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► To cite this version:

Romain Pelletier, Brendan Le Daré, Diane Le Bouëdec, Alexis Bourdais, Pierre-Jean Ferron, et al.. Identification, synthesis and quantification of eutylone consumption markers in a chemsex context. Archives of Toxicology, 2024, 98 (151-158), 10.1007/s00204-023-03615-z . hal-04266563

HAL Id: hal-04266563

<https://univ-rennes.hal.science/hal-04266563>

Submitted on 15 Jan 2024

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Identification, synthesis and quantification of eutylone consumption markers in a chemsex context

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Abstract

Eutylone is a cathinone-derived synthetic amphetamine scheduled by the World Health Organization and European Monitoring Centre for Drugs and Drug Addiction since 2022 due to its growing consumption. We report here an eutylone intoxication involving a 38-year-old man and a 29-year-old woman in a chemsex context. A bag containing a white crystalline powder labelled as research product was found in their vehicle. Nuclear magnetic resonance and liquid chromatography-high-resolution mass spectrometry (LC-HRMS) analyses identified the powder as eutylone and confirmed purity superior to 99%. LC-HRMS data analysis using molecular networking allowed to propose new eutylone metabolites in blood samples in a graphical manner. We described 16 phase I (e.g hydroxylated or demethylated) and phase II metabolites (glucuroconjugates and sulfoconjugates). The same metabolites were found both in the man and the woman blood samples. Toxicological analyses measured eutylone concentration in blood samples at 1374 ng/mL and 1536 ng/mL for the man and the woman, respectively. A keto-reduced metabolite (m/z 238.144) was synthesized to permit its quantification at 67 ng/mL and 54 ng/mL in man and woman blood samples, respectively. Overall, the identification of these metabolites will increase the knowledge of potential drug consumption markers and allow to implement mass spectrometry databases to better monitor future drug abuse or consumption.

Keywords: eutylone, cathinone, chemsex, molecular networking, LC-HRMS

1. Introduction

Synthetic cathinones represent the second largest family of new psychoactive substances (NPS) in terms of consumption and seizures (European Monitoring Centre for Drugs and Drug Addiction. 2022). These compounds are chemically derived from natural cathinone present in the African shrub Khat (*Catha edulis*), and present stimulating properties close to amphetamines (Pieprzyca et al. 2020). Numerous related analogs are identified every year, and their consumption seems to be increasing, especially in the context of Chemsex (Cartiser et al. 2021; Batisse et al. 2022; Kuroпка et al. 2023). Indeed, Chemsex consists on the intake of psychoactive substances while having sex, in order to increase both pleasure and feelings. Legislations concerning cathinone consumption are heterogeneous, depending on the countries. In 2022, the European Monitoring Centre for Drugs and drug Addiction (EMCDDA) recorded 162 synthetic cathinone derivatives, a number which highlights their popular and widespread use (European Monitoring Centre for Drugs and Drug Addiction. 2022). In France, the whole family of synthetic cathinones is classified as narcotic, making its use, possession and sale prohibited (Arrêté du 22 février 1990 fixant la liste des substances classées comme stupéfiants). Among them, eutylone ($C_{13}H_{17}NO_3$), also known as β -keto-1,3-benzodioxolyl-*N*-ethylbutanamine, bk-EBDB or *N*-ethylbutylone, is currently one of the most popular drug used for recreative purposes, in particular for Chemsex in both homosexual and heterosexual environment (Malandain et al. 2021; Krotulski et al. 2021; Kuroпка et al. 2023). Its first detection was made in Poland in 2014 (European Monitoring Centre for Drugs and Drug Addiction (EU body or agency) and Europol (EU body or agency) 2015), and its use and toxicity have been repeatedly reported by drug-monitoring institutions and scheduled by EMCDDA and USA in 2022 (Eutylone critical review report; NFLIS-Drug 2020 Annual Report & Drug Midyear Report).

From a pharmacological point of view, *in vitro* experiments have shown effects of eutylone on monoaminergic neurotransmitter transporters, inhibiting the reuptake of dopamine, serotonin and norepinephrine. The pharmacological effects found *in vitro* are similar to those observed with other known cathinones derivatives, suggesting that eutylone use may pose significant psychostimulation problems (Glatfelter et al. 2021).

