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CASE REPORT

Fatal intoxication with *N*-ethylpentylone: a case report and method for determining *N*-ethylpentylone in biological material

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Abstract

Purpose We present a fully validated method for determination of *N*-ethylpentylone in biological material and a case report of fatal intoxication with *N*-ethylpentylone.

Methods Blood and urine samples were extracted with ethyl acetate from alkaline medium (pH 9). The analysis was carried out using ultra-high-performance liquid chromatography–tandem mass spectrometry. MDMA-d_5 was used as the internal standard. Validation criteria were evaluated for blank blood and urine at concentrations of 10 and 100 ng/mL.

Results The validation parameters were as follows: lower limit of quantification: 1 ng/mL for blood and urine, coefficient of determination: blood > 0.9996, urine > 0.9975, precision for 10 and 100 ng/mL, respectively: blood 4.87% and 4.47%, urine 1.93% and 2.43%, accuracy for 10 and 100 ng/mL, respectively: blood 14.7% and −2.95%, urine 19.1% and 2.10%, recovery for 10 and 100 ng/mL, respectively: blood 91.5% and 100.2%, urine 97.4% and 96.7%, matrix effect in blood was 127% and 117% for 10 and 100 ng/mL, respectively, in urine 124% and 117% for 10 and 100 ng/mL, respectively. In the present case of fatal intoxication with *N*-ethylpentylone, the determined concentration of this substance was 10.6 µg/mL in peripheral blood and 17.6 µg/mL in urine. In both materials, four metabolites of *N*-ethylpentylone were determined qualitatively.

Conclusion The developed method enables the determination of *N*-ethylpentylone with high sensitivity and selectivity. The method was used to make determinations in biological material in the case of fatal intoxication with *N*-ethylpentylone.

Keywords *N*-Ethylpentylone · New psychoactive substances · Forensic toxicology · Case report · Fatal intoxication

Introduction

N-Ethylpentylone (IUPAC name: 1-(2H-1,3-benzodioxol-5-yl)-2-(ethylamino)pentan-1-one) belongs to the group of synthetic cathinones, which in recent years have gained popularity on the illegal drug market. The increased interest in *N*-ethylpentylone among online forum users and others, and alarming reports about victims of intoxication with this substance contributed to the development by the World Health Organization (WHO) of the *Critical Review Report: N-Ethylnorpentylone*, presented in November 2018 [1].

N-Ethylpentylone was synthesized in the 1960s, similarly to other stimulants studied at the time such as pentylone or butylone [2]. The first reports of its appearance on the black market come from 2014 (the USA). According to the data from the United Nations Office on Drugs and Crime (UNODC), identification of *N*-ethylpentylone was reported by one country in 2015, 25 countries in 2016, and 10 countries in 2017. In 2018, three countries had reported identification of *N*-ethylpentylone as of 25 August [3]. The Drug Enforcement Administration (DEA) also draws attention to the increased popularity of *N*-ethylpentylone. In 2016, it was detected in 63 out of 347 cathinone identifications, while in 2017 this number increased to 201 out of 369 cathinone identifications [1]. In Europe, *N*-ethylpentylone was reported for the first time in Slovenia in 2016 [4]. According to reports by the European Union Early Warning System on New Psychoactive Substances, *N*-ethylpentylone has so far been seized in many European countries, including the United Kingdom, Turkey, Slovenia, Spain, Sweden, Romania, Portugal, Malta, Lithuania, Latvia, Ireland, Hungary,

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Italy, Greece, Belgium, Austria, the Czech Republic, and Bosnia and Herzegovina [1].

N-Ethylpentylone appears on the black market and on internet forums under different names, including *N*-ethylnorpentylone, MDEVP, bk-EBDP, bk-ETHYL-K, bk-EPDP, ephylone, and Mercedes [1, 4, 5]. *N*-Ethylpentylone is available in crystal, powder, rock, capsule, and tablet forms, mostly as a racemate. There have been cases in which *N*-ethylpentylone was sold as ecstasy/MDMA (tablets marked with the logo characteristic of MDMA) [1]. *N*-Ethylpentylone, like other synthetic cathinones, can be taken orally, parenterally, and rectally. In addition, based on the information available on internet forums, it can also be taken through intravenous injection, sublingual administration, or nasal insufflation [1].

Previous reports suggest that *N*-ethylpentylone shows similar activity to other ring-substituted synthetic cathinones of the pyrovalerone type, such as alpha-PVP (1-phenyl-2-(pyrrolidin-1-yl)pentan-1-one) or MDPV (1-(2H-1,3-benzodioxol-5-yl)-2-(pyrrolidin-1-yl)pentan-1-one). *N*-Ethylpentylone acts as a psychomotor stimulant [1]. According to Costa et al. [6], *N*-ethylpentylone inhibited the uptake of the monoamine neurotransmitters norepinephrine, serotonin, and especially dopamine. The researchers also demonstrated that *N*-ethylpentylone was not a transporter substrate for inducing the release of dopamine, norepinephrine, or serotonin.

