K42 Analysis of Opioids in Urine Specimens by Solid Phase Extraction (SPE) and Ultra Performance Liquid Chromatography/Tandem Mass Spectrometry (UPLC/MS/MS)

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After attending this presentation, attendees will better understand the benefits of using a method combining Enzymatic Hydrolysis (EH), SPE, and UPLC/MS/MS for the routine analysis of opioids in urine.

This presentation will impact the forensic science community by permitting forensic and analytical toxicology laboratories to decrease turnaround time and to increase productivity by introducing a simple, robust, reproducible, and rapid urine opioid procedure based on EH, SPE, and UPLC/MS/MS.

Introduction: In postmortem and human performance forensic toxicology case investigations, body fluids and tissues are routinely submitted to the laboratory for toxicological analysis to help determine if alcohol, drugs, and poisons played any role in the case under investigation. Forensic and analytical toxicology laboratories must, therefore, employ validated methods to screen, confirm, and quantify drugs and their metabolites in biological samples, if present. This study followed method validation guidelines published by the Scientific Working Group for Forensic Toxicology (SWGTOX) to develop and validate a method for the analysis of seven common opioids (morphine (MOR), codeine (COD), 6-acetylmorphine (6-MAM), hydrocodone (HC), hydromorphone (HM), oxycodone (OC), and oxymorphone (OM)) using SPE and UPLC/MS/MS and apply the new methodology to real human urine specimens.

Method: SPE was performed on large particle-size, mixed-mode cartridges (C_8-SCX) to extract the opioids. Urine aliquots (1.0mL) of calibrators, controls, and real case specimens containing internal standard (500ng/mL of a 10µg/mL opiate stock solution of MOR-d6, COD-d6, HC-d6, OC-d6, HM-d6, and OM-d3) were adjusted to pH5 using acetate buffer (1.0M) and hydrolyzed at 65°C for 60 minutes with 50µL of an enzyme derived from Red Abalone. After cooling to room temperature, the pH was adjusted to 6.0 with 3.0mL of aqueous phosphate buffer (0.1M). Samples were vortexed and added to pre-conditioned SPE columns. The columns were washed with 3.0mL each of Deionized (DI) water, acetic acid (1.0M), and methanol and were dried under vacuum for 15 minutes. Analytes were eluted from the SPE cartridges with 3.0mL of solvent mixture (methylene chloride:isopropanol:ammonium hydroxide (78:20:2)). Eluents were evaporated to dryness at 35°C after which the residues were dissolved in mobile phase (100µL) for analysis.

Analyte separation was performed by LC on a pentafluorophenylpropyl column (50mm x 2.1mm, 1.8µm) aided by a guard column. Mobile phases consisted of methanol containing formic acid (0.05%) and DI water containing formic acid/ammonium formate (5mM) (0.05%/0.1%). The flow rate was 0.3mL/minute in a gradient mode. The total run time was nine minutes.

MS/MS was performed in positive Multiple Reaction Monitoring (MRM) mode for morphine (286.2 to 165.1, 201.1), codeine (300.2 to 165.1, 58.2), HM (286.2 to 185.1, 157.1), OM (302.1 to 284.1, 227.1), HC (300.2 to 199.1, 171.1), OC (316.2 to 298.1, 241.1), and 6-MAM (328.2 to 165.1, 211.1). The quantitation ions are underlined.

Results: The method achieved 0.50ng/mL Limit of Detection (LOD) and 2.0ng/mL Limit of Quantitation (LOQ) for all analytes. Linearity was found to be greater than 0.995 in the range from 2.0ng/mL to 1,000ng/mL. Recoveries were determined to be: MOR: 81.25 ± 13.02%; COD: 90.14 ± 9.47%; 6-MAM: 90.18 ± 10.42%; OC: 90.38 ± 11.70%; OM: 88.90 ± 13.43%; HC: 84.64 ± 17.14%; and, HM: 70.06 ± 18.17%.

The intra-day and inter-day variations of the seven opioids were found to be <15% and <12%, respectively. Matrix effects were determined to be: MOR: -34.14 ± 10.37%; COD: -23.869 ± 14.42%; 6-MAM: -24.563 ± 18.12%; OC: -35.813 ± 13.68%; OM: -37.54 ± 16.48%; HC: -24.619 ± 16.71%; and, HM: -37.39 ± 16.55%.

Several closed human performance cases were analyzed and extracted blind. The data were evaluated and compared to the reported results in the database. The results obtained from the new methodology are shown in the provided table.

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Specimen #	Drug(s) Found/ (Concentration in ng/mL)
1	MOR (3,972), HM (43.0), HC (1,143), COD (3), OC (1)
2	HM (19), HC (10)
3	MOR (962), HM (16), COD (3,520), HC (22)
4	MOR (21), OM (308), HM (97.0), COD (3), OC (1,102)
5	MOR (66), HM (238), HC (722)
6	MOR (59), HM (308), HC (1,722)

The ability of this new method to detect quantifiable amounts of drugs that were not detected with the current in-house method(s) and are now able to be reported to the database attests to the efficiency of this new procedure.

Conclusion: The newly developed method was used to successfully screen opioids in real forensic toxicology case urine specimens. The use of SPE and UPLC/MS/MS as part of an extraction procedure that is capable of screening for seven opioids at once can provide valuable data about their presence in urine and cut down turnaround time and analytical costs in high-functioning toxicology laboratories. The method allows laboratories to employ more efficient analyses to simultaneously screen for multiple compounds.

Opioids, Urine, SPE