Oxycodone is an opioid analgesic metabolized to oxymorphone and noroxycodone by cytochrome P450 (CYP) 2D6 and 3A4/5, respectively. This was a retrospective study to evaluate sex, age, urinary pH and concurrent medication use on oxycodone, oxymorphone and noroxycodone distributions. Urine specimens obtained from patients on chronic opioid therapy were analyzed by LC–MS-MS. There were 108,923 specimens from a subject’s first or single visit, who were at least 18 years of age, and had documented physician-reported oxycodone use. The majority of specimens had detectable oxycodone urine concentrations ($n = 106,852$) resulting in oxycodone mole fractions (arithmetic mean ± SD) of $0.44 ± 0.27$. Ninety-eight percent ($n = 106,229$) and 49% ($n = 53,394$) had detectable oxymorphone and noroxycodone, respectively. Oxycodone and oxymorphone mole fractions were lower in women compared with men ($P < 0.0001$). Mean ± SD age was 49.1 ± 12.9 years. Noroxycodone mole fractions were highest in the 65 years and older age group. Concurrent use of a CYP2D6 inhibitor, but not a CYP3A4/5 inhibitor, altered oxycodone and oxymorphone mole fractions. Dual inhibition of CYP2D6 and CYP3A4/5 did not result in a statistical difference upon comparison with CYP2D6 inhibitor or CYP3A4/5 inhibitor use. Patient factors affect oxycodone and metabolite mole fractions and suggest increased awareness of each contribution when attempting to monitor therapy with urine drug testing.

**Introduction**

Oxycodone is a semi-synthetic opioid analgesic indicated for cancer pain, non-malignant chronic pain, and post-surgical acute pain. Oxycodone is an agonist at the mu, kappa and delta opioid receptors in the central nervous system which mediate antinociceptive activity. The minor metabolite, oxymorphone, is an agonist at the mu receptor, is 14 times more potent than oxycodone and has been speculated to confer the analgesic effect (1, 2). In contrast, in vivo and single-dose crossover studies attribute the analgesic effect to oxycodone (3–6), and less to oxymorphone and other metabolites such as noroxycodone (6). In the USA, oxycodone immediate release (IR) is co-formulated with acetaminophen, and oxycodone alone is marketed as an IR and controlled or extended release formulation. Oxycodone does not undergo rapid first pass metabolism in conjugation with glucuronic acid due to the methyl ester at position three on its aromatic ring (7, 8). Oxycodone is metabolized via hepatic conjugation and oxidative degradation (9) by cytochrome P450 (CYP) 2D6 and CYP3A4/5. CYP3A4/5 is responsible for the N-demethylation of oxycodone to noroxycodone (major metabolite), while CYP2D6 catalyzes the O-demethylation of oxycodone to oxymorphone (minor metabolite) (10) (Fig. 1). These metabolites, as well as the parent drug, are detectable in plasma (11) and are excreted in urine (12). Oxycodone and oxymorphone are active and known to contribute to the side effects of the drug (13).

Numerous factors are known to contribute to interindividual variability in drug response and include genetic, non-genetic and environmental factors. Age, sex and concurrent medication use are known to alter plasma concentrations of drugs. However, data are conflicting regarding effects of concurrent medication use on plasma concentrations of oxycodone (14). In rats, quinidine (a CYP2D6 inhibitor) increased the oxycodone area under the curve 3-fold compared with rats that were not on a CYP2D6 inhibitor (15). In humans, concurrent administration of quinidine and oxycodone resulted in decreased oxymorphone plasma concentrations and increased noroxycodone plasma concentrations (2, 16). Another study reported that the analgesic effect of oxycodone IR did not change upon quinidine co-administration (2). Treatment with paroxetine (a CYP2D6 inhibitor) in humans showed no relevant changes in oxycodone exposure (17, 18). The objective of this retrospective study was to evaluate sex, age, urinary pH and concurrent medication use on oxycodone, oxymorphone and noroxycodone distributions in urine. The implications of this study are that mole fractions may aid in understanding oxycodone urine drug testing variability and in the potential monitoring of clinically significant drug–drug interactions.