Adverse symptoms after taking *N*-ethylpentylone include psychomotor agitation, aggressiveness, confusion, tachycardia, visual hallucinations, hyperthermia, psychosis, inconsistent speech, sleeplessness, and cardiac arrest [2, 7]. Additionally, in the case presented by Thirakul et al. [7], a man developed acidosis (arterial blood pH < 6.7) after taking *N*-ethylpentylone. Laboratory tests show hypoglycaemia, hyperkalemia, elevated troponin, and elevated concentration of lactic acid. In addition, intoxication with *N*-ethylpentylone can lead to liver failure, renal injury, and rhabdomyolysis [1, 7].

The previously described methods for the determination of *N*-ethylpentylone are liquid chromatography–tandem mass spectrometry (LC–MS/MS) [2, 6] and gas chromatography–mass spectrometry (GC–MS) [5, 7].

This study presents a fully validated method for detecting *N*-ethylpentylone in biological material and the first case of death due to intoxication with *N*-ethylpentylone in Poland.

Materials and methods

Chemicals

Water (Chromasolv® LC–MS), acetonitrile (Chromasolv® LC–MS), methanol (Chromasolv® LC–MS), acetone, ethyl acetate, and formic acid were purchased from Sigma-Aldrich (Steinheim, Germany); ammonium formate was purchased from Sigma-Aldrich (Bangalore, India); ammonium carbonate was purchased from Fluka (Buchs, Switzerland); and *N*-ethylpentylone and MDMA-d_5 were purchased from Cerilliant (Round Rock, TX, USA). Standard solutions (at a concentration of 100 µg/mL) of *N*-ethylpentylone and internal standard (IS) (MDMA-d_5) were prepared in methanol.

Biological material

Drug-free blank blood samples used for the development and validation of the method were obtained from a regional blood donation centre (Wrocław, Poland). Drug-free blank urine samples used for the development and validation of the method were obtained from a healthy volunteer. Forensic blood and urine samples were sent to the Institute of Toxicology Research, Borowa, Poland, for routine toxicological analysis.

Working solutions, calibration curve, and quality control samples

The working solutions of different concentrations were prepared by dilution of the standard solution with methanol. The stock solution and standard solutions were stored at −20 °C.

Standard solutions were diluted with methanol to obtain working standard solutions at the following concentrations for *N*-ethylpentylone: 10, 50, 100, 200, 500, and 1000 ng/mL. Calibration points and quality control (QC) samples were prepared by spiking the appropriate working solution into drug-free whole-blood/urine samples. The final concentrations of the calibrators were 1 (lower limit of quantification (LLoQ)), 5, 10, 20, 50, and 100 (upper limit of quantification (ULoQ)) ng/mL blood/urine for *N*-ethylpentylone. QC samples were prepared by spiking blank human blood/urine to yield a final concentration of 10 and 100 ng/mL for *N*-ethylpentylone.

Sample procedure

Two hundred microliters (µL) of blood or urine was transferred into 12-mL plastic vials. Next, 20 µL of methanolic internal standard solution (MDMA-d_5 at a concentration of 1 µg/mL) was added, along with 200 µL of buffer (0.5 M ammonium carbonate, pH 9). Liquid–liquid extraction with 2 mL of ethyl acetate was carried out for 10 min. The samples were centrifuged at 2540×*g* at 4 °C for 10 min. The organic phase was transferred into 2-mL Eppendorf tubes and evaporated to dryness under a stream of inert nitrogen gas (at 40 °C). The dry residues were dissolved in 50 µL of methanol. The solution was then transferred into inserts for

autosampler vials and analysed by ultra-high-performance liquid chromatography–triple-quadrupole tandem mass spectrometry (UHPLC–QqQ-MS/MS). The injection volume was 2 µL. Because the concentrations of *N*-ethylpentylone in both materials were markedly above ULoQ (100 ng/mL), the assay was repeated. Blood and urine were diluted with water (LC–MS grade) twice, and those samples were then diluted 100-fold, finally obtaining a 200-fold dilution for blood and urine.

Chromatographic and spectrometric conditions

Analyses were performed using an ultra-high-performance liquid chromatograph (Nexera X2, Shimadzu, Kyoto, Japan). The separation was done using a Kinetex XB-C18 2.6 µm 2.1 × 150 mm column (Phenomenex, Torrance, CA, USA) with the thermostat set at 40 °C. The mobile phase consisted of a mixture of 10 mM ammonium formate and 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient elution was carried out at a constant flow of 0.4 mL/min. The gradient applied was as follows: 0 min, 5% B; 12 min, 98% B; 14 min, 98% B; and 15 min, 5% B. Return to the initial gradient composition (95% A and 5% B) was performed at 5 min.

