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Cathinone stability in authentic urine specimens

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ABSTRACT

Purpose: Synthetic cathinones are encountered in a variety of antemortem and postmortem forensic toxicology investigations. Earlier experimental studies using fortified urine have evaluated analyte, temperature and pH-dependent variables associated with their stability. The purpose of this study was to compare experimental findings with those obtained using authentic urine from cathinone users.
Methods: In this report we compare cathinone concentrations in 180 authentic unpreserved urine specimens, following known periods of refrigerated storage. These findings are compared with previously published experimental data using fortified drug-free urine. Liquid chromatography quadrupole time-of-flight mass spectrometry (LC-Q/TOF-MS) was used to target 22 cathinones. Quantitative results were compared in urine specimens (pH 4.5–10) following 5–17 months of storage.
Results: The 180 specimens resulted in 164 quantitative findings involving α-PVP, ethylone, methylone, MDPV and pentylone. Initial drug concentrations ranged from 25 ng/mL to over 100,000 ng/mL. Upon reanalysis, the percentage of drug remaining (0–119%) was correlated with storage time and specimen pH. The ability to reconfirm original results was not correlated with storage time. Instead, specimen pH was far more predictive. The relationship between initial and final drug concentration was highly pH-dependent, yielding significant correlations for α-PVP, ethylone and methylone, particularly under acidic conditions.
Conclusions: These results are in good agreement with experimental findings and highlight the critical importance of specimen pH, rather than conventional time dependent variables, when considering cathinone stability in biological samples. The potential for pre-analytical changes in cathinone concentrations must be carefully considered when interpreting their results.

1. Introduction

The escalating use of new psychoactive substances (NPS) has significantly impacted the global landscape of recreational drug use. These increases have been documented through reports issued by the European Monitoring Center for Drugs and Drug Addiction (EMCDDA), the National Forensic Laboratory Information System (NFLIS), poison control center reports, and both antemortem and post-mortem forensic toxicology casework [1–6]. Synthetic cathinones still represent a significant portion of the NPS market, which is significantly fueled by the Internet [7]. The epidemiology of their use is complicated by the rapid emergence of new analogs and derivatives.

As of 2016, the EMCDDA was monitoring as many as 103 synthetic cathinones [3]. According to the 2017 European Drug Report, this number increased to 118, making it the second largest new substance group, having been identified in more than half of the participating European countries [8]. NFLIS reported an increase from 5 cathinones in 2009 to 35 in 2015. The five cathinones reported in 2009 were mephedrone, MDPV, methylone, methcathinone, and 4-MEC, with mephedrone and methcathinone being the most prevalent [1]. By 2015, methcathinone was no longer among the top twenty cathinones, having been largely replaced by methylone, ethylone, and α-PVP. Although NPS use in the US lags behind many of the European countries, they tend to follow the same overall trends. While some cathinones have remained popular, including α-PVP and MDPV, others have decreased (e.g. buphedrone), or have been largely replaced by newer analogs such as brephedrone (4-BMC) and other halogenated species. Despite their evolving nature, surveillance reports from both the United States and Europe confirm that recreational use of these novel psychostimulants continues to be a problem.

Synthetic cathinones are capable of producing stimulant and euphoric effects similar to methamphetamine and cocaine. Sought after effects may include increased sociability, energy, focus, and empathy [9–11]. Synthetic cathinone toxicity has resulted in

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neurological, cardiovascular, and psychopathological symptoms including hyperthermia, paranoid psychosis, organ failure, and death [4,10–13]. Their physical and neurological effects are attributed to their interactions with the monoamine neurotransmitters dopamine, norepinephrine, and serotonin.

Synthetic cathinones have been associated with impairment, intoxication, and fatal overdose. Quantitative determinations have been reported throughout the scientific literature in a variety of biological matrices. In this report, we describe changes in urinary drug concentrations among a population of cathinone users. Cathinone concentrations in antemortem urine have been reported over a very wide range, from tens of nanograms per milliliter to several thousand [14–18]. Therefore, analytical methods must have not only adequate specificity to identify structurally similar drugs and regioisomers, but also high sensitivity. The latter becomes critically important, particularly if pre-analytical changes in concentration take place due to instability or degradation of the drug.

We previously reported a comprehensive synthetic cathinone stability study in urine to address analyte, pH, temperature, concentration and time-dependent variables [19]. Although no concentration dependence was observed, cathinone stability was significantly dependent upon urinary pH, storage temperature, and structural characteristics of the cathinone itself. A total of 22 cathinones fortified in pooled drug-free human urine (pH 4 and 8) were investigated at four temperatures (32, 20, 4, and −20 °C) during six months of storage. Cathinones were less stable in alkaline urine, with significant changes observed within hours for some drugs under certain conditions. In contrast, all drugs remained stable over the entire six month period in acidic urine when refrigerated or frozen. The pyrrolidine (tertiary amine) and methylenedioxy groups exerted significant stabilizing effects. Drugs containing both groups (e.g. methylenedioxypyrovalerone (MDPV) and 3,4-methylenedioxy-α-pyrrolidinobutiophenone (MDPBP)) were the most stable of the twenty-two drugs investigated. Unsubstituted and ring substituted cathinones were considerably less stable, with 3-fluoromethcathinone (3-FMC) exhibiting the greatest instability. Although the approach using fortified preserved urine affords a robust experimental design for the evaluation of long-term stability, authentic urine specimens from cathinone users can also provide valuable information. In this study, we compare cathinone concentrations in 180 authentic unpreserved urine specimens from cathinone users following known periods of storage. These findings are compared with previously published experimental data using fortified drug-free urine.