Nowadays, high-resolution mass spectrometry (HRMS) coupled to liquid chromatography (LC-MS or LC-HRMS) is the gold standard technology in complex mixture analysis and finds useful applications in the field of analytical toxicology. This allows fast and efficient acquisition of data which can be further processed through *ad hoc* softwares. However, one of the main limitations with NPS is their unambiguous identification, given the close structural analogies between compounds of the same chemical family. In this context, exploration of the NPS metabolism profile using innovative bioinformatics approaches represents a promising challenge, since each compound gives a unique fingerprint. Among them, molecular networking has been proposed as a powerful approach to group structurally related molecules into clusters, and thus to group metabolites of a parent substance into a single cluster (Wang et al. 2016; Le Daré et al. 2020b). By propagating information within a molecular network, this methodology makes it possible to identify previously unknown metabolites. To increase confidence in the identification of metabolites, chemical approaches such as nuclear magnetic resonance or metabolite synthesis have already proven useful in toxicology (Hays 2005; Ameline et al. 2019; Bottinelli et al. 2019).

Through a case of eutylone intoxication in a couple in a chemsex context, we aim to use a cross-sectional analytical approach to identify and quantify eutylone and its metabolite in biological samples.

2. Material and Methods

2.1. Case report

We report a case of a couple of drug users (a 38-years-old man and a 29-years-old woman) arrested by the police in a state of agitation, with aggressivity, delusions and hallucinations. The man was arrested and placed in custody while the woman was hospitalized in a nearby hospital for 8 days. The police proceeded to the seizure of a bag containing a white crystalline powder and Vardenafil (VILITRA®) tablets, an erectile dysfunction drug. During the interrogation, they affirmed having used NPS and having sexual intercourse in a context of chemsex. Blood samples were taken from both individuals. The bag containing the powder bore a hard-to-read inscription,

probably eutylone, suggesting NPS or research chemicals (Figure 1A). The seized products and blood samples were sent to the forensic toxicology laboratory of the Rennes university hospital for toxicological analysis.

2.2. Toxicological analysis

Powder Analysis. LC-HRMS was used to identify eutylone in the powder sample found close to the patients. In addition, NMR analysis was performed to unambiguously confirm the chemical structure of eutylone in the powder sample, to check its purity and exclude the presence of other organic compounds (adulterants). The NMR spectra were recorded on a Bruker Avance 300 (Bruker Biospin, France) equipped with a DUL 300 MHz S1 probe (5 mm). The ^1H (zg30) and ^{13}C (JMod) spectra were recorded in deuterated methanol (CD_3OD or MeOD). For this purpose, 20.0 mg of the crystalline powder were dissolved in 0.7 mL of MeOD at room temperature. The data were processed with Bruker Topspin 4.14 software. Chemical shifts (δ) are reported in parts per million (ppm). The signal of the residual non-deuterated solvent was used as the internal standard, 3.31 ppm and 49.00 ppm for ^1H and ^{13}C , respectively. The following abbreviations are used for multiplicities: s, singlet; d, doublet; t, triplet; dd, doublet of doublets; m, multiplet. Coupling constants (J) are reported in Hertz (Hz).

Metabolites synthesis

One of the metabolites identified by LC-HRMS from the blood samples (both male and female) corresponded to the keto-reduced derivative of eutylone (m/z 238.144, $[\text{M}+\text{H}]^+$). To confirm this hypothesis, eutylone which structure was asserted, was treated with the reducing agent NaBH_4 in MeOH to yield the keto-reduced compound which was analyzed by NMR and MS. For this purpose, NaBH_4 (32 mg, 0.851 mmol) was added to a solution of eutylone (182 mg, 0.77 mmol) in MeOH (5 mL) at 0°C . The reaction was completed in 30 min (thin-layer chromatography monitoring). Then, water was added and the reaction mixture was extracted with CH_2Cl_2 (3 times 10 mL). The combined organic layers were dried over MgSO_4 and concentrated *in vacuo*. The crude was purified by flash chromatography (petroleum ether/ethyl acetate 50:50 + 1% triethylamine) to give the corresponding alcohol as a white solid (134 mg, 73% yield).