Detection of the investigated compounds was achieved using a triple-quadrupole mass spectrometer (LCMS-8050, Shimadzu, Kyoto, Japan). The spectrometer was equipped with an electrospray ionisation (ESI) source; determination of the investigated substances was carried out in the multiple reaction monitoring (MRM) mode. The following MS parameters were fixed: nebulising gas flow, 3 L/min; heating gas flow, 10 L/min; interface temperature, 250 °C; desolvation line (DL) temperature, 200 °C; heat block temperature, 350 °C; and drying gas flow, 10 L/min. A summary of precursor and product ions, collision energies, dwell time, Q1–Q3 pre-bias voltages, and retention time for each compound is presented in Table 1.

Validation

Linearity

Linearity was evaluated by analysis of *N*-ethylpentylone working solutions with human blood in final concentrations of 1, 5, 10, 20, 50, and 100 ng/mL. The linearity of the calibration curve was determined by plotting the peak area ratio of *N*-ethylpentylone to IS in human blood/urine against the corresponding concentration ratio for assessment of method performance. The coefficient of determination (R^2) was calculated.

Precision

The precision and accuracy of the method were estimated by replicating analysis (number of samples: 5; $n = 5$) of QC samples. Precision was defined as relative standard deviation (RSD).

Accuracy

The accuracy was expressed as mean relative error [MRE% = (mean of the measured concentration − nominal concentration)/nominal concentration × 100%].

LLoQ

LLoQ was defined as the lowest concentration at which the RSD did not exceed 20%.

Recovery

The recovery ($n = 5$) of the *N*-ethylpentylone was evaluated at concentrations of 10 and 100 ng/mL. The recovery (as a percentage) was determined by comparing the mean peak areas of matrix spiked with *N*-ethylpentylone and IS before extraction to matrix spiked with standards after extraction.

Matrix effect

Matrix effects were examined at concentrations of 10 and 100 ng/mL. They were calculated by comparing the peak areas from neat solutions of *N*-ethylpentylone and IS (A) with those from blood/urine spiked with the same standards after extraction (B). Matrix effect [%] = B/A × 100 (in accordance with [8]).

Table 1 MRM conditions used in the UHPLC/ESI–MS/MS analysis of *N*-ethylpentylone and IS

Compounds	Precursor ion (*m*/*z*)	Product ion (*m*/*z*)	Dwell (ms)	Q1 Pre-bias (V)	Collision energy (V)	Q3 Pre-bias (V)	Retention time (min)
N-Ethylpentylone	250.0	202.2[a] 232.2 174.1	2.0	−11	−20 −15 −32	−20 −22 −16	4.45
MDMA-d_5	199.0	165.2[a]	14.0	−19	−14	−30	3.47

