K63 Determination of Novel and Non-Routine Benzodiazepines and Suvorexant in Whole Blood by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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Learning Overview: After attending this presentation, attendees will understand the benefits and limitations of an efficient analytical method for the determination of select benzodiazepines and suvorexant in whole blood by LC/MS/MS.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by presenting a validated method for the detection of select benzodiazepines and suvorexant in whole blood.

Introduction: Benzodiazepines are frequently encountered in human performance forensic toxicology cases, such as drug-facilitated assaults and impaired driving, due to their effect on the central nervous system. The growing trend of Novel Psychoactive Substance (NPS) -benzodiazepines has increased the need to expand the scope of targeted assays. In the past 19 months, the Palm Beach County Sheriff's Office Crime Lab has received drug seizure submissions containing clonazolam, etizolam, flualprazolam, and flubromazolam.

Objective: Develop and validate an efficient method for quantitative determination and/or qualitative identification of select novel and non-routine benzodiazepines and suvorexant in whole blood by LC/MS/MS.

Method: Two hundred microliters of whole blood was prepared by protein precipitation using LC/MS-grade acetonitrile. Analysis of the samples was performed on a Shimadzu® Prominence XR LC system with a SCIEX® 3200 QTRAP® MS/MS and a Raptor™ Biphenyl column with dimensions of 50 x 2.1mm and a 2.7µm particle size. A gradient LC program with a flow rate of 0.6mL/minute was used with LC/MS-grade water and acetonitrile, both with 0.1% formic acid. The total run time was eight minutes. Stable isotopically labeled internal standards of bromazepam, clonazolam, clobazam, diclazepam, estazolam, etizolam, suvorexant, and triazolam were used. The method was validated by evaluating the calibration model, sensitivity, carryover, selectivity/specificity, repeatability, bias, robustness, a case sample evaluation, ionization suppression/ enhancement, and extract stability.

Results: Two different quantitative ranges were employed based on anticipated concentrations of each analyte in whole blood. The range for Group 1 (clonazolam, diclazepam, flualprazolam, flubromazepam, flubromazolam, loprazolam, lormetazepam, and triazolam) was 5–100ng/mL. The range for Group 2 (bromazepam, clobazam, clotiazepam, estazolam, etizolam, phenazepam, prazepam, suvorexant, and tetrazepam) was 25–500ng/mL. Flubromazepam and prazepam were validated for qualitative identification only. The Limit Of Detection (LOD) was 1ng/mL for Group 1 (except clonazolam, flubromazolam, and triazolam, which was 5ng/mL) and 5ng/mL for Group 2. The Limit Of Quantitation (LOQ) was administratively set to the lowest calibrator. The calibration model for all analytes used a quadratic curve fit with 1/x weighting. Within-run (n=3) and between-run (n=15) precision for the quantitative analytes did not exceed 14% for controls prepared at the LOQ (in five different matrix sources), a midpoint concentration, and a concentration within 20% of the highest calibrator. Bias did not exceed ±14%. An evaluation with previously characterized case samples (n=19) demonstrated interference for one of the ion transitions for triazolam in one case that may interfere with its identification. There was no interference with other common analytes from drug classes, such as stimulants, tetrahydrocannabinols, opioids, and other benzodiazepines. Ionization suppression/enhancement of the target response relative to the internal standard was not greater than ±25% for all analytes, except prazepam at the LOQ and loprazolam at both the LOQ and a concentration within 20% of the highest calibrator. Extract stability of flubromazepam did not exceed 24 hours (peak area varied by ≥20%). Tetrazepam and triazolam demonstrated stability up to 48 hours. Clotiazepam, lormetazepam, and phenazepam demonstrated stability up to 72 hours. All other compounds demonstrated stability up to 96 hours. A certified reference material for suvorexant was not available and therefore will be reported qualitatively even though all validation requirements were met. Flubromazepam and prazepam demonstrated bias >±20% with control replicates prepared in alternate matrix sources at the LOQ. Stable isotope-labeled internal standards may be required for quantitation of those analytes by this method.

Conclusion: The presented method requires a small volume of sample, minimal sample preparation, and demonstrated acceptable performance for the quantitative analysis of 15 of the 17 compounds evaluated. This method allows for the quantitative determination and/or the qualitative identification of 16 novel and non-routine benzodiazepines and suvorexant in whole blood by LC/MS/MS.

Benzodiazepines, Validation, LC/MS/MS

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