

Quantification of Oxycodone and Its Major Metabolites in Human Plasma by HPLC/MS/MS

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Introduction

BASi has developed a sensitive, specific and robust method for detecting and quantifying oxycodone and its two major metabolites, noroxycodone and oxymorphone, in human plasma. The assay is performed using LC-MS/MS to quantify these analytes along with three isotopically-substituted internal standards, d_6 oxycodone, d_3 oxymorphone and d_3 noroxycodone.

This assay addresses an important aspect of oxycodone metabolism. Cytochrome P450 isoforms CYP3A and CYP2D6 catalyze oxidation of oxycodone to noroxycodone and oxymorphone, respectively¹. Oxymorphone itself is a potent analgesic and is thus clinically relevant. It is up to ten times more potent per dose than orally administered morphine. However, the concentration of circulating oxymorphone following oxycodone administration is much lower (2 to 12-fold) than that of noroxycodone. This is especially true among poor metabolizers of oxycodone. Simultaneous quantification of oxymorphone, oxycodone and noroxycodone poses an analytical challenge². The range of the BASi method is 0.1-100 ng/mL for oxycodone and oxymorphone, and 0.5-100 ng/mL for noroxycodone. This method has been validated according to FDA regulations and has been used successfully to measure oxycodone and metabolite concentrations in clinical samples.

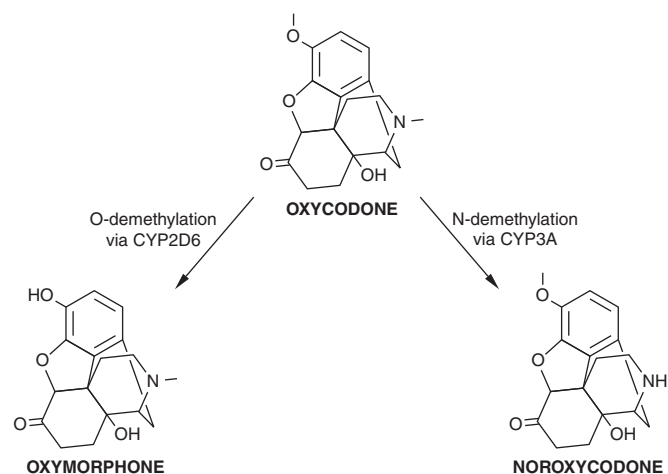


Figure 1. N- and O-demethylation of oxycodone. CYP3A and CYP2D6 are the cytochrome P450 enzymes that oxidize oxycodone into its primary metabolites. N-demethylation occurs primarily via CYP3A4, while O-demethylation is mediated by CYP2D6.

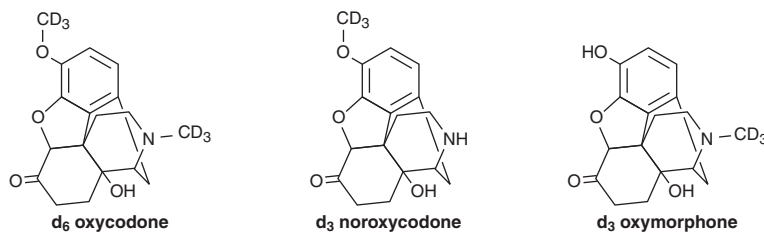


Figure 2. Internal standards d_6 oxycodone, d_3 noroxycodone and d_3 oxymorphone

Materials and Methods

Drugs and Reagents

Standard solutions of oxycodone, oxymorphone, noroxycodone and their deuterated analogs d_6 oxycodone, d_3 oxymorphone and d_3 noroxycodone in methanol were purchased from Cerilliant Inc. (Austin, TX). Naltrexone and 6β -naltrexol in methanol were also purchased from Cerilliant. Human KEDTA plasma was obtained from BioChemed Services (Winchester, VA). Burdick and Jackson solvents were used for extraction and HPLC.

Spiking solutions used for standard calibrators (SC) were prepared by serial dilution of 1.0 mg/mL stocks. Spiking solutions ranging from 10 to 2000 ng/mL were used to make SCs in human plasma from 0.100 to 100 ng/mL for oxycodone and oxymorphone, and from 0.500 to 100 ng/mL for noroxycodone. Quality control samples (QC) were made in a similar fashion at 0.3, 10, and 75 ng/mL for oxycodone and oxymorphone, and 1.0, 10, and 75 ng/mL for noroxycodone.