[a]Ions selected for quantitative analysis

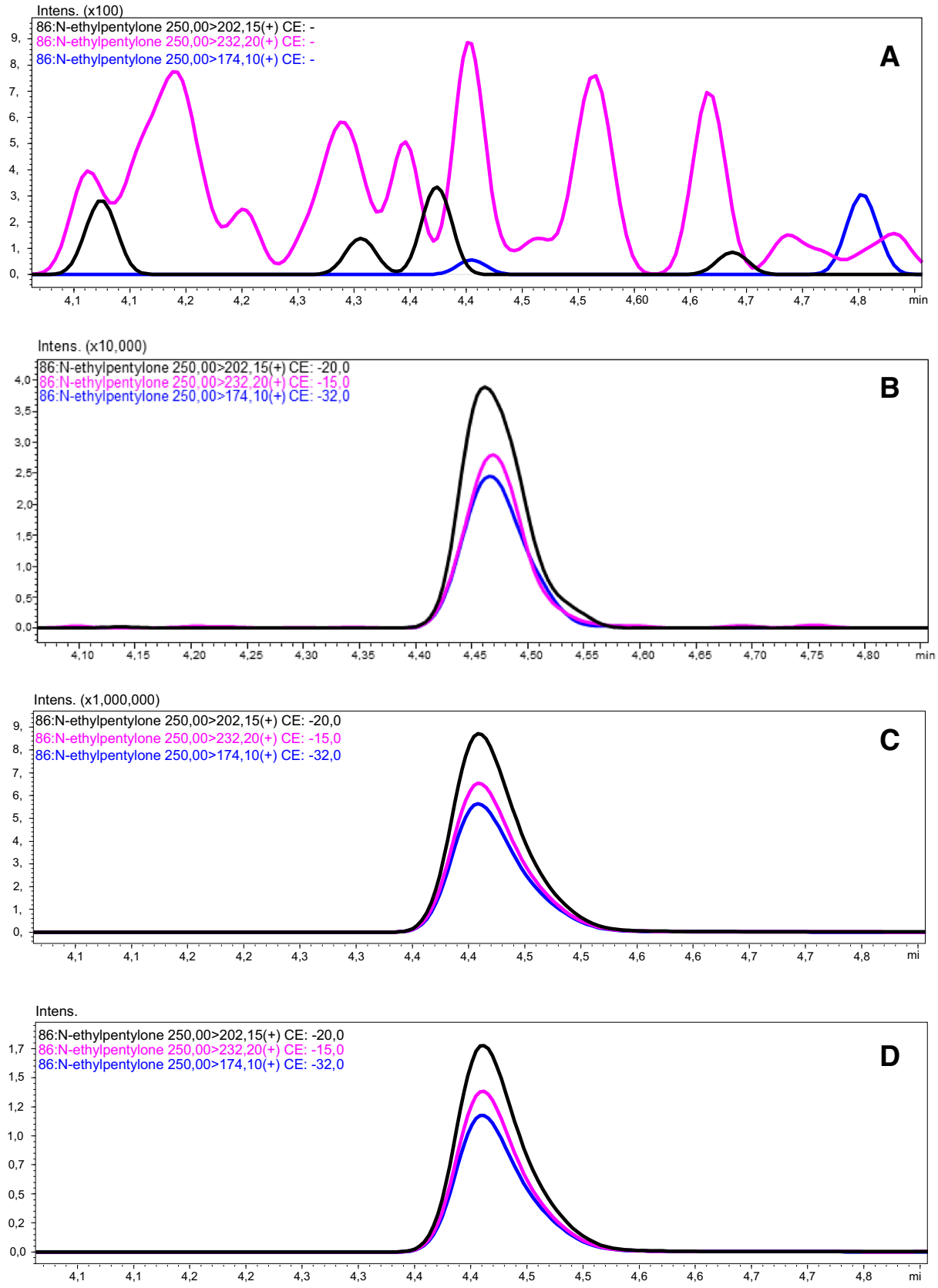

Fig. 1 MRM of *N*-ethylpentylone in blank human blood (**a**), 1 ng/mL (LLoQ) (**b**), 50 ng/mL (**c**), and 100 ng/mL (**d**)

Table 2 Parameters of the method

N-Ethylpentylone		
Biological matrix	Blood	Urine
Linear concentration range (ng/mL)	1–100	1–100
Coefficient of determination (R^2)	> 0.9996	> 0.9975
Calibration line equation	$y=0.876x+0$	$y=1.47x+0$
LLoQ (ng/mL)	1	1
Precision (%RSD)		
10 ng/mL	4.87	1.93
100 ng/mL	4.47	2.43
Accuracy (MRE%)		
10 ng/mL	14.7	19.1
100 ng/mL	−2.95	2.10
Recovery (%)		
10 ng/mL	91.5	97.4
100 ng/mL	100.2	96.7
Matrix effect (%)		
10 ng/mL	127	124
100 ng/mL	117	117

Case history and pathological findings

The naked body of a 30-year-old man (height = 1.77 m; weight = 96 kg) in a state of putrefaction was found in a passenger car. The body was in a semi-recumbent position in the driver's seat. Apart from the deceased, some sex toys were found in the car, including vibrators.

The autopsy showed no obvious pathological changes in the organs. Putrefactive changes were observed in the form distension of the skin by putrid gases, progressive blurring of the structure of the internal organs, greenish discolouration of the skin, diffusion streaks, and desquamation of the epidermis.

Results and discussion

The method employed enabled the determination of *N*-ethylpentylone with high sensitivity and selectivity. Figure 1 shows a chromatogram with MRM transitions typical of *N*-ethylpentylone. Table 2 shows the validation parameters of the method. For both matrices ion enhancement was observed. Figure 2 shows the mass spectrum of *N*-ethylpentylone in the test blood sample after a 25-fold dilution.

In the present case, the concentrations of *N*-ethylpentylone were 10.6 µg/mL in blood and 17.6 µg/mL in urine. In addition, 4-chloromethcathinone (4-CMC) was detected in the biological material at concentrations of 0.6 ng/mL and 14.3 ng/mL in blood and urine, respectively. The concentration of ethyl alcohol in both materials was 0 mg/mL. Because of the determination of high concentrations of *N*-ethylpentylone in both biological materials, it was assumed that the victim was probably addicted to substances from the group of cathinones; hence a tolerance of such high concentration of *N*-ethylpentylone was developed. However, the death occurred when the concentration of *N*-ethylpentylone almost reached the peak in blood, in the phase of the most developed symptoms of the sympathomimetic syndrome. In addition, the body was revealed in a state of putrefaction, which made it difficult to estimate the real concentrations of *N*-ethylpentylone at the time of death. It is also not known whether in the period from the time of death to the performance of toxicological tests there was no degradation of 4-CMC in biological material. Previous reports by Adamowicz and Malczyk [9] and Nowak et al. [10], among others, indicate the instability of this cathinone in the biological matrices.