2. Materials and methods

2.1. Chemicals and reagents

Methcathinone, 3-FMC, 4-fluoromethcathinone (4-FMC, flephedrone), methylone, ethcathinone, ethylone, methedrone, buphedrone, butylone, mephedrone, eutylone, 4-methylethcathinone (4-MEC), 3,4-methylenedioxy-α-pyrrolidinobutiophenone (MDPBP), pentedrone, pentylone, 3,4-dimethylmethcathinone (3,4-DMMC), α-pyrrolidinopentiophenone (α-PVP), 4-ethylmethcathinone (4-EMC), 4-methyl-α-pyrrolidinobutiophenone (MPBP), methylenedioxypyrovalerone (MDPV), pyrovalerone, and naphyrone were purchased from Cerilliant Corporation (Round Rock, TX, USA) in 1.0 mg/mL methanolic solutions. Internal standards methylone-D3, ethylone-D5, butylone-D3, mephedrone-D3, eutylone-D5, pentylone-D3, α-PVP-D8, MDPV-D8, and naphyrone-D5 were also purchased from Cerilliant Corporation in 0.1 mg/mL methanolic solutions. The internal standard solution consisted of all nine deuterated internal standards at a concentration of 0.25 μg/mL in methanol. Pooled drug-free urine, preserved with 1% sodium fluoride, was purchased from Utak Laboratories (Valencia, CA, USA).

Dichloromethane, isopropyl alcohol, and glacial acetic acid were purchased from Mallinckrodt Chemicals (St. Louis, MO, USA). LC–MS grade methanol, concentrated hydrochloric acid, LCMS grade acetonitrile, and dibasic sodium phosphate were purchased from J.T. Baker (Center Valley, MA, USA). Optima® hexane and HPLC grade ethyl acetate were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Formic acid (>99%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Monobasic sodium phosphate was purchased from VWR (Radnor, PA, USA). Deionized water was purified in-house using a Millipore Direct-Q® UV Water Purification system (Billerica, MA, USA). PolyChrom ClinII 3 cc (35 mg) solid phase extraction (SPE) columns were purchased from SPEware (Baldwin Park, CA, USA).

2.2. Instrumentation

An Agilent Technologies 6530 LC-Q/TOF-MS (Santa Clara, CA, USA) equipped with an Agilent 1290 Infinity autosampler and a Series 1200 LC system was used for instrumental analysis. Cathinones were separated using an Agilent Poroshell 120 EC-C18 column (2.1 × 100 mm, 2.7 μm particle size) and an Agilent Poroshell 120 EC-C18 guard column (2.1 × 5 mm, 2.7 μm particle size) maintained at 35 °C. Mobile phase A and B comprised of 0.1% formic acid in deionized water and acetonitrile, respectively, and used the following gradient elution profile: 96% A and 4% B (0–0.5 min); 10% B (0.5–5 min); 40% B (5–11 min); 100% B (11–12 min). Nitrogen was generated using a Genius 3040 Nitrogen Generator (Peak Scientific, Billerica, MA, USA). Solid phase extraction (SPE) was performed using a JT Baker vacuum manifold and extracts were evaporated to dryness under nitrogen using a TurboVap LV® concentration workstation (Caliper Life Sciences, Hopkinton, MA, USA). A FiveEasyTM FiveGoTM pH meter FE20/FG2 was used for pH measurement (Mettler Toledo, Columbus, OH, USA). The LC-Q/TOF-MS procedure used for quantification of cathinones in urine was based on a previously published method and was validated in accordance with generally accepted guidelines [20,21]. Limits of quantitation for the twenty-two target compounds ranged from 0.25 to 5 ng/mL with recovery rates greater than 80% [20].

2.3. Authentic urine specimens

Urine specimens from cathinone users were obtained in accordance with an IRB-approved study. A total of 180 cathinone-positive urine specimens were received from Redwood Toxicology Laboratory (RTL). Following the initial analysis for cathinones using gas chromatography–mass spectrometry (GC–MS) at RTL, specimens were stored at 4 °C at the reference laboratory. Aliquots of urine were transferred into borosilicate glass tubes and transported to our facility on ice for subsequent testing. Storage times for each specimen were known, and was defined as the time between the first analysis at RTL and the second analysis at our facility (5–17 months later). Urinary pH was determined at the time of reanalysis. Quantitative reanalysis was performed using one milliliter of unpreserved urine. Appropriate dilutions using 0.1 M, pH 6 phosphate buffer were performed for samples that exceeded the calibration range of the assay (500 ng/mL). Following SPE, samples were reconstituted in 25 μL of a 50:50 mixture of Mobile Phase A:B and 1 μL was injected onto the LC-Q/TOF-MS for analysis.

2.4. Extraction of cathinones from urine

Internal standard solution (100 μL) was added to 1 mL urine to achieve a final concentration of 25 ng/mL. Urine, diluted with 2 mL