Optical rotation measurement

The optical rotation of eutylone was measured on a Perkin Elmer model 341 polarimeter at 589 nm, in a 1 dm cell and at 19°C. A solution of 10.0 mg in 1.0 mL was prepared and no deviation was observed ($\alpha = 0$).

Sample extraction. Two hundred microliters of each biological samples were supplemented with 500 μL of methanol and 300 μL of a 0.1 mol/L zinc sulfate solution for extraction. After supernatant evaporation, the residue was taken up in 200 μL of mobile phase and transferred to chromatographic vials for analysis using LC-HRMS (Le Daré et al. 2020b).

LC-HRMS/MS analysis for molecular networking. LC-HRMS was performed with an UltiMate 3000 pump coupled to a Q Exactive mass spectrometer (Thermo Scientific, San Jose, CA, USA) with a heated ESI source. The non-targeted LC-HRMS/MS screening method used to build molecular networks has been described elsewhere (Le Daré et al. 2020a, c). Briefly, LC was performed on an Accucore Phenyl Hexyl (100 \times 2.1 mm, 2.6 μm) column (Thermo Scientific) at 40°C, using an injection volume of 10 μL , an elution gradient, and a flow rate of 500 $\mu\text{L}/\text{min}$ for 15 min. The orbitrap mass spectrometer operated in positive ESI mode, with the acquisition range of 100-450 m/z . Ion precursor selection was performed in "data-dependent" mode: the five most intense ions from the previous scan were selected for fragmentation ("top N of 5").

LC-HRMS quantitation

Stock solutions of eutylone and its keto-reduced derivative were prepared at 1.0 g/L in methanol. The stock solutions were stored at -20°C. Linear concentration ranges from 1 to 1,000 ng/mL for eutylone and its keto-reduced derivative were used for quantitation. Eutylone and its keto-reduced derivative were quantified on blood samples using a full scan analysis by LC-HRMS as already published (Le Daré et al. 2020a; Allard et al. 2020).

Routine toxicological analyses

Toxicological screening including psychotropic and cardiotropic agents, illicit drugs, and opioid withdrawal medications was performed using LC-HRMS/MS, as previously reported (Le Daré et al. 2020a; Kernalléguen et al. 2022). Volatile compounds including alcohol, methanol, isopropanol and acetone were quantified on a Thermo

Scientific TRACE 2000 GC including a TriPlus Headspace autosampler (Thermo Scientific) by headspace gas chromatography–flame ionization detector.

Generation of molecular networks. Semi-quantitative approach using molecular network generation has been described elsewhere (Allard et al. 2019, 2020; Le Daré et al. 2020b; Gicquel et al. 2021a). Briefly, spectral raw data (.raw format) were converted into an open MS format (.mzXML) and then preprocessed (deconvolution, deisotoping, alignment, gap-filling, and filtering) with MZmine 2.4.1 software (mzmine.github.io). The single .mgf output file was then loaded on the Global Natural Products Social networking (GNPS) web platform (www.gnps.ucsd.edu), to generate the molecular network. Nodes represented the molecules and were linked and grouped into clusters when they were spectrally close. Biotransformation reactions were identified from mass differences between molecules. Links between nodes were created when the cosine score was greater than 0.7. The molecular network was displayed using Cytoscape 3.8.0 software (San Diego, CA, USA). Nodes were labelled with the exact protonated mass (m/z), and the links between nodes were labelled with the exact mass shift. A color was assigned to each concentration, and the areas of different colors in each node semi-quantitatively represented the concentrations of the corresponding compound in each condition, as described previously (Le Daré et al. 2020b; Gicquel et al. 2021a).

3. Results

3.1. Powder analysis

The seized powder contained in the bag (Figure 1A) was analyzed using a LC-HRMS/MS screening method and identified an $[M+H]^+$ ion of m/z 236.124 with a high intensity and a retention time of 4.9 minutes. This data is in accordance with chemical formula of eutylone: $C_{13}H_{17}NO_3$. In addition, analysis of the 1H (shown) and ^{13}C NMR spectra allowed to confirm the chemical structure of eutylone, as written on the bag (Figure 1) and determine a high purity near to 100%. Measurement of the optical rotation indicated the compound was present in its racemic form ($[\alpha] = 0$).

