K16 Isotopic Dilution in the Analysis of Cocaine, Cocaethylene, and Benzoylecgonine in Whole Blood: Comparison of the "Traditional" to the "Direct" Application of Deuterated Drug Analogs

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After attending this presentation, attendees will obtain a better understanding of mass spectral analyses incorporating isotopically labeled drug analogs as internal standards. A review of the traditional use of isotopically labeled drug analogs and, more importantly, their use in an expanded analytical role is presented. The attendee will obtain an understanding of Response Factors and Relative Response Factors in quantitative mass spectrometric analyses.

This presentation will impact the forensic community and/or humanity by demonstrating that costs can be greatly reduced, without sacrificing analytical reliability, by lowering the number of GC/MS injections necessary, ultimately resulting in decreased instrument time. Costs associated with analyst's time are also greatly reduced because only four extracts are necessary: (1) a single calibration standard to verify the Relative Response Factor (RRF) and to establish the necessary qualitative criteria (retention times, ion ratios, etc), (2) a positive control,
(3) a negative control, and (4) the specimen. This direct analytical approach greatly simplifies analysis of blood samples containing multiple analytes because additional isotopically labeled drug analytes can be added at the beginning of the extraction sequence.

Traditionally, laboratories use isotopically labeled drug analogs as internal standards for quantitating drugs in biological specimens. Calibration curves are constructed by plotting the response ratios, i.e., area abundances of drug-D_o/ area abundances of drug-D_n versus the concentrations of the drug–D_o. For purposes of this study D_o represents the drug analyte, and D_n refers to the tri-deuterated, isotopically labeled, drug analog.

In the "direct" application of deuterated drug analogs, the concentration of D_o in the specimen is determined directly from the amount of D_n used in the analysis by applying the following relationship: ng/mL D_o = (Area abundance D_o/ Area abundance D_n x nmol D_n x 1 nmol D_o/1 nmol D_n x MW D_o) / mL specimen.

To validate this approach, the Relative Response Factor (RRF) for D_o relative to D_n over the range of expected results is established. The RRF is determined by considering the individual Response Factors (RF) for D_o and for D_n, where RF is defined as the magnitude of some measurable parameter divided by the amount giving rise to that measurement, or RF-D_o = area abundance D_o / nmole-D_o, and RF-D_n = area abundance D_n / nmole-D_n. The RRF (RF-D_o / RF-Dn) is determined for each point in the range. Theoretically, an RRF of 1.0 over a given range indicates that the mass spectrometer response to D_o is identical to the response to D_n for that range.

Mathematically, RRF = RF-D_o / RF-D_n ; or RRF = Area D_o / nmole D_o or Area D_o / Area D_n Area D_n / nmole D_n nmole D_o / nmole D_n which, on rearrangement results in (Area D_o / Area D_n) = RRF (nmole D_o / nmole D_n) and represents a linear relationship in the (y = mx + b) format. By plotting (Area D_o / Area D_n) as y; (nmole D_o / nmole D_n) as x; and setting b to 0, the slope (RRF) is obtained.

This study demonstrated that the RRFs for cocaine, cocaethylene and benzoylecgonine were 1.01, 0.99, and 0.99 respectively over the range of 20 – 2000 ng D_o versus 100 ng D_n with R^2 values greater than 0.99 for each, which indicated that the mass spectrometer response to D_o was the same as the response to D_n over that range and allowed for the D_o concentration in a whole blood specimen to be determined directly from the amount of D_n used in the analysis.

Drug Analyte (Drug-D0), Deuterated Drug Analog (Drug-Dn), RRF (Relative Response Factor)

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