K17 A Targeted Qualitative Screen for the Detection of Pesticides in Postmortem Specimens by Ultra High Performance Liquid Chromatography-Ion Trap-Mass Spectrometry (UHPLC-Ion Trap-MS^n)

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Learning Overview: After attending this presentation, attendees will better understand the qualitative identification of 13 pesticides in postmortem samples by UHPLC-Ion Trap-MS^n. Pesticide classes included in the study were common organophosphates, carbamates, triazines, and triazoles. The majority of the analytes used in this method were pesticides found at a local hardware store or used in the agricultural industry specifically for the treatment of insects, weeds, or fungi found on tropical plants or fruits.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing a validated targeted screening method for the qualitative identification of pesticides in postmortem specimens and how to apply the method to several medical examiner cases in which a pesticide is suspected as a cause of death.

In Miami-Dade County, exposure to harmful pesticides is common due to its location, tropical climate, and large agricultural industry. Forms of exposure can include overspray from airplanes or tractors, occupational (farmers and landscapers), or household (improper use, storage, and application). From 2014 to 2017, Florida Poison Information Center Network ranked pesticides among the top ten exposure categories for Miami-Dade County with approximately 400 calls related to pesticide exposure reported annually. Although the number is underreported, the Miami-Dade Medical Examiner Department (MDME) reported approximately 1-2 cases per year since 2004 in which a pesticide was listed in the cause of death or in which pesticide poisoning was suspected in the cause of death. Due to the prevalence of pesticide use in the community and the potential for accidental or intentional fatalities, it is imperative that laboratories are able to identify pesticides in postmortem matrices.

The method validation plan included the evaluation of Limit Of Detection (LOD), carryover, interferences, and autosampler stability. Blank blood was utilized for the method validation studies, whereas medical examiner casework analysis included the evaluation of blood, serum, urine, and/or gastric contents obtained at autopsy. Then 500µL of specimen was fortified with a deuterated internal standard solution containing atrazine-D_5. Protein precipitation was performed by the addition of 2mL of acetonitrile:methanol (95:5), followed by centrifugation at 2,800g for 10 minutes. The supernatant was analyzed using a Thermo Scientific™ UltiMate™ 3000 UHPLC coupled to a Bruker® amaZon™ SL Ion Trap Mass Spectrometer. Chromatographic separation was achieved with a Thermo Scientific™ Acclaim® RSLC 120 C18 column (2.1 x 100mm, 2.2µm) at 40°C with an aqueous mobile phase consisting of 2mM ammonium formate, 0.1% formic acid, and 1% acetonitrile in water, and an organic mobile phase consisting of 2mM ammonium formate, 0.1% formic acid, and 1% water in acetonitrile. Positive electrospray ionization was utilized with a scan range of 50-450 *m/z*. Tandem Mass Spectrometry (MS/MS) data analysis included multi-step identification using a data-dependent scheduled precursor list, which utilizes retention time and molecular ion data to trigger MS^n analysis.

An in-house library containing 13 pesticides of interest and an internal standard, including retention time, parent ion spectra, and daughter ion spectra, was created based on the analysis of neat PESTANAL® standards. Chromatographic separation of all pesticides was achieved within the first ten minutes. The experimental LODs ranged from 0.10mg /L to 1.0mg/L. Carryover was not observed for any pesticide up to 10mg/L, except for diazinon, which was free from carryover up to 5mg/L. No exogenous interferences were observed. Extracts were stable on the autosampler for all pesticides up to 72 hours.

Although forensic toxicology laboratories largely deal with illicit substances, over-the-counter medications, and prescription medications, everyday household chemicals, such as pesticides, are just as fatal. With the ease of availability of pesticides to the general public, it is no surprise that they are the result of accidental or intentional poisonings and/or fatalities. Symptoms of pesticide toxicity vary depending on the pesticide, but many include nausea, vomiting, headache, dizziness, abdominal pain, and central nervous system depression; which are easy to mistake for common ailments. The development of this assay demonstrated that protein precipitation followed by UHPLC-Ion Trap-MS^n analysis was a suitable method for the detection of pesticides in biological matrices. The combination of a qualitative targeted screen for pesticides, pathological findings, and clinical manifestations can be used to aid the medical examiner in determining cause and manner of death in cases where pesticide toxicity is suspected.

Pesticides, Postmortem Forensic Toxicology, UHPLC-Ion Trap MS/MS

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