K76 Validation of Enzyme Linked Immunosorbent Assay (ELISA) for Detection of Synthetic Cannabinoids Metabolites in Urine

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After attending this presentation, attendees will be able to evaluate the use of immunoassay tests for the detection of synthetic cannabinoid metabolites in urine.

This presentation will impact the forensic science community by outlining an approach to method validation for immunoassay, and by providing an assessment of the merits of an immunoassay approach to the detection of these emerging compounds.

The prevalence and popularity of synthetic cannabinoid drugs has created the need for a low-cost option for screening for the presence of these drug metabolites in biological fluids. Liquid Chromatography/Mass Spectrometry (LC/MS/MS), which is in limited use as a method for screening for these compounds, requires sample extraction, lengthy run times, and is an expensive approach for high-volume screening. It also requires additional development for every new analyte or new metabolite discovered. Enzyme Linked Immunosorbent Assay (ELISA) is in widespread use for screening for many classes of drugs, and the development and evaluation of two ELISA tests to detect synthetic cannabinoids is described. ELISA is a rapid procedure that uses low-cost reagents and can be readily automated, making it an optimal technique for this process.

Following the production of antibodies in a rabbit model, antisera was harvested and antibodies isolated. After evaluating the performance of antibodies from several animals, the optimum antibody was used in a homogeneous ELISA plate immunoassay on a 96-well plate.

Two ELISA assays were developed, targeted to JWH-018 (1-naphthyl-(1-pentylindol-3-yl)methanone) and JWH-250 (2-(2-methoxyphenyl)-1-(1-pentylindol-3-yl)ethanone), respectively. At the time of development, JWH-018 and JWH-250 were the most prevalent compounds on the illicit drug market.

The assays were validated to determine their performance at the defined cut-off of 5ng/mL. The 5ng/mL cut-off was selected based on analysis of incurred authentic positive urine samples and LC/MS/MS analysis of JWH-018 and JWH-250 metabolite concentrations in authentic samples. Intraday and interday precision were evaluated by analyzing calibrators and controls over 10 days with 2 runs per day. Cut-off calibrators were run in duplicate and the mean OD used to establish the cut-off. A negative control, positive control (20ng/mL), and near cut-off concentration control (10ng/mL) were evaluated and performed acceptably for both assays under these conditions.

The assays showed significant cross reactivity with other synthetic cannabinoid standards, and metabolites. Several compounds including JWH-018 4-OH pentyl, JWH-018-5OH pentyl, JWH-081, JWH-081 4-OH pentyl, JWH-081 5-OH pentyl, JWH-122, JWH-122 5-OH pentyl, AM-2201, AM-2201 4-OH pentyl, and others generated positives on the JWH-018 assay at concentrations of less than 20ng/mL. Fewer compounds including JWH-250, JWH-250 4-OH pentyl, JWH-250 5-OH pentyl, and others generated positives on the JWH-250 assay at the same threshold. Common drugs of abuse and therapeutic drugs did not react at elevated concentrations (>10,000ng/mL).

Validation controls (positives and negatives) as determined by LC/MS/MS were presented to the ELISA methods. Subject samples testing positive for JWH-018 (5-hydroxypentyl) metabolite using LC/MS/MS with a cutoff concentration of 0.1ng/mL were assessed using the JWH-018 Direct ELISA kit. There were 61 of 63 LC/MS/MS positive samples which tested positive by ELISA, while all 51 pedigreed negative samples tested negative by ELISA. Thus, the sensitivity, specificity, and accuracy for the JWH-018 Direct ELISA kit were 96%, 100%, and 98%, respectively.

Subject samples testing positive for JWH-250 (4-hydroxypentyl) metabolite using LC/MS/MS with a cutoff concentration of 0.5ng/mL were assessed using the JWH-250 Direct ELISA kit. There were 32 of 33 LC/MS/MS positive samples which tested positive by ELISA, while all 51 pedigreed negative samples tested negative by ELISA. Thus, the sensitivity, specificity, and accuracy for the JWH-250 Direct ELISA kit were 97%, 100%, and 99%, respectively.

These ELISA assays have proven effective, sensitive, and specific for the purposes of screening for many of the currently popular synthetic cannabinoid compounds. Continued vigilance is needed to ensure that the assays will cross react with newly emerging drugs in this class.

ELISA, Cannabinoids, Designer Drugs

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