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CASE REPORT

# A case of death caused by abuse of a synthetic cannabinoid *N*-1-naphthalenyl-1-pentyl-1*H*-indole-3-carboxamide

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**Abstract** A man aged in his twenties was found dead on the floor in his room. A package containing dried herbal blend labeled "Fairy evolution" and smoking devices were found in the room. The postmortem interval of the deceased was estimated to be 3 days. The autopsy disclosed no marked findings explaining the cause of death. Toxicological analyses by gas chromatography–mass spectrometry and liquid chromatography–tandem mass spectrometry revealed the presence of *N*-1-naphthalenyl-1-pentyl-1*H*-indole-3-carboxamide (NNEI) in the herbal blend and in specimens taken from the victim. The concentrations in the blood and adipose tissue specimens were 0.64–0.99 and 42.9 ng/ml, respectively. To our knowledge, this is the first report to describe NNEI concentrations in human specimens in the fatal poisoning case.

**Keywords** *N*-1-Naphthalenyl-1-pentyl-1*H*-indole-3-carboxamide · NNEI · Synthetic cannabinoid · Herbal blend · LC–MS–MS · GC–MS

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## Introduction

In recent years, herbal products containing designer drugs, such as synthetic cannabinoids and cathinone derivatives, have become widely distributed and have caused serious social problems [1–6]. Acute fatal poisoning cases caused by abuse of designer drugs have been reported [2, 5–7]. *N*-1-Naphthalenyl-1-pentyl-1*H*-indole-3-carboxamide (NNEI) is one of the synthetic cannabinoids and is an analog of JWH-018. The chemical structure of NNEI is shown in Fig. 1. This compound was first described by Shevyrin et al. [8] in 2013. The disposition and toxic details of NNEI are not known. In this article, we report a fatal case of NNEI poisoning caused by abuse of a herbal blend, and describe the toxicological analyses of NNEI in the deceased's body fluids, solid tissues, and hair. To our knowledge, this is the first report to describe the analysis of NNEI in human specimens obtained in a poisoning case.

## Case history

A man aged in his twenties was found dead on the floor in a supine position in his room. A package containing dried herbal blend labeled "Fairy evolution" and smoking devices were found in the room. A relative reported that he had a poor appetite and that his body weight had decreased by about 15 kg during the past 10 months. The deceased had no medical history. An autopsy was performed about 3 days after the estimated time of death.

The deceased was 174 cm tall, weighed 69 kg, and was of average physical build. No remarkable injuries were observed by macroscopic observation. The lungs showed marked congestion, the left weighing 818 g and the right weighing 932 g. No obvious changes were observed in

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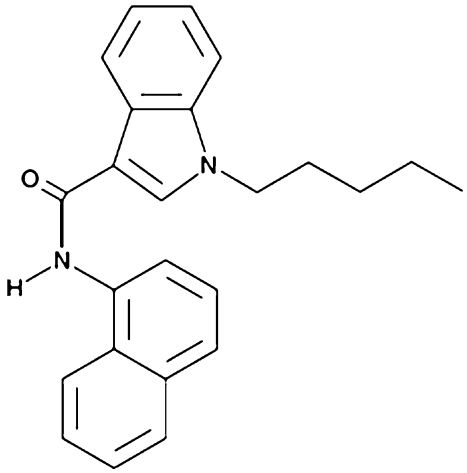


**Fig. 1** Chemical structure of *N*-1-naphthalenyl-1-pentyl-1*H*-indole-3-carboxamide (NNEI)

other organs. By microscopic observation, the organs showed congestion. In the heart, arteriolar wall hypertrophy, slight interstitial fibrosis, and contraction bands were found. The lungs showed marked congestion and edema, and alveolar macrophage infiltrations were also observed. In the liver, slight lymphocytic infiltrations were found in the Glisson's sheath. Arteriolar hyalinizations and severe congestion were found in the spleen. Corpora amylacea were observed in part of the corpus callosum in the brain. From the above-described microscopic observation, it was difficult to explain the cause of death.

