K41 The Quantification of Oxycodone and Its Phase I and II Metabolites in Urine

Michael Truver, PhD, Humble, TX 77346; Gerd Jakobsson, MSc, Linkoping University, Linkoping 58185, SWEDEN; Maria Cherma, PhD, Linkoping University, Linkoping 58185, SWEDEN; Henrik Green, PhD, Linkoping University, Linkoping 58185, SWEDEN; Robert Kronstrand, PhD, National Board of Forensic Medicine, Linkoping, Ostergotland SE 587 58, SWEDEN*

Learning Overview: After attending this presentation, attendees will have a better understanding of the significance of incorporating phase II metabolites into methods to investigate oxycodone use.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by filling a knowledge gap regarding the excretion of oxymorphone-3ß-D-glucuronide and noroxymorphone-3ß-D-glucuronide after controlled dosing and their presence in case samples.

The purpose of this research was to develop and validate a comprehensive analytical method for the detection and quantification of noroxymorphone-3ß-D-glucuronide, oxymorphone-3ß-D-glucuronide, noroxymorphone, oxymorphone, 6α-oxycodol, 6β-oxycodol, noroxycodone, and oxycodone in urine by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) primarily to be used in a human study.

An ACQUITY® UPLC® I-class coupled to a Waters® Xevo-TQD® was used for analysis. Separation was achieved using an ACQUITY® HSS T3 column (1.7µm, 2.1 x 100mm) at 30°C at 0.5mL/min. Mobile phase A was 0.001% formic acid in 10mM ammonium formate (pH 5.2) and mobile phase B was 0.001% formic acid in acetonitrile. The gradient started with 2% mobile phase B for 1.5min, then increased to 25% for the next 4.7min, and ended at 7.1min after high organic wash and re-equilibrium. Two transitions were monitored for each analyte and one for the deuterated internal standards. The method was validated according the Academy Standards Board (ASB) Standard Practices for Method Development in Forensic Toxicology. The method was then applied to a single-dose pilot study of a subject. Urine samples were collected from the subject after ingesting 10mg oxycodone as an immediate release tablet. The time of collections were 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 9, 10, 12, 14, 24, 48, 72, and 96 hours. Additionally, urine specimens (*n*=15) from petty drug offences that had previously been confirmed positive for oxycodone were analyzed using the newly validated method.

The calibration range for noroxymorphone-3ß-D-glucuronide and oxymorphone-3ß-D-glucuronide was 0.05–10µg/mL, while the calibration range for all other analytes was 0.015–10µg/mL. The standard curves for all analytes were fitted using a linear regression with a 1/x weighting within the calibration range, except for noroxymorphone and 6α/β-oxycodol, which were determined using a quadratic regression with a 1/x weighting. The limits of quantification were determined to be 0.015µg/mL for oxycodone, noroxycodone, 6α/β-oxycodol, oxymorphone, and noroxymorphone and 0.050µg/mL for noroxymorphone-3ß-D-glucuronide and oxymorphone-3ß-D-glucuronide. Imprecision ranged between 1.8 and 13%CV. Bias was determined to be between -12.2% and -0.4%. Process efficiency was within ±25% for all analytes except for noroxymorphone-3ß-D-glucuronide (71%). No carryover was observed after the injection of the highest calibrator (10µg/mL). Dilution integrity was acceptable for a ten-fold dilution after analytes quantified within an acceptable limit (98%–111%) of the target concentration.

After the method was validated, urine samples from the pilot study (*n*=1) were analyzed. Urine concentrations were corrected for creatinine concentration. It was observed that oxycodone, noroxycodone, and oxymorphone-3ß-D-glucuronide contained the highest concentrations and were present in either the 0.5h or 1h void. Noroxycodone and oxymorphone-3ß-D-glucuronide were detected until the 48h sample, while oxycodone was only detected up to the 24h sample. T_{max} in urine was achieved within 1.5h for oxycodone and with 3h for noroxycodone and oxymorphone-3ß-D-glucuronide. C_{max} in urine for oxycodone, noroxycodone, and oxymorphone-3ß-D-glucuronide was 3.15, 2.0, and 1.56µg/mg, respectively. From the authentic urine specimens, oxycodone concentrations ranged from 0.015–12µg/mL. Ranges for noroxymorphone-3ß-D-glucuronide and oxymorphone-3ß-D-glucuronide were 0.054–9.7µg/mL and 0.14–67µg/mL, respectively. It was observed that larger concentrations of oxycodone did not always result in larger concentrations of the phase II metabolites. In the future, the excretion of phase I and II metabolites in a controlled dosing study involving both immediate and extended release formulations of oxycodone will be investigated.

A comprehensive method for the quantification of noroxymorphone-3ß-D-glucuronide, oxymorphone-3ß-D-glucuronide, noroxymorphone, oxymorphone, 6α-oxycodol, 6β-oxycodol, noroxycodone, and oxycodone in urine was optimized and met validation criteria. Including noroxycodone and phase II metabolites of oxycodone benefited analyses by extending the window of detection. The concentrations of noroxymorphone-3ß-D-glucuronide and especially oxymorphone-3ß-D-glucuronide presented in this study provide details needed in the forensic community to better comprehend oxycodone pharmacokinetics.

Oxycodone, Pharmacokinetics, Oxymorphone Glucuronide

*Presenting Author