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A fatal case of intoxication from a single use of eutylone: clinical symptoms and quantitative analysis results

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Declarations of interest

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A fatal case of intoxication from a single use of eutylone: clinical symptoms and quantitative analysis results

Abstract

Eutylone is a synthetic cathinone that is becoming an increasingly popular drug in the US and Europe. This report describes a fatal case of eutylone intoxication. A 32-year-old man went into cardiac arrest after several minutes of abnormal behavior. Rectal temperature was 37.0°C at 5 h after death. Autopsy revealed no remarkable injuries apart from several small abrasions and no signs of rhabdomyolysis. Toxicological examination revealed only aripiprazole in the therapeutic range and eutylone. The eutylone concentration in cardiac blood was 4,290 ng/g. This case is valuable because it involved fatal intoxication from a single use of eutylone and quantitative analysis, whereas most previous reports of eutylone intoxication have involved a mixture of drugs with limited quantitative analysis.

Keywords

Eutylone, intoxication, new psychoactive substances, synthetic cathinone, forensic toxicology

1. Introduction

New psychoactive substances (NPS) are defined by the United Nations Office on Drugs and Crime as “substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat” [1] and have spread around the world since the 2000s. NPS are classified into numerous groups, such as synthetic cannabinoids, which have high affinity for cannabinoid receptors, synthetic cathinones, which are based on a β-ketophenethylamine structure, synthetic phenethylamines, which include multiple 2C molecules and show high affinity for 5-HT_{2A} receptors, synthetic cocaine, synthetic opioids, and GABA-A/B receptor agonists [2].

Eutylone (β-keto-1,3-benzodioxolyl-*N*-ethylbutanamine, also known as bk-EBDB and *N*-ethylbutylone) is a synthetic cathinone with a structure similar to that of 3,4-methylenedioxymethamphetamine (MDMA) and was first synthesized at Germany in 1967 along with butylone, pentylone, dibutylone, and *N*-ethyl pentylone [3]. In Europe, use of eutylone as a drug of abuse has been reported since the 2000s, and it has been found as a contaminant in pills sold as “Ecstasy” [1]. Eutylone was also detected as an ingredient in drugs sold as “Ecstasy”, “Molly”, and “MDMA” in the US from 2014 to 2017 [4]. The popularity of eutylone in the US increased during 2019, and the Center for Forensic Science Research & Education has recently cited it as one of the drugs most frequently identified during forensic investigations [5].

Although eutylone is drawing attention, there have been few reports on eutylone intoxication that include quantitative analysis. Here we report a fatal case of intoxication from a single use of eutylone determined by quantitative analysis. This is the first such case reported in Japan.

Abbreviations: MDMA, 3,4-methylenedioxymethamphetamine; NPS, new psychoactive substances

2. Case Report

One day in October, a 32-year-old man came out of an apartment in his underwear. He was shouting words such as ‘Ouch!’ and ‘I’m sorry’, running around, rolling on the ground in a parking space, and jumping on a truck. When a police officer arrived at the scene, the man was sitting on a bench but unable to communicate. About 20 min after the first observation of abnormal behavior, the man lay down prone on the ground and stopped moving. The police officer turned him over and found him to be unconscious. The man was transported to the hospital by ambulance but was in cardiac arrest (pulseless electrical activity at the scene and asystole in the emergency department) and he was pronounced dead on arrival, about 50 min after the emergency call was made.

The man had a four-year history of schizophrenia for which he had been receiving an intramuscular injection of aripiprazole 400 mg once a month without any oral medication. His last visit to the hospital was almost a month before his death.

The police found a grinder, glass pipe, and filter for smoking of cannabis, three empty plastic bags labeled “MDMA”, two plastic bags labeled “HOP” and filled with white powder, two paper bags labeled “Arigato”, two unlabeled microtubes filled with white powder, and a cup containing white powder.

2.1. Autopsy findings

Autopsy was performed 2 days after death. Height was 176.0 cm and weight was 87.0 kg. Rectal temperature measured 5 h after pronouncement of death was 37.0°C (ambient temperature 22°C). There were no remarkable injuries apart from several small abrasions on the face and extremities. His skeletal muscles were intact without macroscopic liquefactive necrosis. Cardiac blood was solid without coagulation and the internal organs were congested. Both lungs were severely congested and edematous (weight, 743.4 g on the left

and 877.6 g on the right). Microscopic examination revealed moderate fatty liver and proximal and distal convoluted tubular necrosis in the kidneys but no inflammatory cell infiltrates.

