K13 Analysis of Buprenorphine, Norbuprenorphine, Naloxone, and Their Glucuronides From the Urine Obtained in Drug and Driving Cases

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After attending this presentation, attendees will better understand choosing the most efficient method for extracting buprenorphine, norbuprenorphine, naloxone, and related glucuronides from urine employing available Solid Phase Extraction (SPE) cartridges and Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS). Buprenorphine is being requested more often as a test in drug and driving cases. Buprenorphine does not cross-react with typical opiate immunoassays and cannot be detected in basic drug screens using Gas Chromatography/Mass Spectrometry (GC/MS) without derivatization. LC/MS/MS does offer an efficient alternative to GC/MS.

This presentation will impact the forensic science community by offering analysts operating in forensic facilities information regarding the extraction and analysis of buprenorphine, norbuprenorphine, naloxone, and the related glucuronides in urine samples obtained in drug and driving cases using SPE and LC/MS/MS. These drugs are now regularly being encountered in drug and driving casework and this method will greatly assist analysts in offering better interpretation to submitting agencies.

Method: 1mL samples of urine (calibrators, controls, and test samples each containing deuterated internal standards) were diluted with 3mL of 0.1M aqueous phosphate buffer (pH 6), vortex mixed and centrifuged. The supernatant liquid was applied to a pre-conditioned SPE mixed mode (C8/SCX) column. The SPE columns were conditioned with methanol, Deionized (DI) water and 0.1M phosphate buffer (3mL, 3mL, 1m, respectively). After loading samples at 1mL/ minute, the SPE cartridges were washed with DI water, 1.0M acetic acid, and methanol (3mL of each, respectively). The SPE columns were dried and eluted with 3mL of a solution containing methylene chloride-isopropanol-ammonium hydroxide (78-20-2) and 3mL of a solution of methanol containing 4% ammonium hydroxide. The eluates were collected separately and combined to form one solution. The eluate solutions were evaporated to dryness under nitrogen at 35°C. The dried residues were dissolved in 100µL of mobile phase for LC/MS/MS. LC was performed in gradient mode employing a 50mm x 2.1mm (2.1µm) aromatic phase LC column using mobile phase consisting of acetonitrile and 0.1% aqueous formic acid at a flowrate of 0.5mL/minute.

Tandem mass spectrometry was performed in positive Multiple Reaction Mode (MRM). The following transitions were monitored (quantification transition ions underlined): buprenorphine (468.3 to 396.2, 414.3), buprenorphine-d4 (472.5 to 400.1, 415.3), Norbuprenorphine (414.3 to 340.1, 326.0), norbuprenorphine-d3 (417.3 to 343.4, 326.0), naloxone (328.2 to 253.0, 212.1), respectively. The glucuronides were monitored as follows: buprenorphine glucuronide (644.3 to 468.1, 396.3), norbuprenorphine glucuronide (590.3 to 414.3, 396.2), naloxone glucuronide (335.2 to 299.1, 273.1), naloxone-d5 (332.1 to 258.1, 273.1), respectively. In this presentation, representative chromatograms are shown to illustrate the efficiency of the chromatography and analysis of buprenorphine, norbuprenorphine, naloxone, and related glucuronides from 20 (completed) drug and driving cases

Results: The limits of detection/quantification for this method were determined to be 5ng/mL and 10ng/mL, respectively, for all of the analytes (i.e., buprenorphine/norbuprenorphine/naloxone, and glucuronides). The method was found to be linear from 10ng/mL to 1,000ng/mL (r2>0.999). The analyte recoveries were found to be greater than 95% for all of the noted compounds. Interday and Intraday variation of the method were found to <8% and <10 %, respectively. Matrix effects were determined to be <6%. Details regarding the concentrations of buprenorphine/norbuprenorphine/naloxone found in 20 genuine urine cases are presented.

Conclusion: This method demonstrates the efficient use of both SPE coupled with the use of LC/MS/MS for the analysis of buprenorphine/norbuprenorphine/naloxone and their related glucuronides in cases of driving under the influence of drugs. The ability to analyze buprenorphine, norobuprenorphine, naloxone ,and their glucuronides in drug and driving cases will greatly assist toxicologists in offering the appropriate interpretation to the submitting agencies.

Buprenorphine Glucuronide, Naloxone, SPE