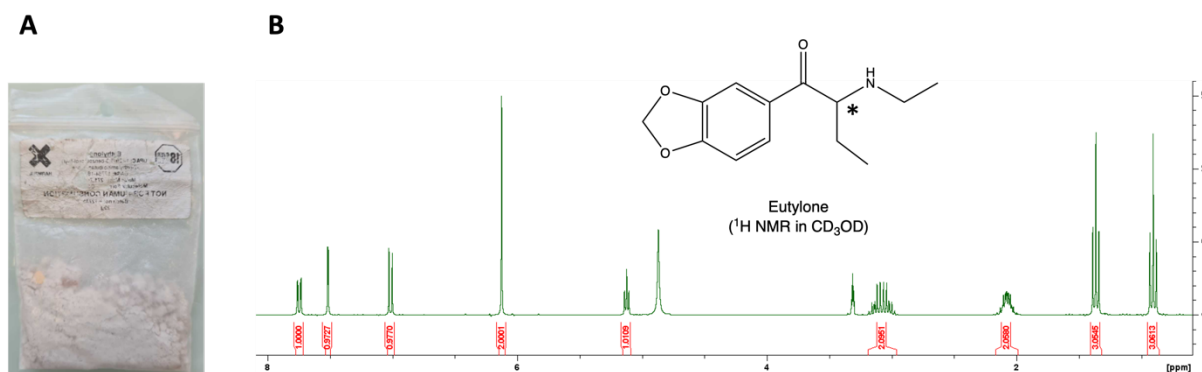


Figure 1. A) seized bag containing white powder. B) ^1H NMR spectrum of the powder (300 MHz, MeOD) : [δ : 7.74 (dd, 1H, $J = 8.6, 1.8$ Hz), 7.51 (d, 1H, $J = 1.8$ Hz), 7.01 (d, 1H, $J = 8.6$ Hz), 6.12 (s, 2H), 5.12 (t, 1H, $J = 5.2$ Hz), 3.18-2.97 (m, 2H), 2.15-1.99 (m, 2H), 1.36 (t, 3H, $J = 7.3$ Hz), 0.95 (t, 3H, $J = 7.5$ Hz)].

3.2. Biological samples analysis

Neither narcotic drugs (cocaine, opiates, heroin, cannabis, amphetamines) nor alcohol (ethanol) were detected in blood samples. Toxicological screening by LC-HRMS/MS report the presence of etylone in both man and woman blood. Vardenafil, a treatment for erectile function was found in man blood sample. Nordazepam, doxylamine and paracetamol were also quantified at therapeutic concentrations in woman blood (respectively 540 ng/ml, 0.1 ng/ml and 20,000 ng/ml).

3.3 Metabolism study using molecular networking

To confirm etylone intoxication, molecular networking was used to identify etylone consumption markers. Blood samples from both individuals were collected, as well as the powder suspected to have been consumed. Data processing of the LC-HRMS results allowed to compare the etylone metabolism profiles in men and woman together with the authentic seized powder to obtain a multi-matrix molecular network (Figure 2).

Within the overall network (Figure 2A), a cluster containing etylone (structurally identified by NMR) and structurally related compounds was found (Figure 2B). Etylone node (m/z 236.128) was identified in three samples: male blood (blue), female blood (pink) and the seized powder (at 1 mg/L) (white). Etylone intensity was similar between the male and female samples. The distribution of nodes structurally

related to eutylone was similar between woman and men, but not found in the powder, suggesting that they are putative metabolites of eutylone.

Overall, 16 nodes were proposed as eutylone metabolites (Figure 2B). Among them, numerous compounds showed a mass shift of -14.015 or -28.03 from the neighboring compound such as m/z 190.086, m/z 208.097, m/z 210.113 and m/z 224.128, which may be consistent with a demethylation or di-demethylation reaction, respectively (Table 1). Also, hydroxylated + dehydrogenated (m/z 250.108) and dihydrogenated *i.e.* keto-reduced (m/z 238.144) metabolites were identified. Taken together, these results suggest that these compounds are phase I metabolites.

