Method for quantification of opioids and their Metabolites in autopsy blood by liquid Chromatography-Tandem Mass Spectrometry

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A method using LC/ESI/MS/MS was developed and validated for the determination of morphine, codeine, hydromorphine, dihydrocodeine, oxycodone, buprenorphine, and naloxone with their metabolites morphine-3-glucuronide, morphine-6-glucuronide, normorphine, 6-acetylmorphine, 6-acetylcodeine, codeine-6-glucuronide, norcodeine, hydromorphine-3-glucuronide, dihydrocodeine-6-glucuronide, norbuprenorphine, buprenorphine-3-glucuronide, norbuprenorphine-3-glucuronide, and naloxone-3-glucuronide in human whole blood. Polar metabolites (glucuronides) and other analytes were extracted by SPE using Bond Elut . Chromatographic separation was performed on a Synergi reverse phase column with gradient elution based on a mobile phase consisting of 10 mM ammonium formate adjusted to pH 3 (A) and acetonitrile (B) at flow rate 0.3 mL/min in the first 13 min, following by 0.2 mL/min for the next 13 min, after that the initial flow rate was applied until the end of analysis. The gradient conditions were: initially, 97% of solution A for 3 min, decreasing to 84.5% at 8 min, to 74% at 13 min and to 20% at 26 min. 5% of solution A was maintained for the next 3 min before returning to 97% for 7 min prior to the next injection. Intra-day and inter-day precision for all analytes were between 0.6 to 13.8% and recoveries were between 82% and 101.4%. Calibration curves were linear for all analytes over the concentration range 5-400 ng/mL and correlation coefficients (R^2) were better than 0.999. Limits of detection and limits of quantitation were 0.16 - 1.2 ng/mL and 0.5 - 4.09 ng/mL, respectively.

The method described consolidates previous work on opioids and their metabolites published in the literature and is the first to include the detection of naloxone-3-glucuronide. The method has been applied in routine post-mortem cases after opiate overdose with three

AIMS: it has been used to interpret the cause of death, to determine type of death (rapid or immediate death, sub-acute death, or delayed death) and to distinguish between heroin, morphine and codeine users.

KEYWORDS: *Opioids; Glucuronides; LC/MS/MS; Cause of death*

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