The results of a blood chemistry panel in the emergency department were as follows: white blood cell count 18,500/μL, C-reactive protein 11.88 mg/dL, total protein 9.3 g/dL, albumin 6.00 g/dL, blood urea nitrogen 32.5 mg/dL, creatinine 3.30 mg/dL, sodium 156.5 mEq/L, and hemoglobin A_{1c} 8.7%.

Alcohol examination performed using gas chromatography (GC-14B-FID; Shimazu Corporation, Kyoto, Japan) revealed no alcohol in blood obtained in the emergency department or in blood and urine collected at autopsy.

2.2. Toxicological methods and findings

2.2.1 Preliminary toxicological investigation

The preliminary toxicological investigation was performed using STAT CASSETTE™ (Items Inc., Tokyo, Japan) and Instant-view® K2 (Alpha Scientific Designs, CA) drug screening kits with a urine sample collected during autopsy. The results were positive for methylenedioxymethamphetamine and synthetic marijuana, respectively.

2.2.2 Sample preparing and LC-MS/MS conditions

Blood, urine samples, and gastric contents collected at autopsy as well as peripheral blood obtained in the emergency department were stored at −20°C until instrumental toxicological examination could be performed. Analytical standards for eutylone hydrochloride and aripiprazole were purchased from Supelco® (Merck, Darmstadt, Germany) and Cerilliant (Round Rock, TX), respectively.

Samples were prepared using the QuEChERS method [6]: 0.5 g of a pre-packaged extraction

preparation of a QuEChERS extraction kit (AOAC method; Agilent Technologies, Santa Clara, CA) and 1.5 mL of acetonitrile were added to samples diluted with pure water and stirred for 20 s using a stainless steel ball. After centrifugation (3000 rpm, 10 min), 1 mL of the acetonitrile layer was added to a dispersive-solid phase extraction sorbent for fruits and vegetables (AOAC method; Agilent Technologies), stirred for 30 s, and centrifuged (12000 rpm, 5 min). The final solution was filtered using an Ultrafree-MC-GV centrifugal filter (Merck) and injected into a liquid chromatography-tandem mass spectrometry (LCMS) system.

LCMS was performed using a Prominence UFLC™ (Shimazu Corporation) and an LTX XL™ (Thermo Fisher Scientific, MA) with an L-Column2 ODS (Chemicals Evaluation and Research Institute, Tokyo, Japan) at 40°C. The mobile phase was 10 mM ammonium acetate (A) and methanol (B). The solvent gradient was started at 95% A/5% B, was linearly changed to 5% A/95% B over 7.5 min, was held for 5 min, and was then returned to 95% A/5% B over 0.1 min. Finally, this ratio was maintained for 5.4 min. The flow rate was 0.2 mL/min and injection volume was 2 µL.

2.2.3 Results of screening assay and qualitative analysis

A screening assay was performed using the original database of the National Research Institute of Police Science and the results were positive for eutylone and aripiprazole in the cardiac blood.

Figure 1 shows the extracted ion chromatogram at m/z 236 from the cadaveric blood sample and the analytical standard for eutylone, while Figure 2 shows a comparison of the product ion spectrum between the blood sample and the analytical standard for eutylone. Eutylone and aripiprazole were also detected in the urine and fat tissue specimens collected at autopsy, and in the peripheral blood obtained in the emergency department. The gastric contents were

positive for eutylone alone.