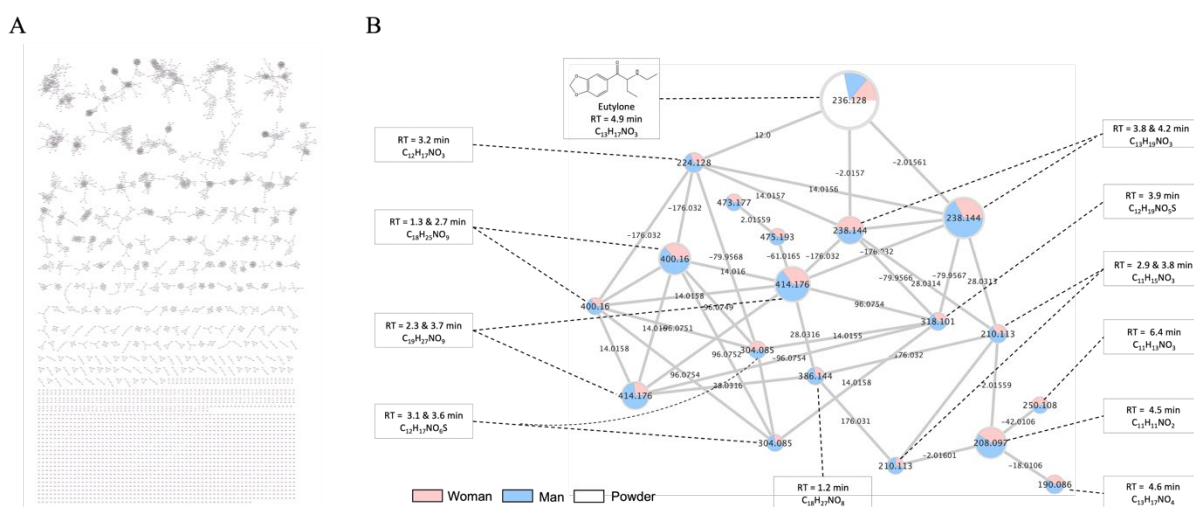


Figure 2A: Visualization of the multi-matrix molecular network B: Eutylone-containing cluster to visualize structurally related eutylone compounds in blood samples from two authentic intoxication cases. Nodes are labelled with the exact protonated mass (m/z) and the links are labelled with the exact mass shift. Three matrices are studied: man blood (blue), woman blood (pink) and eutylone powder (diluted at a concentration of 1 mg/L) (white). RT = retention time (minutes).

Interestingly, we found compounds with a mass shift of +176.032 and +79.956, which may correspond to glucuroconjugation (m/z 386.144, m/z 400.160 and m/z 414.176) or sulfoconjugation (m/z 304.085 and m/z 318.101) reactions, respectively. All these putative phase II metabolites were derived from a molecule that has previously undergone a phase I biotransformation reaction.

Eutylone metabolites also highlighted 5 pairs of isomers (m/z 210.113, m/z 238.144, m/z 304.085, m/z 400.160 and m/z 414.176) presenting the same fragmentation pattern. These isomers were well separated by chromatography but presented identical fragmentation spectra corresponding probably to stereoisomer due to the presence of asymmetric carbon(s).

Using ionization intensity data of the putative metabolites through the node size, we found that two metabolites were more intensely detected within the spectrometer: m/z 400.160 and m/z 414.176. These two metabolites correspond to putative glucuronoconjugated derivatives and were present in both men and woman. The other metabolites have nearly identical intensities between man and woman samples.