**Methods**

**Patient selection**

Urine specimens were collected at pain physician practices from patients on chronic opioid therapy for routine drug monitoring purposes. Our study received Institutional Review Board exempt status by the University of California, San Diego Human Research Protection Program. 1,025,136 de-identified specimens collected between September 2010 and May 2011 were available for analysis. Inclusion criteria were specimens from a subject’s first or single visit, subjects who were at least 18 years of age and physician-reported oxycodone use. Exclusion criteria were as follows: specimens with oxycodone urinary concentrations <50 ng/mL (lower limit of quantitation), specimens with urine creatinine concentrations of <20 mg/dL, missing patient demographic data (e.g., date of birth, sex), physician-reported oxymorphone use, physician-reported CYP2D6 inducer ($n = 35$) use and physician-reported CYP3A4/5 inducer use ($n = 1,160$). Specimens that had a creatinine concentration of <20 mg/dL were excluded because these were considered to be potentially tampered (19).

**Analytical procedure**

Specimens were prepared for injection by incubating 25 μL of urine with 50 units of β-glucuronidase Type I-II from *Patella*...
oxymorphone remained at 50 °C, the flow was 12 L/min, nebulizer gas (nitrogen) was 40 PSi and the capillary voltage was 3,000 V. Dwell times were 50 ms. HPLC water, acetonitrile, methanol and formic acid HPLC grade were obtained from VWR (Westchester, PA, USA). Calibration solutions of 100, 200, 3,200 and 6,400 ng/mL were prepared by diluting the standards into synthetic urine provided by Microgenics Corporation (Fremont, CA, USA). The deuterated internal standards were added to the calibration solutions and to the patient specimens so that the final solution contained 1,000 ng/mL. Upper limits of linearity were 100,000 ng/mL for all of the analytes by the dilutions on the Cerilliant certified standards into synthetic urine (negative control, also provided by Microgenics Corporation). Quantitation of specimens >100,000 ng/mL was estimated through linear regression extrapolation from data <100,000 ng/mL as previously described (20). There were 1,177 oxycodone, 91 oxymorphone and 968 noroxycodone specimens with concentrations >100,000 ng/mL, which represents 1.1, 0.08 and 1.8% of the total specimens included in the current study.

The interassay coefficient of variation for all the analytes at the low and high ends of the quantitation curve were <10%. In addition, at 1,000 ng/mL, the coefficients of variation for oxycodone, oxymorphone and noroxycodone were 6, 6 and 7%, respectively. At 100 ng/mL, the coefficients of variation for oxycodone, oxymorphone and noroxycodone were 9.4, 8.7 and 9.5%, respectively. All quantitative data were obtained from calibration curves with \( R^2 > 0.95 \), where most curves had an \( R^2 \) value of 0.99.

**Data and statistical analysis**

Statistical analyses were conducted using OriginPro 8.5.1 Student Version (Origin Lab, Northampton, MA, USA) and SAS v9.3 (Cary, NC, USA). Specimens were creatinine normalized to ng/g creatinine units in order to account for subject variability due to muscle mass and hydration status (21). In order to compare the parent drug and metabolite amount in the urine, creatinine normalized data were converted to moles (22). Molecular weights used are as follows: oxycodone 315.36 g/mol, noroxycodone 301.33 g/mol and oxymorphone 301.33 g/mol. Mole fractions of oxycodone, oxymorphone and noroxycodone were calculated by the following formula:

\[
\text{mole fraction} = \frac{(\text{mole parent drug or metabolite/g creatinine})}{(\text{mole oxycodone/g creatinine}) + (\text{mole oxymorphone/g creatinine}) + (\text{mole noroxycodone/g creatinine})}.
\]  

Mole fractions were used for data analysis purposes since there is less interindividual variability versus urine concentration (20, 23) and to determine if the proportions of each metabolite change with total exposure or with concurrent use of CYP2D6 and/or CYP3A4/5 inhibitors.

For the age analysis, specimens were divided into three groups: 18–39, 40–64 and 65 years and older (24). For the evaluation of CYP2D6 and CYP3A4/5 substrate and inhibitor use, specimens were separated based on physician-reported medication use. Specimens that did not include CYP2D6 and CYP3A4/5 substrates and inhibitors represented the control group. A list of **Chemical structure and metabolic pathway of oxycodone.**

*Chemical structure and metabolic pathway of oxycodone.*

*Figure 1.*

*Figure 1.* Chemical structure and metabolic pathway of oxycodone.
known CYP2D6 and CYP3A4/5 substrates and inhibitors was obtained from the University of Indiana, School of Medicine (25). The medication history for each individual was evaluated for the listed 48 CYP2D6 substrates, 75 CYP3A4/5 substrates, 36 CYP2D6 inhibitors and 31 CYP3A4/5 inhibitors. Mole fractions based on the presence of CYP2D6 and/or CYP3A4/5 substrate and/or inhibitor use were compared with mole fractions from the control group.

