mislabeled as *Tabernanthe iboga*. It highlights the worldwide problem of counterfeit products available over the internet and the danger associated with their consumption.

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LEGENDS

Figure 1. Analysis of alkaloids in unknown powder (A) and blood samples (B) : Chromatograms of protonated ajmaline, yohimbine, and reserpine

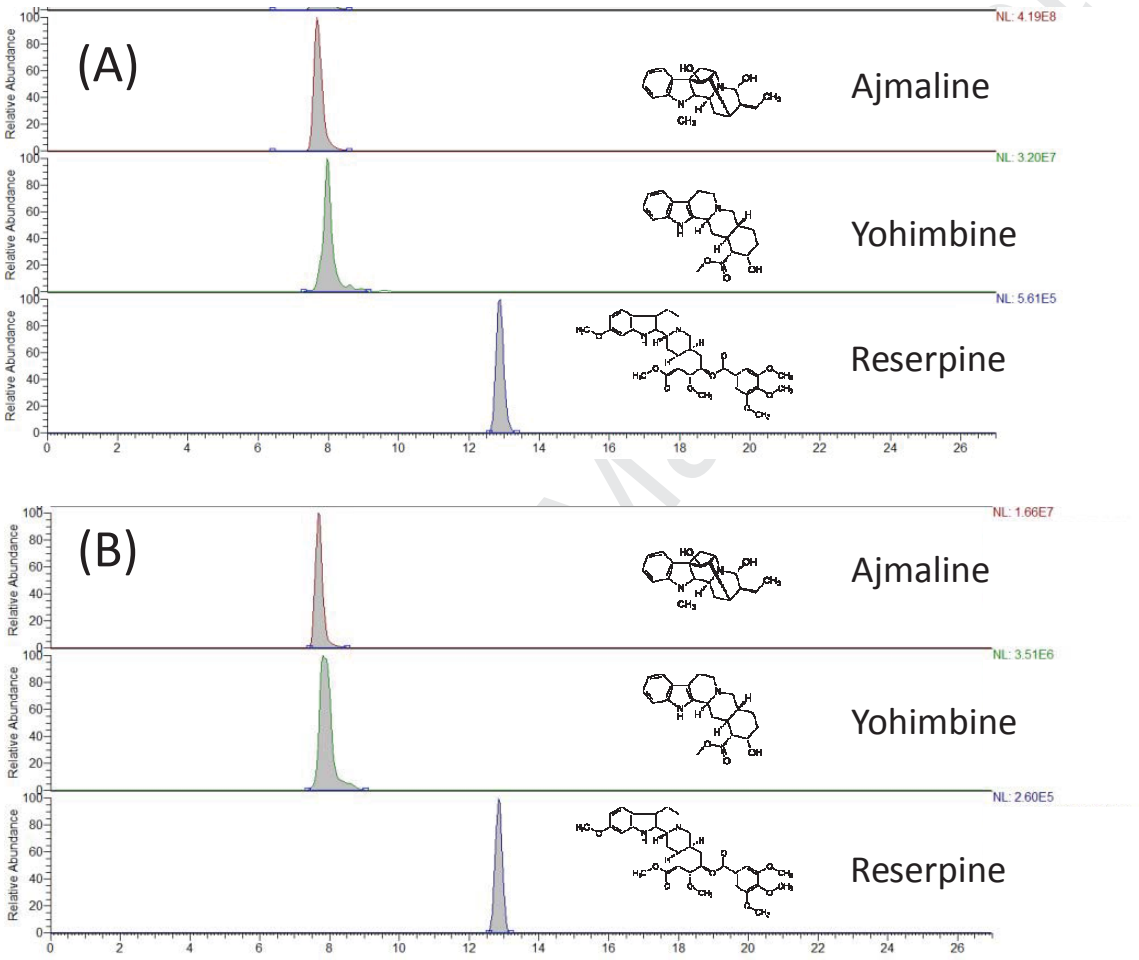

Table 1. Reaction monitoring parameters for ajmaline, yohimbine, flurazepam and reserpine. (RT = retention time, NCE = Normalized Collision Energy)

Compound	**Chemical formula**	**RT (min)**	**Precursor ion $[M+H]^+$ (*m/z*)**	**NCE (%)**	**Product ion (*m/z*)**
Ajmaline	$C_{20}H_{26}N_2O_2$	3.0	327.2060	50	144.0805
				50	158.0963
Yohimbine	$C_{21}H_{26}O_3N_2$	3.2	355.2050	50	212.1277
				50	144.0805
Flurazepam	$C_{21}H_{23}ClFN_3O$	3.8	388.1586	50	315.0686
Reserpine	$C_{33}H_{40}N_2O_9$	4.6	609.2796	50	174.0911
				50	195.0650

Table 2. Concentrations in hair segments. Segment 1 is the most recent and segment 8 is the oldest. (ND = not detected)
(6-MAM = 6-MonoAcetylMorphine ; MDMA=3,4-methylenedioxy-methamphetamine ; EDDP=2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine)

Concentration (ng/mg)	Segment 1	Segment 2	Segment 3	Segment 4	Segment 5	Segment 6	Segment 7	Segment 8
Morphine	15.6	2.0	1.7	1.8	1.7	2.1	1.5	1.8
6-MAM	0.1	0.2	0.2	1.2	0.2	0.3	0.2	0.2
Amphetamine	7.0	6.1	3.5	2.2	1.8	1.7	1.7	1.7
MDMA	4.2	3.8	6.0	4.1	2.3	2.2	2.1	2.5
Cocaine	1.4	2.4	3.5	4.7	5.2	6.8	5.9	8.7
Benzoylecgonine	0.4	0.6	1.0	1.3	1.7	3.2	2.7	4.4
Methadone	6.5	8.4	6.7	4.8	3.4	3.6	2.9	2.1
EDDP	0.7	1.1	0.8	0.6	0.2	0.6	0.4	0.3
Buprenorphine	ND	ND	ND	ND	ND	ND	ND	ND
Norbuprenorphine	ND	ND	ND	ND	ND	ND	ND	ND
Acetaminophen	13.0	13.5	13.8	13.7	13.6	13.7	13.6	13.6
ketamine	0.92	0.33	0.31	0.41	0.49	0.47	0.5	0.56