## Toxicological analysis

### Sample collection

Samples of whole blood (right atrium, left atrium, right femoral vein, left femoral vein), urine, brain, heart, lung, liver, kidney, and abdominal subcutaneous adipose tissue were collected at autopsy and kept frozen at −30 °C. To prepare plasma, the whole blood samples (right atrium, left atrium) were centrifuged at 3,000 rpm for 10 min and the supernatant was transferred to a new tube; the plasma samples were stored at −30 °C until use. Black hair, 40 cm long, was collected from the parietal region at autopsy and kept at 4 °C.

### Chemicals

NNEI and [1-(5-fluoropentyl)-1*H*-indol-3-yl-2,4,5,6,7-$d_5$]-(4-methyl-1-naphthalenyl)-methanone (MAM-2201-$d_5$) (500 μg/500 μl methanolic solution) were purchased from Cayman Chemical (Ann Arbor, MI, USA); the MonoSpin $C_{18}$ columns from GL Sciences (Tokyo, Japan); methanol (HPLC grade) and acetonitrile (HPLC grade) from Sigma Aldrich (St. Louis, MO, USA). All other chemicals were analytical grade and purchased from Wako Pure Chemical (Osaka, Japan).

### Analysis of the herbal blend by gas chromatography–mass spectrometry

The herbal blend was analyzed by slight modification of a previous method [2] using gas chromatography–mass spectrometry (GC–MS). About 5 mg of the herbal blend was immersed in 1 ml of methanol. The mixture was vortexed and left at room temperature for 10 min. After centrifugation, the supernatant was analyzed by GC–MS using an Agilent 6890 N GC system with a 5975B mass-selective detector (Agilent, Santa Clara, CA, USA) with an HP-5MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent) under the conditions described previously [9].

### Analysis of NNEI in body fluids, solid tissues, and hair of the victim

NNEI in the deceased's blood, plasma, urine, the brain, heart, lung, liver, kidney, and abdominal subcutaneous adipose tissue were determined by the previously described method [2] using liquid chromatography–electrospray ionization–tandem mass spectrometry (LC–ESI–MS–MS). MAM-2201-$d_5$ was used as internal standard (IS). The body fluids were extracted with a MonoSpin $C_{18}$ column. The tissues were homogenized and extracted with acetonitrile [2].

To confirm whether NNEI attached to the hair of the victim during smoking of the herbal blend, the wash solvent used on the hair of the victim was also analyzed. Several hair shafts that were not washed were weighed, and washed with methanol by vortex mixer. The methanol layer was transferred, and dried under a nitrogen stream. The residue was dissolved in acetonitrile and injected into the LC–ESI–MS–MS.

For direct analysis of the hair, the strand of hair was cut into 13 segments. Segments 1–10 were cut into 2-cm sections from the hair root; segments 11 and 12 were 5 cm long, and segment 13 was the strand from the distal end of segment 12 to the hair end. Each sample was washed with methanol and pure water, extracted using the method reported by Kim et al. [10], and analyzed by LC–ESI–MS–MS.

LC–ESI–MS–MS analysis was conducted with an Agilent 1200 LC system with a 6410 Triple Quad tandem mass spectrometer (Agilent). Chromatographic separation was performed on an Inert-Sustain $C_{18}$ HP 3-μm (100 × 3 mm)

column at 40 °C. Gradient elution was used for chromatographic separation with mobile phase A of 0.1 % acetic acid aqueous solution, and mobile phase B of acetonitrile. Linear gradient elution started from 80 % A/20 % B, changed to 100 % B over 5 min with a 1-min hold, and returned to 80 % A/20 % B over 4 min for the next run. The flow rate was 0.6 ml/min. The injection volume was 3 µl. The ESI–MS–MS system was operated in the positive-ionization mode, with ions monitored in the selected reaction monitoring (SRM) mode. The ESI source parameters were: high-purity drying gas, nitrogen; flow rate, 6 l/min; temperature, 300 °C; capillary voltage, 4,000 V; nebulizer, 103 kPa; fragmentor and collision energies, 140 and 21 V for NNEI and 170 and 25 V for IS, respectively; ion transitions for selected reaction monitoring, *m/z* 357.2 → 214.1 for NNEI and *m/z* 379.2 → 169.1 for IS.