Table 1: exact mass shift corresponding to known biotransformation reaction

| Biotransformation | Exact mass shift (Da) | Formula change |
|-----------------------------------|-----------------------|---|
| Dihydrogenation (keto reduction) | + 2.015 | + H ₂ |
| Demethylation + hydrogenation | - 12.000 | - C |
| Demethylation | - 14.015 | - CH ₂ |
| Hydrolysis | - 18.010 | - H ₂ O |
| Deethylation or 2 x demethylation | - 28.031 | - C ₂ H ₄ |
| Desisopropylation | - 42.010 | - C ₃ H ₆ |
| Sulfation | + 79.956 | + O ₃ S |
| Glucuronidation | + 176.032 | +C ₆ H ₈ O ₆ |

3.4. Structural confirmation of the dihydrogenated metabolite found in vivo

To increase our confidence in the identification of the dihydrogenated metabolite found *in vivo* (m/z 238.144), and to quantify it in biological matrices, this derivative was synthesized. Purity was > 99% and structure was confirmed by NMR (Figure 3).

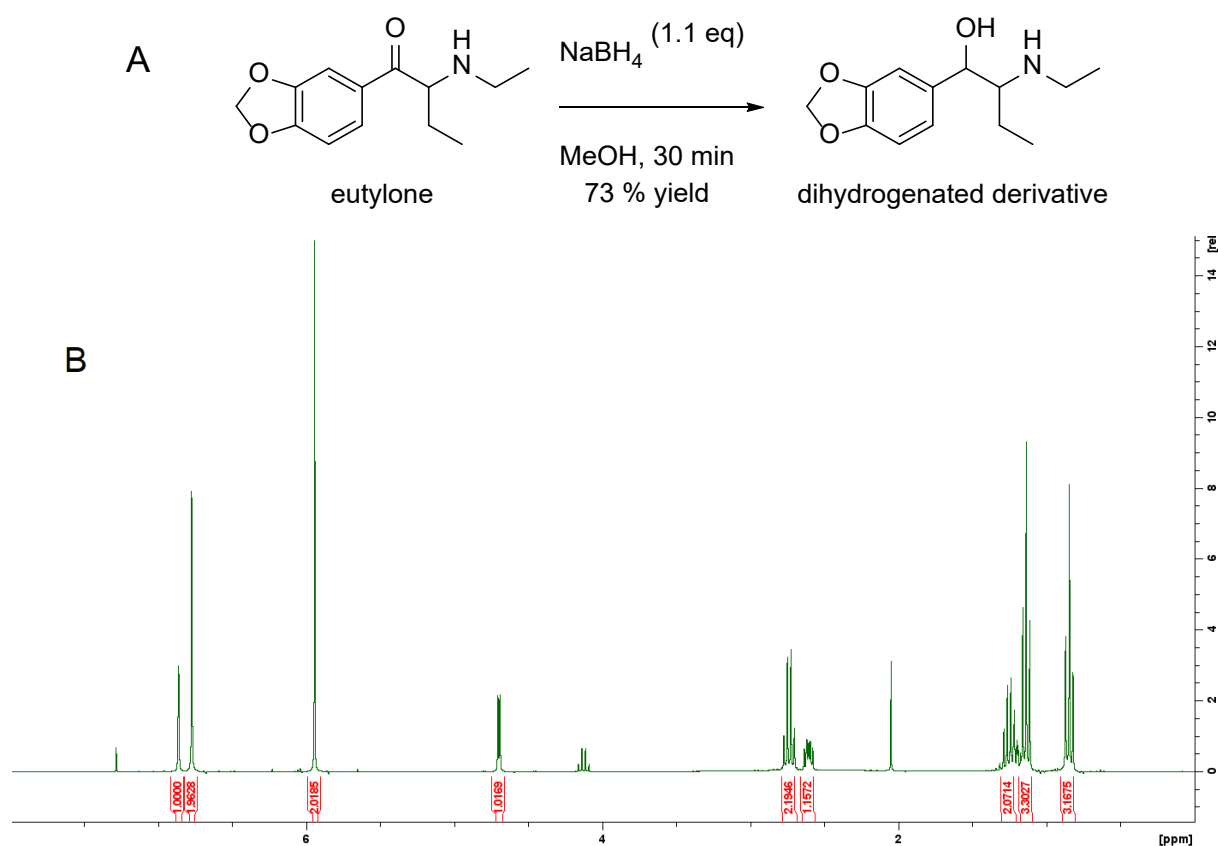


Figure 3: A) Preparation of the dihydrogenated derivative by NaBH₄-promoted reduction of eutylone. B) ¹H NMR spectrum of the dihydrogenated derivative (300 MHz, CDCl₃): [δ :6.87 (s, 1H), 6.77 (bs, 2H),

5.94 (s, 2H), 4.70 (d, 1H, J = 4.3 Hz), 2.72 (q, 2H, J = 7.2 Hz), 2.64-2.58 (m, 1H), 1.30-1.17 (m, 2H), 1.14 (t, 3H, J = 7.3 Hz), 0.85 (t, 3H, J = 7.5 Hz)].

