Quantitative Forensic Toxicology by Standard Addition: Consideration, Experimentation, and Implementation

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After attending this presentation, attendees will be able to assess the value of standard addition in comparison to traditional external calibration approaches and be able to implement a standard addition protocol in their laboratory.

This presentation will impact the forensic science community by expanding knowledge of types of acceptable quantitative analyses and by providing an avenue that is effective and efficient with the possibility of changing forensic quantitative approaches in the future.

Quantitative determination of drug concentrations in forensic toxicology samples, especially blood, can assist a forensic toxicologist with interpretation of analytical findings in the context of an investigation. Knowing a drug concentration allows a forensic toxicologist to compare results from a specific sample or case to previously published literature or per se limits (e.g. blood ethanol concentrations). Most commonly, quantitative forensic toxicological analyses are performed using external calibration curves and control samples to establish a concentration gradient and to test the accuracy and precision of the analytical process, respectively. The AAFS Standards Board (ASB) has adopted a standard practices document for evaluation of this type of calibration model through method validation. This process generally takes at least five days to complete a long series of experiments to prove a developed method is acceptable for quantitation of a drug in a specific matrix and within a certain concentration range. However, the use of an external calibration model and validation of that process may not be the most effective, efficient, or cost-conscious approach depending on the frequency with which a drug is detected, its prevalence among given populations, and/or its life cycle.

Standard addition is an alternative quantitative approach which has been used for many years among various analytical fields for determination of concentration for a species in a matrix. Standard addition has been previously used in the field of forensic toxicology; however, its current implementation is scarce, and many scientists are not familiar with the approach. The method of standard addition uses an internal calibration model where drug standard is fortified to case samples aliquoted in replicate. Comparison of spiked concentration and peak area ratios (PAR) between analyte and internal standard allow for the analyst to calculate the concentration of drug in the unfortified sample. There are several pros and cons to using standard addition over traditional calibration curves which will be discussed during this presentation.

The method of standard addition can scientifically be applied to various drug classes and matrix types but lends itself especially useful for quantitation of emerging novel psychoactive substances (NPS) since analytical methods typically do not exist for these drugs and their lifespan can be quite short. Our laboratory has employed standard addition for the quantitation of several different NPS over the last two years. In each scenario, an analytical method was developed using a Waters Xevo TQ-S micro liquid chromatograph tandem mass spectrometer (LC-MS/MS). Drugs were extracted from matrix by liquid-liquid extraction (LLE) using borax buffer (0.1 M, pH 10.4) and N-butyl chloride and ethyl acetate (70:30, v:v). Prior to testing of authentic case samples, the analytical method and standard addition approach were verified using

a series experiments adopted from the ASB standard. Experiments assessed linearity over the target range, limit of detection, recovery, and interference from matrix, analyte, internal standard, and commonly encountered drugs, as well as mock standard addition on fortified control samples.

For quantitation, four replicate samples were aliquoted and prepared by fortification with drug standard: one sample remained "blank" with no drug standard added and three samples were "up-spiked" at appropriate concentration for the specific drug. After LC-MS/MS analysis, resulting analyte-internal standard PAR were plotted against the up-spike concentration. A linear trendline between all data points was implemented. Correlation (R^2) between the data points was required to be greater than 0.98. The concentration of drug in the sample was determined by calculating the x-intercept of the plotted line.

This approach has been successfully implemented for the quantitative determination of NPS in forensic casework, including isotonitazene (opioid), brorphine (opioid), eutylone (stimulant), 2F-deschloroketamine (hallucinogen), hydroxy-PCP (hallucinogen), and flualprazolam (benzodiazepine). Specific drug and case results will be discussed further during this presentation. Our laboratory has had overwhelming success with standard addition. It is recommended that forensic toxicologists consider standard addition as an acceptable alternative quantitative approach for certain drugs and sample types, when appropriate.

Keywords: Quantitation, Analytical, Toxicology, LC-MS/MS