## Results and discussion

No alcohol was detected in the blood and urine samples using head-space gas chromatography with flame-ionization detection. Triage Drugs of Abuse Panel Plus TCA (Biosite, San Diego, CA, USA) showed negative results for the urine sample. A drug screening test was also performed by GC–MS with NAGINATA software (Nishikawa Keisoku, Tokyo, Japan) for the plasma and urine samples; the result was negative.

The total ion chromatogram (TIC) obtained from the extract of the herbal blend by GC–MS is shown in Fig. 2. The mass spectrum of the peak in the TIC is shown in Fig. 3a. The spectrum showed the same fragmentation pattern as the spectrum of the NNEI reference standard provided by the Cayman Chemical Company library (Fig. 3b). No other drugs could be detected from the herbal blend by GC–MS. Therefore, we focused our attention on the quantitative analysis of NNEI in body fluids, solid tissues, and hair by LC–MS–MS.

Typical SRM chromatograms of LC–MS–MS for blank whole blood spiked with 1 ng/ml of standard NNEI and for whole blood from the left femoral vein of the deceased are shown in Fig. 4. The concentrations of NNEI in the victim's body fluids and tissues are shown in Table 1. The NNEI concentration in the abdominal subcutaneous adipose tissue was higher than those of other specimens; it was considered that the high fat solubility typical of synthetic cannabinoids allowed NNEI to remain there without decomposition or metabolism. The concentrations of NNEI in blood and tissues except abdominal subcutaneous adipose tissue were very low; it was not detected in urine. Therefore, it was considered that NNEI was metabolized rapidly in most specimens.

NNEI was detected in the solvent used to wash the deceased's hair, which was 40 cm long; the calculated concentration of NNEI present on the surface of the hair was 93.5 pg/mg. It is suggested that the smoke of the herbal blend including NNEI became attached to the victim's hair. Much higher concentrations of NNEI were detected in all segments of the hair that were divided and washed (Table 2). The concentrations in the different segments were similar except for segment 11. If the NNEI detected in the washed hair was derived from the body, it was considered that he had abused it for about 40 months, because hair usually grows by 1 cm for 1 month. However, this was considered unlikely because NNEI was not distributed in Japan more than 2 years ago. It is suggested that NNEI in the smoke was absorbed into the inside of the hair from the hair surface. We assumed that the hair was tied back and covered with a rubber tie at the length corresponding to segment 11, thereby explaining the low concentration at that length. Analyses of drugs in hair are often used to prove ingestion of them, but, in the present case, it