3.5. Eutylone and its metabolites quantitation

LC-HRMS/MS analyses confirmed that the peak (m/z 238.144) at 3.8 min corresponds to a dihydrogenated metabolite (= keto-reduced) identified by molecular networking. Eutylone was quantified in man and woman blood samples at 1,374 ng/mL and 1,536 ng/mL, respectively. Eutylone dihydrogenated derivative was quantified at 67 ng/mL in man blood sample and at 54 ng/mL in woman blood sample.

3. Discussion

We presented two cases of eutylone intoxication in a heterosexual chemsex context, described eutylone metabolism and quantified eutylone and one of its phase I metabolite in blood samples.

Firstly, the most important step was to confirm the eutylone intoxication in the two individuals. NMR analysis confirmed the chemical structure of eutylone at > 99% purity in the seized powder. Also, the use of LC-HRMS/MS analysis and multi-matrix molecular networking allowed us to confirm that the compound present in the powder was also found in the blood of the woman and the man, suggesting that eutylone powder was consumed by both individuals. These results are also consistent with the chemsex context associated with the use of this product in the literature (Malandain et al. 2021), and are supported by what the individuals said during interrogation and the use of vardenafil in man. Therefore, the combination of NMR, LC-HRMS and molecular networking appears to be a robust method for the identification of NPS powder consumption (Gicquel et al. 2021b).

Secondly, eutylone concentrations in the peripheral blood were respectively 1,536 ng/mL and 1,374 ng/mL for the woman and the man, which is consistent with the limited data reported in the literature. Krotulski *et al.* (2020) described 83 cases of eutylone intoxications between 2019 to 2020 in 13 states of the USA, 72 of which were *post-mortem*, with 67 blood samples, 3 urine samples and 7 other matrices (Krotulski et al.

2021). The mean *post-mortem* eutylone blood concentration was $1,020 \pm 2.242$ ng/mL (range between 1.2 and 11,000 ng/ml). In cases of driving under the influence of psychotropic substances, the average eutylone blood concentration was measured at 942 ± 1.407 ng/ml (17 – 3,600 ng/ml) (Krotulski et al. 2021). In Taiwan, Chen *et al.* (2021) described 11 patients who presented to the emergency department involving eutylone use. Eutylone concentrations in the only fatal case were 406 and 15,149 ng/ml in his blood and urine respectively (Chen et al. 2021). Lastly, Nakamura *et al.* (2022) reported on a fatal case of intoxication from a single use of eutylone in a 32-year-old man, with eutylone concentration in cardiac blood at 4,290 ng/g (Nakamura et al. 2022). Here, the synthesis of the keto-reduced (= dihydrogenated) metabolite (m/z 238.144, RT = 3.8 min) allowed us to confirm its structure and to quantify it in blood samples (67 ng/mL and 54 ng/mL, in man and woman blood samples, respectively) bringing valuable insight on eutylone pharmacokinetics.

Thirdly, molecular networking approach has allowed the identification of molecules structurally close to eutylone in the blood of men and women. Because of the known biotransformation reactions linking these molecules to each other and to eutylone, and the fact that these molecules are not present in the analyzed powder, they are most probably metabolites of eutylone. Metabolic pathway mainly included phase I biotransformation reactions (dihydrogenation (= reduction), demethylation, deethylation or 2 x demethylation and desisopropylation) as well as phase II biotransformation reactions (glucuroconjugaison and sulfation). Thus, we found similar phase I biotransformation reactions to those reported for butylone (Meyer et al. 2010), dibutylone (Krotulski et al. 2018a) and N-ethyl pentylone (Krotulski et al. 2018b), allowing to better understand the biotransformation reactions that the compounds of this family undergo. Here, we were able to describe 16 putative eutylone metabolites using molecular networking, 11 of these putative metabolites were not described until now corresponding to 6 phase I metabolites (m/z 190.086 RT= 4.6 min; m/z 250.108 RT= 6.4 min; m/z 304.085 RT= 3.1 & 3.6 min; m/z 318.101 RT= 3.9 min; m/z 318.144 RT= 3.8 & 4.2 min) and 5 phase II metabolites (m/z 386.144 RT= 1.2 min; m/z 400.160 RT= 1.3 & 2.7 min, m/z 414.176 RT= 2.3 & 3.7 min). Interestingly, the metabolite distribution and intensities were similar in men and woman blood samples.

