K65 The Detection and Quantification of Amphetamine and Norephedrine in Rat Brain, Heart, and Liver Tissues at Different Stages of Decomposition After Internment

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Learning Overview: After attending this presentation, attendees will be aware of the possible relationship between antemortem amphetamine dose and the concentration of amphetamine and its metabolite norephedrine in buried remains at various stages of decomposition.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing information regarding the effect of decomposition on the identification and quantification of amphetamine and norephedrine in postmortem tissue samples. This will aid death investigators in determining a possible drug contribution to cause of death when amphetamine is present.

In forensic toxicology casework, the primary matrices include biological fluids, such as blood and urine. However, some forensic casework includes the testing of decomposed postmortem tissue samples. Forensic toxicologists, therefore, need to know if decomposition has an effect on the identification and quantification of a drug because any analysis completed by the toxicologist assists the forensic pathologist in determining the impact of the drug(s) on the cause of death. This involves the understanding of taphonomy and its effects. Due to this intersection of toxicology and taphonomy, this research aims to determine the relationship between antemortem amphetamine dose, the concentration of amphetamine, its metabolite norephedrine, and the stage of decomposition of buried remains.

This relationship is important for amphetamine as it is a drug that is seen in fatal drug cases, so a possible relationship between decomposition and the concentration detected could have an impact on its interpretation in postmortem samples. To determine this relationship, 17 male Long Evans rats were dosed with amphetamine at concentrations of 10mg/kg, 6mg/kg, 2mg/kg, 1mg/kg. 0.6mg/kg, and 0.2mg/kg once a day for ten days. The rats dosed at the 10mg/kg, 6mg/kg, and 2mg/kg concentrations were euthanized with CO_2 ten days after the final injection while the other doses were euthanized with CO_2 immediately following the final dose. The rats were then interred in the New Jersey Pine Barrens, then later exhumed at different stages of decomposition in accordance with the Megyesi method. Following exhumation, liver, heart, and brain tissue samples were collected during dissection, but when decomposition was more advanced, samples were collected from the general location at which the organ would have been found. The samples were stored at -20°C until analysis. During analysis, the samples were tested for amphetamine and its metabolite norephedrine.

The samples were prepared by homogenizing the tissues with saline using a Biotage® Bead Ruptor 24 (1:1 for brain and liver, 2:1 for heart) and performing a protein precipitation with acetonitrile followed by a Liquid-Liquid Extraction (LLE) for amphetamine and norephedrine. The LLE method involved an initial organic extraction using dichloromethane/ethyl-acetate/isopropyl alcohol (3:1:1), a back extraction using a 1M phosphate buffer at pH 2.5, and a final organic extraction using dichloromethane/isopropyl alcohol/ammonium hydroxide (78:20:2). The analytes were identified and quantified using a Perkin Elmer Clarus® SQ 8T Gas Chromatograph/Mass Spectrometer (GC/MS) in Selected Ion Monitoring (SIM) mode with amphetamine-D11 (m/z 194) and norephedrine-D3 (m/z 283) as the internal standards. The SIM ions for amphetamine are 190, 118, and 91; the SIM ions for norephedrine are 280, 190, and 91. The column used in this research is an Agilent® DB-5ms Ultra column (30m, 0.25mm ID, 0.25µm df). This method was validated using the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines for method validation.

The analysis of the postmortem tissue samples showed that amphetamine and norephedrine can be detected in soft tissue through the different stages of decomposition. A dose-related concentration was seen within the two groups of rats.

Amphetamine, Buried Remains, Postmortem Tissue