Mole fractions are reported as arithmetic means, medians and 95% confidence intervals. Mole fraction data did not follow a normal distribution. Therefore, the Wilcoxon–Mann–Whitney and Kruskal–Wallis non-parametric statistical tests were conducted to evaluate sex, age and CYP2D6 and CYP3A4 or CYP2D6 and CYP3A4 inhibitor use. Spearman correlations were performed to evaluate associations between urinary pH and mole fractions. Statistical significance was defined as $P \leq 0.05$.

Results
There were 108,923 specimens that met all inclusion and exclusion criteria. Descriptive statistics for oxycodone, oxymorphone and noroxycodone are summarized in Table I. The majority of specimens had detectable oxycodone urine concentrations ($n = 106,852$) resulting in oxycodone mole fractions (arithmetic mean $\pm$ SD) of $0.44 \pm 0.27$. Ninety-eight percent ($n = 106,229$) of specimens had detectable oxymorphone urine concentrations, while 49% ($n = 53,394$) of specimens had detectable noroxycodone urine concentrations. However, mean oxymorphone mole fractions were lower versus noroxycodone mole fractions ($0.31 \pm 0.22$ vs. $0.54 \pm 0.18$; Table I).

Oxycodone, oxymorphone and noroxycodone mole fractions were compared between women ($n = 55,655$) and men ($n = 53,268$). Oxycodone mole fractions were slightly lower in women versus men ($0.43 \pm 0.44$; $P < 0.0001$). Oxymorphone mole fractions were also lower in women versus men ($0.29 \pm 0.32$; $P < 0.0001$). In contrast, women had higher noroxycodone mole fractions versus men ($0.57 \pm 0.5$; $P < 0.0001$).

Mean $\pm$ SD age was $49.1 \pm 12.9$ years. Data are summarized in Table II. Significant differences in oxycodone, oxymorphone and noroxycodone mole fractions were observed. Oxycodone mole fractions were highest in the 40–64 year age group. The oxymorphone mole fractions were highest in the 18–39 year age group, while noroxycodone mole fractions were highest in the 65 years and older age group. Mean $\pm$ SD urinary pH was $6.2 \pm 0.93$. Spearman correlations ($r$) were $-0.16$ ($P < 0.001$), $0.19$ ($P < 0.001$) and $0.12$ ($P < 0.001$) for oxycodone, oxymorphone and noroxycodone mole fractions.

Table I
Summary Statistics for Oxycodone, Oxymorphone and Noroxycodone Mole Fractions

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Oxycodone</th>
<th>Oxymorphone</th>
<th>Noroxycodone</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N$</td>
<td>106,852</td>
<td>106,229</td>
<td>53,394</td>
</tr>
<tr>
<td>Arithmetic mean</td>
<td>0.44</td>
<td>0.31</td>
<td>0.5</td>
</tr>
<tr>
<td>Median</td>
<td>0.37</td>
<td>0.26</td>
<td>0.54</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.27</td>
<td>0.22</td>
<td>0.18</td>
</tr>
<tr>
<td>95% confidence interval</td>
<td>0.43–0.44</td>
<td>0.3–0.31</td>
<td>0.53–0.54</td>
</tr>
<tr>
<td>%CV</td>
<td>61%</td>
<td>71%</td>
<td>33%</td>
</tr>
</tbody>
</table>

Discussion
This was a retrospective study evaluating oxycodone, oxymorphone and noroxycodone distributions in urine. Noroxycodone had the highest mean mole fraction, followed by oxycodone and oxymorphone. These results are consistent with studies reporting higher urine concentrations of noroxycodone and support the observation that noroxycodone is the major metabolite of oxycodone (26, 27). Although noroxycodone is the major metabolite, we observed a prevalence of 49%. The reported prevalence of noroxycodone is higher compared with a previous study (28).