Atherton et al. [5] presented four fatal cases in which *N*-ethylpentylone was detected. In these cases, the peripheral blood concentrations of *N*-ethylpentylone ranged from 31 to 1210 ng/mL. In one case, the cause of death was complications due to acute intoxication with *N*-ethylpentylone (953 ng/mL in blood); in another case, it was caused by complex intoxication with narcotic drugs, including *N*-ethylpentylone (1210 ng/mL in blood), while in the other two cases the cause of death was homicide (gunshot wounds). Krotulski et al. [2] identified *N*-ethylpentylone in postmortem blood collected from 26 people. In 25 cases, *N*-ethylpentylone concentrations ranged from 12 to 1200 ng/mL (mean 313 ng/mL, median 125 ng/mL). In one case, the concentration determined was 50,000 ng/mL. In most cases, determinations in blood were not limited to *N*-ethylpentylone, but included other substances such as new psychoactive substances butylone, dibutylone, eutylone, U-47700, U-49900, carfentanil, and 4-FIBF (4-fluoroisobutyrylfentanyl). Costa et al. [6] determined *N*-ethylpentylone in samples collected from five hospitalized patients and one deceased subject. The concentrations of *N*-ethylpentylone in the serum collected from living persons ranged between 7 and 149 ng/mL ($n=4$). For one patient, the concentration of *N*-ethylpentylone was qualitatively determined in the urine at 48 h after ingestion, but no presence of *N*-ethylpentylone was found in the serum. In addition, in one of the hospitalized subjects (concentration in serum 7 ng/mL), the concentration of *N*-ethylpentylone in the urine was qualitatively determined. The concentration of *N*-ethylpentylone in the blood of the deceased, who died a few minutes after its ingestion, was 170 ng/mL. Costa et al. [6] suggest that concentrations of *N*-ethylpentylone above 100 ng/ml can cause a life-threatening condition that can eventually lead to death.

Krotulski et al. [2] conducted in vitro studies using human liver microsomes (HML) and detected four metabolites of *N*-ethylpentylone: M1

