K64 Stability of Seven Benzodiazepines Together With Zolpidem, Methodone, and Propoxyphene in Bloodstains

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After attending this presentation, attendees will learn about another aspect of bloodstain analysis. The goal of this presentation is to emphasize the potential interest in toxicological analysis of bloodstains.

This presentation will impact the forensic science community by highlighting another aspect of bloodstain analysis that should be valued in forensic practice.

Blood ranks among the most usual kind of physical evidence encountered on a crime scene. Individualization of human blood has been performed for decades by using the ABO system and, more recently, DNA typing granted the forensic scientist a high-performance tool for this purpose. Forensic toxicology, however, also followed a continuous progress, currently providing the possibility to detect various drugs in very small blood samples. This purportedly offers the opportunity to assay bloodstains for toxicological analysis, which could be of interest in some situations, e.g., determination of the victim's toxicological status even if no corpse is found at the crime scene, or of the perpetrator's status if he/she bled in the surroundings of the crime scene. Moreover, when DNA typing cannot be compared to a reference (the victim and/or any biological element for DNA comparison was not found), detecting drugs in bloodstains could contribute to the victim's identification. Until now, only a few works have dealt with the detection of drugs in such samples, and the stability of drugs in bloodstains under different storage conditions have never been studied before. The stability of seven benzodiazepines (diazepam, bromazepam, clonazepam, alprazolam, clobazam, tetrazepam, and triazolam) were investigated, together with zolpidem, methadone, and propoxyphene over periods ranging from 24 hr to one month under various environmental conditions (drugs were chosen because of their frequent prescription in France).

Drug-free 50µL blood samples were spiked with an amount of 10ng/ml of each analyte (500pg per blood sample) and deposited on a glass slide. After storage at -20°C, +4°C, and +35°C away from light, at +20°C in daylight, away from light, and in "extreme" conditions (outside the laboratory exposed to daylight, wind, and variable temperature), bloodstains were collected after 24hr, 48hr, 72hr, one week, and one month by scratching and by swabbing. When scratched, the bloodstain was weighed and rehydrated for 45 min in 0.5mL ammonium buffer (pH 9.5). Swabbing was performed with swabs previously moistened with saline. Swabs were placed in 0.5mL ammonium buffer, sonicated 15 min, and stored away from light for 45 min before swabs were removed. Liquid/liquid extraction was performed using methylenechloride, N-Heptane, isopropanol (65:25:10, v/v) with Prazepam as an internal standard. Then toxicological analyses were carried out by Ultra Performance Liquid Chromatography/Liquid Chromatography with Tandem Mass Spectrometry (UPLC/MS/MS) under previously reported conditions.

The validation of the method was performed on dried bloodstains stored away from light at room temperature for six hours spiked with the different analytes. Under these analytical conditions, the method appeared sensitive (0.0005<LOQ<0.005ng/mg), linear (LOQ-10ng/mg), and accurate (CV<20%).

Results showed a good stability of all drugs tested even after one month of storage in each condition, except for clonazepam at -20°C (sometimes undetected), and for all drugs tested at +35°C and in "extreme" condition with sometimes up to 50% loss after one month. By scratching or swabbing, each analyte could be detected, except for clonazepam at -20°C. This study shows an acceptable stability of most benzodiazepines, zolpidem, methadone, and dextropropoxyphene in dried bloodstains. It opens the way to a new analytical approach which may enhance the bloodstain pattern analysis of a crime scene.

Bloodstain, Drugs, Stability

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