Table II
Mean Oxycodone, Oxymorphone and Noroxycodone Mole Fractions Based on Age

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mole fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oxycodone</td>
</tr>
<tr>
<td>18–39 years</td>
<td>0.46</td>
</tr>
<tr>
<td>$N$</td>
<td>25,861</td>
</tr>
<tr>
<td>40–64 years</td>
<td>0.51</td>
</tr>
<tr>
<td>$N$</td>
<td>70,460</td>
</tr>
<tr>
<td>65 years and older</td>
<td>0.5</td>
</tr>
<tr>
<td>$N$</td>
<td>12,482</td>
</tr>
<tr>
<td>$P$-value$^a$</td>
<td>0.001</td>
</tr>
</tbody>
</table>

$^a$P-values determined from Kruskal–Wallis non-parametric test.
study reporting a prevalence of 36.2% (27). One possible reason for the difference could be CYP2D6-mediated metabolism of noroxycodone to noroxymorphone. However, the current study did not have noroxymorphone data and was unable to determine the fraction of noroxymorphone for subjects. Patients with a CYP2D6 genetic polymorphism resulting in an ultra-rapid metabolizer phenotype would be expected to have little noroxycodone in urine. Another possible reason for the lower prevalence could be concurrent use of a CYP2D6 inducer. This is unlikely as physician-reported CYP2D6 inducer use was excluded. Concurrent use of a CYP3A4/5 inhibitor may also be another reason, but this is unlikely due to the relatively small number of subjects concurrently using oxycodone and a CYP3A4/5 inhibitor (Table III).

Oxycodone, oxymorphine and noroxycodone mole fractions were significantly different based on sex. Women had lower oxycodone and oxymorphine mole fractions compared with men. Women also had higher noroxycodone mole fractions. Women might have increased CYP3A4/5-mediated metabolism of oxycodone to noroxycodone versus men as there is suggestion that women have higher CYP3A4/5 activity (28). These results add to the current conflicting literature reported in plasma that support or negate sex-related differences in CYP2D6 activity (29, 30). The magnitude of these differences in the current study was small, which suggests lack of clinical significance. However, due to the narrow therapeutic index of oxycodone, close monitoring of oxycodone analgesic effects and toxicity is warranted.

Hepatic function, renal function, liver mass and hepatic blood flow are known to decrease with age (31). These factors contribute to oxycodone, oxymorphine and noroxycodone pharmacokinetic changes in plasma (29, 30). In one study, oxycodone and noroxycodone plasma concentrations and/or exposure were significantly higher in older patients compared with patients aged 20–40 years of age (30). To our knowledge, this is the first study to evaluate the effects of age on urinary oxycodone, oxymorphine and noroxycodone. Our results are consistent with previous findings in plasma regarding higher oxycodone and noroxycodone mole fractions in the 65 years and older age group (Table II). Age-related changes due to decreased CYP2D6 and CYP3A4/5 activity may not be contributory factors as one study reported a lack of age-dependent changes in CYP2D6 and CYP3A4/5 activity (32).

Urinary pH was associated with oxycodone and metabolite mole fractions. Although statistical significance was observed, the $r$ values were weak ($r = -0.16–0.19$). Caution is needed in the interpretation of statistically significant, but weak $r$ values as correlation coefficients describe the strength and direction of an association between independent and dependent variables. Correlation coefficients are not a measure of predictive performance. Taken together, the association of urinary pH to oxycodone and metabolite mole fractions is minor.

Interindividual variability of oxycodone pharmacokinetics and pharmacodynamics is due, in part, to CYP2D6- and CYP3A4/5-mediated drug–drug interactions (2, 18, 33). In theory, oxymorphine metabolism formation would decrease upon concurrent administration of a CYP2D6 substrate due competition for binding sites on the CYP2D6 enzyme between oxycodone and the CYP2D6 substrate. However, there was no difference in oxycodone and oxymorphine mole fractions compared with the control group (Table III). Oxycodone and oxymorphine mole fractions may not be robust enough to detect subtle perturbations in CYP2D6 activity with a CYP2D6 substrate. In contrast, noroxycodone mole fractions increased. This is probably due to a compensatory mechanism involving CYP3A4/5-mediated metabolism to form noroxycodone. Upon administration of oxycodone and a CYP2D6 inhibitor, we observed higher oxycodone and noroxycodone fractions and lower oxymorphine fractions (Table III). Upon concurrent use of a CYP2D6 substrate and CYP2D6 inhibitor, the magnitude of change in mole fractions were further altered, suggesting an additive impact on CYP2D6 metabolism.

