K8 Short-Term Stability of Mephedrone in Blood and the Impact of Storage Conditions on Concentrations Detected by GC/MS

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After attending this presentation, attendees will understand the implications of different storage conditions upon mephedrone concentrations in blood.

This presentation will impact the forensic science community by offering forensic toxicologists, chemists involved with drug analysis, and forensic pathologists more information about appropriate sample storage conditions to reduce potential sample degradation and loss between the time samples arrive into the laboratory and when they are tested.

Introduction: The time between sample collection and sample analysis varies greatly from case to case; therefore, knowledge of analyte stability is of extreme importance. Not only does it help the toxicologist select the most suitable sample for analysis, it also ensures that optimum storage conditions and preservatives are used to limit any sample degradation. This in turn aids the interpretation of concentrations of any drugs detected and their significance.

Mephedrone first appeared on sale to the public in 2007, and remains one of the most routinely detected "bath salts" or Novel Psychoactive Substances (NPS) in the United Kingdom and the United States. At the time, the majority of toxicology laboratories were not equipped to test for this compound; however, since developing methods for its analysis, many have performed retrospective testing on samples to investigate the presence of this drug. Previous work has shown mephedrone to be unstable in biological matrices. This research was intended to ascertain the effects of various storage temperatures and preservatives in preventing analyte degradation and determine the optimum conditions.

Method: Preservative-free bovine blood was spiked with mephedrone (1mg/L). This was divided into separate aliquots (1mL), enabling the examination of the stability of the drug in blood stored: (1) without preservative; (2) with citric acid preservative (8%); and, (3) with sodium fluoride (1.67%)/potassium oxalate (0.20%) at three different temperatures (-20°C, -4°C, and 20°C). Aliquots were tested daily for seven days and then weekly over a period of ten weeks. Samples were analyzed in triplicate and calibrations and controls were run during each analysis. Samples were extracted using Solid Phase Extraction (SPE) prior to derivatization with Pentafluoroprionionic Acid-Ethyl Acetate (PFPA:EtOAc (2:1)) and analyzed using a Bruker® Gas Chromatograph-Tandem Mass Spectrometer (GC/MS/MS) with a DB5 column (30m x 0.25mm, 0.25µm) in splitless mode. The total run time was 25 minutes, and mephedrone-D_3 was used as the internal standard.

Results: Samples stored at 20°C were the most effected with mephedrone becoming undetectable after a period of 21 days regardless of any preservative present. After one day, samples stored at 20°C saw a loss of on average 19%, ranging from 17% with citrate solution to 21% with fluoride oxalate. Refrigerated samples preserved with fluoride/oxalate and citrate preservatives were initially stable; however, fluoride/oxalate rapidly decreased after five weeks, with a total loss of 96% over the ten-week period. Refrigerated samples preserved with citrate solution showed no significant decrease over the ten-week period. Refrigerated samples stored with no preservative saw a 41% drop after five weeks and a 74% reduction in concentrations across the ten-week period. Samples stored at -20°C were stable under all conditions over the ten-week period.

Conclusion: To maximize stability of mephedrone, samples should be stored at -20°C and preserved using citrate as this prevents any oxidative losses occurring. Although fluoride/oxalate was shown to preserve samples when stored at -20°C, degradation was still problematic at 4°C after five weeks, seeing a 36% decrease. This in turn would affect reported concentrations from laboratories which store samples at 4°C prior to analysis before archiving them at -20°C. Regarding retrospective analysis, analysts should be cautious in interpreting negative results in cases where the history indicates mephedrone use. The sample history must be taken into consideration. Further work is underway to investigate potential degradation products; cross validation with human blood is also being planned.

Stability, Mephedrone, Preservatives

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