K7 Study of L-2-Aminothiazoline-4-Carboxylic Acid as a Biomarker for Cyanide Poisoning by LC-MS/MS Analysis

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After attending this presentation, attendees will understand how cyanide poisoning can be detected by measuring the amount of a derivative of cyanide, L-2-Aminothiazoline-4-Carboxylic Acid (ATCA) in biological fluids. ATCA can be easily measured in blood, urine, and organs from a subject.

This presentation will impact the forensic science community, as well as the Army, by improving the detection of cyanide from poisoning in various ways.

One threat of cyanide poisoning is the use of cyanide as a chemical warfare agent (CWA). Once exposure is identified, the amount of poison can be quantified and a more accurate treatment distributed. Identification of cyanide or its metabolites in biological fluids is necessary for many purposes in forensic, clinical, military, research, and veterinary fields. However, because of the volatility of cyanide and the difficulty of establishing steady-state cyanide levels with time, methods of directly evaluating cyanide levels are limited.

These studies focus on a chemically stable urinary metabolite of cyanide, 2-aminothiazoline-4-carboxylic acid (ATCA), which is an effective biomarker for cyanide exposure, specifically in mice liver samples. ATCA was used because it is stable over time, unlike cyanide, and its concentration level is directly proportional to the amount of cyanide from exposure. After using a method previously developed to dissect, preserve organs, and homogenize the livers, the organs were spiked with an internal standard, 2-aminothiazole-4-carboxylic acid (ATZA). The similarity between ATCA and ATZA is advantageous because ATZA is co-eluted with ATCA and detected at the same time by LC-MS/MS, therefore experiencing the same magnitude of ion suppression. ATCA was then extracted by solid phase extraction (SPE). Endogenous levels of ATCA were determined by comparing the non- exposed livers to calibrators containing known concentrations of ATCA, both of which were evaluated by the LC-MS/MS.

Mice were later exposed to various doses of cyanide and liver ATCA contents were compared to the dose of cyanide mice were given. An optimal method was developed to detect ATCA, with a recovery of 40-50%. Endogenous levels of ATCA in liver were found to be at least 100 ng/ml and were measured multiple times. This study indicates that this method can continue to be used for other organs, such as kidney, lung, and heart, to detect endogenous ATCA. Future studies will also concentrate on determining the concentration of ATCA in organs obtained from mice that were previously exposed to cyanide and compare the differences between endogenous ATCA levels and levels after exposure.

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