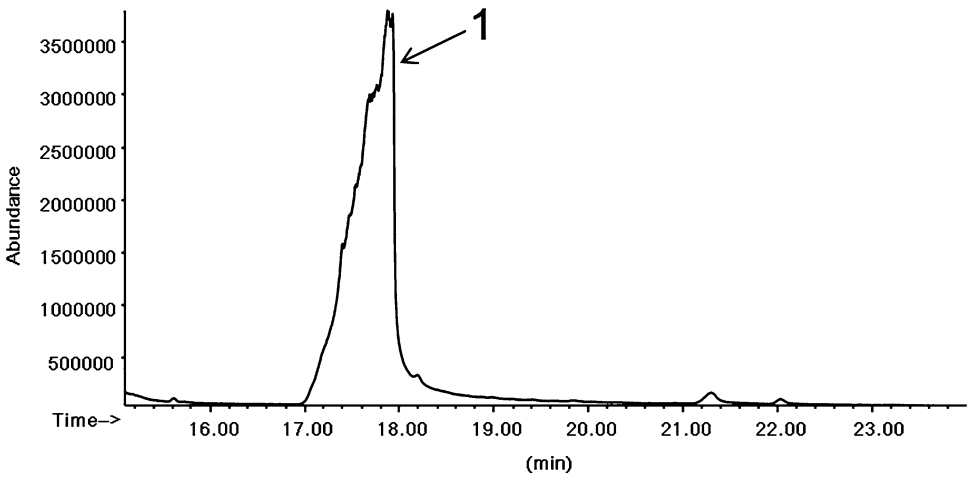


**Fig. 2** Total ion chromatogram obtained from the extract of the herbal blend by gas chromatography–mass spectrometry

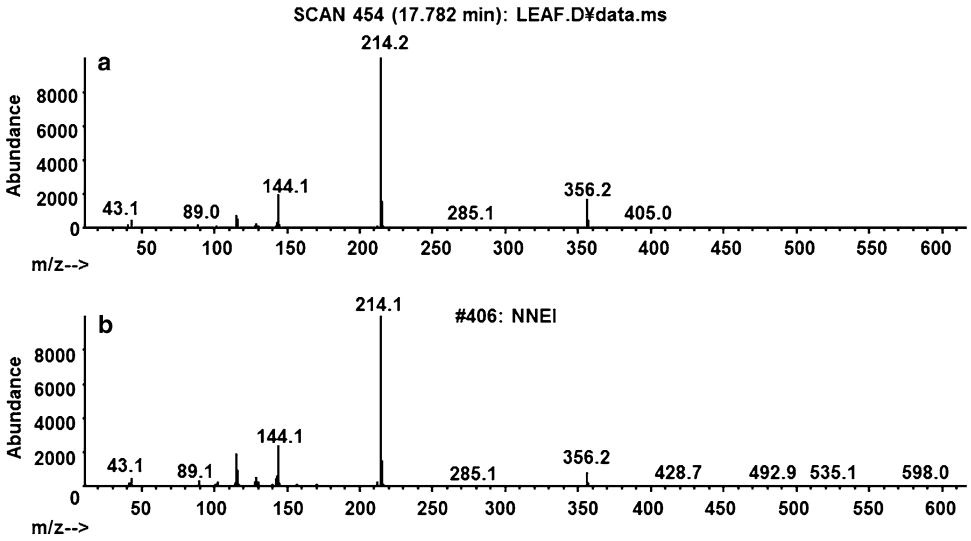


**Fig. 3** Electron ionization mass spectrum of peak 1 in Fig. 2 (**a**), and the spectrum of the reference standard NNEI (**b**)

