K23 Oxycodone and Oxymorphone Glucuronide Conjuagtes in Urine From Pain Management Patients

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Oxycodone is generally present in urine as readily extractable free, unconjugated form; however, its oxymorphone metabolite is extensively conjugated. The goal of this presentation therefore, is to assure detection or extend the detection time for oxycodone, urine should be hydrolyzed prior to analysis.

This presentation will impact the forensic community and/or humanity by assisting forensic toxicologist in the detection of oxycodone and its oxymorphone metabolite in urine specimens and in evaluation of oxycodone urine concentrations.

The concentrations of free and glucuronide conjugates oxycodone and its oxymorphone metabolite in urine specimens collected from 200 different pain management patients will be presented. These patients were receiving oxycodone as the sole opiate derivative used for pain control either in combination with acetaminophen or in a sustained release dosage form. Daily doses ranged from 20 to 80 mg of oxycodone. Urine specimens were analyzed initially fluorescence polarization opiate immunoassay (FPIA) at a cut-off of 100 ng/mL and oxycodone enzyme immunoassay (EIA) at a cut-off of 100 ng/mL before and after enzymatic hydrolysis with beta-glucuronidase. Following the addition of hydroxylamine, oxycodone and oxymorphone oxime derivatives were isolated from urine by solid phase extraction in a Detectabuse™ Gravity GV-65 column as described by the manufacturer (Biochemical Diagnostics Corp.). Oxycodone and oxymorphone oximes were derivatized by the addition of BSTFA [N,O-bis (trimethylsilyl)trifluoroacetamide]. The residues were analyzed on a Hewlett Packard (Palo Alto, CA) 6890 gas chromatograph with a split/splitless injection port, a 7673 auto-sampler and a 5973A mass selective detector (MSD). The column was an HP-5 capillary column (5.0 m x 0.1 mm id x 0.40 um film thickness). Column flow rate was 1.0 mL/min; inlet pressure at 60.53 psi; and injection was in the 4:1 split mode with a split flow of 4.0 mL/min. The oven temperature program was: initial 170^{0}C for 0 min., then ramped at 30^{0}C/min. to 280^{0}C that was held for 0.33 min. Under these conditions the retention times in minutes of TMS derivatives were oxycodone-oxime, 3.32; and oxymorphone-oxime, 3.62. The MSD was operated in the SIM mode using the following ions: oxycodone-oxime-TMS, 459, 444 and 368; oxymorphone-oxime-TMS, 517, 502 and 412; $^2H_3$-oxycodone-oxime-TMS 462 and 447; and $^2H_3$-oxymorphone-oxime-TMS, 520 and 503. Oxycodone and oxymorphone were quantitated using a single point calibrator of each at 500 ng/mL with internal standards of the respective deuterated analogs. The assay was linear from 50 to 10,000 ng/mL of oxycodone and oxymorphone. The lower limit of quantification of each analyte was 100 ng/mL. In the 200 unhydrolyzed urine specimens, the mean oxycodone concentration was 2,450 ng/mL (range, 119-7,600 ng/mL) and the mean oxymorphone concentration was 131 ng/mL (50-1,000 ng/mL). Following glucuronidase hydrolysis of these specimens, the mean oxycodone concentration was 4,000 ng/mL (range, 149-18,600 ng/mL) and the mean oxymorphone concentration was 2,900 ng/mL (range, 172-54,000 ng/mL). The mean percent of oxycodone and oxymorphone as glucuronides in the urine specimens were 29% (range, <1-85%) and 95.5% (range, 66-99%), respectively. These data demonstrate that oxycodone is generally present in urine as the readily extractable free, unconjugated form; however, its oxymorphone metabolite is extensively conjugated. Therefore, to assure detection or extend the detection time for oxycodone, urine should be hydrolyzed prior to analysis.

Oxycodone, Glucuronides, Oxymorphone

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