2.2.4 Quantitative analysis

Quantitative analysis of the blood sample and gastric contents obtained during autopsy was performed using the standard addition method with a six-point calibration curve. Samples for quantitative analysis were prepared as follows: 40 μL of blood was measured with a precision mass scale and diluted to 20 mL for eutylone quantification; 400 μL of blood was diluted to 20 mL for aripiprazole quantification; and 200 μL of gastric supernatant was diluted to 50 mL for eutylone quantification. Methanol solutions of eutylone were prepared at concentrations of 0, 1, 2, 3, 4, and 5 mg/L and of 0, 0.8, 1.6, 2.4, 3.2, and 4.0 mg/L for quantitative standards of blood and gastric contents, respectively. Aripiprazole solutions were prepared similarly at concentrations of 0. 0.2, 0.4, 0.6, 0.8, and 1.0 mg/L. Then, 10 μL of each quantitative standard was added to 0.5 mL of diluted blood sample before addition of a pre-packaged extraction preparation, using the QuEChERS method described above. The concentration values of the compounds in diluted samples were measured by standard addition calibration curves, then respective concentration values of target compounds in authentic samples were calculated according to magnitudes of each dilution. The concentrations of urine, fat tissue, and peripheral blood obtained in the emergency department were calculated using one of the calibration curves of the blood analysis as the standard curve due to the limited amount of the samples. The results and the representative equation of the calibration curves are shown in Table 1.

Table 1. Eutylone and aripiprazole concentrations in each sample

Sample	Eutylone concentration	Aripiprazole

	(ng/g ± S.D.**)	concentration (ng/g ± S.D.**)
Cardiac blood (autopsy)	4,290 (±167)	49.1 (±8.82)
Representative equation	y = 4252.8x + 22356 (R^2 = 0.9996)	y = 106267x + 4025.9 (R^2 = 0.9994)
Urine*	192,000	34.5
Gastric contents*	2,120 (±63.4)	-
Representative equation	y = 13.434x + 28747 (R^2 = 0.9996)	
Fat tissue	1,310	358
Peripheral blood (emergency department)	2,500	26.7

*Calculated with specific density as 1

**Results for the cardiac blood (autopsy) and gastric contents were determined from the mean of three trials

3. Discussion

We encountered a fatal case of eutylone intoxication in which quantitative analysis revealed only eutylone and aripiprazole. The blood concentration of aripiprazole was 49.1 ng/g in the sample obtained at autopsy and 26.7 ng/g in the sample obtained in the emergency department; both these values are lower than the reported mean concentration on day 28 after intramuscular injection of aripiprazole 400 mg [7]. In addition, the gastric contents included eutylone without aripiprazole, indicating the ingestion of eutylone alone before death. Therefore, this case seems to involve intoxication by eutylone alone. We diagnosed the cause of death as eutylone intoxication and judged the manner of death to be accidental

intoxication.

According to the police investigation, eutylone was also detected in a cup and in one of the plastic bags at the scene. Unfortunately, information about the precise amount of eutylone was not shared by the police. Although the presence of a grinder, glass pipe, and filter in the man's room suggested that he used illegal substances such as cannabis habitually, it is unclear whether this was his first time using eutylone or not.

Eutylone has been detected as one of the substances in drugs sold as "Ecstasy" [1,4]. Despite the increasing popularity of eutylone, there are few case reports of intoxication with this drug or reports that include quantitative analysis. Chen et al. reported 11 clinical cases of eutylone intoxication, including one that was fatal, in which there was a wide range of drug concentrations in urine [8]. The large case series reported by Krotulski et al. was the first to include a quantitative analysis [9]. Their series included 83 fatalities, and a detailed history was available in 22 cases of these cases, most of which involved multi-drug intoxication with ethanol, cocaine, fentanyl, methamphetamine, or an NPS other than eutylone. Three of the 22 cases were diagnosed as death due to eutylone intoxication and there was only one case in which eutylone alone was detected; the blood eutylone concentration was 3,100 ng/mL in that case. Although the interaction with aripiprazole in our case cannot be ignored, the blood concentration of 4,290 ng/g provides further information on intoxication from a single use of eutylone.

The concentrations of both eutylone and aripiprazole were 1.7 to 1.8 times higher in the cardiac blood collected during autopsy, which was performed 2 days after death, than in the peripheral blood taken during the resuscitation procedure. This disparity may be due to postmortem redistribution. Because none of the cases reported by Krotulski et al. [9] involved eutylone quantification at different postmortem intervals, our case is the first to suggest postmortem changes in blood concentration. Therefore, further studies should be conducted

to better understand postmortem redistribution. Skov et al. compared the concentration of aripiprazole between cadavers and living patients and concluded that the concentration of aripiprazole does not increase due to postmortem redistribution [10]. However, our results show that the blood concentration of aripiprazole, at least given intramuscularly, increases after death which is reasonable considering its high distribution in fat tissue [11].