In the literature, Yeh *et al.* (2022) described eutylone metabolism using a cell-free

model (human liver microsomes) and compared the results to 8 urine samples collected from eutylone consumers. Five eutylone metabolites were found in the urine of patients of 3 different masses: m/z 238.144, m/z 210.113, m/z 240.159. Their experiments did not reveal any phase II metabolites (Yeh and Wang 2022). In addition, Krotulski *et al.* (2021) described 3 eutylone metabolites obtained *in vivo* through N-deethylation (M1, m/z 208.097), demethylenation (M2, m/z 224.128) and hydrogenation or reduction (M3, m/z 238.144) as well as 2 putative metabolites found *in vitro*, namely M4 (m/z 196.097 obtained through N-deethylation + demethylenation) and M5 (m/z 252.123 obtained through hydroxylation) (Krotulski *et al.* 2021). Overall, all metabolites found *in vivo* in the literature were found in our study, with the exception of m/z 240.159 (Yeh and Wang 2022). Chemical synthesis characterized one isomer m/z 238.144 at RT: 3.8min. The second isomer (m/z 238.144; RT: 4.2min) could correspond to an oxidation of the methylenic bridge carbon and demethylenation as reported by Yeh and Wang (2022). Furthermore, we first report the detection of phase II eutylone derivatives. These two glucuroconjugate derivatives (m/z 400.160 and m/z 414.176) had an important peak area in blood samples and could constitute relevant eutylone consumption markers. Thus, it does not always seem appropriate to perform preanalytical treatments with glucuronidase as described in the literature (Yeh and Wang 2022), which would make the detection of these glucuronate compounds impossible.

The implementation of these metabolites in the mass spectrometry databases will allow to add interesting consumption markers in cases of eutylone intoxication. This is all the more interesting as we only have plasma samples here, whereas urine samples would have a much higher concentration of metabolites.

4. Conclusion

We reported a case of chemsex under the influence of the new cathinone eutylone in a heterosexual couple. Study of the eutylone metabolism profile *in vivo* using molecular networking allowed the identification of 16 putative metabolites. Among these metabolites, a phase I metabolite (m/z 238.144) and 2 phase II metabolites (m/z 400.160 and m/z 414.176) were proposed as eutylone consumption markers in

biological matrix, and could be added in mass spectrometry libraries. The chemical synthesis of a metabolite, produced from a powder whose purity was verified by NMR, allowed us to strengthen our level of evidence in the identification of this compound and to quantify it in biological matrices. Consequently, the association of LC-HRMS coupled to molecular networking as well as the use of chemistry (NMR, synthesis) appears as a robust method in the NPS metabolism study from a seized powder.

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Acknowledgments

This project was supported by financial allowance “Défi Scientifique” Université Rennes 1.

Declarations

Competing interests

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Ethical standards

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of University Hospital of Rennes.

Author contributions

Conceptualization Pelletier Romain, Le Bouëdec Diane, Le Daré Brendan, Ferron Pierre-Jean, Porée François-Hugues, Gicquel Thomas; **Methodology**: Le Bouëdec Diane, Le Daré Brendan, Pelletier Romain, Ferron Pierre-Jean, Gicquel Thomas; **Formal analysis and investigation**: all authors; **Writing - original draft preparation**: Pelletier Romain, Le Bouëdec Diane, Porée François Hugues, Le Daré Brendan, Thomas Gicquel; **Writing - review and editing**: All authors; **Supervision**: Gicquel Thomas.

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