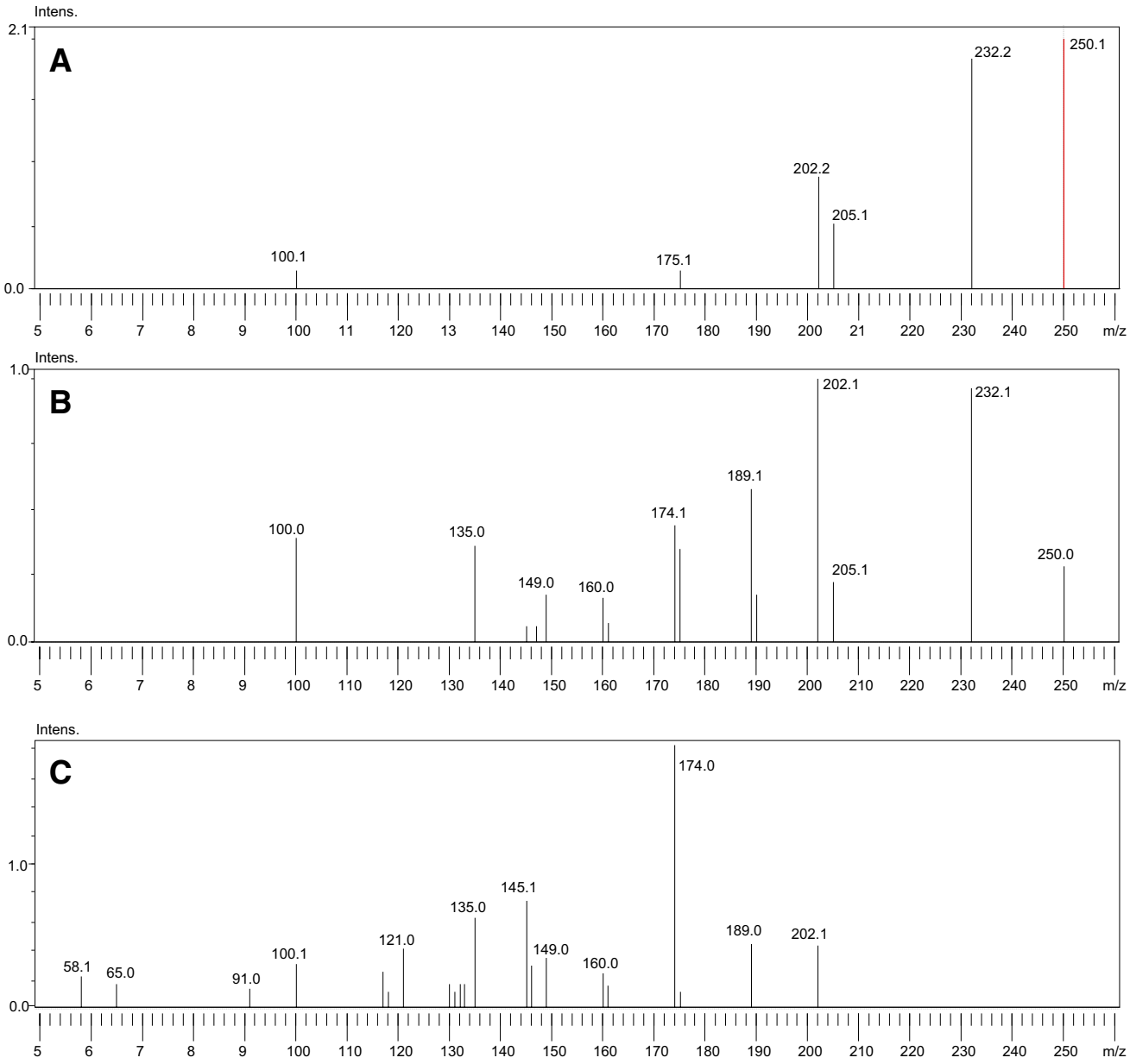

Fig. 2 Mass spectra of *N*-ethylpentylone in authentic human blood (25-fold sample dilution); collision energy: **a** –10, **b** –20, and **c** –35

(1-(2H-1,3-benzodioxol-5-yl)-2-(ethylamino)pentan-1-ol), 252 *m/z* (hydrogenation); M2 (2-amino-1-(1,3-benzodioxol-5-yl)pentan-1-one), 222 *m/z* (*N*-deethylation); M3 (1-(3,4-dihydroxyphenyl)-2-(ethylamino)pentan-1-one), 238 *m/z* (demethylation); and M4 (1-(1,3-benzodioxol-5-yl)-2-(ethylamino)-hydroxy-pentan-1-one), 266 *m/z* (hydroxylation). In addition, in both, the *N*-ethylpentylone standard and the HML samples, the researchers identified eutylone (1-(1,3-benzodioxol-5-yl)-2-(ethylamine)buthane-1-one), 236 *m/z*, which was considered an impurity.

Krotulski et al. [2] detected *N*-ethylpentylone, eutylone, and all four metabolites in the tested blood and saliva samples. In our case, we also determined *N*-ethylpentylone, eutylone, and all four metabolites of *N*-ethylpentylone in the blood and urine of the deceased subject. Figure 3 shows the mass spectra of eutylone and all four metabolites of *N*-ethylpentylone in the urine sample of the deceased subject.

