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Assessment of the stability of mephedrone in ante-mortem and post-mortem blood specimens

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ABSTRACT

Aims: The aim of this work is to test the stability of mephedrone added to whole blood collected from alive and dead mephedrone free-users and stored at three different temperatures (−20, +4 and +20 °C) with and without preservatives up to 6 months, trying to establish the best storage condition in order to reduce possible analyte loss/degradation during the storage period.

Materials and methods: Different sources of blood were obtained as follow: 10 samples of blood came from 10 alive mephedrone free-users (mean age 34 ± 15.8 years old) (Group 1), whereas 10 post mortem blood samples were obtained from 10 cadavers, in which the post mortem interval was between 24 and 36 h (Group 2). The cause of death in post mortem cases (mean age 45 ± 14.2 years old) was not drug related. Pools of blood were spiked with mephedrone at the concentration of 1 mg/L and 1 mL aliquots were transferred in 2 mL Eppendorf capped tubes with and without preservatives as follow: with ethylenediaminetetraacetic acid (EDTA) 3%; with sodium fluoride/potassium oxalate (NaF/KOx) 1.67%/0.2%, respectively; without preservatives. All samples were stored at three different temperatures: −20 °C, 4 °C and 20 °C and extracted and analyzed in duplicate by GC–MS according to a previously published method by Dickson et al., every other day during the first month and then weekly up to 6 months.

Results and conclusions: our study allow us to affirm that −20 °C is the best storage temperature for mephedrone stability in ante-mortem and post-mortem blood samples in comparison to the other two tested temperatures (+4 and +20 °C), showing higher values in both groups in samples stored with and without preservatives ($p < 0.0001$).

The comparison of Group 1 (samples coming from alive subjects) and Group 2 (post-mortem samples) highlights a better stability of mephedrone in Group 1 ($p < 0.001$) at all tested storage conditions.

Finally, the analysis of blood specimens stored with and without preservatives in both groups suggests that specimens stored with NaF/KOx maintain mephedrone stability better than those stored with EDTA ($p < 0.001$) and those stored without preservatives ($p < 0.0001$), therefore, we strongly recommend in order to maintain the highest mephedrone stability in blood, to store specimens at −20 °C adding NaF/KOx as preservative.

1. Introduction

Mephedrone (4-methylmethcathinone) is a β-ketoamphetamine structurally similar to cathinone derivatives and substituted amphetamines (Fig. 1). It is also known as "meow meow", "miaow", "M-Cat", "meph", "spice E", "plant food" and others and it is the most popular substance among cathinone derivatives [1]. Mephedrone is a psychoactive research chemical that produces stimulant and empathogenic effects similar to the ones that amphetamine, MDMA, methylamphetamine and cocaine elicit [2].

After khat-extracted cathinones were outlawed, 4-methylmethcathinone was specifically synthesized by altering cathinone's chemical structure, aiming at a related unscheduled substance to avoid existing drug misuse laws [3]. Mephedrone can be found as a white, occasionally off-white or even slightly yellowish powder or fine crystals. Less frequently it is marketed as tablets or

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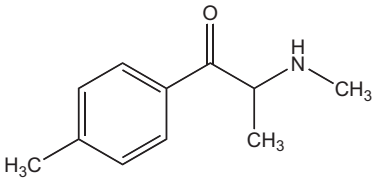

Fig. 1. Chemical structure of 4-methyl-N-methylcathinone (mephedrone).

capsules with different colors, shapes and thickness, either with or without logo. In recent years, its abuse has increased dramatically due to its broad purchase availability both in head shops and online and its promotion by aggressive web-based marketing thus becoming a significant public health problem both in Europe and US [1,3]. Moreover, the increased prevalence of mephedrone on the UK market may have been the result of the unprecedented decrease of cocaine and MDMA purity [1]. In April 2010 mephedrone and substances structurally related were classified as Class B substances in the UK under the Misuse of Drugs Act. Legislation [4]. The drug has been also classified in some other countries as a measure for the control of its availability.

The prevalence of mephedrone use is difficult to be defined. However, the results of an online survey of club-goers in the UK revealed that 41% had used mephedrone. A third had used the substance in the last month and 14% reported weekly use [5]. According to self-reported data obtained from high school and college students in the UK; 20% had used mephedrone at least once, while 4% reported daily use. It is worth pointing out that all of those using the drug daily were under 21 years old [6]. Users report obtaining the drug from both local dealers and Internet sources [6]. However, after regulatory measures that restrict possession, sale, and manufacture of synthetic cathinones passed in the UK, the number of users who purchased the drug from dealers increased considerably, while its price increased, almost two times higher than its price before legislation [4].

