B88 Game's Up - The Presence of Tranquilizers and Stimulants in Venison Detected by HPLC-MS

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Attendees will learn of an unusual forensic case in which tranquilzers and stimulants were detected in processed tissue. In the absence of blood and urine, the detection of these drugs was quite challenging. The sensitivity that HPLC-MS provides was advantageous and will be discussed.

This presentation will impact the forensic community and/or humanity by highlighting the advantage of HPLC-MS in the forensic laboratory setting. The sensitivity that LC-MS provides combined with the added sensitivity of the instrument in MS/MS mode permits the detection of drugs of interest in difficult matrices at low ppb range levels.

The preferred matrices for detection of drug residues in biological fluids are blood and urine. While these matrices can prove to be quite challenging, in their absence, with only processed tissue available for sampling, the challenge can be amplified for the analytical chemist. This paper will discuss an unusual forensic dilemma in which the sensitivity of HPLC-MS proved to be advantageous.

The sale of a specific deer for hunting is illegal in most states. Once a deer has been taken in violation of State law and transported in interstate commerce, it becomes a violation of Federal law under the U.S. Fish and Wildlife's Lacey Act. In a recent case farm raised deer were being hunted at a private preserve in violation of State laws. In most instances, the clients traveled to the preserve from out of state, and the hides, meat, and antlers were then shipped across state lines to the hunters in their home states. The case became a joint effort between the state Department of Natural Resources and U.S. Fish and Wildlife. The Food and Drug Administration's Forensic Chemistry Center became involved in the case when it was learned that once the deer were selected by the client, they were unlawfully tranquilized, moved to a small pen, and "reversed" (i.e., revived with stimulants) to then be killed by the client/hunter. The presence of tranquilizing and/or stimulant drugs in meat is a violation of FDA law whereby meat intended for consumption containing these compounds is considered to be adulterated.

The drugs that were specifically targeted for analysis were xylazine, tiletamine, zolazepam, and tolazoline. Xylazine is an α_2-adrenergic receptor agonist and acts as a sedative. Tiletamine is a dissociative agent and is pharmacologically similar to ketamine. It produces immobilization and acts as an analgesic. Zolazepam is a benzodiazepine sedative and muscle relaxant. Zolazepam also prevents seizures associated with tiletamine and is therefore marketed as the combination drug Telazol®. The drug mixture of xylazine and Telazol is commonly referred to as a "cocktail" and is considered an effective way to sedate large animals. The Telazol can be purchased as a powder and rather than reconstituting the powder with 5 mL of sterile water, 5 mL of xylazine drug solution is used and the mixture is injected intramuscularly. Tolazoline is a mixed α_1 and α_2 adrenergic receptor agonist and is used to reverse the effects of xylazine sedation.

A number of factors made the detection of these drugs challenging for the analytical chemist. The drug dosages ranged from 250mg to 400mg and the half-lives of the drugs spanned from approximately 50 minutes to 4.5 hours. However, the laboratory did not have any information regarding the time frame in which the deer were drugged before being killed. Average adult bucks range in weight from 100lbs to 250lbs, and most of the drugs readily distribute to all body tissues though tolazoline tends to concentrate in the liver and kidneys.

The initial samples received in the laboratory consisted of highly processed meat. The samples were prepared using in part a method published in the "Handbook of Analytical Toxicology" which generates residues for weak acid/neutral drugs, strong acid drugs, and basic drugs. Initial sample preparation was done by weighing approximately 10g of meat and making a slurry with water. The slurry was acidified, saturated with salt, and placed in a hot water bath for 30min. The solution was then filtered and extracted with an equal volume of ethyl ether. The drugs of interest are basic and therefore only the aqueous extract was taken through further extraction procedures while the ethyl ether layer was discarded. The aqueous extract was made basic and extracted with equal volumes of chloroform. The resulting basic extract was first analyzed using GC-MS and no drugs were detected. Control deer meat was obtained to conduct spiking experiments. Based on these experiments, between 5 and 10ug/mL was the detection limit for the drugs of interest operating this instrumentation in full scan mode. Due to the sensitivity limitations and the absence of data regarding the stability of the drugs during the meat processing, detection by GC-MS did not appear to be feasible. The original sample extracts resulted in approximately 300 mL of chloroform. These extracts were taken to dryness and then reconstituted with 200µL of 10% MeOH/0.5% formic acid in water, thereby increasing the concentration by approximately 1500 fold. The samples were then analyzed using HPLCMS. The samples were analyzed with a gradient mobile phase of 0.1% formic acid and acetonitrile on a C18 column and were introduced into the MS by electrospray ionization. The sensitivity that the LC-MS provided combined with the added specificity of the instrument in MS/MS mode permitted detection of the drugs of interest in highly processed venison at low ppb range levels.

Stimulants, Tranquilizers, HPLC-MS

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