K73 A Food and Drug Administration (FDA) -Cleared Immunoassay Screen and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Confirmation for Benzodiazepines in Hair

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Learning Overview: After attending this presentation, attendees will have learned the performance characteristics of an FDA-cleared immunoassay for benzodiazepines in hair, and the concentrations of selected benzodiazepines in hair samples after an extended aqueous wash. The concentrations are determined via LC/MS/MS.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing knowledge regarding methods useful for determining the concentrations of selected benzodiazepines in hair samples.

Hypothesis: Benzodiazepines could be detected in hair samples using a patented digestion method to remove the drug from hair, followed by a qualitative enzyme immunoassay screen. The benzodiazepine concentrations could then be determined quantitatively in hair using LC/MS/MS.

Hair samples were screened by: (1) drug extraction from hair using a patented method (United States Patent 8,084,215), and (2) performing an in-house developed microplate immunoassay using a selected monoclonal antibody with an oxazepam cutoff at 1ng/10mg hair and controls at -50/+100% of the cutoff. The immunoassay is designed for qualitative use and has been cleared by the FDA (k163590). Cross-reactivity of the immunoassay with selected benzodiazepines using oxazepam calibrator was alprazolam (277%), lorazepam (13%), diazepam (550%), nordiazepam (220%), oxazepam (100%), and temazepam (312%). The effects of cosmetic treatments (permanent wave, dye and bleach, relaxer, and shampoo) using benzodiazepine negative and positive samples in the immunoassay were determined. In this study, all negative hair samples remained negative after treatment, with all positive samples remaining positive after treatment. A comparison study (n=392) of the immunoassay with LC/MS/MS results after an extended aqueous wash was conducted with stored samples previously received for workplace drug testing by the Psychemedics laboratory. All samples identified as negative by the immunoassay were confirmed as negative by LC/MS/MS (n=234). One hundred thirty-four samples identified as positive by the immunoassay were confirmed positive by LC/MS/MS. Twenty-four samples identified as positive by the immunoassay were confirmed below the cutoff but containing drug above Lower Limit of Quantification (LLOQ) by LC/MS/MS after an extended aqueous wash.

The confirmation process consisted of a new hair aliquot that was first washed using an extended aqueous method followed by solid phase extraction and quantitation using an AB SCIEX™ API 3200 for LC/MS/MS confirmation using primary and secondary ions for each analyte in positive multiple reaction mode. The benzodiazepines confirmed were alprazolam, lorazepam, diazepam, nordiazepam, oxazepam, and temazepam. The cutoff for the confirmation was set at 0.2ng benzodiazepine/10mg hair. The LC/MS/MS method was linear from 0.05 to 20ng/10 mg with LLOQ of 0.05ng/10mg hair for all analytes. The LC/MS/MS method was reviewed and cleared by the FDA as a part of the benzodiazepine 510k process (k163590).

Conclusion: The first-of-its-kind, FDA-cleared, immunoassay screen and LC/MS/MS confirmation for benzodiazepines in hair has been presented. This study has provided the hair concentrations of six benzodiazepines analyzed by the assay.

Benzodiazepines, Immunoassay, Hair

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