K37 An Investigation of the Binding of Benzodiazepines to Human Serum Albumin and the Effect on Quantitation in Blood Samples

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After attending this presentation, attendees will understand how the binding of benzodiazepines to Human Serum Albumin (HSA) can affect the quantitation of benzodiazepines in blood samples. Attendees will also be made aware of how the varying binding affinities of different benzodiazepines for human serum albumin can affect quantitation within specific sample preparation methods.

This presentation will impact the forensic science community by providing further pharmacological/toxicological information on benzodiazepines, a class of drug that is commonly used therapeutically and is increasingly being abused in social settings.

Benzodiazepines are commonly prescribed central nervous system depressants which are found in a wide variety of different medications, from sedatives and hypnotics to amnesiatics and anticonvulsants. Benzodiazepines are increasingly being used as recreational drugs, often in combination with other drugs such as opiates and alcohol. HSA is the most abundant plasma protein in humans. Many drugs, including benzodiazepines, bind reversibly to albumin with albumin then acting as a carrier for the drug. This binding can increase the apparent solubility of the drug in the plasma and can influence the distribution, metabolism, and excretion of the drugs. Quenching of albumin fluorescence can be used to study the interactions of these drugs with albumin and characterize the binding affinities and other important binding characteristics. In a preliminary investigation, the binding affinities and other binding characteristics for alrazolam, bromazepam, diazepam, flunitrazepam, flurazepam, lorazepam, oxazepam, temazepam, and triazolam to HSA were tabulated. The binding constants of the nine benzodiazepines ranged from $1.14x10^{2}$M for diazepam, having the lowest binding affinity, to $8.05x10^{6}$M for flunitrazepam, with the highest binding affinity. The binding of these drugs to HSA and the binding affinity of each benzodiazepine derivative may affect the quantitation of these drugs in blood. In the current research, different preparation methods were utilized on samples spiked with known amounts of benzodiazepine. Quantitation was accomplished using an Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) method with Multiple Reaction Monitoring (MRM) which utilized a C18 column and isocratic elution with 0.1% formic acid in methanol (60%) and 0.1% formic acid (40%) at a total flow rate of 0.3500mL/min. The temperature range was 40°C – 95°C. Within a preparation method, the effect of differing binding affinities on the quantitation was studied. In a dilute and shoot method, flunitrazepam, a benzodiazepine which was shown to have a high binding affinity for albumin, showed a significant difference when quantitated in samples containing human serum albumin compared to samples without human serum albumin. Samples containing HSA had calculated concentrations that were 31% – 50% lower than samples without HSA. Diazepam, which was shown to have a lower binding affinity for albumin, also showed a significant difference when quantitated in samples containing HSA compared to those without. Samples containing HSA had a calculated concentration that was 40% – 60% lower than samples without HSA. Other preparation methods were used; a comparison of these results will also be presented.

Benzodiazepines, Human Serum Albumin, Quantitation

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