K48 The Quantification of Loperamide by Gas Chromatography/Mass Spectrometry (GC/MS)

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After attending this presentation, attendees will be able to: (1) understand the abuse potential of loperamide; (2) explain the loperamide method-validation study summary; and, (3) describe loperamide abuse cases in Alabama.

This presentation will impact the forensic science community by providing additional analytical options to laboratories and increase awareness of loperamide as a drug of abuse.

Hypothesis: GC/MS may be used to quantitate loperamide when it is abused in postmortem toxicology cases.

Statement of Content/Methods: Loperamide is a member of the opioid drug classification. At therapeutic concentrations (≤5ng/mL), loperamide is restricted to the gastrointestinal tract where it functions as an anti-diarrheal. Loperamide is abused as a replacement for legally controlled opioids, to mitigate withdrawal symptoms, and in monitored known abusers. In this validation, loperamide was quantitated in whole blood by GC/MS following a basic drug extraction (liquid-liquid extraction) using n-butyl chloride. Loperamide (m/z 239, 72, 266) and loperamide-d_6 (m/z 245, 78) ions were monitored and data was collected using both Selected Ion Monitoring (SIM) and scan modes in a 10-minute method. In accordance with the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines, validation studies included: selectivity, reproducibility, specificity, stability, Limit Of Detection (LOD), regression model analysis, and matrix enhancement/suppression.

Summary of Results: Method selectivity was evaluated through inter- and intra-day accuracy, precision, Coefficient of Variation (CV), and reproducibility. Inter-day accuracy, precision, and CV were measured at three concentrations (200ng/mL, 400ng/mL, and 650ng/mL) over the course of the validation (seven batches). Result ratios were calculated by dividing the measured result by the intended result and then averaged. The inter-day average result ratio was 1.05 ± 0.09 with CV=8.87%. The intra-day accuracy and precision were determined at three concentrations in replicates of three over three days with an average result ratio of 1.03 ± 0.09 with CV=8.53%. Reproducibility of the method was evaluated through standard addition and comparisons to previous results. The reproducibility result ratio was 0.95 ± 0.10 with a CV=10.84% across ten standard addition samples. Specificity assessment included analysis of ten different blank matrices, 60 commonly encountered drugs, and the individual analysis of loperamide (4,000ng/mL) and deuterated loperamide at high concentrations (1,000ng/mL). No interference was observed. Stability of the extracts was evaluated at room temperature over the course of five days. The loperamide to loperamide-d_6 ratio remained consistent between days one to five with a CV=6.59%. The method was determined to be transferable across two analysts. The LOD was set at 100ng/mL. A linear range from 100ng/mL to 1,000ng/mL was determined through regression analysis. The regression analysis was calculated from analyte area responses for both loperamide and loperamide-d_6. Matrix enhancement, suppression, and recovery were evaluated through a modified Matuszewski study. Neither matrix enhancement nor suppression was observed for loperamide at 750ng/mL and loperamide-d_6 at 300ng/mL (-6.5% and -4.2%); however, both analytes at 300ng/mL exhibited some suppression (-57% and -55%). Recovery ranged from 31% to 36% for all concentrations of loperamide and loperamide-d_6.

Conclusion: Results of this validation demonstrate that the designed method is highly selective and precise, <10% CV and specific, and no interference was detected. The LOD and Limit Of Quantitation (LOQ) linear range are suitable for the seven loperamide abuse deaths that have been observed in Alabama (concentration range from 130ng/mL to 1,400ng/mL) in the last two years. Using the loperamide to loperamide-d_6 ratio corrected for matrix suppression and recovery. Per this research, the presented method sufficiently meets the needs of postmortem toxicology laboratories.

GC/MS, Loperamide, Overdose