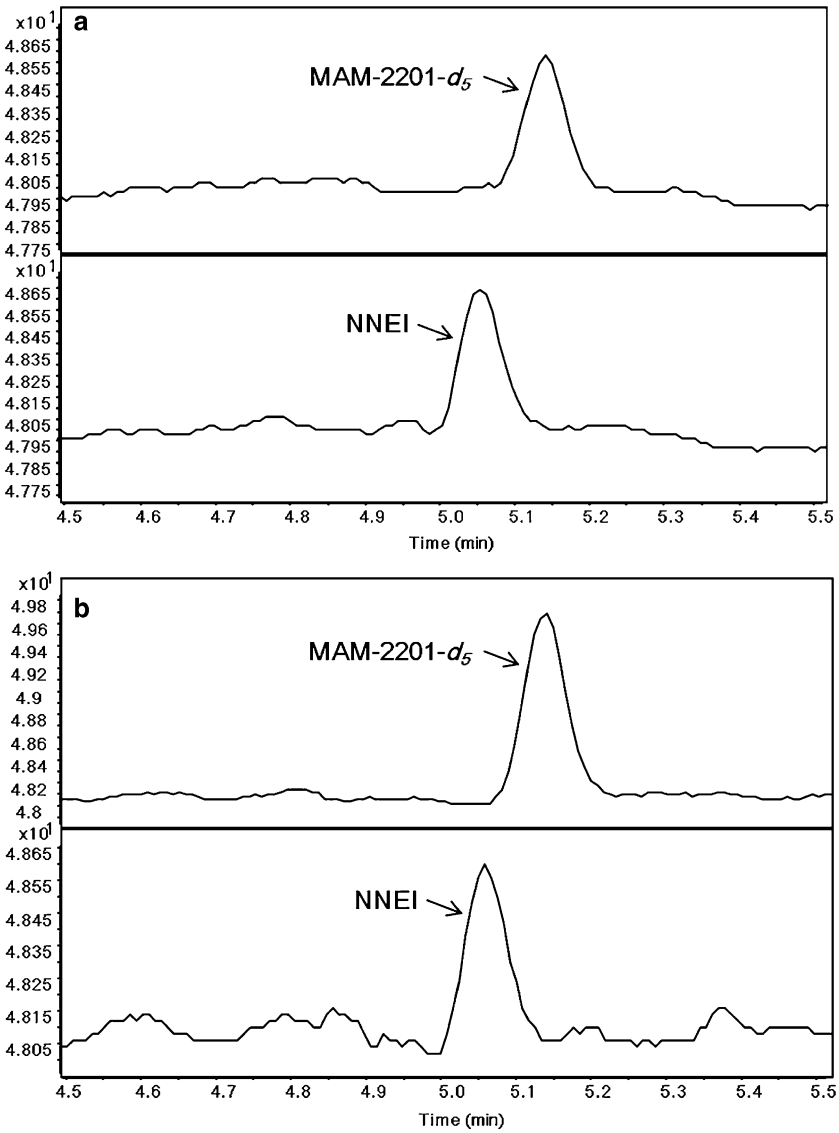


**Fig. 4** Typical selected reaction monitoring chromatograms of liquid chromatography–tandem mass spectrometry for the extracts of blank whole blood spiked with 1 ng/ml of standard NNEI and 2 ng of MAM-2201-$d_5$ as internal standard (IS) (**a**) and the deceased's whole blood sample (left femoral vein) spiked with IS (**b**). The ions monitored were: $m/z$ 357.2 → 214.1 for NNEI and $m/z$ 379.2 → 169.1 for IS

**Table 1** NNEI concentrations in the body fluids and tissues of the victim

| Sample | Concentration (ng/ml or g) |
|---|---|
| Plasma | |
| Right atrium | 0.92 |
| Left atrium | 0.77 |
| Whole blood | |
| Right atrium | 0.75 |
| Left atrium | 0.64 |
| Right femoral vein | 0.99 |
| Left femoral vein | 0.84 |
| Urine | n.d.[a] |
| Brain | 0.76 |
| Heart | 0.82 |
| Lung | 1.06 |
| Liver | 1.31 |
| Kidney | 0.92 |
| Abdominal adipose tissue | 42.9 |

[a] Not detected

**Table 2** Segmental analysis of NNEI concentrations in the deceased's hair after washing

| Segment | | Concentration (pg/mg) |
|---|---|---|
| 1 | 0 (scalp side)–2 cm | 235 |
| 2 | 2–4 cm | 385 |
| 3 | 4–6 cm | 612 |
| 4 | 6–8 cm | 679 |
| 5 | 8–10 cm | 740 |
| 6 | 10–12 cm | 781 |
| 7 | 12–14 cm | 684 |
| 8 | 14–16 cm | 539 |
| 9 | 16–18 cm | 584 |
| 10 | 18–20 cm | 432 |
| 11 | 20–25 cm | 163 |
| 12 | 25–30 cm | 561 |
| 13 | 30+ cm | 530 |

is necessary to consider that it may be derived from external contamination by the smoke of the herbal blend.

## Conclusions

On macroscopic examination of the deceased, no marked changes were observed except for congestion. Histological examination showed arteriolar hyalinizations, which tend to be observed in aged and hypertensive patients, in the spleen, and slight interstitial fibrosis was observed in the heart. Because the deceased was still in his twenties, it was considered that his long abuse of herbal blends caused hypertension and hyperactivity of cardiac function. Poisoning cases with synthetic cannabinoids have been reported [2, 3]. In the present case, no obvious changes were observed on either macroscopic or microscopic examination that could explain the cause of death, and only NNEI was detected in the specimens collected from the victim. Thus, we concluded that the acute circulatory disturbance was induced by NNEI poisoning. To our knowledge, this is the first report describing a fatal case of poisoning by NNEI.

**Conflict of interest** There are no financial or other relations that could lead to a conflict of interest.

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