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Opioid Quantitation using DPX Tips and Semi-Automated Integra Pipetting System on an Agilent Triple Quadrupole Mass Spectrometer

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Background/Introduction: According to the Centers for Disease Control and Prevention, overdose deaths involving prescription opioids were five times higher in 2016 than 1999. The age-adjusted rate of drug overdose deaths involving synthetic opioids other than methadone (e.g. fentanyl, tramadol) doubled between 2015 and 2016, from 3.1 to 6.2 per 100,000. The increase in opioid related incidences in DUI/DUID and overdose related deaths has resulted in an increase in cases needing confirmatory testing. It is desirable to have a method that is efficient, robust, and capable of detecting and quantitating a large number of opioid analytes.

Objective: To develop a semi-automated dispersive pipette extraction (DPX) method for the analysis of opioids in whole blood using an LC-QQQ in an effort to replace an existing Solid Phase Extraction (SPE) and Gas Chromatography/Mass Spectrometry (GC/MS) analysis, and expand the laboratory's detection and quantitation capabilities to include oxymorphone, hydromorphone, and tapentadol.

Method: Drug standards were purchased from Cerilliant Corporation and Lipomed. DPX WAX tips were purchased from DPX Labs, LLC (Columbia, SC). DPX WAX tips use a reverse phase and anionic exchange resin with styrene dibenzene to remove non-polar substances and phospholipids that are sources of ion suppression in whole blood. The extraction procedure was performed using an Integra semi-automated pipetting unit with DPX WAX tips. Instrumental analysis was performed using an Agilent 1290 LC coupled to a 6430 QQQ equipped with a Restek Raptor™ Biphenyl 2.1 x 100 mm, 2.7 µm column. The following analytes were included: codeine, morphine, 6-monoacetylmorphine (6-MAM), hydrocodone, hydromorphone, oxycodone, oxymorphone, fentanyl, tapentadol, tramadol, methadone, and meperidine. Mobile phases consisted of 5 mM Ammonium Acetate with 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). Injections of 10 uL were introduced into a gradient elution from 98% A to 98% B over 9 minutes. SWGTOX method development and validation guidelines were followed and included accuracy and precision, limits of detection, linearity, calibration model, matrix and analyte interference, carryover, matrix effects/ion suppression, dilution integrity, and stability.

Results: Between run and within run accuracy was acceptable and within +/- 20% of the intended target concentration. The lower and upper limits of quantitation were determined to be 0.5 and 100 ng/mL respectively for oxymorphone, fentanyl, hydromorphone, and 6-MAM, and 5 and 1,000 ng/mL for morphine, meperidine, tapentadol, codeine, oxycodone, hydrocodone, tramadol, and methadone. Linearity was assessed and all targets were best fit with a quadratic fitting algorithm with a weighting of 1/x. No carryover was detected following concentrations of 100 ng/mL for oxymorphone, fentanyl, hydromorphone, and 6-MAM, and 1,000 ng/mL for morphine, meperidine, tapentadol, codeine, oxycodone, hydrocodone, tramadol, and methadone.

Conclusion/Discussion: A semi-automated sample preparation and quantitation method for the analysis of opioids in whole blood was successfully developed. This allows for a more comprehensive scope of opioid analytes within a single method, a wider linearity range, and combines separate methodologies commonly employed for the analysis of opioids in DUI/DUID and post mortem analyses. A high percentage of our monthly caseload currently produces presumptive positives within the aforementioned analytes. This methodology will decrease extraction/analysis time by more than 50% and in combination with LC-QQQ technology, allows for an overall more efficient analysis than is currently employed. The work presented here will benefit other Forensic Laboratories in the analysis of opioids using semi-automated technology coupled with LC/QQQ analysis.

Keywords: Semi-automation, dispersive pipette extraction, Opioid

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