Lower oxymorphine and higher noroxycodone mole fractions were observed with CYP3A4/5 substrate use (Table III). These results are surprising, as one would expect noroxycodone metabolite formation to decrease upon concurrent administration of a CYP3A4/5 substrate due competition for enzymatic-binding sites. A compensatory mechanism would also be expected to occur involving CYP2D6-mediated metabolism to
form more oxymorphone. However, the magnitude of mean mole fraction change with oxymorphone (0.31 vs. 0.33) and noroxycodone (0.52 vs. 0.51) is small compared to the control group (Table III) and may not be of clinical significance. Upon administration of oxycodone and a CYP3A4/5 inhibitor, there was no difference in any of the evaluated mole fractions (Table III). These results were based on a low sample size (n = 22–44) and should be interpreted with caution. We speculate that with an appropriate sample size, one would observe lower noroxycodone mole fractions upon concurrent CYP3A4/5 inhibitor use.

In the current study, dual CYP2D6 and CYP3A4/5 inhibitor use significantly affected oxycodone and oxymorphone, but not noroxycodone mole fractions compared with control (Table III). Dual CYP2D6 and CYP3A4/5 inhibitor use versus CYP2D6 inhibitor use resulted in no difference in oxycodone, oxymorphone and noroxycodone mole fractions. Upon comparison with CYP3A4/5 inhibitor use, lack of statistical significance was also observed. These results contrast with a study reporting dual inhibition of oxycodone by paroxetine (a CYP2D6 inhibitor) and itraconazole (CYP3A4/5 inhibitor) results in a substantial increase in oxycodone exposure (18) compared with exclusive CYP2D6 inhibition. However, comparisons between studies are difficult due to different subject populations (patients versus healthy adults) and differences in oxycodone administration (single dose versus presumed multiple dose).

The current study lacked dosing administration times of oxycodone. There may also be variation in the timing of oxycodone relative to CYP substrate or inhibitor administration. Whether these factors impacted oxycodone, oxymorphone and/or noroxycodone mole fractions is unknown. Another study limitation was the presumption of medication use by documentation from physician-reported medication lists. Some subjects included in the study may have been taking oxymorphone with undocumented use on the medication list. Additionally, subjects who were taking oxycodone may have been study exclusions on the basis of undocumented use on the medication list.

Other study limitations include lack of data regarding a subject’s CYP2D6 and CYP3A4/5 genotype and lack of qualitative and/or quantitative analysis of medications that were listed on the physician-reported medication lists as CYP2D6 and CYP3A4/5 substrates and inhibitors. We acknowledge an ideal situation would be to have information on an individual’s CYP2D6 and CYP3A4/5 genotype as well as qualitative and/or quantitative analysis of all medications a patient is reported to be taking. However, this is unrealistic. With few exceptions, CYP2D6 and/or CYP3A4/5 genotyping is performed on a reactive, rather than screening basis. In examples with other medications such as antidepressants and tamoxifen, with known CYP2D6 genotype as well as qualitative and/or quantitative analysis of all medications a patient is reported to be taking. However, this is unrealistic. With few exceptions, CYP2D6 and/or CYP3A4/5 genotyping is performed on a reactive, rather than screening basis. In examples with other medications such as antidepressants and tamoxifen, with known alterations in efficacy based on CYP2D6 genotype (34, 35), there are no formal recommendations to genotype all patients prior to starting or who are currently taking these medications. Qualitative and/or quantitative analysis of medications that were listed on the physician-reported medication list is also unrealistic. Medication history is a tool to evaluate drug–drug interaction potential and has been reported. One study evaluated the drug–drug interaction potential of immunosuppressants in a hematopoietic stem cell transplant population. Concurrent medication use was identified by medication history reporting and not by any qualitative and/or quantitative analysis (36).

Conclusions
This was a retrospective study to evaluate sex, age, urinary pH and concurrent medication use on oxycodone, oxymorphone and noroxycodone distributions in urine. Noroxycodone had the highest mole fraction, followed by oxycodone and oxymorphone. Women had lower oxycodone and oxymorphone, but higher noroxycodone mole fractions. However, the magnitude of these differences was small. Oxycodone and noroxycodone mole fractions where higher in the 65 years and older age group compared with 18–39 year age group. Increased awareness of concurrent use of oxycodone and other medications that are metabolized by CYP2D6 and/or CYP3A4/5 is recommended, in light of the observed differences in oxycodone and metabolite mole fractions during various CYP2D6 and/or CYP3A4/5 conditions.

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