100 ng/mL solutions of naltrexone and 6β -naltrexol were made and analyzed according to the method in order to evaluate potential chromatographic interference with the analytes of interest. Naltrexone is sometimes pre-administered to subjects in order to prevent formation of dependence on oxycodone and related drugs. In addition, multiple lots (n=6) of human plasma were selected at random, individually spiked and analyzed to account for variation that could reasonably be expected among subjects in a clinical study. Blanks from each lot were evaluated for their potential to generate false positives.

HPLC-MS/MS Parameters

The HPLC setup consisted of a Shimadzu Prominence Series binary pump and autosampler. Chromatographic separations were performed with a C18 column. Mobile phase A was 10 mM ammonium bicarbonate, and Mobile Phase B was 100% methanol. A gradient of 20-60% MPB over 7 minutes was used to elute all three analytes, followed by a short re-equilibration interval. Typical retention times were 5.5 minutes for oxycodone, 3.8 minutes for oxymorphone, and 2.6 minutes for noroxycodone.

Solid Phase Extraction

200 μ l aliquots of human plasma were buffered and vortexed. Samples were then transferred to a conditioned MCX plate (Waters, Wexford, Ireland), washed and eluted in basic solution. Extracts were evaporated to dryness and reconstituted in 10 mM ammonium bicarbonate.

Analysis was performed on an Applied Biosystems API 4000 triple quadrupole mass spectrometer using TurboIonSpray® in positive ion mode. The following ion transitions were monitored: oxycodone, 316/241; oxymorphone and noroxycodone, 302/284; d_6 oxycodone, 322/304; d_3 oxymorphone and d_3 noroxycodone, 305/287. Tuning and other parameters were adjusted as needed to optimize signal.

Analytical Parameters

Peak area was used to perform standard regression with a quadratic fit and a weighting factor of $1/X^2$.

Results

Oxycodone, oxymorphone and noroxycodone in human plasma were separated and quantified via HPLC-MS/MS following cleanup and solid phase extraction. Oxycodone and oxymorphone were quantified with a lower limit of quantitation (LLOQ) of 0.1 ng/mL and an upper limit of quantitation (ULOQ) of 100 ng/mL. Noroxycodone was quantified with a LLOQ of 0.5 ng/mL and an ULOQ of 100 ng/mL. Baseline separation was achieved for all three analytes and their internal standards. Neither naltrexone nor 6β -naltrexol interfered with detection or quantitation of oxycodone or any of its metabolites. Results were reproducible with each of the six matrix lots used, and none of the lots generated false positive results.

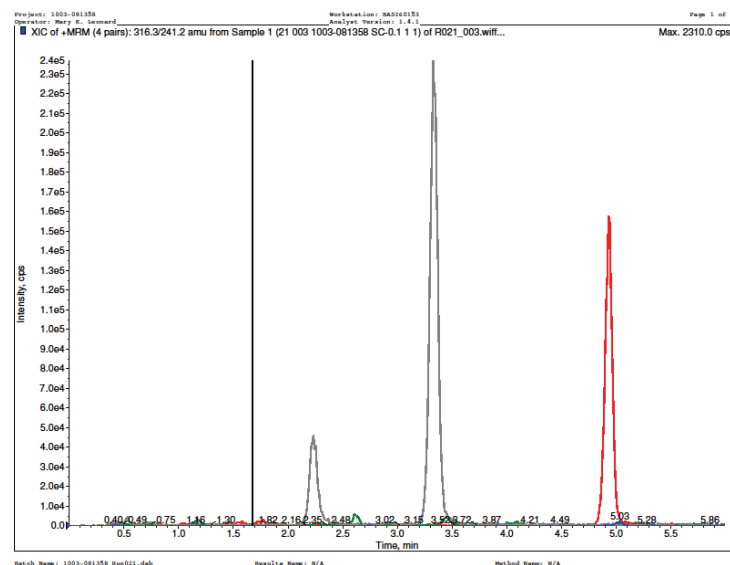


Figure 3. Chromatogram at lower level of quantitation for oxycodone (blue trace at 5.03 min.) and oxymorphone (green trace at 3.5 min.), 0.1 ng/mL. Red and grey traces are for d_6 oxycodone, d_3 oxymorphone and d_3 noroxycodone.