The clinical history and physical findings in this case are limited but interesting. Abnormal behavior, implying delirium or agitation, was observed before death in this case. Rectal temperature was 37.0°C at 5 h after death, suggesting severe hyperthermia before death. Although there were no obvious findings of rhabdomyolysis, elevated blood sodium, total protein, and albumin levels suggested severe dehydration. The psychiatric symptoms and hyperthermia in this case are similar to the symptoms observed in previously reported cases of eutylone intoxication [8]. Moreover, dehydration and renal failure are clinical features consistent with intoxication involving synthetic cathinone [12].

4. Conclusions

This is the first report of fatal eutylone intoxication in Japan. This case is valuable because it involved fatal single-drug intoxication with eutylone and quantitative analysis, whereas most of the previous reports have involved mixed-drug intoxication. The supply of cathinones has increased worldwide over the past decade [13] and new NPS are expected to emerge. As in Europe and South America, eutylone is becoming a frequently encountered drug in Asia. In cases of intoxication, it may be detected alone or in combination with a mixture of drugs. Accumulation of further cases is required to evaluate its toxicity in more detail.

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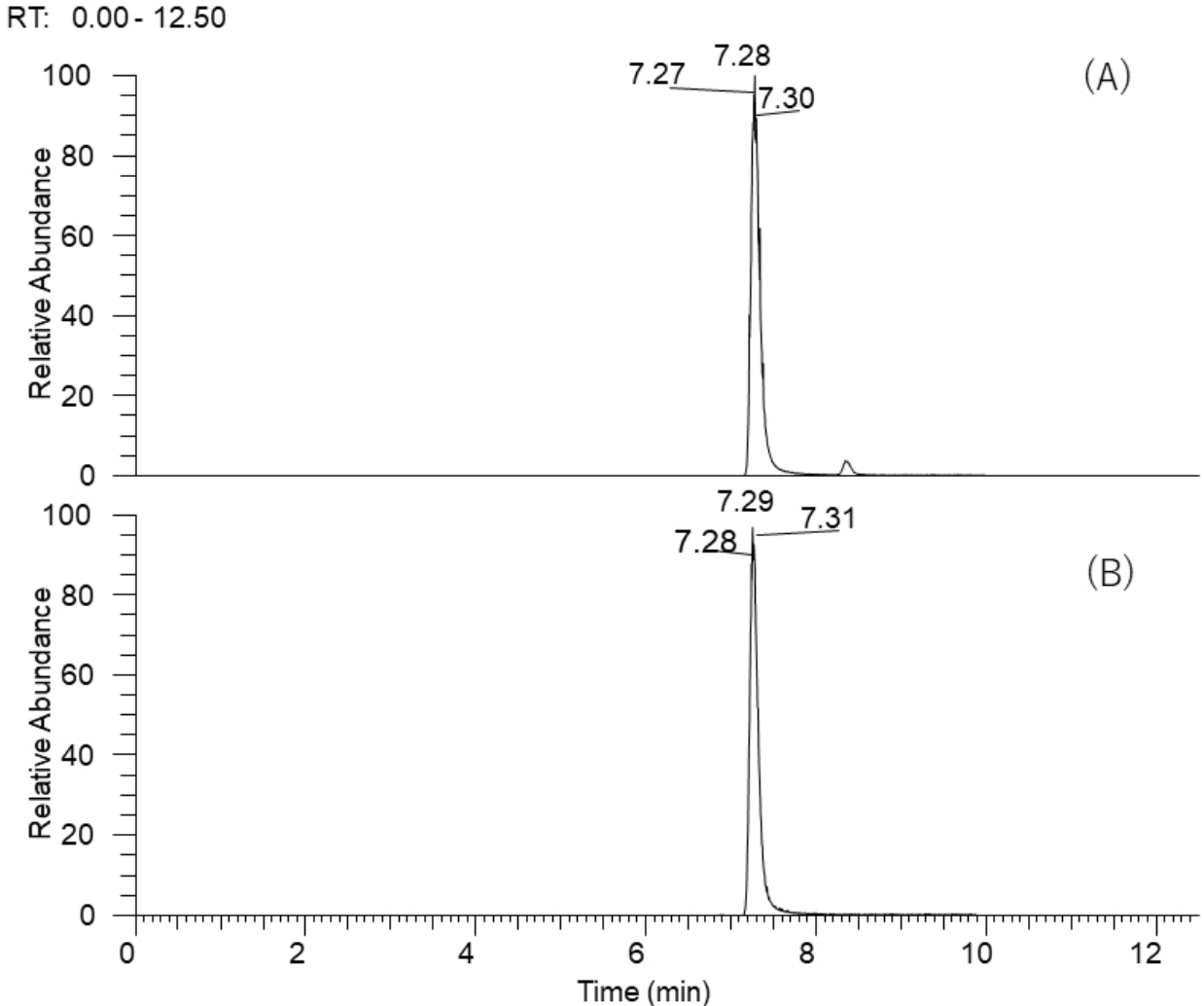
Figure captions