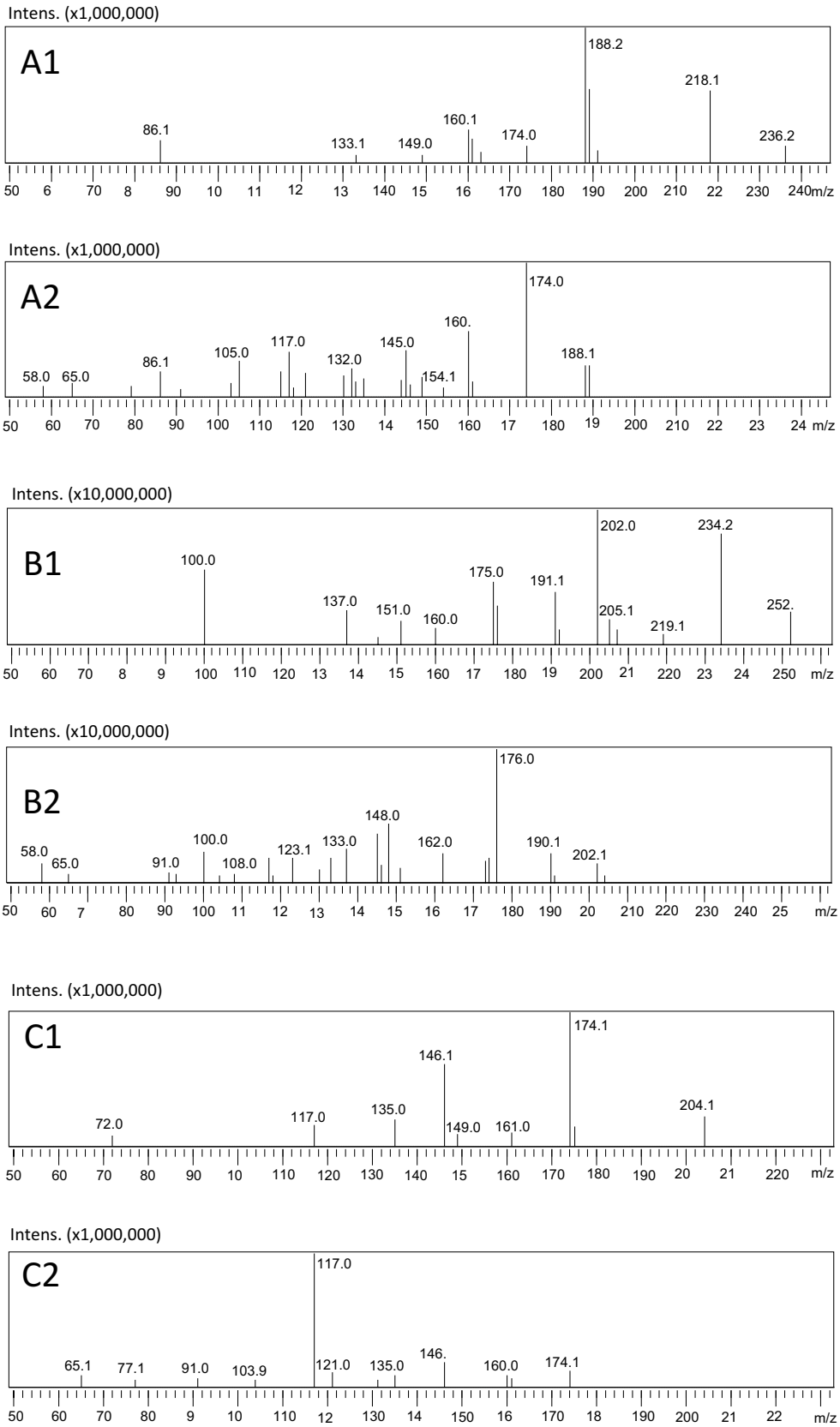

Fig. 3 Mass spectra of eutylone: A1 (CE −20), A2 (CE −35) and metabolites of *N*-ethylpentylone: M1: B1 (CE −20), B2 (CE −35); M2: C1 (CE −20), C2 (CE −35), M3: D1 (CE −20), D2 (CE −35), M4: E1 (CE −20), E2 (CE −35). Analyses performed on a urine sample. *CE* collision energy