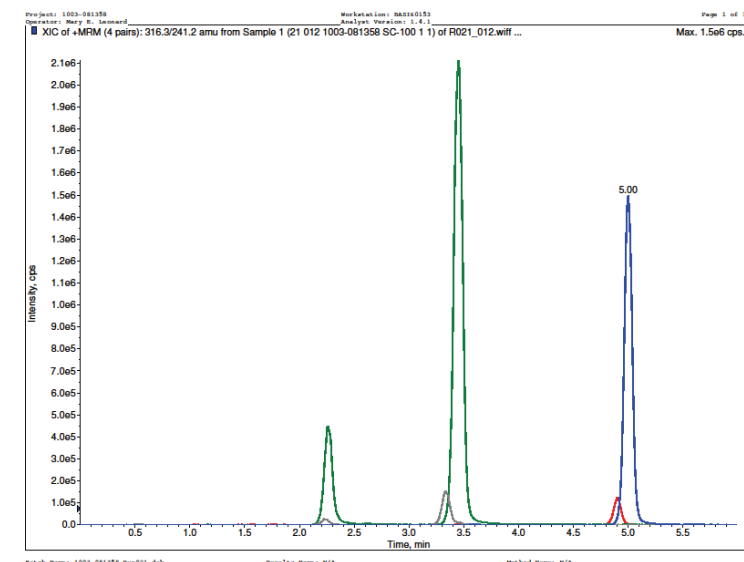


Figure 4. Chromatogram at upper level of quantitation for oxycodone (blue trace), oxymorphone and noroxycodone (green traces at 3.5 min. and 2.3 min., respectively), 100 ng/mL.

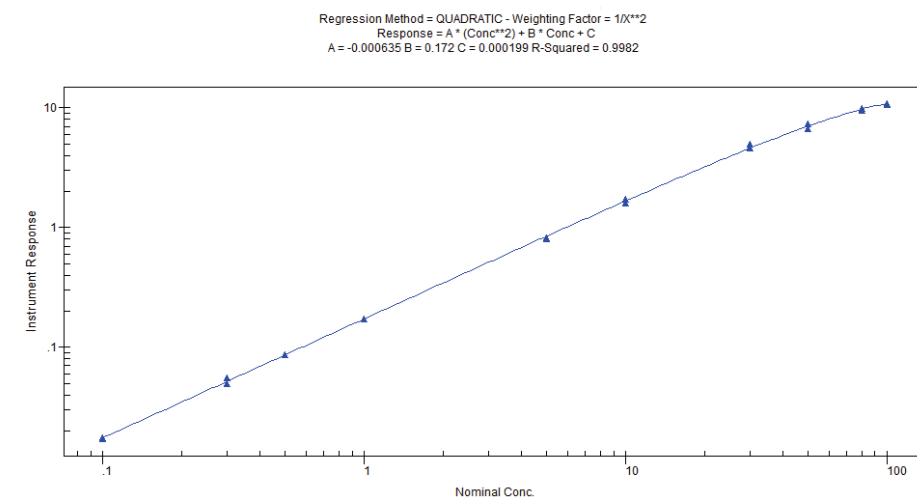


Figure 5. Standard calibration curve for oxymorphone, 0.1 ng/mL to 100 ng/mL.

Conclusions

BASi has developed a fast, efficient, sensitive and robust method for quantification of oxycodone and oxymorphone in human plasma over a range of 0.1 to 100 ng/mL, and for noroxycodone over a range of 0.5 to 100 ng/mL. The method is specific with regard to these analytes. Evaluation of multiple matrix lots generated no false positives, and no interference from naltrexone was observed. The assay is also reliable, yielding results that are highly reproducible as indicated by incurred sample repeat, short term and long term stability analyses. Although published methods exist for accurate quantitation at concentrations as low as 1 ng/mL, the BASi method addresses the need for higher sensitivity, especially with regard to oxymorphone in clinical samples.

References

- Lalovic, B.; Phillips, B.; Rislis, L.L.; Howald, W.; Shen, D.D. *Drug Metab. Dispos.* **2004**, *32*, 447-454.
- Edwards, S.R.; Smith, M.T. *J. Chromatogr.* **2007**, *848*, 264-270.