Figure 1. Comparison of the extracted ion chromatogram at m/z 236 from the cadaveric blood sample (A) and the analytical standard for eutylone (B).

Figure 2. Comparison of the product ion spectrum between the blood sample (A) and the analytical standard for eutylone (B).

Figure 1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(B)
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4
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8
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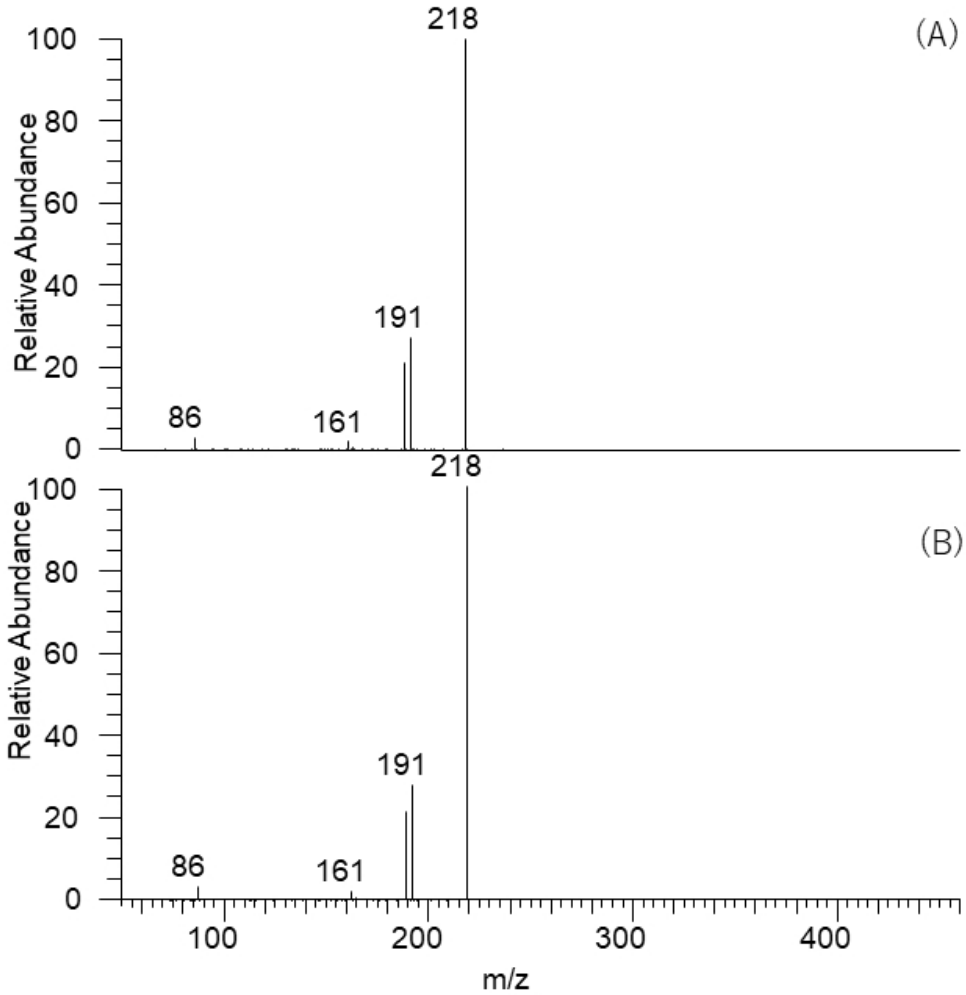
Time (min)

Figure 2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(B)
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