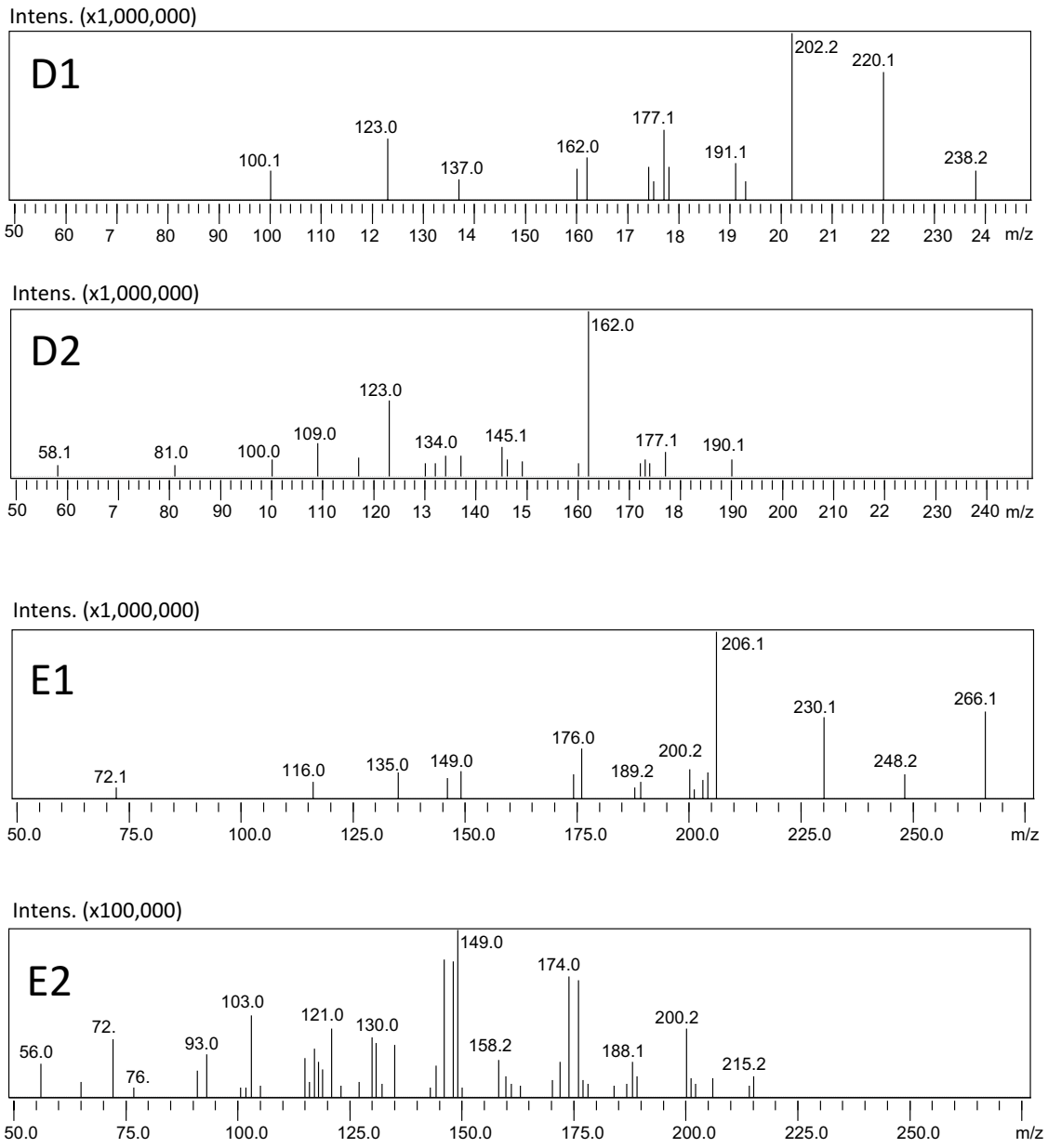

Fig. 3 (continued)

Conclusions

N-Ethylpentylone belongs to the group of synthetic cathinones, which are popular on the European and global black market. The case of fatal intoxication with *N*-ethylpentylone presented by the authors is the first reported case of its kind in Poland. The work presents the mass spectra of *N*-ethylpentylone and its metabolites determined in biological material, which confirm previous reports of possible metabolic pathways of *N*-ethylpentylone.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement All procedures performed in this study involving human participants were in accordance with the ethical standards of the international and/or national committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Blood and urine collections from cadaver was made by judicial authorities, and the samples were sent to our institute for toxicological analysis to their request. This article does not contain any studies with living human participants or animals performed by any of the authors.

References

1. World Health Organisation (2018) Critical review report: *N*-ethylnorpentylone. https://www.who.int/medicines/access/controlled-substances/N-Ethylnorpentylone.pdf. Accessed 12 Dec 2018
2. Krotulski AJ, Papsun DM, De Martinis BS, Mohr ALA, Logan BK (2018) *N*-ethyl pentylone (ephylone) intoxications: quantitative confirmation and metabolite identification in authentic human biological specimens. J Anal Toxicol 42:467–475. https://doi.org/10.1093/jat/bky025
3. UNODC Early Warning Advisory on New Psychoactive Substances. https://www.unodc.org/LSS/Home/NPS. Accessed 12 Dec 2018
4. European Monitoring Centre for Drugs and Drug Addiction (2017) EMCDDA–Europol 2016 Annual Report on the implementation of Council Decision 2005/387/JHA. http://www.emcdda.europa.eu/system/files/publications/4724/TDAN17001ENN_PDFWEB.pdf. Accessed 12 Dec 2018
5. Atherton D, Dye D, Robinson CA, Beck R (2019) *N*-Ethyl pentylone-related deaths in Alabama. J Forensic Sci 64(1):304–308. https://doi.org/10.1111/1556-4029.13823
6. Costa JL, Cunha KF, Lanaro R, Cunha RL, Walthers D, Baumann MH (2018) Analytical quantification, intoxication case series, and pharmacological mechanism of action for *N*-ethylnorpentylone (*N*-ethylpentylone or ephylone). Drug Test Anal 11:461–471. https://doi.org/10.1002/dta.2502
7. Thirakul P, Hair LS, Bergen KL, Pearson JM (2017) Clinical presentation, autopsy results and toxicology findings in an acute *N*-ethylpentylone fatality. J Anal Toxicol 41:342–346. https://doi.org/10.1093/jat/bkx004
8. Matuszewski BK, Constanzer ML, Chavez-Eng CM (2003) Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC–MS/MS. Anal Chem 75:3019–3030. https://doi.org/10.1021/ac020361s
9. Adamowicz P, Malczyk A (2018) Stability of synthetic cathinones in biological materials. Forensic Sci Int 295:36–45. https://doi.org/10.1016/j.forsciint.2018.12.001
10. Nowak K, Szpot P, Marcin Z (2019) Unstability of 4-CMC in human serum specimen. Forensic Toxicol 37:261–264. https://doi.org/10.1007/s11419-018-0455-4 **letter to the editor**