Sometimes mephedrone is sold as either cocaine or ecstasy while cut-agents such as paracetamol, caffeine, amphetamine, ketamine and even cocaine may be found in mephedrone [1,7]. As reported by users and drug-orientated websites, mephedrone is commonly used recreationally either via oral ingestion or insufflation.

Series of fatalities have been thought to be linked with mephedrone misuse. A number of fatalities that have been attributed to either lethal mephedrone intoxication or multi-drug toxicity (involving mephedrone) were reported in the literature [8–10]. Adamowicz et al. [11] reported a case where the death was attributed to mephedrone intoxication with blood and vitreous humor concentrations of 5.5 and 7.1 μg/mL, respectively. Dickson et al. [12] stated a case where the medical examiner reported the cause of death as multiple-drug toxicity. Heroin and mephedrone were detected in the biological samples of the deceased; mephedrone was confirmed in the decedent's blood and urine at 0.50 and 198 μg/mL, respectively. Moreover, blood and urine mephedrone concentrations of 1.33 and 144 μg/mL, respectively, were reported in another fatal multidrug intoxication case involving mephedrone [13]. Torrance and Cooper [14] detected mephedrone in four fatalities in Scotland; in two of the cases the cause of death was attributed to mephedrone intoxication. As evidence suggests, Forensic Toxicology laboratories must assess their current testing protocols to ensure they can detect mephedrone in biological samples.

The aim of this work is to test the stability of mephedrone added to whole blood collected from alive and dead mephedrone free-users and stored at three different temperatures (−20, +4 and +20 °C) with and without preservatives up to 6 months, trying to establish the best storage condition in order to reduce possible analyte loss/degradation during the storage period.

2. Materials and methods

2.1. Reagents

All solvents used were high-performance liquid chromatography grade and purchased from Carlo Erba (Milan, IT). Mephedrone HCl, 1.0 mg/mL and mephedrone-d_3 HCl, 100 μg/mL were purchased from Cerilliant–Sigma–Aldrich (St. Louis, MO, USA); pentafluropropionic anhydride (PFAA) was purchased from Restek (Bellefonte, PA); concentrated HCL was purchased from Carlo Erba (Milan, IT) and potassium hydroxide (KOH) pellets were purchased from Carlo Erba (Milan, IT).

2.2. Preparation of standard, calibrators and controls

A stock standard of mephedrone in methanol was prepared and stored at −15 °C *in an amber vial*. Certified drug-free blood was used for the preparation of calibrators. Blood (2 mL) was spiked with mephedrone at the following concentrations: 0.05, 0.10, 0.20, 0.50, 1.00 and 2.00 mg/L. Stock standard solution and certified drug-free blood were used for the preparation of positive blood control at concentration of 0.5 mg/L. Both controls and calibrators were prepared prior to each extraction. Internal standard (mephedrone-d_3) spiking solutions were prepared at concentration of 0.01 mg/mL. Both positive and negative controls were included and extracted with each batch.

2.3. Samples' preparation and storage conditions

Different sources of blood were obtained as follow: 10 samples of blood came from 10 alive mephedrone free-users (mean age 34 ± 15.8 years old) (Group 1), whereas 10 post mortem blood samples were obtained from 10 cadavers, in which the post mortem interval was between 24 and 36 h (Group 2). The cause of death in post mortem cases (mean age 45 ± 14.2 years old) was not drug related (see Table 1). Pools of blood were spiked with mephedrone at the concentration of 1 mg/L and 1 mL aliquots were transferred in 2 mL Eppendorf capped tubes with and without preservatives as follow: with Ethylenediaminetetraacetic acid (EDTA) 3%; with sodium fluoride/potassium oxalate (NaF/KOx) 1.67%/0.2%, respectively; without preservatives. All samples were stored at three different temperatures: −20 °C, 4 °C and 20 °C and extracted and analyzed in duplicate, every other day during the first month and then weekly up to 6 months. All specimens were not exposed to light and kept in the dark.

2.4. Instrumentation

The GC/MS analysis was carried out using an Agilent Technologies (AT) model 6890 N GC coupled with an AT mod. 5973 Inert mass selective detector and an AT 7683 Series automatic sampler at the following chromatography conditions: DB5-MS capillary column (30 m, 0.32 mm i.d.) with helium carrier gas maintained at constant flow of 1.0 mL/min. The MS source and quadrupole temperatures were held at 230 °C and 150 °C, respectively. The transfer line was set at 280 °C. Full scan EI MS data was collected over a mass range of *m/z* 42–550 and a detection threshold of 150. Identification of mephedrone was accomplished using selected ion monitoring (SIM). The SIM ions collected were *m/z* 204, 160 and 119 for mephedrone and 207, 163 for mephedrone-d3; the quantitative ions are underlined.